

Safety and efficacy of antiviral therapy for prevention of cytomegalovirus reactivation in immunocompetent critically ill patients

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Title Page

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Title

Reactivation of cytomegalovirus (CMV) in critically ill patients: randomised clinical trial of antiviral prophylaxis

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Key Points

Question

Reactivation of latent cytomegalovirus (CMV) has been detected in up to 30% of critically ill patients. The clinical importance of this observation is unanswered.

Findings

This randomised controlled trial assessed the efficacy, safety and feasibility of antiviral prophylaxis to prevent CMV reactivation in critically ill patients. Valganciclovir and valaciclovir both suppressed CMV reactivation compared to control (Kaplan-Meier: 35% vs. 8%, $p=0.002$), although the valaciclovir arm was stopped early because of higher mortality.

Meaning

Prophylaxis prevents CMV reactivation in critically ill patients; further research would be needed to determine clinical efficacy and safety.

Abstract

Importance:

Latent Cytomegalovirus (CMV) infection is present in over half of the adult population and viral reactivation, where virus becomes measurable in body fluids such as blood, can occur in up to a third of these individuals during episodes of critical illness.

Objective:

To determine whether antiviral therapy is safe and effective for preventing CMV reactivation in a general population of critically ill patients.

Design:

Single centre, open-label, randomised, controlled trial recruiting between January 2012 and January 2014.

Setting:

Large 100 bedded intensive care unit in England, UK

Participants:

124 CMV-seropositive patients undergoing mechanical ventilation for at least 24 hours in intensive care. Baseline characteristics were similar between groups with mean age 57 years and mean APACHE II score 17.6.

Interventions:

Patients were randomised to receive anti-CMV prophylaxis with valaciclovir (N=34) or low dose valganciclovir (N=46) for up to 28 days to suppress CMV reactivation, or to a control group with no intervention (N=44).

Main Outcome Measure:

Time to first CMV reactivation in blood within the 28 day follow up period following initiation of study drug.

Results:

Viral reactivation in blood occurred in 12 patients randomised to the control group, compared with one reactivation in the valganciclovir group and two reactivations in the valaciclovir group (HR=0.1, 95% CI: 0.04-0.5 for combined treatment groups vs. control). Although this trial was not powered to assess clinical endpoints, the valaciclovir arm was halted prematurely because of higher mortality; 14 of 34 patients (41%) had died by 28 days, compared with 5 of 37 (13.5%) patients in the control arm at the point of the decision to halt this arm. Other safety endpoints showed similar outcomes between groups.

Conclusion and Relevance:

Antiviral prophylaxis with valaciclovir or low dose valganciclovir suppresses CMV reactivation in the setting of critical illness. However, given the higher mortality, a large-scale trial would be needed to determine the clinical efficacy and safety of CMV suppression.

Trial Registration:

Clinicaltrials.gov, NCT01503918, <https://clinicaltrials.gov/ct2/show/NCT01503918>

Introduction

Herpesvirus infections are widely prevalent within the human population and establish a state of chronic infection. Primary infection with Cytomegalovirus (CMV) is usually clinically silent and most individuals become chronically infected during their lifetime.¹⁻³ The presence of measurable CMV virus in body fluids such as blood is prevented by a competent host immune system acting to suppress the virus. If this response is inadequate, reactivation occurs (i.e. CMV is present in body fluids). CMV is thus often detectable in immunosuppressed patients. Viral reactivation is associated with a wide range of clinical problems and antiviral prophylaxis has become well established as therapy in high risk settings, such as transplantation and therapeutic immunosuppression.⁴⁻⁸

Critical illness impairs host defence mechanisms, particularly in those patients with a systemic inflammatory response, and this increases the risk of CMV reactivation which has been reported as affecting up to 30% of critically ill patients.⁹⁻²²

Clinical risk factors associated with reactivation of CMV include the duration of intensive care unit (ICU) stay, pneumonia, sepsis, and high disease severity.^{18,23} There are also many biological factors that act to increase the frequency of CMV reactivation in critical illness, including direct stimulation of viral replication resulting from endotoxin and inflammatory cytokine release and the increased levels of catecholamines.²⁴⁻²⁹ In addition to direct tissue damage, CMV viremia may itself serve to suppress normal immune function and increase the risk of secondary infective complications.^{30,31} Indeed, systematic reviews have demonstrated that mortality in patients with CMV reactivation was on average doubled compared to those without viremia.^{23,32} Limaye *et al.* have further demonstrated a direct correlation between CMV viral load and mortality.⁹

A number of antiviral agents are available with activity against CMV. Both valganciclovir and valaciclovir (orally active forms of ganciclovir and aciclovir) are used for prophylaxis against CMV reactivation in organ transplant recipients. However, despite the potential for benefit, there are

currently no data evaluating their efficacy as prophylaxis for viral reactivation in non-immunosuppressed patients in the intensive care unit.^{25,33}

We have performed a proof of principle study, designed to assess the efficacy, safety and feasibility of antiviral prophylaxis for suppressing CMV reactivation in critically ill patients receiving support in the ICU. Two active treatment arms were chosen, one using low dose valganciclovir and the other using valaciclovir. Both regimens have been used widely outside critical care settings. Valganciclovir has been shown to have a less favourable side effect profile but demonstrates increased activity against CMV. In contrast, valaciclovir requires high dosage administration because of its relatively limited activity against CMV but is generally well tolerated. Here we report the results of this study.

Methods

Study Design

We conducted a single centre, proof of principle, open-label, randomised, controlled, three armed study of two anti-CMV prophylaxis treatments and standard care (no antiviral prophylaxis; control group) in CMV-seropositive patients in the ICU at Queen Elizabeth Hospital Birmingham, UK between January 2012 and January 2014. The study was approved by the National Research Ethics Service Committee, London. The trial was registered with clinicaltrials.gov, study identifier NCT01503918.

We adopted a two-stage contingent consent process: first for a screening sample to determine CMV positivity, and second for recruitment to the interventional trial in the event of positive screening. Generally, sedated patients did not have capacity to give informed consent, and so consent was sought from a personal or professional legal representative prior to randomisation. Patient consent to continue as a trial participant was sought once capacity had been regained.

Participants

Patients were eligible for the study if CMV seropositive, already in the ICU for more than 24 hours and mechanically ventilated, with this anticipated to continue for at least 48 hours.

As the study was designed to examine patients without pre-existing immune suppression, the following exclusions were applied: known or suspected congenital or acquired immunodeficiency; receipt of immunosuppressive medication within 30 days; and chemotherapeutic agents within 6 months. Corticosteroids were not an exclusion criterion if the dose was less than 10mg/day prednisolone (or equivalent), short courses of up to 1mg/kg prednisolone (or equivalent) for exacerbations of COPD for up to 14 days, or 'stress dose' hydrocortisone up to 400mg/day as part of intensive care support. Patients were also excluded from randomisation if they tested as CMV IgG seronegative, were under 18 years, if onset of acute illness was more than 7 days at the point of randomisation, they were in receipt of systemic antiviral medication within 7 days (use of oseltamivir allowed), expected survival less than 48 hours, neutropenia $<1.0 \times 10^9/L$, they had suffered an isolated brain injury, known allergy to any study drugs, or if known to be pregnant or breast feeding.

Intervention and randomisation

Eligible participants were randomised in a 1:1:1 ratio to receive valganciclovir, valaciclovir or control by telephone access to a computer-generated random treatment allocation sequence (Birmingham Clinical Trials Unit (BCTU), UK). The randomisation was stratified by age (age ≤ 50 years or >50 years). Although patients and treating physicians were not masked to the assigned treatment group, CMV quantitative PCR (QPCR) results were not available during the study period and were block processed at a later date; laboratory staff were blinded to treatment allocation. Interim safety analyses were performed at 6 months, and at intervals thereafter by the independent Data Monitoring Committee (DMC).

Participants randomised to one of the two study drug arms received either valganciclovir or valaciclovir prophylaxis. Low dose valganciclovir prophylaxis has been established as the mainstay of

prophylaxis in other groups. High dose valaciclovir has the benefit of activity against a wider group of viruses, as well as a low toxicity profile. Both low dose valganciclovir and high dose valaciclovir have been used successfully in trials of immunosuppressed patients, and subsequently in the clinical setting. Patients randomised to valganciclovir received 450mg valganciclovir, once a day by the enteral route. Patients in this group unable to receive enteral medication received intravenous ganciclovir 2.5mg/kg ideal bodyweight, once a day until enteral absorption was established. Patients randomised to receive valaciclovir received 2g valaciclovir, four times a day by enteral route. Patients unable to receive enteral medication received intravenous aciclovir 10mg/kg ideal bodyweight, three times a day until enteral absorption was established.

In both arms, the study drug was initiated on the day of randomisation and continued for a period of 28 days in the ICU. The drug was discontinued after a minimum of 14 days if patients were discharged from the ICU to the ward. The drug was discontinued if patients were discharged from hospital. Treatment dosing was modified in the presence of renal impairment (eTable S1). Study drug was withdrawn in the presence of severe neutropenia ($<1.0 \times 10^9/L$), requirement for G-CSF therapy, or at the request of the clinical team overseeing patient care.

The patient group randomised to receive no antiviral prophylaxis received standard care. Antiviral medication could be initiated if the clinical team overseeing the care of the patient deemed it necessary for therapeutic reasons.

Data Collection

We obtained patients' acute physiology and chronic health evaluation (APACHE II) scores from our local case mix programme database of the Intensive Care National Audit and Research Centre (ICNARC). Physiological and routine blood test results, including data allowing calculation of daily sequential organ failure assessment (SOFA) score^{34,35}, was collected for 28 days or until death or discharge from hospital if this was sooner. Patients were followed up until death or hospital discharge.

Specimens of plasma, urine, throat swab and non-directed bronchiolar lavage (NDBL) fluid were collected on day one, and at 5 (\pm 1) day intervals during the 28 day study period (on days 1, 6, 11, 16, 21 and 26), or until patient death or discharge from hospital, if this was sooner. Anonymised specimens were sent to the Microbiology Department, University Hospitals Birmingham, to be analysed for the presence of CMV DNA using a QPCR assay (Abbott™ Diagnostics, Maidenhead, UK. limit of detection 20 copies/ml). NDBL samples were performed on patients with no contraindication, whose trachea remained intubated at the required sampling time point. Blood specimens underwent analysis for tumour necrosis factor (TNF) and interleukin-6 (IL-6) levels using Proseek® Multiplex, Olink Bioscience, Uppsala, Sweden. Protein concentrations were evaluated using assay specific units; NPX units (Normalised Protein eXpression), on Log2 scale, normalised to minimise intra- and inter-assay variation, in which a high value corresponds to a high protein concentration.

Outcomes

The primary outcome measure was time to first reactivation of CMV in blood (defined as above the lower limit of the QPCR assay which was 20 copies/ml) from initiation of study drug until day 28, excluding those patients who had already reactivated on the day of enrolment. Secondary outcomes were time to first reactivation of CMV by day 28 in urine, throat swab and NDBL specimens. Time to >1,000 and >10,000 copies/ml, peak CMV viral load and area under the curve (AUC) were also planned analyses. Secondary clinical outcome measures included mortality by 28 days after randomisation; organ failure free days (SOFA score <2) and moderate organ dysfunction free days (SOFA score <5) at 28 days^{36,37}; time to discharge from ICU and time to discharge from hospital. Assessments of drug safety were time to neutropenia ($<1.0 \times 10^9$ /L), time to thrombocytopenia (platelet count $<50 \times 10^9$ /L), requirement for 'rescue' G-CSF therapy or premature cessation of study drug, number of platelet transfusions, and development of renal insufficiency (defined as both: creatinine clearance of <60 ml/min; and creatinine clearance of <30 ml/min or requirement for renal

replacement therapy). Cytokine analysis was performed on blood, with change in TNF- α and IL-6 from time of randomisation to day 14 and 28 selected *a priori* as exploratory outcome measures.

Statistical Analysis

The sample size was based on studies of CMV reactivation rates in CMV seropositive patients within the ICU, where reactivation rates of up to 30% have been observed, and high drug efficacy seen in other patient populations. The target sample of 141 patients (47 patients in each group) was determined using 90% power at $p=0.05$ to detect a difference in CMV reactivation from 30% in the control group to 5% in the treatment group in CMV seropositive critically ill patients.

Primary analyses compared the combined treatment groups with the control group. As the valganciclovir arm was closed early due to safety concerns, it was decided by the trial team and BCTU statisticians (who were blind to the data at this time) to also compare valganciclovir with the control group. Time to event analyses were performed using survival analysis methods to compare time to first CMV reactivation between groups. Kaplan-Meier plots were produced and unadjusted Cox proportional hazard models used to report hazard ratios (HR) and 95% confidence intervals (95% CI). All analyses were performed on an intention to treat principle, whereby patients included in the analysis were analysed according to the treatment group to which they were randomised regardless of whether they received this treatment. As the primary outcome of the study was to measure the efficacy of antiviral drugs to prevent CMV reactivation, patients were excluded from the analyses of CMV viral load if viral reactivation had already taken place before initiation of study drug on the day of recruitment. In the event of patient discharge from hospital or death, the results were censored at the most proximate blood CMV QPCR sample point. Analysis of clinical and safety outcomes included all patients and so included those who had reactivated CMV in any body fluid at baseline. All analyses were undertaken using SAS version 9.2 (Cary, NY, USA).

Role of the Funding Source

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Results

Patients

Between January 2012 and February 2014, 124 patients were randomised into the trial; 44 in the control group, 34 in the valaciclovir group (recruitment stopped prematurely to this arm, see next section) and 46 in the valganciclovir group (Figure 1). Baseline characteristics were similar across the three groups (eTable S2). Disease severity scoring at ICU admission was similar between groups, with mean APACHE II score of 17.5 in the control group, 17.9 in the valaciclovir group and 17.4 in the valganciclovir group. Main category of diagnosis at enrolment is shown in eTable S2.

Cessation of Valaciclovir Arm

Recruitment into the valaciclovir arm was ceased in September 2013, following an interim analysis presented to the independent DMC, who advised that this arm be closed because of significantly higher mortality in this group. At this point, 34 participants had been recruited into the valaciclovir arm, 14 (41%; 95% CI: 25%-58%) of whom had died by 28 days, compared with 5 of 37 (13.5%; 95% CI: 2%-25%) participants in the control arm, and 7 of 34 (20.6%; 95% CI: 7%-34%) participants in the valganciclovir arm. To investigate potential associations between study drug use and cause of death, an independent case record review was performed. Reviewers were intensive care doctors independent of the study team; each set of case notes was examined by two reviewers blinded to

group allocation. The reviewers identified all deaths as expected and attributable to the underlying disease. By the end of the study, the control group mortality increased from 13.5% to 15.9% for 28 day mortality, and to 20.5% for hospital mortality.

Study drug adherence

Nine participants had the study drug stopped prematurely during the 28 day trial period (4 in valaciclovir group and 5 in valganciclovir group). Two patients in the valaciclovir group had the study drug stopped after 5 and 7 days by the supervising clinician because of a change to palliative care. One patient in the valganciclovir group had the study drug stopped after 6 days at the request of their personal legal representative (with permission to continue sampling and data collection following withdrawal of study drug). The other six patients had the study drug stopped prematurely because of possible adverse events (n=2) or serious adverse events (SAE; n=4). In the valaciclovir group, two patients had the study drug stopped due to rashes, whilst four patients stopped drug early in the valganciclovir group, one due to allergic reaction, one due to a rash and two due to clinical concerns related to low platelet counts. No patients had study drug stopped because of the predefined stopping points of neutropenia or use of G-CSF.

Primary Outcome

Figure 2 shows CMV blood viral load over time, and includes all enrolled patients split into two groups; those receiving antiviral prophylaxis of any form, and those in the control arm receiving no antivirals. 14 patients had CMV viremia on the day of enrolment (shown in red in figure 2) and were therefore excluded from the primary analysis of drug efficacy (time to reactivation). Viral reactivation in blood occurred in 12 patients randomised to the control group compared with 3 reactivations in the combined active treatment group (Kaplan-Meier: 35% vs. 8%, HR=0.1, 95% CI: 0.04-0.5, p=0.002 for combined treatment group vs. control) (Table 1, Figure 3a). There was one reactivation in the low dose valganciclovir group (HR=0.08, 95% CI: 0.01-0.6, p=0.01 for valganciclovir vs. control; Figure 3b).

Secondary Outcomes

CMV reactivation in blood, urine, NDBL, and throat swab are presented in Table 1. Blood was the most sensitive body fluid for the demonstration of CMV reactivation in this study, although this may reflect the more complete dataset, with 83% patients (15 out of 18) who reactivated in any body fluid, at least doing so in blood. NDBL data were available beyond baseline in only 28% of patients due to difficulties in obtaining subsequent samples (compared to $\geq 85\%$ for blood, urine and throat), meaning this data should be interpreted with caution.

Clinical endpoints are shown in Table 2. In total, 9 of 44 patients died in hospital in the control group compared to 15 of 34 patients in the valaciclovir group and 12 of 46 patients in the valganciclovir group. The relative risk for hospital mortality was 1.3 (95% CI: 0.6-2.7) in the valganciclovir group compared to control, and 2.2 (95% CI: 1.1-4.3) for the valaciclovir group compared to control. Seven (16%) patients reported SAEs in the control group, compared to 10 (29%) in the valaciclovir group and 16 (35%) in the valganciclovir group. The relative risk for a patient experiencing an SAE was 1.8 (95% CI: 0.8-4.4) when comparing the valaciclovir with control group, 2.2 (95% CI: 1.0-4.8) when comparing the valganciclovir with control group, and 2.0 (95% CI: 1.0-4.3) when comparing the combined treatment groups with the control group. The time to renal impairment (Creatinine clearance < 60 ml/min) and severe renal impairment (creatinine clearance < 30 ml/min or requirement for renal replacement therapy) was similar between the combined treatment groups and control: HR: 1.2 (95% C.I: 0.7-2.0), and HR: 1.0 (95% C.I: 0.6-1.8) respectively. Comparing valaciclovir with control gave similar results: HR: 1.5 (95% C.I: 0.9-2.8) and HR: 1.2 (95% C.I: 0.6-2.4) for renal impairment and severe renal impairment respectively. There was no evidence of any difference in levels of bone marrow suppression between groups; there were no reports of neutropenia, and the risk of thrombocytopenia was similar between combined treatment groups and control (HR: 1.0 (95% C.I: 0.5 to 2.2)).

Exploratory Outcomes

A pre-planned exploratory analysis assessing changes in TNF- α and IL-6 from time of randomisation to day 14 and day 28 was undertaken. To accommodate variations in sampling times, we used time windows rather than a fixed time point: if samples for the pre-specified days were not available, samples from days 13 and 15 were included in the day 14 analysis, and samples from days 21 to 28 were included in the day 28 analysis. These data were only available for patients who were in hospital at the time the sample was taken and as such the analysis is based on a limited group of patients. Results from patients allocated to valganciclovir were compared with control. The mean difference in change in TNF between day 0 and day 13-15 was -0.01 (95% C.I.: -0.16-0.14) and between day 0 and day 21-28 was -0.05 (95% C.I.: -0.13-0.02) (Table 2). The mean difference in change in IL-6 between day 0 and day 13-15 was -0.25 (95% C.I.: -1.48-0.98) and between day 0 and day 21-28 was -0.11 (95% C.I.: -1.41-1.20).

Planned Analyses Not Presented

Time to >1,000 and >10,000 copies/ml and AUC were also planned analyses for the blood, urine, NBDL and throat sample data. There were very few samples with >1,000 copies/ml (3 in total): (1 in blood, 2 in NBDL) or >10,000 copies/ml (none), and incomplete sample profiles caused by death or discharge from hospital or non-availability of sampling access (patients whose tracheas were extubated preventing NBDL) limited the utility of AUC analyses.

Discussion

The results of this study, designed to assess the efficacy, safety and feasibility of antiviral prophylaxis in CMV seropositive non-immunosuppressed critically ill patients, demonstrate that CMV reactivates in around a third of critically ill patients, and that reactivation can be suppressed from 35% to 3%, through the use of antiviral prophylaxis with low-dose valganciclovir. Blood was the most sensitive body fluid for the detection of CMV reactivation in this study, with 83% of patients who reactivated

doing so in at least blood. This may reflect the more complete dataset for this body fluid, as it was not possible to collect other specimens as consistently because of patient constraints including anuria (urine), or tracheal extubation (NDBL). Viral loads for patients with CMV viraemia were generally low (range 25 – 1382 copies/ml).

Although valaciclovir was effective at suppressing CMV, it was difficult to administer (high frequency of administration of both routes, inability to crush and administer valaciclovir enterally via nasogastric tube, and potential risk of thrombophlebitis from intravenous administration).

Valaciclovir was associated with increased mortality, although it is unclear why. All study deaths were classed as expected and attributable to the underlying disease by independent blinded reviewers. The incidence of renal impairment and bone marrow suppression, both potential side effects of the treatment drugs, were similar between groups, although this study was not powered to identify differences. It is possible that critically ill patients, commonly developing significant organ dysfunction, are more susceptible to the potential side effects of antiviral drugs, although these drugs have long been used safely for CMV reactivation suppression in the transplant population.

Herpesviruses have the capacity to modify host immune defences including TNF-regulated signalling pathways.³⁸ Elevated levels of TNF and IL6 may mediate the higher mortality rates associated with CMV antibody response in an elderly Latino population³⁹ Survivors of critical illness with CMV reactivation are reported to have a more marked pro-inflammatory biomarker profile (including elevated IL6 levels) than seronegative survivors three months after ICU discharge.⁴⁰ However, in our exploratory analysis, we find no consistent pattern in trends for IL6 or TNF between the study groups (Table 2). We suspect that if CMV-related cytokine changes occur, they are likely to be masked by the pro-and anti-inflammatory components of acute critical illness.

There are a number of limitations to our study. Although this was a single centre study, and the representativeness of patients and generalizability of the results would have been improved by recruiting from multiple sites, this study was conducted in the largest ICU in Europe, with 100 beds,

including both general and specialty Intensive care beds. For practical reasons, the study was necessarily open-label. The primary outcome measure of time to CMV reactivation in blood was felt to be robust, since laboratory staff were blinded to treatment allocation. The lack of blinding could however have influenced other data, such as the recording of adverse events. Although a third of patients in the control arm demonstrated CMV viremia, the viral loads were generally low when compared to those found in immunosuppressed patients, and this may be relevant when assessing the importance of viral suppression with drugs. The valaciclovir arm was terminated early because of safety concerns and this led to a smaller sample size than planned in this group. Valaciclovir has been used for many years and examined in other studies outside of the critical care setting with few side effects, with the additional benefit over valganciclovir of activity against other herpesviruses.⁴¹⁻
⁴³ However, the challenges of administering the drug to critically ill patients make it an unsuitable option for any subsequent trial powered to assess clinical outcomes. It could be questioned whether prophylactic therapy, as chosen in this study, is preferable to early treatment of active reactivation. Prophylaxis is conceptually more attractive, as it prevents viral reactivation before direct systemic and tissue injury takes place, and is simpler when many ICUs do not have access to rapid CMV assay.^{44,45} Prophylaxis has been chosen as the standard in many other populations, with more effective CMV suppression, although at the expense of a higher incidence of side effects.⁶ However, treatment following reactivation minimises population drug exposure, which may be particularly important in the setting of polypharmacy associated with critical illness.

In conclusion, this study demonstrates that antiviral prophylaxis effectively suppresses CMV reactivation in critically ill patients, and is best achieved through the use of low dose valganciclovir, administered enterally, or intravenously as ganciclovir. Valaciclovir was associated with an increased mortality, although it was not possible to identify a causal link between the drug administration and death. The safety and efficacy of antiviral prophylaxis to prevent CMV reactivation in this setting can only be determined by conducting a large-scale trial with close monitoring of clinical and patient-centred outcomes.

Contributions

NC, JB, PM and HO designed the study.

SS, JM, NC, AO enrolled patients.

NC, AO, JM, SS oversaw data collection.

NC wrote the first draft of the report.

NC, JB, PM and AO analysed data, and provided interpretation, and edited drafts of the report.

RW and NI performed the statistical analysis of the data, provided interpretation, and edited drafts of the report.

All authors contributed to edits of the final manuscript and NC served as the corresponding author.

Declaration of Interests

None declared

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and Dr Nicholas Cowley had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Figures/Tables

Table 1: Reactivation and peak viral load data for CMV viral load by PCR (copies/ml) for each body fluid.

	Control	Valaciclovir	Valganciclovir	HR (95% CI) (combined vs. control)	HR (95% CI) (valganciclovir vs. control)
Blood					
Number of Reactivations	N=44 12	N=34 2	N=46 1	0.1 (0.04 to 0.5) p=0.002	0.08 (0.01 to 0.6) p=0.01
Median viral load for first positive PCR (range)	33.0 (22 – 95)	29.5 (27 – 32)	37 (-)		
Median peak viral load in patients who reactivated (range)	37.5 (25 – 1382)	29.5 (27 – 32)	60 (-)	-	-
Urine					
Number of Reactivations	4	0	0	-	-
Median peak viral load in patients who reactivated (range)	48.5 (28 – 278)	-	-	-	-
NDBL					
Number of Reactivations	2	0	2	0.2 (0.02 to 2.6) p=0.2	0.3 (0.03 to 3.5) p=0.3
Median peak viral load in patients who reactivated (range)	843 (22 – 1664)	-	423.5 (25 – 822)	-	-
Throat*					
Number of Reactivations	4	2	1	0.5 (0.1 to 2.3) p=0.4	0.3 (0.03 to 2.4) p=0.2
Any Body Fluid**					
Number of Reactivations	14	2	2	0.1 (0.03 to 0.5)	0.2 (0.05 to 0.5)

HR=Hazard Ratio; 95% CI=95% Confidence Interval; NDBL=non-directed bronchiolar lavage

Patients who reactivated at baseline (on day 1) are excluded from this analysis (n=14 for blood, n=2 for urine, n=6 for NDBL, n=6 for throat swab), patients with only data at baseline are censored at day 1 (n=12 for blood, n=11 for urine, n=47 for NDBL, n=11 for throat swab), and patients are censored at discharge from hospital or death if this occurred before the end of scheduled sampling. Patients with no samples (blood n=0, urine n=7, NDBL n=38, throat swab n=0) are censored at day 1.

* Throat swabs are either positive or negative, so viral loads are not presented.

** Reactivation in blood, urine, NDBL, or throat swab at any point excluding baseline.

Table 2: Clinical, safety & exploratory cytokine outcomes.

		Control (N=44)	Valaciclovir (N=34)	Valganciclovir (N=46)
Secondary Clinical Outcome Measures				
Organ failure free days (SOFA score<2)	Median	3.5	1.5	2.0
	IQR	(0 - 18)	(0 - 13)	(0 - 11)
	Range	(0 - 31)	(0 - 24)	(0 - 36)
Moderate organ failure free days (SOFA score<5)	Median	18.0	11.0	16.5
	IQR	(2 - 24)	(0 - 22)	(4 - 21)
	Range	(0 - 41)	(0 - 28)	(0 - 44)
Discharged from ICU by 3 months†	N (%)	36 (82)	21 (62)	34 (74)
Discharged from Hospital by 3 months†	N (%)	30 (68)	17 (50)	28 (61)
ICU Duration of Stay (days)	Median	11.5	12.0	16.0
	IQR	(7 - 16)	(7 - 31)	(11 - 27)
Number of SAEs forms returned	N	7	12	18
Number of Patients reporting SAEs	N (%)	7 (16)	10 (29)	16 (35)
Mortality (28 day)	N (%)	7 (16)	14 (41)	10 (22)
Mortality (Hospital)	N (%)	9 (20)	15 (44)	12 (26)
Safety Outcomes				
Requirement for G-CSF therapy	N (%)	0 (0)	0 (0)	0 (0)
Neutropenia (<1 x10 ⁹ /L)	N (%)	0 (0)	0 (0)	0 (0)
Platelet count < 50 x10 ⁹ /L)	N (%)	10 (23)	9 (26)	10 (22)
Platelet transfusions	N	44	32	42
	Median	0	0	0.2
	IQR	(0 - 0)	(0 - 0.5)	(0 - 1)
Renal Insufficiency				
CrCl<60ml/min	N (%)	23 (52)	22 (64)	24 (52)
CrCl<30ml/min or required dialysis	N (%)	19 (43)	16 (47)	18 (39)
Exploratory Cytokine Analyses				
TNF (NPX units)		Control		Valganciclovir
Mean change (SD) between day 0 and day 14		N = 23 0.07 (0.18)		N = 26 0.05 (0.33)
Mean difference (95% CI)				-0.01 (-0.16 to 0.14)
Mean change (SD) between day 0 and day 28		N = 23 0.04 (0.15)		N = 22 -0.01 (0.10)
Mean difference (95% CI)				-0.05 (-0.13 to 0.02)
IL-6 (NPX units)		Control		Valganciclovir
Mean change (SD) between day 0 and day 14		N = 23 -1.09 (2.46)		N = 26 -1.34 (1.82)
Mean difference (95% CI)				-0.25 (-1.48 to 0.98)
Mean change (SD) between day 0 and day 28		N = 23 -1.91 (2.14)		N = 22 -2.01 (2.18)
Mean difference (95% CI)				-0.11 (-1.41 to 1.20)

SOFA=Sequential organ failure assessment; CrCl=Creatinine clearance; G-CSF=Granulocyte-colony stimulating factor; SAEs=Serious adverse events. IQR=Interquartile range. SD=Standard deviation; TNF=tumour necrosis factor; IL-6=interleukin-6. NPX – normalised protein expression. For day 14, samples taken between days 13-15 were used. For day 28, samples taken between days 21-28 were used. †Based on patients who remained alive and could be discharged from hospital. By three months, all patients (except one) who could have been discharged (i.e. alive patients) had been discharged from hospital.

Figure footnotes:

Figure1: Trial flowchart

Figure 2: CMV viral load in blood in combined treatment groups (valaciclovir and valganciclovir arms) versus control group over the 28 day period of data collection. Each line represents a single patient. Lines shown in red represent patients who had CMV viremia on day of enrolment, and thus were excluded from primary analysis of time to CMV reactivation. All enrolled patients are shown here to show differences in viral load over time with or without antiviral prophylaxis.

Figure 3a: Time to CMV viral reactivation in blood in combined treatment groups (valaciclovir and valganciclovir arms) versus control group.

Figure 3b: Time to CMV viral reactivation in blood in valganciclovir group versus control group