JAMA | Preliminary Communication

Effect of Ganciclovir on IL-6 Levels Among Cytomegalovirus-Seropositive Adults With Critical Illness
A Randomized Clinical Trial

Ajit P. Limaye, MD; Renee D. Stapleton, MD, PhD; Lili Peng, MS; Scott R. Gunn, MD; Louise E. Kimball, PhD; Robert Hyzy, MD; Matthew C. Exline, MD; D. Clark Files, MD; Peter E. Morris, MD; Stephen K. Frankel, MD; Mark E. Mikkelsen, MD, MSCE; Duncan Hite, MD; Kyle B. Enfield, MD; Jay Steingrub, MD; James O'Brien, MD, MSc; Polly E. Parsons, MD; Joseph Cuschieri, MD; Richard G. Wunderink, MD; David L. Hotchkin, MD; Ying Q. Chen, PhD; Gordon D. Rubenfeld, MD; Michael Boeckh, MD

IMPORTANCE The role of cytomegalovirus (CMV) reactivation in mediating adverse clinical outcomes in nonimmunosuppressed adults with critical illness is unknown.

OBJECTIVE To determine whether ganciclovir prophylaxis reduces plasma interleukin 6 (IL-6) levels in CMV-seropositive adults who are critically ill.

DESIGN, SETTING, AND PARTICIPANTS Double-blind, placebo-controlled, randomized clinical trial (conducted March 10, 2011-April 29, 2016) with a follow-up of 180 days (November 10, 2016) that included 160 CMV-seropositive adults with either sepsis or trauma and respiratory failure at 14 university intensive care units (ICUs) across the United States.

INTERVENTIONS Patients were randomized (1:1) to receive either intravenous ganciclovir (5 mg/kg twice daily for 5 days), followed by either intravenous ganciclovir or oral valganciclovir once daily until hospital discharge (n = 84) or to receive matching placebo (n = 76).

MAIN OUTCOMES AND MEASURES The primary outcome was change in IL-6 level from day 1 to 14. Secondary outcomes were incidence of CMV reactivation in plasma, mechanical ventilation days, incidence of secondary bacteremia or fungemia, ICU length of stay, mortality, and ventilator-free days (VFDs) at 28 days.

RESULTS Among 160 randomized patients (mean age, 57 years; women, 43%), 156 received 1 or more dose(s) of treatment, and 132 (85%) completed the study. The mean between-group change in IL-6 level was not significantly different. Among secondary outcomes, CMV reactivation in plasma was significantly lower in the ganciclovir group. The ganciclovir group had more VFDs in both the intention-to-treat population and in the prespecified sepsis subgroup. There were no significant between-group differences in other secondary outcomes.

	Placebo Group (n = 72)	Ganciclovir Group (n = 84)	Absolute Difference (95% CI)[a]	*P* Value
Primary Outcome at Day 14				
Difference in IL-6 level, $\log_{10}$ units, mean (95% CI)	−0.79 (−2.14 to 0.56)	−0.79 (−2.06 to 0.48)	0 (−0.3 to 0.2)	>.99
Secondary Outcomes at Day 28				
Any CMV reactivation, No. (%)	28 (39)	10 (12)	−27 (−40 to −14)	<.001
Mechanical ventilation days, median (IQR)	6 (3 to 12)	5 (3 to 9)	−1 (−3 to −1)	.16
VFDs, median (IQR)	20 (8 to 24)	23 (16 to 25)	3 (0 to 6)	.05
Sepsis subgroup analysis	20 (9 to 24)	23 (16 to 25)	3 (0 to 4)	.03
ICU length of stay, median (IQR), d	8 (5 to 15)	8 (4 to 14)	0 (−4 to 2)	.76
Secondary bacteremia or fungemia, No. (%)	11 (15)	13 (15)	0 (−10 to 10)	.67
Mortality, No. (%)	11 (15)	10 (12)	−3 (−14 to 7)	.54

CONCLUSIONS AND RELEVANCE Among CMV-seropositive adults with critical illness due to sepsis or trauma, ganciclovir did not reduce IL-6 levels and the current study does not support routine clinical use of ganciclovir as a prophylactic agent in patients with sepsis. Additional research is necessary to determine the clinical efficacy and safety of CMV suppression in this setting.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01335932

JAMA. 2017;318(8):731-740. doi:10.1001/jama.2017.10569

Editorial page 709

Supplemental content

CME Quiz at jamanetwork.com/learning

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Ajit P. Limaye, MD, University of Washington, PO Box 356174, 1959 NE Pacific St, Seattle, WA 98195-6174 (limaye@u.washington.edu).

Cytomegalovirus (CMV) is a widely prevalent human herpesvirus that is found in 50% to 80% of otherwise healthy adults. In immunocompetent persons, primary infection is usually asymptomatic and is followed by lifelong latent infection in multiple sites, including the lung.[1-3] Latent CMV commonly reactivates during immunosuppression or critical illness, and multiple observational studies have found an association of CMV reactivation with worse clinical outcomes, including increased mortality, longer duration of mechanical ventilation, increased intensive care unit (ICU) and hospital length of stay, and higher rates of secondary infections.[4-8] An animal model of sepsis-induced CMV reactivation has identified lung inflammation and injury, and demonstrated that these effects can be attenuated by antiviral drug (ganciclovir) given as prophylaxis.[9-11] A controlled clinical trial of CMV prevention or treatment is ultimately required to definitively assess a causal relationship between CMV reactivation and adverse clinical outcomes.

ARDS *acute respiratory distress syndrome*

BAL *bronchoalveolar lavage*

CMV *cytomegalovirus*

IL-6 *interleukin 6*

Before proceeding to definitive phase 3 efficacy studies of CMV prevention in the ICU setting, several issues need to be addressed including preliminary assessment of toxicity of the primary CMV antiviral drug ganciclovir and feasibility of rapidly identifying CMV-seropositive status. Additionally, identification of specific clinical outcomes that might be affected and estimates of the relative effect of CMV suppression by ganciclovir are necessary to rationally select end points and sample sizes for future phase 3 studies.

The Ganciclovir/Valganciclovir for Prevention of Cytomegalovirus Reactivation in Acute Injury of the Lung (GRAIL) study was designed as a phase 2 clinical trial to assess the safety and feasibility of an antiviral drug given as CMV prophylaxis, define the study population, and explore potential clinical end points for future definitive phase 3 trials. Interleukin 6 (IL-6) was chosen as the primary end point because it has been associated with mortality in prior ICU studies,[12] there are preliminary data linking CMV reactivation with increased IL-6 levels,[13-15] and a phase 2 study could be sufficiently powered to detect a significant difference for a biomarker end point.

Key Points

Question Does prevention of cytomegalovirus (CMV) reactivation with ganciclovir reduce plasma interleukin 6 (IL-6) levels among CMV-seropositive adults with critical illness due to sepsis or trauma?

Findings In this multicenter randomized clinical trial of 160 adults, treatment with ganciclovir vs placebo did not significantly reduce plasma IL-6 levels (mean change from days 1 to 14, −0.79 and −0.79 $\log_{10}$ units, respectively).

Meaning Treatment with ganciclovir in adults who were critically ill did not significantly reduce IL-6 levels, and these findings do not support routine prophylactic use in this setting.

Methods

Study Design

The investigator-initiated randomized clinical trial was conducted to compare ganciclovir or valganciclovir vs placebo for preventing CMV reactivation in adults with respiratory failure, including those with acute respiratory distress syndrome (ARDS). All protocol modifications and amendments for this study were approved by both the central institutional review board (Fred Hutchinson Cancer Center) and the data and safety monitoring board, then by each site's local institutional review board (Supplement 1 and Supplement 2). Initial written informed consent was obtained from the patient's legally authorized representative. Subsequent written consent from the patient was obtained when possible. The study was conducted with the Fred Hutchinson Cancer Research Center as the coordinating center, and its Statistical Center for HIV/AIDS Research and Prevention (SCHARP) provided internet-based randomization and data capture, assurance of data accuracy and completeness, and biostatistical analysis.

Study Participants

Nonimmunocompromised adults hospitalized with respiratory failure and severe sepsis were screened for eligibility. On July 9, 2012, because of slower-than-anticipated accrual, the inclusion criteria were amended to add patients with respiratory failure and severe trauma (Injury Severity Score >15 within a 24-hour period), based on prior studies demonstrating similar rates of CMV reactivation in this population.[16] Patients were enrolled from March 10, 2011, to April 29, 2016, with follow-up of the last enrolled patient on October 27, 2016.

Key inclusion criteria included patient or next of kin informed consent, being older than 18 years, CMV IgG-seropositive, intubated and requiring mechanical positive pressure ventilation, meeting criteria for either severe sepsis or trauma with respiratory failure and an Injury Severity Score of more than 15 within a 24-hour period, and not eligible for a spontaneous breathing trial or failed a spontaneous breathing trial on the day of randomization. Exclusion criteria included body mass index (calculated as weight in kilograms divided by height in meters squared) more than 60, known or suspected immunosuppression, survival expectation of less than 72 hours, hospitalized for more than 120 hours, neutropenia (absolute neutrophil count <1000/mm^3), use of anti-CMV drugs, tracheostomized patients on continuous 24-hour positive pressure chronic mechanical ventilation, diagnosis of Child class C cirrhosis, or preexisting interstitial lung disease.

The study was reviewed biannually by an independent data and safety monitoring board. Reporting of race, ethnicity, and sex is required for all National Institutes of Health–supported clinical research. Because the participant was intubated at the time of enrollment, self-reporting of race and ethnicity was not feasible. The designation of race was obtained through family members or medical records. The categorization was based on fixed categories as listed on the case report form reflecting established federal categories.

ARDS was not a study inclusion criterion, although the majority of patients would likely have met ARDS criteria. The America-European Consensus Conference on ARDS definition for ARDS was used because this study was begun in mid-2011, which was before the Berlin definition was published in June 2012. It is unlikely to have any significant effect on the trial outcome because the only significant difference in definitions pertaining to this trial is the requirement for a minimal level of positive end-expiratory pressure and none of our patients were enrolled with a positive end-expiratory pressure measurement less than 5 mm Hg.

Randomization

Enrolled patients were randomized in a 1:1 ratio via a web-based system at SCHARP to receive either study drug or placebo. Randomization was concealed and stratified by study site and trauma vs severe sepsis.

Study Interventions

Intravenous ganciclovir was provided initially by Genentech and later was purchased commercially by the study; matching placebo (saline) was formulated by the study site pharmacies. Valganciclovir and matching placebo were also provided initially by Genentech and distributed to each clinical site from the central site pharmacy. Study drug was started within 24 hours of randomization. The initial study design mandated at least 14 days of study drug, with a maximum of 28 days. For the initial 5 days of treatment, the dose was 5 mg/kg of intravenous ganciclovir (based on total body weight at the time of ICU admission) or intravenous placebo every 12 hours. After 5 days, the dose was reduced to 5 mg/kg of intravenous ganciclovir or intravenous placebo once daily. Ganciclovir doses were adjusted according to estimated renal function as per package insert. After day 5, patients could be switched to 900 mg of oral valganciclovir or placebo by mouth until day 14 or until hospital discharge (maximum of 28 days), whichever occurred later.

The study was modified on March 31, 2014, to include only intravenous ganciclovir (purchased commercially) because the initial batch of oral study drug had expired. Patients enrolled after this date received only intravenous study drug and only until the time of hospital discharge. Patients who were discharged prior to day 14 would have received less than 14 days of study drug.

Other Study Methods

CMV serostatus was assessed during screening using commercially available enzyme-linked immunoassays for IgG antibodies to CMV, and interpreted according to manufacturer recommendations. CMV viral load was quantitated in plasma, bronchoalveolar lavage (BAL), and throat swabs using a previously validated assay.[17]

Study Outcomes

In the absence of definitively accepted surrogate end points for ARDS clinical trials, and for the reasons outlined previously, we used a biological primary end point (plasma IL-6 level) as recommended in the funding opportunity announcement (RFA-HL-10-003) in studies of ventilator-induced lung injury.[18] The primary end point was the change in plasma IL-6 levels between baseline and day 14 between the ganciclovir and placebo groups. Secondary clinical outcomes included incidence of CMV reactivation in plasma, throat, endotracheal tube aspirate samples, and BAL; duration of mechanical ventilation; ventilator-free days during the first 28 days after ICU admission; incidence of bacteremia or fungemia; ICU and hospital length of stay; organ system failure scores at day 7 and 14; and mortality though day 180. Ventilator-free days were calculated as previously published.[19] Additional prespecified analyses of primary and secondary outcomes included the subset of patients with sepsis, patients who survived at least 7 days after randomization, and patients who were mechanically ventilated for at least 7 and 14 days after randomization. Specified key clinical outcomes according to the intention-to-treat (ITT) population and the sepsis subgroup (as described in the protocol and statistical analysis plan) are reported except for functional assessment or quality of life at day 180 and the multiple organ dysfunction scores at day 14 and 28. The multiple organ dysfunction score was not able to be assessed because total bilirubin (a component of the score) was not collected as planned.

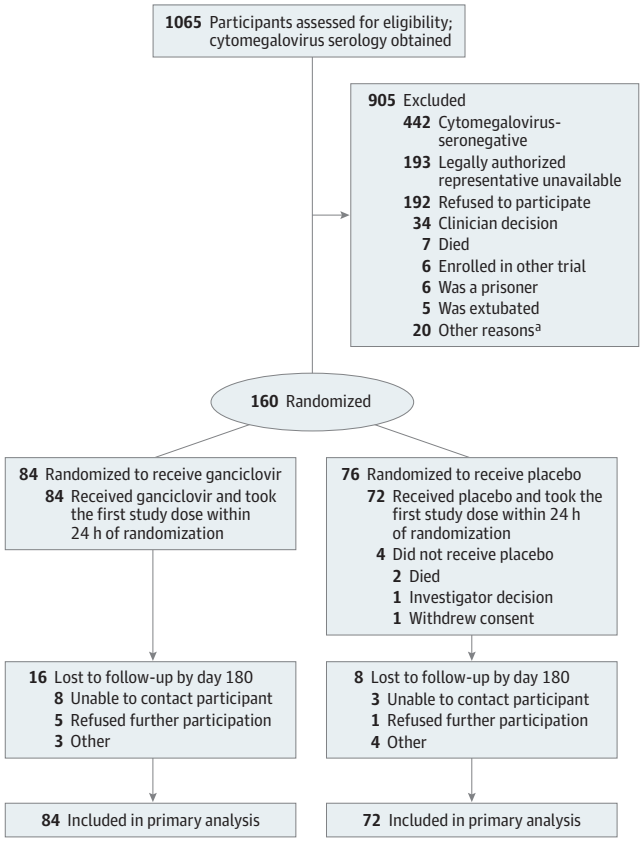
Given the known toxicity profile of ganciclovir, safety was assessed by comparing transfusion requirements and incidence of neutropenia between groups. Exploratory post hoc analyses included the ratio of arterial oxygen partial pressure to fractional inspired oxygen (Pa_{O_2}/Fi_{O_2} ratio) and duration of mechanical ventilation stratified by survival status by 28 days, as previously described.[20,21]

Statistical Analysis

This phase 2 randomized trial planned to enroll a total of 160 study patients. The ITT population included all randomized patients who received at least 1 dose of study drug. For the primary end point of the change in plasma IL-6 level from day 1 to 14, this trial was expected to have 80% power to detect at least a 16% difference between the 2 trial groups, with a 2-sided *t* test and an α of .05, assuming 10% of study dropout by day 14. As a benchmark, the effect size (measured in percentage of reduction in mean plasma IL- 6 levels from days 1 to 3) in a large randomized trial[12] of standard vs lung protective ventilation was used. In that trial, 26% reduction in mean plasma IL-6 levels between enrollment and day 3 was associated with a 22% reduction in mortality between study groups. The mean and SD in IL-6 levels at day 1 and day 3 were estimated from log-transformed data from the 6 mL/kg/min groups of the trial. The rate of decline in IL-6 levels was assumed to be linear over time.

For the GRAIL study, a semiparametric calculation was used to calculate the power based on the difference in the change in IL-6 levels, under the assumption of a 10% dropout rate with a 2-sided test and an α of .05. The SDs at different days and the interperson correlations were used to calculate the SD of the difference between baseline and day 14. With the sample size of 160 patients, there was also at least 82% power to detect a relative risk of 0.3 in the incidence of CMV reactivation between the ganciclovir group and the placebo group,

Figure 1. Flow of Patients in the Ganciclovir to Prevent Reactivation of Cytomegalovirus in Adults With Critical Illness (GRAIL) Randomized Clinical Trial



[a] Other reasons included out of time window, do-not-resuscitate or do-not-intubate order, no longer septic, non-English speaking, transfer to other hospital, home ventilation, and unable to perform study procedures.

assuming the rate of CMV reactivation was 25% for placebo. The sample size of 160 patients also provided meaningful minimal detectable difference in some key secondary clinical end points between the 2 groups. For example, for the outcome of ventilation-free days, this trial would have been able to detect a difference of 4.5 days between the 2 groups with 80% power, and a difference of 5.2 days with 90% power. To handle missing data, we verified that missing IL-6 level values at day 14 occurred at random. For the primary end point (between-group change in IL-6 level from day 1 to 14), only those patients who had values at both time points were included. We opted against imputation in accordance with the protocol's statistical analysis plan.

An interim safety analysis was planned when about 50% of the expected deaths of 48 patients occurred, via the Lan-Demets implementation of the Pocock lower boundary.[22] Specifically, for the total of 24 events at the interim analysis, to reach the Pocock boundary for a lower death rate in the ganciclovir group, the placebo group would need to have at least 10 excess events (7 in the ganciclovir group vs 17 in the placebo group); to reach the Pocock boundary for a lower death rate in the placebo group, the ganciclovir group would need to have at least 10 excess events (7 in the placebo group vs 17 in the ganciclovir group).

Summary statistics were calculated in proportions, means, or event rates in person-years, depending on the variables being binary, continuous, or count, respectively. The primary data analysis was performed for all enrolled patients on an ITT basis. To test for whether the mean difference in the primary end point (ganciclovir vs placebo) differed from 0, a 2-sample t test was used. Secondary and safety end points were compared by t test, χ^2 test, and log-rank test, when they were continuous, categorical, and time-to-event type, respectively. Kaplan-Meier estimates were calculated to summarize the distribution of cumulative incidences during the trial follow-up. A P value less than .05 was considered statistically significant. All testing was 2-sided. In accordance with the statistical plan, we did not include specific correction for multiple comparisons for the prespecified secondary outcomes or other exploratory analyses. Thus, results of the secondary analyses should be considered exploratory. Additional methods are shown in Supplement 1. The statistical software was SAS (SAS Institute), version 9.4 TS Level 1M2, and R (R Foundation), version 3.3.2.

Results

Participants

The flow of patients in the clinical trial is shown in **Figure 1**. The baseline characteristics of the ITT and sepsis subgroup populations are shown in **Table 1**. The prespecified subgroup

Table 1. Baseline Characteristics of Patients With Critical Illness Overall and in the Sepsis Subgroup

Characteristic	Intention-to-Treat Group (n = 156)		Sepsis Subgroup (n = 137)	
	Placebo Group (n = 72)	Ganciclovir Group (n = 84)	Placebo Group (n = 66)	Ganciclovir Group (n = 71)
Age, median (IQR), y	58 (51-68)	57 (45-70)	57 (48-66)	61 (46-72)
Men, No. (%)	40 (56)	49 (58)	37 (56)	37 (52)
Race, No. (%)				
White	62 (86)	71 (85)	57 (86)	62 (87)
Nonwhite	10 (14)	13 (15)	9 (14)	9 (13)
Severe sepsis or septic shock, No. (%)	66 (92)	71 (85)	66 (100)	71 (100)
Trauma, No. (%)	6 (8)	13 (15)		
Apache III score, mean (SD)[a]	71.12 (24.55)	77.10 (28.86)	73.44 (24.26)	77.56 (28.62)
MODS score on day 1, mean (SD)[b]	12.63 (3.33)	12.77 (3.23)	12.57 (3.41)	12.91 (3)
Baseline plasma IL-6 levels, mean (SD), $\log_{10}$ units	1.7 (0.8)	1.48 (0.61)	1.71 (0.82)	1.45 (0.63)
Time from ICU admission to enrollment, median (IQR), d	2 (2-4)	3 (2-4)	2 (2-3)	3 (2-3)
CMV reactivation in plasma at enrollment, No. (%)[c]	3 (4)	7 (8)	3/66 (5)	7/71 (10)
CMV reactivation in endotracheal aspirate or BAL sample at enrollment, No./Total No. (%)	12/60 (20)	14/70 (20)	10/56 (18)	14/59 (24)
Pao_2/Fio_2 ratio at enrollment, median (IQR), mm Hg	200 (149-251)	186 (141-242)	189 (135-244)	200 (160-268)

Abbreviations: BAL, bronchoalveolar lavage; CMV, cytomegalovirus; ICU, intensive care unit; IL-6, interleukin 6; IQR, interquartile range; MODS, multiple organ dysfunction score.

[a] Apache III score is used to calculate predicted mortality risk of patients who are critically ill in an ICU setting (score range, 0 to 299; higher scores indicate more severe disease and a higher risk of death).

[b] MODS is used to predict mortality and duration of ICU stay by patients with multiorgan dysfunction (score range, 0 to 24; higher scores indicate ICU stay and a higher risk of mortality).

[c] CMV detected by polymerase chain reaction assay at any level above the level of detection was considered a positive result.

with severe sepsis composed 88% of the study population, and 6% of all patients already had CMV reactivation in plasma at the time of randomization. Of 156 enrolled and dosed patients, 132 (85%) completed the study (ie, either died before or had vital status confirmed at day 180) and were evaluable for vital status at day 180.

Primary Outcome

Of the 129 patients who survived and did not withdraw consent by day 14, 88 (68%; 48 in the ganciclovir group and 40 in the placebo group) had samples available for both day 1 and day 14 to assess the primary end point. Details of missing patient specimens for the primary end point are shown in Supplement 3. The mean plasma IL-6 levels on day 1 for the ganciclovir and placebo groups are shown in Table 1. The mean plasma IL-6 level on day 14 in the ganciclovir group was 0.69 $\log_{10}$ units (95% CI, 0 to 1.79) and placebo group was 0.91 $\log_{10}$ units (95% CI, 0 to 2.13), also shown in eFigure 3 in Supplement 3. The mean within-group change in plasma IL-6 levels (from day 1 to day 14) was −0.79 $\log_{10}$ units (95% CI, −2.06 to 0.48) in the ganciclovir group and −0.79 $\log_{10}$ units (95% CI, −2.14 to 0.56) in the placebo group (point estimate of difference, 0 [95% CI, −0.29 to 0.29]; $P > .99$).

Secondary Outcomes

CMV Reactivation

The cumulative incidence of CMV reactivation and effect of ganciclovir are shown in **Figure 2**; and eFigure 1 in Supplement 3. CMV reactivation in plasma was significantly lower in the ganciclovir group than in the placebo group: 12% (10 of 84 patients) in the ganciclovir group vs 39% (28 of 72 patients) in the placebo group; absolute risk difference, −27 (95% CI, −40 to −14). Ganciclovir was associated with a reduction in both low-grade and high-grade (>1000 IU/mL) reactivation in plasma, lung (defined as endotracheal tube aspirate or BAL), and throat specimens. The effect of ganciclovir on CMV viral load in different compartments (at any site, lung, throat) is shown in eFigure 2 in Supplement 3.

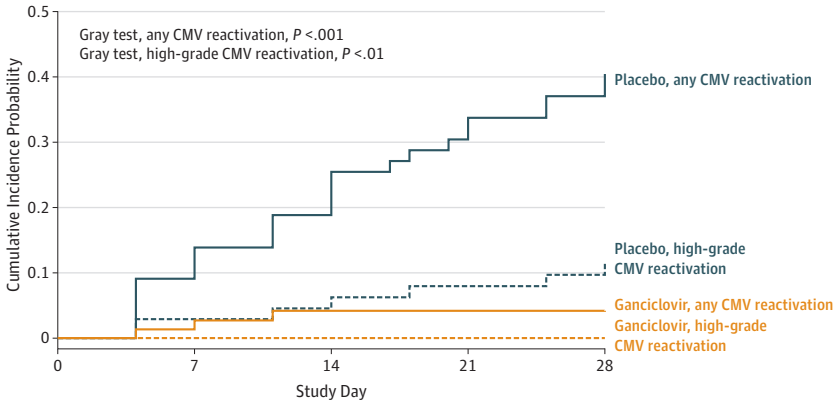
Clinical Outcomes

The key secondary clinical outcomes for the ITT population and the prespecified sepsis subgroup are shown in **Table 2**. The median ventilator-free days in the ITT group (23 days [95% CI, 16 to 25] in the ganciclovir group vs 20 days [95% CI, 8 to 24] in the placebo group; $P = .05$) and sepsis subgroup (23 days [95% CI, 16 to 25] in the ganciclovir group vs 20 days [95% CI, 9 to 24] in the placebo group; $P = .02$) were significantly higher in the ganciclovir group. A Kaplan-Meier mortality analysis is shown in **Figure 3**. Box and whisker plots of the duration of mechanical ventilation for the ITT group and sepsis subgroup are shown in **Figure 4**A. Ventilator-free days for the ITT group and sepsis subgroup are shown in Figure 4B.

Post Hoc Exploratory Analyses

The duration of mechanical ventilation, stratified by survival status at day 28, separately for the ITT group and sepsis subgroup, is shown in eTable 1 in Supplement 3. Among those who died, there was no significant difference in the duration of mechanical ventilation for both the ITT group and sepsis subgroup who received ganciclovir. In contrast, among survivors,

Figure 2. Cumulative Incidence of Any Cytomegalovirus (CMV) Reactivation[a] and High-Grade CMV Reactivation in Plasma[b] Through Day 28



No. of participants					
Any CMV reactivation					
Ganciclovir	77	72	62	60	60
Placebo	69	57	49	42	37
CMV reactivation >1000 IU/mL (high-grade reactivation)					
Ganciclovir	83	79	70	68	67
Placebo	72	62	56	54	51

Baseline positive DNA detections were excluded from this analysis. The median duration of follow-up for CMV reactivation at any level was 28 days (range, 1 to 28) for the ganciclovir group and 28 days (range, 1 to 28) for the placebo group. The median duration of follow-up for high-grade CMV reactivation was 28 days (range, 1 to 28) for the ganciclovir group and 28 days (range, 1 to 28) for the placebo group.

[a] CMV DNA was quantified in specimens using a previously published polymerase chain reaction assay method. The frequency of assessment was every 3 days until day 35 for plasma; day 1 and then every fourth day while intubated for endotracheal tube aspirate samples; days 1 and 7 only for the first 10 patients for bronchoalveolar lavage; and every 3 days until day 35 for throat.

[b] Excluding baseline positive DNA detection.

Table 2. Primary and Secondary Outcomes Among Patients With Critical Illness Receiving Ganciclovir vs Placebo Overall and in the Sepsis Subgroup

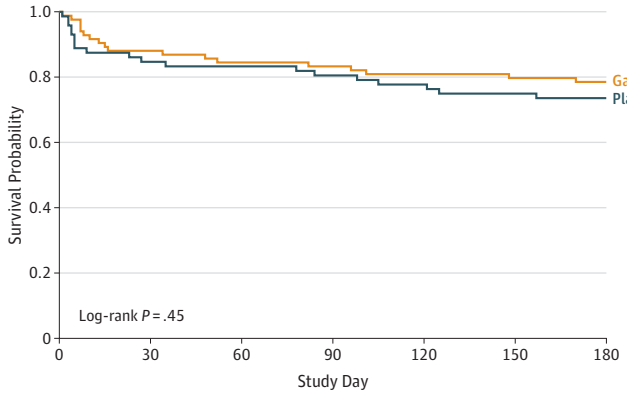
	Intention-to-Treat Group (n = 156)				Sepsis Subgroup (n = 137)			
	Placebo Group (n = 72)	Ganciclovir Group (n = 84)	Absolute Difference (95% CI)	*P* Value	Placebo Group (n = 66)	Ganciclovir Group (n = 71)	Absolute Difference (95% CI)	*P* Value
Primary Outcome at Day 14								
Difference in plasma IL-6 level, mean, $\log_{10}$ units	−0.79 (−2.14 to 0.56)	−0.79 (2.06 to 0.48)	0 (−0.3 to 0.2)	>.99	−0.88 (−2.23 to 0.47)	−0.81 (−2.20 to 0.58)	0.1 (−0.2 to 0.2)	.83
Secondary Outcomes at Day 28								
Cumulative incidence of any plasma CMV reactivation, No. (%)	28 (39)	10 (12)	−27 (−40 to −14)	<.001	26 (39)	10 (14)	−25 (−40 to −11)	<.001
Mechanical ventilation duration, median (IQR), d[a]	6 (3 to 12)	5 (3 to 9)	−1 (−3 to −1)[b]	.16	6 (3 to 11)	5 (3 to 8)	−1 (−4 to 0)	.06
Ventilator-free duration, median (IQR), d[a]	20 (8 to 24)	23 (16 to 25)	3 (0 to 6)	.05	20 (9 to 24)	23 (16 to 25)	3 (0 to 4)	.03
ICU length of stay, median (IQR), d[a]	8 (5 to 15)	8 (4 to 14)	0 (−4 to 2)	.76	8 (5 to 14)	7 (4 to 12)	−1 (−4 to 1)[b]	.36
Hospital length of stay, median (IQR), d[a]	13 (8 to 23)	14 (8 to 22)	1 (−1 to 1)	.92	13 (8 to 22)	13 (8 to 20)	0 (−1 to 1)	.76
Secondary bacteremia or fungemia, No. (%)	11 (15)	13 (15)	0 (−10 to 10)	.97	9 (14)	10 (14)	0 (−10 to 10)	.96
Mortality, No. (%)	11 (15)	10 (12)	−3 (−14 to 7)	.54	10 (15)	9 (13)	−2 (−14 to 9)	.68
Composite end point of mortality and >7 d of mechanical ventilation or >50% increase in IL-6 level, No. (%)	49 (68)	42 (50)	−18 (−33 to −3)	.02	44 (67)	34 (48)	−19 (−35 to −3)	.04

Abbreviations: CMV, cytomegalovirus; ICU, intensive care unit; IL-6, interleukin 6; IQR, interquartile range.

[a] For the specific outcomes that were measured in days, the status of that outcome was assessed in a standardized manner across all study sites at 8 AM local time, and expressed as days in whole numbers (ie, fractions of days were not assessed). For example, for mechanical ventilation duration, a patient who had been mechanically ventilated for the prior 4 days and was assessed to not be mechanically ventilated at the 8 AM assessment on day 5 would be recorded as 4 days, even if discontinuation occurred between assessments (ie, assessment at durations <1 day were not recorded).

[b] The 95% CI for the between-group difference was estimated by bootstrapping methods.

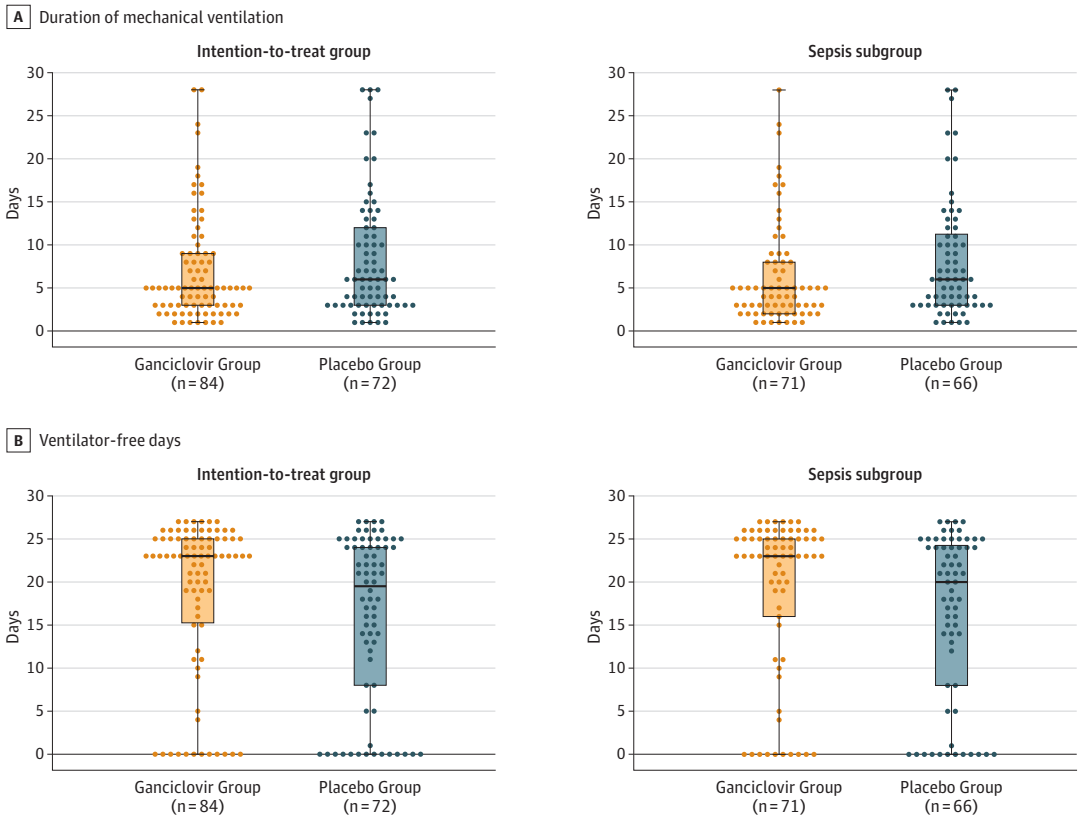
Figure 3. Kaplan-Meier Curve of Mortality by Treatment Group Through Day 180



No. of participants							
Ganciclovir	84	74	71	70	68	67	66
Placebo	72	61	60	58	56	54	53

Median (interquartile range) duration of follow-up was 180 days (89-180) for the ganciclovir group and 180 days (91-180) for the placebo group.

Figure 4. Duration of Mechanical Ventilation and Ventilator-Free Days Through Day 28[a]



The *P* value was .16 for panel A, .06 for panel B, .05 for panel C, and .03 for panel D.

[a] Error bars indicate minimum and maximum values; boxes, the interquartile range; horizontal lines, median. Dots indicate individual patients.

there was a statistically significantly shorter duration of mechanical ventilation in the ganciclovir group. To explore a potential effect of CMV suppression on the degree of pulmonary dysfunction, the serial Pao_2/Fio_2 ratio during the first 7 days of mechanical ventilation was assessed (eFigure 4 in Supplement 3).

Safety and Tolerability

The rates of the predefined key hematological and other safety assessments are shown in **Table 3**. There was no statistically significant increase in transfusion requirements, neutropenia, or prespecified study medication-related adverse events in the ganciclovir group.

Table 3. Safety Assessments Among Patients With Critical Illness Receiving Ganciclovir vs Placebo

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

Abbreviation: IQR, interquartile range.

[a] Absolute neutrophil count less than 1000/mm^3.

[b] Defined as glomerular filtration rate less than 60 mL/min.

[c] Defined per protocol, see Supplement 1 and 2.

Discussion

In this phase 2 clinical trial of CMV-seropositive adults who were critically ill with sepsis or trauma and respiratory failure, administration of ganciclovir compared with placebo did not significantly reduce IL-6 levels, the primary outcome. Among the secondary outcomes, ganciclovir was associated with a statistically significant reduction in the proportion of patients with CMV reactivation in blood, and ganciclovir was also associated with a significant increase in ventilator-free days in the ITT group and in the prespecified sepsis subgroup. There were no significant differences between ganciclovir and placebo in overall mortality, secondary bacteremia or fungemia, or ICU or hospital length of stay.

The seroprevalence of CMV in the screened population was approximately 60%, which is similar to estimates in US adults based on data from the National Health and Nutrition Examination Survey III, and suggests that CMV-targeted interventions would be applicable to a large proportion of adult patients in the ICU.[23] The selected ganciclovir dosing regimen significantly prevented new-onset CMV reactivation (>80% effective) as shown in a recent small trial[24] and was not associated with an increased transfusion requirement, nephrotoxicity, or an increase in other predefined selected adverse events or serious adverse events. Thus, future phase 3 studies could appropriately use the selected antiviral drug regimen. There are several other potent and well-tolerated newer CMV-active antiviral drugs under development that could also be considered for future studies.[25]

Multiple biologically plausible mechanistic links between CMV reactivation and adverse outcomes have been proposed based on human, animal, and in vitro studies, including virally mediated lung injury, immunosuppression leading to secondary infections, and enhanced pulmonary or systemic inflammation.[3,5,26] The failure to detect changes in IL-6 (a key inflammatory cytokine previously linked to mortality in patients who are critically ill) might have been because the observed clinical effects in the ganciclovir group were mediated by pathways other than IL-6. Future studies should explore non-IL-6-mediated mechanisms underlying the observed effect of ganciclovir on mechanical ventilation outcomes. There is no universally accepted or validated biomarker in early-phase ICU studies, and future efficacy studies should be designed to demonstrate benefits in clinical outcomes.

Among the prespecified secondary clinical outcomes examined, there was a statistically significant increase in ventilator-free days in both the ITT group and in the sepsis subgroup in the ganciclovir group. This observation supports the hypothesis that ganciclovir might attenuate CMV-mediated lung injury through 1 of the mechanisms postulated above. Supporting this hypothesis, in a post hoc analysis, there was a statistically significant reduction in duration of mechanical ventilation days among those who survived for 28 days (eFigure 4 in Supplement 3). Consistent with prior studies, there was a high rate of local lung CMV reactivation in the placebo group and a significant reduction in lung viral load in the ganciclovir group (eFigure 1 in Supplement 3). Collectively, these findings point toward attenuation of CMV-mediated lung injury as a potential mechanism to explain the significant increase in ventilator-free days among those in the ganciclovir group. Future studies should include analyses of inflammatory cytokines, clinical measures of lung function or injury, and measurement of CMV-directed immune responses in the lung to more precisely define the mechanisms underlying the observed effect on mechanical ventilation. The observed effect on mechanical ventilation identifies a patient-oriented, clinically meaningful, and objective outcome that could be used as an end point in a future phase 3 CMV suppression study. The use of VFDs as a clinical end point has been recommended in ICU trial design guidelines and used as an end point in prior ICU trials.[27,28]

There were several strengths of the study, including the placebo-controlled, double-blind, multicenter study design;

the use of sensitive and quantitative virologic methods to assess the antiviral effect of the ganciclovir intervention; and the inclusion of objective and clinically relevant prespecified secondary outcomes.

Limitations

This study has several limitations. First, there were too few trauma patients to assess treatment effects in this subgroup. Second, the enrollment window length allowed inclusion of some patients with baseline CMV reactivation, and this might have diminished the ability to detect a treatment effect. Third, discontinuation of the study-specific BAL for feasibility or logistical reasons precluded assessment of BAL inflammatory markers and lung CMV replication as potential mechanisms for the observed effects on mechanical ventilation. Fourth, the change from the initial intravenous and oral ganciclovir regimen to an all-intravenous regimen might have affected the efficacy of the intervention. However, drug exposure with intravenous ganciclovir and oral valganciclovir regimens is similar,[29] and the overall use of oral drug was limited. Fifth, detection of spurious associations might have resulted from the multiple outcomes analyzed, so the analyses focused on prespecified secondary outcomes and other findings should be viewed as exploratory.

Conclusions

Among CMV-seropositive adults with critical illness due to sepsis or trauma, ganciclovir did not reduce IL-6 levels and the current study does not support routine clinical use of ganciclovir as a prophylactic agent in patients with sepsis. Additional research is necessary to determine the clinical efficacy and safety of CMV suppression in this setting.

ARTICLE INFORMATION

Accepted for Publication: July 26, 2017.

Author Contributions: Drs Limaye and Boeckh had full access to all of the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Limaye, Stapleton, Chen, Rubenfeld, Boeckh.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Limaye, Boeckh.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Peng, Chen.
Obtained funding: Limaye, Boeckh.
Administrative, technical, or material support: Limaye, Kimball, Boeckh.
Supervision: Limaye, Kimball, Boeckh.

Author Affiliations: Division of Allergy and Infectious Diseases, University of Washington, Seattle (Limaye, Boeckh); Pulmonary and Critical Care Division, University of Vermont College of Medicine, Burlington (Stapleton, Parsons); Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington (Peng, Kimball, Chen, Boeckh); Critical Care Division, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania (Gunn); Division of Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor (Hyzy); Division of Pulmonary, Critical Care and Sleep Medicine, Ohio State University Wexner Medical Center, Columbus (Exline, O'Brien); Department of Pulmonary and Critical Care Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina (Files, Morris); Division of Pulmonary, Critical Care and Sleep Medicine, National Jewish Sleep Center, Denver, Colorado (Frankel); Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Mikkelsen); Department of Critical Care Medicine, Cleveland Clinic, Cleveland, Ohio (Hite); Department of Medicine, University of Virginia, Charlottesville (Enfield); Division of Critical Care Pulmonary Medicine, Baystate Medical Center, Springfield, Massachusetts (Steingrub); Department of Surgery, University of Washington, Seattle (Cuschieri); Division of Pulmonary and Critical Care, Northwestern Feinberg School of Medicine, Chicago, Illinois (Wunderink); Division of Pulmonary and Critical Care Medicine, Oregon Clinic, Portland (Hotchkin); Interdepartmental Division of Critical Care Medicine, Sunnybrook Health Sciences Center, Toronto, Ontario, Canada (Rubenfeld).

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Limaye reports personal fees from Merck and Helocyte; serving on the advisory board for Artemis; and being a site investigator for trials with Astellas and Oxford Immunotech. Dr Files reports being a principal investigator for Ferring Pharmaceuticals. Dr O'Brien reports being an investor in Quorum Therapeutics and receiving personal fees from Quorum Therapeutics, GenEndeavor, Tenax, Abbott, General Electric, and the Medical Simulation Corporation. Dr Hotchkin reports receiving personal fees from Intermune and Boehringer Ingelheim. Dr Boeckh reports receiving personal fees from Merck, Chimerix, Shire, and Artemis and grant funding from Chimerix and Shire. No other disclosures were reported.

Funding/Support: This investigator-initiated study was supported by grant UO1 HL 102547 from the US National Institutes of Health's National Heart, Lung, and Blood Institute. Genentech supplied the initial batch of study drug (ganciclovir, valganciclovir, and placebo).

Role of the Funder/Sponsor: The funders of the study had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript or the decision to submit for publication.

Additional Contributions: We thank Taylor Thompson, MD (Massachusetts General Hospital), David Schoenfeld, PhD, (Harvard Medical School), Mitchell Levy, MD (Brown University), Lorraine Ware, MD (Vanderbilt University), and Paul Griffiths, MD (University College, London), who served on the data and safety monitoring board. They received payment from grant UO1 HL 102547. We thank the following study coordinators: Lesley De Souza (Baystate Medical Center), Helen Donnelly, BSN (Northwestern University), Michelle Ferrari (Cleveland Clinic), Luke Herren (Ohio State University), Meg Day (Oregon Clinic), Jeff McKeehan, MSN (National Jewish Health), Kristine Nelson (University of Michigan), Pam Fazio, BS (University of Pittsburg), Sara Arden, BS (University of Vermont), Lori Flores, BSN (Wake Forest University), Mary Marshall, MSN (University of Virginia), Michaela Kusumi, BS (University of Washington), Fatima Ali, BS (University of Washington), and Laura Hennessy (University of Washington) for site research coordination; Lisa Chung, BS (Fred Hutchinson Cancer Research Center), Terry Stevens-Ayers, MS (Fred Hutchinson Cancer Research Center), Michaela Kusumi, BS (University of Washington), and Fatima Ali, BS (University of Washington), for their help with preparing the figures and manuscript; and Hu Xie, MS (Fred Hutchinson Cancer Research Center) for assistance with statistical analysis. We also thank the staff and coordinators at the Statistical Center for HIV/AIDS Research and Prevention for data management and Sheree Miller, PharmD (University of Washington), for the management and dispensing of the study drug and placebo. They did not receive compensation for their contribution.

REFERENCES

1. Balthesen M, Messerle M, Reddehase MJ. Lungs are a major organ site of cytomegalovirus latency and recurrence. *J Virol*. 1993;67(9):5360-5366.

2. Toorkey CB, Carrigan DR. Immunohistochemical detection of an immediate early antigen of human cytomegalovirus in normal tissues. *J Infect Dis*. 1989;160(5):741-751.

3. Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. *J Pathol*. 2015;235(2):288-297.

4. Kalil AC, Florescu DF. Prevalence and mortality associated with cytomegalovirus infection in nonimmunosuppressed patients in the intensive care unit. *Crit Care Med*. 2009;37(8):2350-2358.

5. Limaye AP, Boeckh M. CMV in critically ill patients: pathogen or bystander? *Rev Med Virol*. 2010;20(6):372-379.

6. Al-Omari A, Aljamaan F, Alhazzani W, Salih S, Arabi Y. Cytomegalovirus infection in immunocompetent critically ill adults: literature review. *Ann Intensive Care*. 2016;6(1):110.

7. Ong DS, Spitoni C, Klein Klouwenberg PM, et al. Cytomegalovirus reactivation and mortality in

patients with acute respiratory distress syndrome. *Intensive Care Med*. 2016;42(3):333-341.

8. Papazian L, Hraiech S, Lehingue S, et al. Cytomegalovirus reactivation in ICU patients. *Intensive Care Med*. 2016;42(1):28-37.

9. Cook CH, Trgovcich J, Zimmerman PD, Zhang Y, Sedmak DD. Lipopolysaccharide, tumor necrosis factor α, or interleukin-1β triggers reactivation of latent cytomegalovirus in immunocompetent mice. *J Virol*. 2006;80(18):9151-9158.

10. Cook CH, Zhang Y, McGuinness BJ, Lahm MC, Sedmak DD, Ferguson RM. Intra-abdominal bacterial infection reactivates latent pulmonary cytomegalovirus in immunocompetent mice. *J Infect Dis*. 2002;185(10):1395-1400.

11. Cook CH, Zhang Y, Sedmak DD, Martin LC, Jewell S, Ferguson RM. Pulmonary cytomegalovirus reactivation causes pathology in immunocompetent mice. *Crit Care Med*. 2006;34(3):842-849.

12. Parsons PE, Eisner MD, Thompson BT, et al; NHLBI Acute Respiratory Distress Syndrome Clinical Trials Network. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med*. 2005;33(1):1-6.

13. Botto S, Streblow DN, DeFilippis V, et al. IL-6 in human cytomegalovirus secretome promotes angiogenesis and survival of endothelial cells through the stimulation of survivin. *Blood*. 2011;117(1):352-361.

14. Geist LJ, Dai LY. Cytomegalovirus modulates interleukin-6 gene expression. *Transplantation*. 1996;62(5):653-658.

15. Humar A, St Louis P, Mazzulli T, et al. Elevated serum cytokines are associated with cytomegalovirus infection and disease in bone marrow transplant recipients. *J Infect Dis*. 1999;179(2):484-488.

16. Limaye AP, Kirby KA, Rubenfeld GD, et al. Cytomegalovirus reactivation in critically ill immunocompetent patients. *JAMA*. 2008;300(4):413-422.

17. Boeckh M, Huang M, Ferrenberg J, et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. *J Clin Microbiol*. 2004;42(3):1142-1148.

18. Ranieri VM, Suter PM, Tortorella C, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA*. 1999;282(1):54-61.

19. Schoenfeld DA, Bernard GR, Network A; ARDS Network. Statistical evaluation of ventilator-free days as an efficacy measure in clinical trials of treatments for acute respiratory distress syndrome. *Crit Care Med*. 2002;30(8):1772-1777.

20. Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A; Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*. 2000;342(18):1301-1308.

21. Schweickert WD, Pohlman MC, Pohlman AS, et al. Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomised controlled trial. *Lancet*. 2009;373(9678):1874-1882.

22. DeMets DL, Lan KK. Interim analysis: the α spending function approach. *Stat Med*. 1994;13(13):1341-1352; discussion 1353-1346. https://eclass.uoa.gr/modules/document/file.php/MATH301/PracticalSession3/LanDeMets.pdf. Accessed July 27, 2017.

23. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004. *Clin Infect Dis*. 2010;50(11):1439-1447.

24. Cowley NJ, Owen A, Shiels SC, et al. Safety and efficacy of antiviral therapy for prevention of cytomegalovirus reactivation in immunocompetent critically ill patients: a randomized clinical trial. *JAMA Intern Med*. 2017;177(6):774-783.

25. Abad CL, Razonable RR. Treatment of α and β herpesvirus infections in solid organ transplant recipients. *Expert Rev Anti Infect Ther*. 2017;15(2):93-110.

26. Varani S, Landini MP. Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpesviridae*. 2011;2(1):6.

27. Young P, Hodgson C, Dulhunty J, et al; ANZICS Clinical Trials Group. End points for phase II trials in intensive care: recommendations from the Australian and New Zealand Clinical Trials Group consensus panel meeting. *Crit Care Resusc*. 2012;14(3):211-215.

28. Kawazoe Y, Miyamoto K, Morimoto T, et al; Dexmedetomidine for Sepsis in Intensive Care Unit Randomized Evaluation (DESIRE) Trial Investigators. Effect of dexmedetomidine on mortality and ventilator-free days in patients requiring mechanical ventilation with sepsis: a randomized clinical trial. *JAMA*. 2017;317(13):1321-1328.

29. Caldés A, Colom H, Armendariz Y, et al. Population pharmacokinetics of ganciclovir after intravenous ganciclovir and oral valganciclovir administration in solid organ transplant patients infected with cytomegalovirus. *Antimicrob Agents Chemother*. 2009;53(11):4816-4824.