Intensive strategy to prevent cytomegalovirus disease in seropositive umbilical cord blood transplant recipients

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Keywords: Cytomegalovirus, umbilical cord blood transplant, prophylaxis, prevention


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Running Title: Intensive CMV prevention for cord blood recipients
Abstract

Seropositive umbilical cord blood transplant (UCBT) recipients are at increased risk for CMV complications. To reduce CMV complications, we adopted an intensive strategy which consists of pre-transplant ganciclovir (5mg/kg IV daily from day -8 to day -2), post-transplant high-dose acyclovir (2 grams 3 times daily) and bi-weekly monitoring using a serum CMV PCR for preemptive therapy. Hazard rates and cumulative incidence of CMV complications along with days treated were compared in high-risk CMV seropositive UCBT recipients who received the intensive strategy and a historical cohort who received a standard strategy. Of 72 seropositive patients, 29 (40%) received standard prophylaxis and 43 (60%) the new intensive approach. The hazard rate for CMV reactivation was lower for patients receiving the intensive strategy (HR 0.27, 95% CI: 0.15-0.48; p<0.001), and led to fewer cases of CMV disease by 1 year (HR 0.11, 95% CI: 0.02-0.53; p=0.006). In patients who reactivated, the intensive strategy also led to fewer days on CMV-specific antiviral therapy (median 42% [IQR 21, 63] vs. 70% [IQR 54, 83], p<0.001). Use of an intensive CMV prevention strategy in high-risk CMV seropositive UCBT recipients results in a significant decrease in CMV reactivation and disease.
Introduction

Cytomegalovirus (CMV) infection remains one of the most important infectious complications after hematopoietic cell transplantation (HCT). CMV frequently reactivates in the post-transplant period and can lead to life threatening invasive disease, particularly in high-risk seropositive recipients. Additional negative effects, including increased rates of bacterial and fungal infections and graft failure have also been shown to be associated with CMV reactivation in HCT recipients. Current preemptive prevention strategies mitigate but have not eliminated life-threatening CMV disease, and the virus continues to be a cause of increased morbidity and mortality in multiple transplant populations.

Umbilical cord blood transplant (UCBT) recipients in particular are at increased risk for CMV complications due to significant delay in immune reconstitution. Cord blood grafts are naïve and have impaired functional recovery which may be more permissive to viral reactivation and less apt to control replication. Since high viral loads have been shown to be strong predictors for CMV disease development, UCBT recipients may also be at increased risk for the development of viral invasion. Incidence rates of CMV in UCBT vary, with reported rates of reactivation fluctuating from 21% to 100% and CMV disease between 6 to 21%, but different prevention methods and the inclusion of low-risk seronegative recipients limit comparisons.

Due to a concern for high rates of CMV complications in UCBT recipients at our institution, we instituted a new preemptive strategy with pre-transplant ganciclovir, primary prevention with high-dose acyclovir/valacyclovir and preemptive screening bi-weekly for CMV DNA. Previous studies have demonstrated that both high-dose acyclovir and pre-transplant ganciclovir are effective in reducing the rate of CMV reactivation and disease among allogeneic transplant recipients. In order to assess the safety and efficacy of this strategy on CMV outcomes, we compared a cohort of
high-risk CMV seropositive recipients of UCBT that underwent standard institutional CMV prevention and those who underwent this new intensive approach.

Materials and Methods

Patients

All patients that received a UCBT at the Fred Hutchinson Cancer Research Center (FHCRC) between 2006-2010 and who were seropositive for CMV were eligible for inclusion in this study; only patients undergoing their first UCBT were included in these analyses. Patients were excluded if they died prior to day 14 post-transplant or had participated in primary CMV antiviral prevention trials. Patients were also excluded if they were receiving anti-CMV therapy at the time of transplantation for pre-transplant reactivation; all patients underwent pre-transplant CMV testing within 2 weeks prior to the start of conditioning.

Transplant practices

Patients received a double cord blood transplant (dUCBT) if a suitable single cord blood graft could not be found, as determined by institutional criteria. Selected cord blood (CB) units were required to be matched to the recipient at ≥ 4 of the 6 HLA loci based on intermediate resolution typing at HLA-A and –B and allele-level for HLA-DRB1 typing; recipients of 2 CB units, units must be at least 3/6 HLA-matched to each other.

Decisions regarding the use of myeloablative or non-myeloablative conditioning regimens were made by the primary transplant team; CMV serostatus was not used as criteria in selecting patient specific conditioning regimens. Myeloablative conditioning typically consisted of cyclophosphamide (Cy) 60 mg/kg intravenously (IV) daily for 2 days, total body irradiation (TBI) 1320 or 1200 cGy, and fludarabine (Flu) 25 mg/m² IV
daily for 3 days. Other patients received Flu 30 mg/m² IV daily for 5 days, Treosulfan (Treo) 14 g/m² IV daily for 3 days, and a single fraction of TBI 200 cGy, or reduced-intensity conditioning consisting of Flu 40 mg/m² IV daily for 5 days, a single dose of Cy 50 mg/kg IV, and a single fraction of TBI 200. Patients who received either no prior chemotherapy or no chemotherapy in the three months preceding UCBT were given higher dose of TBI at 300cGy or had equine anti-thymocyte globulin at 15mg/kg every 12 hours for 3 days added.

All patients received prophylactic immunosuppressive therapy for prevention of GVHD consisting of cyclosporine-A (CSA) and mycophenolate mofetil (MMF). Acute graft-versus-host disease (GVHD) was assessed using standard criteria based on organ involvement and categorized as acute GVHD grades 0–IV. The patient’s underlying disease was categorized as standard or high-risk based upon previously described criteria. All patients received standard prophylactic antimicrobial and antifungal agents during follow-up.

**Antiviral prevention strategies during transplantation**

UCBT patients in this study underwent two different prevention strategies. In the first historical cohort (“standard”), patients received our standard allogeneic regiment consisting of acyclovir 800 mg or valacyclovir 500 mg twice daily (during periods of mucositis 250 mg/m² IV acyclovir every 12 hours, adjusted for renal insufficiency) for varicella zoster virus (VZV) and herpes simplex virus (HSV) prophylaxis. Patients were started on anti-CMV therapy if they developed ≥ 500 copies/mL or any antigenemia during weekly screening. A threshold for preemptive therapy of ≥100 copies/mL was used in patients receiving ≥ 1mg/kg of steroids. After day 100, weekly PCR surveillance and preemptive therapy with valganciclovir (900 mg twice daily or appropriate dosing for pediatric patients) started if patients had > 1000 copies/mL. A
small number of patients underwent preemptive screening with pp65 antigenemia, and in order to compare prophylactic groups, these patients had their weekly clinical samples retrospectively retested for CMV DNA by PCR. These samples, which had been frozen at -20 °C at the time of collection, were thawed and retested for CMV DNA using the same methods.27

Due to observed rates of CMV related complications in our UCBT recipients, an intensified strategy for CMV prophylaxis was implemented in June 2008; this strategy became standard for UCBT recipients in August 2008. In this second cohort ("intensive") CMV seropositive patients received pre-transplant ganciclovir at 5mg/kg IV daily day -8 to day -2 during conditioning followed by high dose acyclovir (2 grams valacyclovir every 8 hours or 500 mg/m² IV acyclovir every 8 hours adjusted for renal insufficiency until tolerating oral meds) for the first 100 days. For patients < 40 or ≥20 kg the dose of valacyclovir was 1 gram every 8 hours; for those < 20 kg 500 mg/m² IV every 8 or 600 mg/m² acyclovir every 6 hours. Patients in this cohort were tested bi-weekly by PCR, with a threshold for preemptive therapy at ≥ 25 copies/mL (limit of detection). After day 100, it was recommended that patients be placed on valganciclovir 900 mg once daily (dose adjusted for pediatric patients according standard guidelines) until 1 year; patients who could not tolerate valganciclovir had high-dose acyclovir continued.

For the purposes of preemptive therapy, patients were started on IV ganciclovir or foscarnet. Patients who were pre-engraftment or had intolerance to ganciclovir were given foscarnet, otherwise patients preferentially received ganciclovir. All patients received either ganciclovir 5 mg/kg IV or foscarnet 90 mg/kg twice daily 7 to 14 days as induction therapy, followed by maintenance therapy with once daily dosing until routine surveillance testing was negative. Patients who did not respond after the second week of induction therapy continued on twice daily dosing until CMV PCR levels started to decline. Patients who rapidly cleared their CMV received at least one week of induction
and 1 week of maintenance therapy. Resistance testing and decisions to change to alternate therapy (i.e. foscarnet from ganciclovir) were at the discretion of the primary team and the Infectious Diseases consult service. Appropriate dose adjustments were made for patients with renal dysfunction.

Definitions
CMV reactivation was defined as any detection of CMV DNA in serum and CMV disease was defined by standardized criteria. Initial CMV PCR level was defined as the CMV DNA copies/mL in serum at first detection, and maximum CMV PCR was highest recorded level during the first 100 days; total days of CMV were considered cumulative. For the purposes of analyses, a binary outcome for high-viral load defined as any CMV DNAemia level >1000 copies/mL. Total days of CMV-specific antiviral use (ganciclovir and/or foscarnet) were calculated from start date to final dose administered during the first 100 days; days of multiple episodes of reactivation were summed cumulatively. Induction therapy was considered to be the period during which patients received the equivalent of twice daily dosing of anti-CMV therapy. Acute kidney injury was assessed up to 100 days and was classified as a serum creatinine concentration that was two or three times as high as the baseline value.

Statistical Methods
Patient and transplant characteristics were compared using Fisher’s exact test and Wilcoxon rank-sum test where applicable. We estimated the probability of CMV reactivation and disease for each treatment cohort using cumulative incidence (CI) methods, with death considered a competing risk in analyses; similar CI methods were used to estimate the rate of engraftment and acute GVHD. Statistical differences in CI curves between groups were assessed using Gray’s test. A multivariable Cox
A proportional hazards model was used to evaluate the impact of the prevention strategy on CMV reactivation and disease; separate hazard ratios (HR) were determined for high-viral load and for early/pre-engraftment or late/post-engraftment reactivation. For the purposes of multivariable analyses we defined two separate periods during follow-up: early/pre-engraftment (≤30 days after transplant) and late/post-engraftment (day >30 to day 100); patients who reactivated during early/pre-engraftment were excluded for late/post-engraftment analyses. Factors identified \textit{a priori} for inclusion in the multivariate model for CMV reactivation were: myeloablative vs. non-myeloablative conditioning, donor number (1 vs. 2 cord blood grafts) and acute GVHD (grade >2) as a time-dependent covariate.

To compare the amount of exposure to antiviral therapy, between treatment groups the percentage of time on CMV antiviral therapy in the first 100 days was determined by dividing the number of days on anti-CMV treatment by the total survival days in the first 100 days. The percentage of days that patients had exposure to anti-CMV therapy was compared between the two prevention strategies using the Wilcoxon-rank sum test. All p-values were two sided and considered significant at the $\alpha=0.05$ level.

All study activities were approved by the FHCRC institutional review board, and all participants provided written informed consent according to the principles of the Declaration of Helsinki.

\textbf{Results}

\textbf{Patient characteristics}
Of 135 who underwent UCBT, 78 patients (58\%) were CMV seropositive (\textbf{Figure 1}). Of these 78, a total of 6 patients were excluded because they died prior to day 14 post transplant (n=2), were on anti-viral therapy at time of transplantation (n=3) or were enrolled in a CMV prevention trial (n=1). Of the remaining 72 patients, 29 (40\%)
received standard prophylaxis and 43 (60%) received the intensive prevention strategy. Patient, transplantation and graft characteristics stratified for the two cohorts are summarized in **Table 1**. The major difference between the two groups was that those who received the more intensive prophylactic strategy had higher risk disease (p=0.02). The two groups were otherwise similar with respect to HLA disparity, intensity of conditioning regimen, sex, total nucleated cells infused and diagnosis, although there was a trend toward an increased age among those who received the more intensive prophylaxis (p=0.10).

**Incidence and Timing of CMV reactivation**

As part of the intervention, patients in the intensive strategy had more frequent CMV testing in the first 100 days during follow-up (intensive, total 948 tests [median 24 tests per patient (IQR 17,28)] vs. standard, total 559 tests [median 18 tests (IQR 16,25)], p=0.049). In patients receiving the intensive strategy, first reactivation occurred at a median of 27 days (range 3-77), as compared to a median of 17 days (range 3-65) to first reactivation in those treated with our standard strategy (p=0.29), and the mean duration of serum CMV PCR detection was significantly shorter among patients who received the intensive approach (16.7 days [range 2-95] vs. 46.7 days [range 4-91]; p<0.001).

The cumulative incidence estimate of CMV reactivation was lower in those that received the more intensive approach compared to the standard group (26/43 [60%] vs. 29/29 [100%], p<0.001) (**Figure 2**). The intensive strategy was also associated with a significant reduction in CMV reactivation in time to event analyses (HR 0.27; 95% confidence interval [CI] 0.15-0.48; p<0.001) (**Table 2**). Interestingly, a total of 15/29 (52%) in the standard cohort and 8/43 (19%) in the intensive cohort developed CMV reactivation prior to engraftment (p=0.003). The hazards of early / pre-engraftment CMV
reactivation were less in those receiving the more intensive strategy (HR 0.25; 95% CI: 0.13-0.49; p<0.001), but the risk was no different during the late / post-engraftment period (HR 0.39; 95% CI:0.11-1.35; p=0.14).

All but 6/72 patients (8%) were tested weekly or bi-weekly using CMV PCR. These 6 patients were all in the standard cohort, and 3 were tested with a mix of PCR and antigenemia testing while the 3 others had testing for the entire post transplant period by antigenemia only. All 6 of these patients were documented to have developed CMV reactivation prior to retrospective testing and were treated with standard CMV preemptive therapy. PCR testing on frozen blood collected at the time of antigenemia determination demonstrated similar positive and negative results upon re-testing except in 2 patients. These two patients were tested post-transplant by antigenemia only and were found on retrospective PCR testing to be positive 7 and 11 days prior to their first positive antigenemia test.

During the first 100 days post-transplant the mean PCR viral load in the intensive strategy cohort was significantly less than in the standard cohort at every week, except for the first (Figure 3). When comparing viral loads in those who developed CMV reactivation, the initial and the maximum level of PCR viral load were significantly lower for patients who received intensive prophylaxis as compared to those who received the standard prevention; median initial viral load: 88 copies/mL (IQR 67-100) vs. 210 (IQR 63–649, p=0.01) and median maximum viral load: 170 copies/mL (IQR 88-310) vs. 3200 (IQR 1400–11000, p<0.001). The hazards of developing a viral load of ≥1000 copies was significantly less in the intensive strategy (HR 0.04; 95% CI: 0.01 – 0.15; p<0.001) (Table 2).

CMV Disease
CMV disease was documented in a total of 8 patients during the first 100 days, 2 in the intensive group and 6 in the standard group (p=0.054) (Table 3). An additional 2 patients in the standard cohort developed CMV disease after day 100 (both pneumonia, Day 165, 191). The overall cumulative incidence of CMV disease at 1 year was 4.7% for patients treated with the new strategy and 27.6% for those treated with the standard strategy. When evaluating CMV disease in time to event analyses, the aggressive strategy was associated with a significant reduction in CMV disease (HR 0.11; 95% CI 0.02-0.53; p=0.006) (Table 2).

When considering all 8 occurrences of CMV disease (both early and late CMV disease) in the standard cohort, 4 developed pneumonia, 3 gastrointestinal and 1 disseminated disease (Table 3). The median time to early CMV disease (≤ 100 days post-transplant, n=6) was 33 days (range 11-92 days); two of whom (33%) died secondary to CMV disease. One of the two patients in the standard group who developed disease during the late period (day >100 to one year) also died from CMV pneumonia.

In the intensive cohort, 2 patients developed CMV disease (Table 3). The first patient was a pediatric patient transplanted for Langerhan’s Cell Histiocytosis. While on IV acyclovir, the patient reactivated at day 3 and a bronchoalveolar lavage was positive for CMV by shell vial and PCR at day 11 post transplant. He was treated with foscarnet and had a full recovery. In the second patient CMV was isolated in BAL by shell vial at day 42 at which time he had numerous other co-infections (vancomycin-resistant Enterococcus, gram negative bacteremia); CMV was never detected in serum. The patient died of multi-organ failure in the setting of relapse at day 47.

**CMV specific antiviral therapy**
Patients who had documented reactivation in the intensive cohort had a smaller percentage of days in the first 100 days post-transplant on active anti-CMV therapy (median 42% [IQR 21, 63] vs. 70% [IQR 54, 83], p<0.001) and fewer days on induction dosing (median 16% [IQR 8, 24] vs. 29% [IQR 18, 42], p<0.001) when compared to those who reactivated in the standard cohort (Figure 4). Of those that reactivated, a total of 4 patients developed CMV resistance during follow-up. In total 3/29 (10.3%) in the standard group developed UL97 mutations associated with ganciclovir resistance (day 30, 36, 113), while only 1/26 (3.8%) developed a UL97 mutation (day 246) (p=0.61). No patient developed a UL54 mutation during follow-up.

**Other Transplant Outcomes**

The time to engraftment and platelet recovery between those who received the intensive and standard strategies was similar (Table 4). Two patients in each cohort developed graft failure; one patient with secondary graft failure was observed in the standard cohort. In time to event analyses, the cumulative incidences of engraftment and graft-versus-host disease (grade III-IV) were not significantly different (p=0.20 and p=0.07, respectively). In the first 100 days non-relapse mortality was similar between the 2 groups (p=0.30, log-rank), and at 1 year did not significantly differ between the 2 treatments groups (p=0.63, log-rank). Importantly, high dose acyclovir/valacyclovir did not appear to lead to additional renal toxicity during the first 100 days post-transplant (Table 4).

**Discussion**

In this study, an intensive strategy of pre-transplant ganciclovir followed by primary prophylaxis with high-dose acyclovir and frequent preemptive screening was highly effective in preventing CMV reactivation and disease in a high-risk cohort of CMV seropositive UCBT recipients. When compared to a standard prophylaxis with moderate
dose acyclovir used at our center, this new strategy was associated with fewer episodes of both CMV reactivation and invasive disease as well as lower levels of viral replication. In addition, this strategy led to fewer days on CMV specific antiviral therapy, fewer cases of drug resistance, and was not associated with kidney dysfunction, delayed engraftment or other transplant related outcomes.

The most important finding in our study was that use of this intensive approach decreased the risk of developing CMV disease in the first 100 days to 4.6%, a figure similar to rates seen using conventional bone marrow and peripheral blood stem cell sources. The outcomes from this intensive strategy were likely a cumulative effect of different interventions aimed at CMV prevention, one of which was high-dose acyclovir/valacyclovir prophylaxis. Acyclovir’s low side effect profile make it an attractive option for prevention, and since high-dose acyclovir/valacyclovir has been shown to decrease CMV reactivation in other HCT populations, some centers use this method of prevention as a standard in their UCBT recipients. However, high-dose acyclovir alone may have limited ability to decrease the risk of CMV disease in UCBT and other HCT recipients. Other antiviral options may offer protection from early CMV disease, but are known to cause additional toxicities. Due in part to delayed recovery of CMV specific T-cell immunity, primary prophylaxis has also been shown to be associated with a higher rate of late CMV disease.

In combination with high-dose acyclovir, the application of twice weekly CMV PCR testing in this strategy allowed for improved identification of early CMV reactivation and intervention at lower viral loads during episodes of reactivation. CMV replicates with a doubling time of approximately 1 day in HCT recipients, suggesting that more frequent testing may have the advantage of detecting low levels of CMV DNA before the development of rapid logarithmic growth. CMV viral load predicts the development of
CMV disease\textsuperscript{14, 39}, therefore interventions at a lower viral load threshold could also partially explain our decreased rate of CMV disease.

Patients also received pre-transplant ganciclovir and late valganciclovir as part of our intensive prevention strategy. Pre-transplant ganciclovir has been shown to decrease CMV complications in other HCT populations\textsuperscript{23, 34, 40-41}, and is hypothesized to decrease the risk of early post-transplant CMV reactivation.\textsuperscript{41} Perhaps due to this intervention, we found that patients in our intensive strategy were significantly less likely to have pre-engraftment CMV. Most importantly, early reactivation appeared to have a negative effect on the rates of CMV disease in our study, and therefore the addition of pre-transplant ganciclovir may have contributed to lower rates of invasive disease. Additionally, although there are too few cases to evaluate in this study, this pre-transplant intervention may also provide some protection against pre-engraftment disease which is known to be associated with increased mortality.\textsuperscript{42} Since other studies have shown safety and efficacy of higher pre-transplant dosing\textsuperscript{34}, an increase to treatment levels (5 mg/kg twice daily) pre-transplant may have provided additional benefits. The use of valganciclovir likely led to less late CMV disease events, but due to limited late disease events in either cohort, we were not able to show a statistically significant benefit to it use.

The incidence of CMV reactivation in our standard cohort are consistent with prior studies in which seropositive UCBT recipients not receiving high-dose acyclovir or anti-CMV antiviral prophylaxis had reactivation rates reported to be between 70 and 100%.\textsuperscript{15-16, 43} However, reactivation rates in our intensive strategy were slightly higher than those reported in CMV seropositive UCBT recipients who received high dose of acyclovir prophylaxis.\textsuperscript{35} The use of CMV PCR as the screening method for preemptive therapy may have provided additional advantages in the UCBT population and may help explain differences in rates of CMV reactivation between studies. Antigenemia detects fewer
cases of CMV reactivation\textsuperscript{44-45}, may necessitate higher viral loads for detection\textsuperscript{46}, and positive results are more likely to be delayed until after the presence of symptoms of disease when compared to patients screened by PCR\textsuperscript{47}. In fact, even when given identical prophylactic regimens, UCBT recipients tested by PCR for preemptive therapy developed fewer episodes of invasive disease when compared to those screened using an antigenemia based strategy\textsuperscript{19}.

In both cohorts CMV was detected by a highly sensitive quantitative double-primer PCR assay that has been shown to be superior to pp65 antigenemia with regard to sensitivity, specificity and predictive values for CMV detection in serum specimens\textsuperscript{27}. Interestingly, 2 patients who were tested by antigenemia only, developed pre-engraftment CMV, where diagnosis was delayed by over a week when compared to retrospective PCR testing. This increased sensitivity may have improved early detection in our study and allowed for prompt intervention. Combined with early detection, our lower threshold also enhanced the use of early CMV specific antiviral preemptive therapy.

The increased rate of identification and the use of lower thresholds in our intensive cohort had the potential to increase the use of CMV specific antiviral therapy. The intensified strategy, however, did not lead to increased utilization of antiviral therapy. In fact, numbers of days on ganciclovir or foscarnet were decreased significantly in patients who had documented reactivation (Figure 4). In addition, patients in the intensive cohort needed less days of induction therapy. The use of less CMV specific antiviral therapy also likely contributed to fewer cases of CMV resistance in this cohort. Finally, since standard anti-CMV drugs used in preemptive therapy have toxicities which can in lead to increased mortality\textsuperscript{37}, the significant reduction seen in our intensive strategy likely provided additional benefits.
The exposure to high dose acyclovir/valacyclovir also had the potential to increase the rate of drug-specific side effects, as acyclovir has been shown to be associated with nephrotoxicity\(^4\) and neurologic complications.\(^5\) During study follow-up there were no difference in renal outcomes between the two study cohorts and no reports of drug associated neurologic complications were noted in those treated with high-dose acyclovir. Additionally, there appeared to be no effect on engraftment or non-relapse mortality when comparing the two cohorts. Perhaps most importantly, the reductions in IV ganciclovir/foscarnet use and CMV disease seen when using this intensive strategy likely outweigh any excess costs and or potential drug side effects from the increased use of these agents as primary prophylaxis.

As with any retrospective study, there are limits that are imposed by our data. We acknowledge that our two populations were not entirely comparable, as patients in our more intensive prophylactic strategy were higher risk transplant recipients due to age and pre-transplant risk stratification. The most important limitation to our study is the small sample size of our study cohort. The majority of studies that assess CMV risk focus on entire cohorts of UCBT recipients, and often include very low-risk CMV seronegative patients.\(^1\) As recipient seropositivity remains the most important risk factor for CMV in HCT and since others have demonstrated increased rates of disease and reactivation in CMV seropositive UCBT recipients\(^1\), we limited our analyses to this high-risk population. Although a smaller cohort size, this allowed us to assess a higher incidence of adverse CMV endpoints and demonstrate significant differences between our two strategies.

Finally, by implementing multiple components in this intensive approach, it is not possible to unravel the benefit of each specific intervention. For example, the protection from early reactivation (<day 30) could be due to pre-transplant ganciclovir, high-dose acyclovir or a combination of both components. Based on these data, we can only
recommend this strategy as a combination of therapies, but future prospective randomized trials could better clarify the importance of each respective intervention.

In conclusion, our data show that an intensive approach to CMV prevention in seropositive UCBT recipients leads to decreased rates of CMV complications. Through the use of pre- and post-transplant antiviral prophylaxis, increased frequency of preemptive screening and lowered thresholds for the institution of pre-emptive therapy, we were able to demonstrate additional protection against CMV disease and the development of pre-engraftment CMV reactivation. This intensive approach was well tolerated and led to a significant reduction in the use of preemptive antiviral therapy. Until the development of less toxic antiviral prophylaxis for CMV prevention, this aggressive approach may be used to provide enhanced protection from CMV in high risk UCBT recipients and could be considered in other populations that are at increased risk of CMV complications.

**Acknowledgements**

The authors would like to thank Terry Stevens-Ayers, Tracy Santo and Meei-Li Huang for their assistance with late CMV PCR testing. The authors would also thank Denise Ziegler and Mary Joy Lopez for their assistance in care of the patients.

**Authorship**

F.M. and S.P. participated in research design, data collection, performed statistical analyses and wrote the manuscript. H.X. performed statistical analyses under direction of W.L. and both contributed to the research design, writing and review of the manuscript. V.C. participated in data collection and contributed to the writing and review of the manuscript. J.G., I.R., M.B., and C.D. participated in research design and contributed to the writing and review of the manuscript.
Grant support: This work was supported by NIH grants K23HL077446, R24 HL74445, 1RC2HL101844, K23HL096831, 1K24HL093294, CA18029 and CA15704. SP is also supported by an ASBMT/Viropharma New Investigator Award. V.C. was supported by the Infectious Diseases Society of America’s Medical Scholars Program. C.D. is a Damon Runyon Clinical Investigator supported in part by the Damon Runyon Cancer Research Foundation (CI# 35-07).
Disclosures / Conflicts of Interest

S.P. has received research support from Chimerix Inc. and Viropharma, Inc; he has received consulting fees from Chimerix, Inc. J.G. has received research support and consulting fees from Chimerix Inc. M.B. has received research support from Chimerix Inc., GlaxoSmithKline, Roche Laboratories, Vical Inc., and Viropharma Inc.; he received consulting fees from Astellas, Boehringer Ingelheim, Chimerix Inc., Novartis, Roche/Genentech, Vical Inc., Viropharma Inc. All other authors report no conflicts.
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Table 1. Characteristics of CMV seropositive recipients undergoing UCBT (n=72)

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<td></td>
<td></td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>12 (28)</td>
<td>7 (24)</td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>21 (49)</td>
<td>17 (59)</td>
<td>0.60</td>
</tr>
<tr>
<td>CML</td>
<td>3 (7)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (16)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td><strong>Disease Risk†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Risk</td>
<td>29 (67)</td>
<td>26 (90)</td>
<td>0.02</td>
</tr>
<tr>
<td>High risk</td>
<td>14 (33)</td>
<td>3 (10)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: UCBT = umbilical cord blood transplantation, CMV = cytomegalovirus, IQR = interquartile range, HLA = human leukocyte antigen, CML = chronic myelogenous leukemia. * For recipients of 2 UCB units the HLA matching reflects the worse matched of the 2 units. † Disease risk: standard refers to aplastic anemia, chronic myeloid leukemia in chronic phase, myelodysplastic syndromes without excess blasts, and leukemia and lymphoma in remission. High refers to all other hematologic malignancies. 25 Fisher’s exact and Wilcoxon rank-sum analyses were used to calculate categorical and continuous variables respectively.
Table 2. CMV outcomes in seropositive recipients undergoing UCBT by prevention strategy (n=72)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intensive n=43</th>
<th>Standard n=29</th>
<th>Unadjusted HR (95% CI)</th>
<th>p-value</th>
<th>Adjusted HR (95% CI)*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CMV reactivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timing†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early / Pre-engraftment</td>
<td>15 (58)</td>
<td>25 (86)</td>
<td>0.26 (0.14 – 0.50)</td>
<td>&lt;0.001</td>
<td>0.25 (0.13 – 0.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Late / Post-engraftment</td>
<td>11 (42)</td>
<td>4 (14)</td>
<td>0.29 (0.09 – 0.93)</td>
<td>0.038</td>
<td>0.39 (0.11 – 1.35)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-level viremia‡</td>
<td>3 (7)</td>
<td>24 (83)</td>
<td>0.05 (0.01 – 0.16)</td>
<td>&lt;0.001</td>
<td>0.04 (0.01 – 0.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistant CMV</td>
<td>1 (2)</td>
<td>3 (10)</td>
<td>- - - - - - - -</td>
<td></td>
<td>0.30§</td>
<td></td>
</tr>
<tr>
<td><strong>CMV disease</strong></td>
<td>2 (5)</td>
<td>8 (28)</td>
<td>0.19 (0.04 – 0.91)</td>
<td>0.038</td>
<td>0.11 (0.02 – 0.53)§</td>
<td>0.006‖</td>
</tr>
<tr>
<td><strong>CMV associated death</strong></td>
<td>0 (0)</td>
<td>3 (10)</td>
<td>- - - - - - - -</td>
<td></td>
<td>0.06§</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CMV = cytomegalovirus, UCBT = umbilical cord blood transplant, CI = confidence interval; *multivariable model includes myeloablative vs. non-myeloablative conditioning, number of donor grafts, and graft-versus-hosts disease (grade ≥ 2, and time-dependent covariate) except where indicated; † includes only patients who reactivated, early ≤ 30 days post-transplant and late >30 – 100 days post-transplant; ‡ high-level viremia is a PCR value ≥1000 copies at any point of time in the first 100 days post-transplant, §calculated using Fisher’s exact probability test; ‖ adjusted only for acute GHVD in the multivariable model
Table 3. Characteristics and outcomes of UCBT recipients who developed CMV disease (n=10)

<table>
<thead>
<tr>
<th>Age, (years)</th>
<th>Prophylaxis</th>
<th>Diagnosis</th>
<th>Conditioning Regimen</th>
<th># of donors</th>
<th>aGVHD grade (days)</th>
<th>Time to CMV reactivation (days)</th>
<th>Viral load at 1st reactivation (copies/mL)</th>
<th>Time to CMV disease (days)</th>
<th>Sites of CMV disease</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Disease (Day 0-100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Standard</td>
<td>AML</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>2 (21)</td>
<td>57</td>
<td>3354</td>
<td>92</td>
<td>GI</td>
<td>Alive</td>
</tr>
<tr>
<td>64</td>
<td>Standard</td>
<td>AML</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>2 (34)</td>
<td>18</td>
<td>22000</td>
<td>34</td>
<td>Lung</td>
<td>Dead</td>
</tr>
<tr>
<td>23</td>
<td>Standard</td>
<td>ALL</td>
<td>Cy+Flu+ATG+TBI(200)</td>
<td>2</td>
<td>3 (15)</td>
<td>17*</td>
<td>35</td>
<td>17*</td>
<td>Lung</td>
<td>Alive</td>
</tr>
<tr>
<td>28</td>
<td>Standard</td>
<td>AML</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>NE</td>
<td>21*</td>
<td>63</td>
<td>33</td>
<td>Disseminated</td>
<td>Dead</td>
</tr>
<tr>
<td>21</td>
<td>Standard</td>
<td>ALL</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>NE</td>
<td>8*</td>
<td>6000</td>
<td>11*</td>
<td>GI</td>
<td>Dead</td>
</tr>
<tr>
<td>42</td>
<td>Standard</td>
<td>ALL</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>2 (35)</td>
<td>3*</td>
<td>100</td>
<td>66</td>
<td>GI</td>
<td>Alive</td>
</tr>
<tr>
<td>1</td>
<td>Intensive</td>
<td>Hystiocytosis</td>
<td>Campath+Mel+Flu</td>
<td>1</td>
<td>0</td>
<td>3*</td>
<td>47</td>
<td>11*</td>
<td>Lung</td>
<td>Alive</td>
</tr>
<tr>
<td>53</td>
<td>Intensive</td>
<td>AML</td>
<td>Treo+Flu+TBI(200)</td>
<td>2</td>
<td>NE</td>
<td>-</td>
<td>-</td>
<td>42†</td>
<td>Lung</td>
<td>Dead</td>
</tr>
<tr>
<td>Late Disease (Day 101-365)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Standard</td>
<td>AML</td>
<td>Cy+Flu+ATG,TBI(200)</td>
<td>1</td>
<td>0</td>
<td>21</td>
<td>1053</td>
<td>191</td>
<td>Lung</td>
<td>Dead</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>AML</td>
<td>Cy+Flu+TBI(1320)</td>
<td>2</td>
<td>3 (10)</td>
<td>8*</td>
<td>3200</td>
<td>165</td>
<td>Lung</td>
<td>Alive</td>
</tr>
</tbody>
</table>

Abbreviations: UCBT = umbilical cord blood transplant, CMV = cytomegalovirus, aGVHD = acute GVHD, AML = acute myelogenous leukemia, ALL = acute lymphoblastic leukemia, Cy = cyclophosphamide, Flu = fludarabine, TBI = total body irradiation, Me = melphalan, ATG = anti-thymocyte globulin, GI = gastrointestinal, NE = not evaluable, Standard = standard prophylactic strategy, Intensive = Intensive prophylactic strategy, *complication developed pre-engraftment, †developed early relapse.
Table 4. Other Transplant Related Outcomes in UCBT Recipients by Prevention Strategy (n=72)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Intensive n=43</th>
<th>Standard n=29</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Time to engraftment†</td>
<td>20 (46)</td>
<td>20 (69)</td>
<td>0.49†</td>
</tr>
<tr>
<td>median days (IQR)</td>
<td>(17-28)</td>
<td>(14-29)</td>
<td></td>
</tr>
<tr>
<td>Time to platelets ≥ 20000†</td>
<td>36 (84)</td>
<td>34 (116)</td>
<td>0.37</td>
</tr>
<tr>
<td>median days (IQR)</td>
<td>(31-50)</td>
<td>(29-45)</td>
<td></td>
</tr>
<tr>
<td>Acute GVHD§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II – IV</td>
<td>28 (74)</td>
<td>25 (86)</td>
<td>0.21</td>
</tr>
<tr>
<td>Grade III – IV</td>
<td>8 (21)</td>
<td>10 (34)</td>
<td>0.22§</td>
</tr>
<tr>
<td>Renal Function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean max creatinine (SD)</td>
<td>1.8 (1.2)</td>
<td>1.6 (1.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>Acute Kidney Injury†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X 2 baseline</td>
<td>28 (65)</td>
<td>18 (62)</td>
<td>0.81</td>
</tr>
<tr>
<td>X 3 baseline</td>
<td>16 (37)</td>
<td>7 (24)</td>
<td>0.31</td>
</tr>
<tr>
<td>Non-Relapse Mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 100 days</td>
<td>8 (19)</td>
<td>2 (7)</td>
<td>0.30§</td>
</tr>
<tr>
<td>1 Year</td>
<td>11 (26)</td>
<td>6 (21)</td>
<td>0.63§</td>
</tr>
</tbody>
</table>

Abbreviations: UCBT = umbilical cord blood transplant, IQR = interquartile range, GVHD = graft-versus-host disease, SD = Standard deviation; *p-values calculated using Fisher’s exact probability test for categorical variables and Wilcoxon-rank sum for continuous variables, unless otherwise specified; † only in patients who engrafted; ‡ when calculated by time to event analysis p=0.20 (Gray’s test); § when calculated by time to event analysis p=0.07 (Gray’s test); † acute kidney injury as defined by ≥ 2 times baseline creatinine or ≥ 3 times baseline, during the first 100 days; ‡ calculated by log-rank
**Figure Legends**

**Figure 1**

Title: Schema of seropositive umbilical cord blood transplant study population.

Legend: Abbreviations: UCBT, umbilical cord blood transplant; CMV, cytomegalovirus

**Figure 2**

Title: Cumulative incidence of CMV reactivation to day +100 by prevention strategy in seropositive UCBT recipients (n=72)*

Legend: Abbreviations: CMV, cytomegalovirus; UCBT, umbilical cord blood transplant; *Competing risk for CMV reactivation considered death or re-transplantation; p-value determined by Grey’s test [30]

**Figure 3**

Title: Mean observed CMV viral load in UCBT recipients during the first 100 days post-transplant by type of prevention strategy (n=72)

Legend: Whiskers equal 95% confidence intervals for weekly mean value.

**Figure 4**

Title: Use of anti-CMV antiviral therapy by type of prevention strategy

Legend: *Anti-CMV therapy considered ganciclovir and foscarnet; induction therapy the period during which patients received the equivalent of twice daily dosing of anti-CMV therapy. Only patients with proven CMV reactivation included. Total percentage
determined by days on anti-CMV therapy divided by total days alive during the first 100
days post-transplant. Whiskers equal 10\textsuperscript{th}-90\textsuperscript{th} percentile and solid dots equal outliers.
Figure 1

UCBT patients n=135

CMV seronegative n=57 (43%)

CMV seropositive n=78 (58%)

Eligible patients n=72 (54%)

Enrolled in CMV prevention trial n=1

Active CMV therapy at time of HCT n=3

Death ≤ 14 days post-transplant n=2

Standard n=29 (40%)

vs.

Intensive n=43 (60%)

CMV Prophylactic Strategy
Figure 2

Cumulative Incidence

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Days Post-transplant

0 10 20 30 40 50 60 70 80 90 100

Standard (n=29)

Intensive (n=43)

$p = <0.0001$
Figure 3

CMV Log10 Viral Load

-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Weeks Post-Transplant

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Standard
Intensive
Intensive strategy to prevent cytomegalovirus disease in seropositive umbilical cord blood transplant recipients

Filippo Milano, Steven A. Pergam, Hu Xie, Wendy M. Leisenring, Jonathan A. Gutman, Ivy Riffkin, Victor Chow, Michael J. Boeckh and Colleen Delaney