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

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Seropositive umbilical cord blood transplant (UCBT) recipients are at increased risk for CMV complications. To reduce CMV complications, we adopted an intensive strategy that consisted of ganciclovir administered before transplantation (5 mg/kg intravenously daily from day −8 to day −2), high-dose acyclovir (2 g, 3 times daily) after transplantation, and biweekly monitoring with 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 (HR) for CMV reactivation was lower for patients receiving the intensive strategy (HR 0.27, 95% confidence interval 0.15-0.48; *P* < .001) and led to fewer cases of CMV disease by 1 year (HR 0.11, 95% confidence interval 0.02-0.53; *P* = .006). In patients who reactivated, the intensive strategy also led to fewer days on CMV-specific antiviral therapy (median 42% [interquartile range 21-63] vs 70% [interquartile range 54-83], *P* < .001). Use of an intensive CMV prevention strategy in high-risk CMVseropositive UCBT recipients results in a significant decrease in CMV reactivation and disease. (*Blood*. 2011;118(20):5689-5696)

Introduction

CMV infection remains one of the most important infectious complications after hematopoietic stem cell transplantation (HSCT). CMV frequently reactivates in the posttransplantation period and can lead to life-threatening invasive disease, particularly in high-risk seropositive recipients.[1] Additional negative effects, including increased rates of bacterial and fungal infections[2,3] and graft failure,[4] have also been shown to be associated with CMV reactivation in HSCT 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 transplantation populations.[3,5,6]

Umbilical cord blood transplant (UCBT) recipients in particular are at increased risk for CMV complications because of significant delay in immune reconstitution.[7-10] Cord blood grafts are naive and have impaired functional recovery that may be more permissive to viral reactivation and less apt to control replication.[11,12] Because high viral loads have been shown to be strong predictors for the development of CMV disease,[13,14] 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%[15-17] and CMV disease between 6% and 21%,[17-20] but different prevention methods and the inclusion of low-risk seronegative recipients limit comparisons.

Because of a concern for high rates of CMV complications in UCBT recipients at our institution, we instituted a new preemptive strategy that consists of the administration of ganciclovir before transplantation, primary prevention with high-dose acyclovir/valacyclovir after transplantation, and preemptive screening biweekly for CMV DNA. The authors of previous studies have demonstrated that the administration of both high-dose acyclovir and ganciclovir before transplantation are effective in reducing the rate of CMV reactivation and disease among allogeneic transplant recipients.[21-23] To assess the safety and efficacy of this strategy on CMV outcomes, we compared a cohort of high-risk CMV-seropositive recipients of UCBT who received the standard institutional CMV prevention strategy and those who underwent this new intensive approach.

Methods

Patients

All patients who received a UCBT at the Fred Hutchinson Cancer Research Center between 2006 and 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 before day 14 after transplantation 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 pretransplantation reactivation; all patients underwent pretransplantation CMV testing within 2 weeks before the start of conditioning.

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Transplantation practices

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

Decisions regarding the use of myeloablative or nonmyeloablative conditioning regimens were made by the primary transplantation team; CMV serostatus was not used as a criterion in selecting patient-specific conditioning regimens. Myeloablative conditioning typically consisted of cyclophosphamide 60 mg/kg intravenously daily for 2 days, total body irradiation (TBI) 1320 or 1200 cGy divided over 4 days, and fludarabine (Flu) 25 mg/m^2 intravenously daily for 3 days. Other patients received Flu 30 mg/m^2 intravenously daily for 5 days, treosulfan 14 g/m^2 intravenously daily for 3 days, and a single fraction of TBI 200 cGy, or reduced-intensity conditioning consisting of Flu 40 mg/m^2 intravenously daily for 5 days, a single dose of cyclophosphamide 50 mg/kg intravenously, and a single fraction of TBI 200 cGy. Patients who received either no previous chemotherapy or no chemotherapy in the 3 months preceding UCBT were given a greater dose of TBI at 300 cGy or had equine antithymocyte globulin at 15 mg/kg every 12 hours for 3 days added.

All patients received prophylactic immunosuppressive therapy for the prevention of GVHD consisting of cyclosporine A and mycophenolate mofetil. Acute GVHD was assessed by the use of standard criteria on the basis of organ involvement and categorized as acute GVHD grades 0-IV.[24] The patient's underlying disease was categorized as standard or high risk on the basis of previously described criteria.[25] All patients received standard prophylactic antimicrobial and antifungal agents during follow-up.[26]

Antiviral prevention strategies during transplantation

UCBT patients in this study underwent 2 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 (and, during periods of mucositis, 250 mg/m^2 intravenously acyclovir every 12 hours, adjusted for renal insufficiency) for varicella zoster virus and 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 ≥ 1 mg/kg of steroids.[26] After day 100, weekly PCR surveillance and preemptive therapy with valganciclovir (900 mg twice daily or appropriate dosing for pediatric patients) was started if patients had > 1000 copies/mL. A small number of patients underwent preemptive screening with pp65 antigenemia, and 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 by use of the same methods.[27]

Because of 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"), before transplantation CMV-seropositive patients received intravenous ganciclovir at 5 mg/kg daily from day -8 to day -2 during conditioning followed by high-dose acyclovir (2 g of valacyclovir every 8 hours or 500 mg/m^2 acyclovir intravenously every 8 hours adjusted for renal insufficiency until tolerating oral medications) for the first 100 days. For patients < 40 kg and ≥ 20 kg, the dose of valacyclovir was 1 g every 8 hours; for those < 20 kg, the dose was 500 mg/m^2 intravenously every 8 or 600 mg/m^2 acyclovir every 6 hours. Patients in this cohort were tested biweekly 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) for 1 year; patients who could not tolerate valganciclovir had high-dose acyclovir continued.

For the purposes of preemptive therapy, patients were started on intravenous ganciclovir or foscarnet. Patients who were pre-engraftment or had intolerance to ganciclovir were given foscarnet. All patients received either ganciclovir 5 mg/kg intravenously or foscarnet 90 mg/kg twice daily 7-14 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 began to decrease. Patients who rapidly cleared their CMV received at least 1 week of induction and 1 week of maintenance therapy. Resistance testing and decisions to change to alternate therapy (ie, 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.[28] The 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 2 or 3 times as high as the baseline value.[29]

Statistical methods

Patient and transplantation characteristics were compared by use of the Fisher exact test and Wilcoxon rank-sum test where applicable. We estimated the probability of CMV reactivation and disease for each treatment cohort by using cumulative incidence methods, with death considered a competing risk in analyses; similar cumulative incidence methods were used to estimate the rate of engraftment and acute GVHD. Statistical differences in cumulative incidence curves between groups were assessed by use of the Gray test.[30] A multivariable Cox proportional hazards model was used to evaluate the impact of the prevention strategy on CMV reactivation and disease; separate hazard ratios (HRs) were determined for high-viral load and for early/preengraftment or late/postengraftment reactivation.

For the purposes of multivariable analyses, we defined 2 separate periods during follow-up: early/pre-engraftment (≤ 30 days after transplantation) and late/postengraftment (day > 30 to day 100); patients who reactivated during early/pre-engraftment were excluded for late/postengraftment analyses. Factors identified a priori for inclusion in the multivariate model for CMV reactivation were myeloablative versus nonmyeloablative 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, we determined the percentage of time on CMV antiviral therapy between treatment groups in the first 100 days 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 were exposed to anti-CMV therapy was compared between the 2 prevention strategies by use of the Wilcoxon-rank sum test. All P values were 2-sided and considered significant at the $\alpha = 0.05$ level. All study activities were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board, and all participants provided written informed consent according the principles of the Declaration of Helsinki.

Results

Patient characteristics

Of 135 who underwent UCBT, 78 patients (58%) were CMV seropositive (Figure 1). Of these 78, a total of 6 patients were

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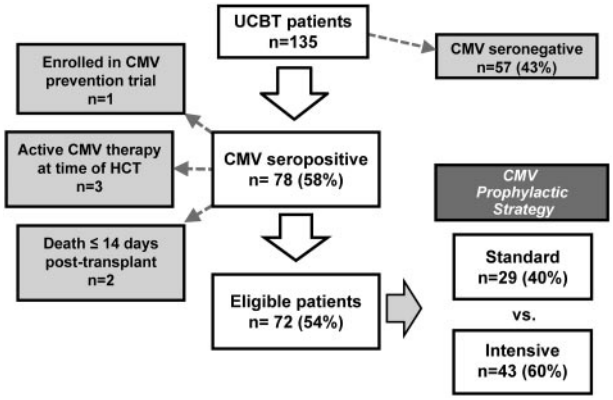

Figure 1. Schema of seropositive UCBT study population.

excluded because they died before day 14 after transplantation (n = 2), were on antiviral 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 2 cohorts are summarized in Table 1.[25] The major difference between the 2 groups was that those who received the more intensive prophylactic strategy had greater risk of disease ($P = .02$). The 2 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 ($P = .10$).

Table 1. Characteristics of CMV-seropositive recipients undergoing UCBT (n = 72)

Characteristic	Intensive strategy, n = 43, n (%)	Standard strategy, n = 29, n (%)	P
Median age, y (IQR)	31.7 (16-57)	21.4 (10.1-41.9)	.10
Sex			.74
Female	22 (51)	16 (55)	
Male	21 (49)	13 (45)	
No. of donors			.50
1	5 (12)	5 (17)	
2	38 (88)	24 (83)	
HLA disparity*			.14
4/6	25 (58)	15 (52)	
5/6	14 (32)	14 (48)	
6/6	4 (10)	-	
Transplantation type			.98
Myeloablative	34 (79)	23 (79)	
Nonmyeloablative	9 (21)	6 (21)	
Total nucleated dose (× 10^7/kg)			.82
median (IQR)	3.9 (3.1-5.1)	4.2 (2.5-6.0)	
Diagnosis			.60
Acute lymphoblastic leukemia	12 (28)	7 (24)	
Acute myeloid leukemia	21 (49)	17 (59)	
CML	3 (7)	3 (9)	
Other	7 (16)	2 (6)	
Disease risk†			.02
Standard risk	29 (67)	26 (90)	
High risk	14 (33)	3 (10)	

CML indicates chronic myelogenous leukemia; IQR, interquartile range; and UCBT, umbilical cord blood transplantation.

*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] The Fisher exact test and Wilcoxon rank-sum analyses were used to calculate categorical and continuous variables, respectively.

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}], $P = .049$). In patients receiving the intensive strategy, first reactivation occurred at a median of 27 days (range, 3-77 days), compared with a median of 17 days (range, 3-65 days) to first reactivation in those treated with our standard strategy ($P = .29$), 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 days] vs 46.7 days [range, 4-91 days]; $P < .001$).

The cumulative incidence estimate of CMV reactivation was lower in those who received the more intensive approach compared with the standard group (26/43 [60%] vs 29/29 [100%], $P < .001$; Figure 2).[30] 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; $P < .001$; Table 2). Interestingly, a total of 15 of 29 (52%) in the standard cohort and 8 of 43 (19%) in the intensive cohort developed CMV reactivation before engraftment ($P = .003$). The hazards of early/preengraftment CMV reactivation were less in those receiving the more intensive strategy (HR 0.25; 95% CI 0.13-0.49; $P < .001$), but the risk was no different during the late/postengraftment period (HR 0.39; 95% CI 0.11-1.35; $P = .14$).

All but 6 of 72 patients (8%) were tested weekly or biweekly with CMV PCR. These 6 patients were all in the standard cohort, and 3 were tested with a mix of PCR and antigenemia testing whereas the 3 others had testing for the entire after transplantation period by antigenemia only. All 6 of these patients were documented to have developed CMV reactivation before 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 on retesting except in 2 patients. These 2 patients were tested after transplantation by antigenemia only and were found on retrospective PCR testing to be positive 7 and 11 days before their first positive antigenemia test.

During the first 100 days after transplantation, 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 we compared viral loads in those who developed CMV reactivation, we found that the initial and the

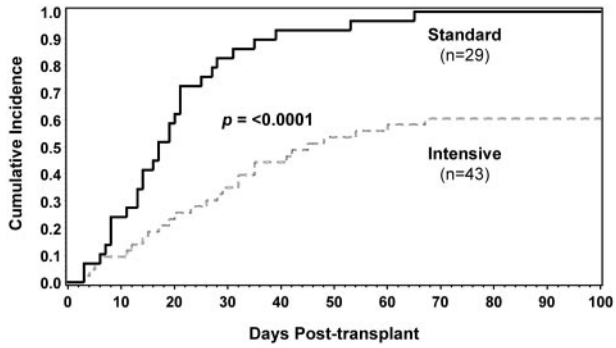

Figure 2. Cumulative incidence of CMV reactivation to day +100 by prevention strategy in seropositive UCBT recipients (n = 72). Competing risk for CMV reactivation considered death or retransplantation; *P* value determined by the Gray test.[30]

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Table 2. CMV outcomes in seropositive recipients undergoing UCBT by prevention strategy (n = 72)

Outcome	Intensive, n = 43 n (%)	Standard, n = 29 n (%)	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)*	*P*
CMV reactivation	26 (60)	29 (100)	0.27 (0.15-0.47)	< .001	0.27 (0.15-0.48)	< .001
Timing†						
Early/pre-engraftment	15 (58)	25 (86)	0.26 (0.14-0.50)	< .001	0.25 (0.13-0.49)	< .001
Late/postengraftment	11 (42)	4 (14)	0.29 (0.09-0.93)	.038	0.39 (0.11-1.35)	.14
Complications						
High-level viremia‡	3 (7)	24 (83)	0.05 (0.01-0.16)	< .001	0.04 (0.01-0.15)	< .001
Resistant CMV	1 (2)	3 (10)			–	.30§
CMV disease	2 (5)	8 (28)	0.19 (0.04-0.91)	.038	0.11 (0.02-0.53)‖	.006‖
CMV-associated death	0 (0)	3 (10)			–	.06§

CI indicates confidence interval; HR, hazard ratio; and UCBT, umbilical cord blood transplantation.

*Multivariable model includes myeloablative vs nonmyeloablative conditioning, number of donor grafts, and GVHD (grade ≥ II ,and time-dependent covariate) except where indicated.

†Only patients who reactivated, early ≤ 30 days after transplantation and late > 30-100 days after transplantation.

‡High-level viremia is a PCR value ≥ 1000 copies at any point of time in the first 100 days after transplantation.

§Calculated by the use of the Fisher exact probability test.

‖Adjusted only for acute GHVD in the multivariable model.

maximum levels of PCR viral load were significantly lower for patients who received intensive prophylaxis compared with those who received the standard prevention; median initial viral load: 88 copies/mL (IQR, 67-100) versus 210 (IQR 63-649, $P = .01$) and median maximum viral load: 170 copies/mL (IQR 88-310) versus 3200 (IQR 1400-11 000, $P < .001$). 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; $P < .001$; 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 ($P = .054$; Table 3). Two other patients in the standard cohort developed CMV disease after day 100 (both pneumonia, days 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 we evaluated CMV disease in time-to-event analyses, we found that the aggressive strategy was associated with a significant reduction in CMV disease (HR 0.11; 95% CI 0.02-0.53; $P = .006$; Table 2).

When considering all 8 occurrences of CMV disease (both early and late CMV disease) in the standard cohort, we found that 4 developed pneumonia, 3 gastrointestinal, and 1 disseminated disease (Table 3). The median time-to-early CMV disease (≤ 100 days after transplantation, n = 6) was 33 days (range, 11-92 days), 2 of whom (33%) died secondary to CMV disease. One of the 2 patients in the standard group who developed disease during the late period (day > 100 to 1 year) also died from CMV pneumonia.

In the intensive cohort, 2 patients developed CMV disease (Table 3). The first patient was a pediatric patient who underwent transplantation because of Langerhans cell histiocytosis. While on intravenous acyclovir, the patient reactivated at day 3, and a bronchoalveolar lavage was positive for CMV by shell vial and PCR at day 11 after transplantation. 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 several other coinfections (vancomycin-resistant *Enterococcus*, gram negative bacteremia); CMV was never detected in serum. The patient died of multiorgan 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 after transplantation on active anti-CMV therapy (median 42% [IQR 21-63] vs 70% [IQR 54-83], $P < .001$) and fewer days on induction dosing (median 16% [IQR 8-24] vs 29% [IQR 18-42], $P < .001$) compared with those who reactivated in the standard cohort (Figure 4). Of those who reactivated, a total of 4 patients developed CMV resistance during follow-up. In total 3 of 29 (10.3%) in the standard group developed UL97 mutations associated with ganciclovir resistance (day 30, 36, 113), whereas only 1 of 26 (3.8%) developed a UL97 mutation (day 246; $P = .61$). No patient developed a UL54 mutation during follow-up.

Other transplantation outcomes

The time-to-engraftment and platelet recovery between those who received the intensive and standard strategies was similar (Table 4).[29] 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 GVHD (grade III-IV) were not significantly different ($P = .20$ and $P = .07$, respectively). In the first 100 days nonrelapse mortality was similar between the 2 groups ($P = .30$, log-rank) and at 1 year did not significantly differ between the 2 treatments groups ($P = .63$, log-rank). Importantly, high-dose acyclovir/valacyclovir did not appear to lead to additional renal toxicity during the first 100 days after transplantation (Table 4).

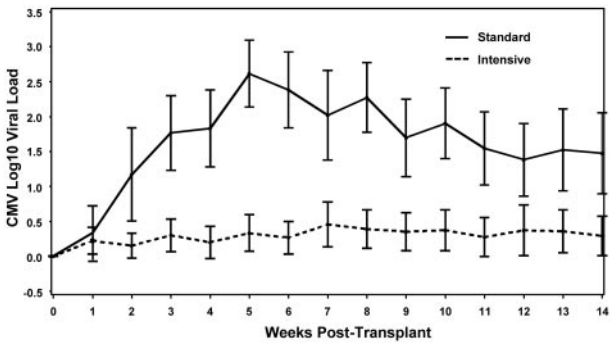

Figure 3. Mean observed CMV viral load in UCBT recipients during the first 100 days after transplantation by type of prevention strategy (n = 72). Whiskers equal 95% CIs for weekly mean value.

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Table 3. Characteristics and outcomes of UCBT recipients who developed CMV disease (n = 10)

Age, y	Prophylaxis	Diagnosis	Conditioning regimen	No. donors	aGVHD grade, days	Time to CMV reactivation, days	Viral load at first reactivation, copies/mL	Time to CMV disease, days	Sites of CMV disease	Out-come
Early disease (days 0-100)										
42	Standard	AML	Cy + Flu + TBI (1320 cGy)	2	2 (21)	57	3354	92	GI	Alive
64	Standard	AML	Cy + Flu + TBI (1320 cGy)	2	2 (34)	18	22 000	34	Lung	Dead
23	Standard	ALL	Cy + Flu + ATG + TBI (200 cGy)	2	3 (15)	17*	35	17*	Lung	Alive
28	Standard	AML	CY + Flu + TBI (1320 cGy)	2	NE	21*	63	33	Disseminated	Dead
21	Standard	ALL	Cy + Flu + TBI (1320 cGy)	2	NE	8*	6000	11*	GI	Dead
42	Standard	ALL	Cy + Flu + TBI (1320 cGy)	2	2 (35)	3*	100	66	GI	Alive
1	Intensive	Hystiocytosis	Campath + Mel + Flu	1	0	3*	47	11*	Lung	Alive
53	Intensive	AML	Treo + Flu + TBI (200 cGy)	2	NE	-	-	42†	Lung	Dead
Late disease (days 101-365)										
54	Standard	AML	Cy + Flu + ATG, TBI (200 cGy)	1	0	21	1053	191	Lung	Dead
2	Standard	AML	Cy + Flu + TBI (1320 cGy)	2	3 (10)	8*	3200	165	Lung	Alive

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; ATG, antithymocyte globulin; Cy, cyclophosphamide; Flu, fludarabine; GI, gastrointestinal; Intensive, intensive prophylactic strategy; Mel, melphalan, NE, not evaluable; Standard, standard prophylactic strategy; TBI, total body irradiation; and UCBT, umbilical cord blood transplantation.

*Complication developed preengraftment.

†Developed early relapse.

Discussion

In this study, an intensive strategy of ganciclovir before transplantation 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. Compared with a standard prophylaxis with a moderate dose of acyclovir that is 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 transplantation-related outcomes.

The most important finding in our study was that use of this intensive approach decreased a patient's risk of developing CMV disease in the first 100 days to 4.6%, a figure similar to rates observed when conventional BM and peripheral blood stem cell sources are used.[31] 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 makes it an

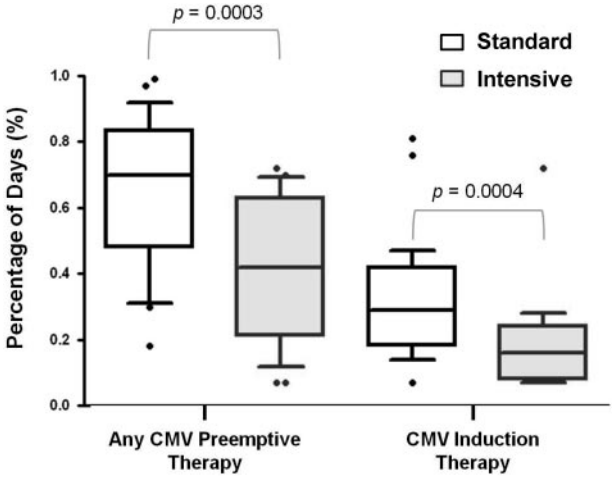

Figure 4. Use of anti-CMV antiviral therapy by type of prevention strategy. Anti-CMV therapy was considered ganciclovir and foscarnet; induction therapy was the period during which patients received the equivalent of twice-daily dosing of anti-CMV therapy. Only patients with proven CMV reactivation included. Total percentage was determined by days on anti-CMV therapy divided by total days alive during the first 100 days after transplantation. Whiskers equal 10th to 90th percentile, and solid dots equal outliers.

Table 4. Other transplantation-related outcomes in UCBT recipients by prevention strategy (n = 72)

Outcomes	Intensive, n = 43 n (%)	Standard, n = 29 n (%)	*P**
Time to engraftment†	20	20	.49§
Median days (IQR)	(17-28)	(14-29)	
Time to platelets ≥ 20 000†	36	34	.37
Median days (IQR)	(31-50)	(29-45)	
Acute GVHD‡			
Grade II-IV	28 (74)	25 (86)	.21
Grade III-IV	8 (21)	10 (34)	.22§
Renal function			
Mean max creatinine (SD)	1.8 (1.2)	1.6 (1.0)	.47
Acute kidney injury‡			
× 2 baseline	28 (65)	18 (62)	.81
× 3 baseline	16 (37)	7 (24)	.31
Nonrelapse mortality			
First 100 days	8 (19)	2 (7)	.30¶
1 year	11 (26)	6 (21)	.63¶

IQR indicates interquartile range; UCBT, umbilical cord blood transplantation.

**P* values calculated with the Fisher 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 $P = .07$ (Gray test).

§When calculated by time to event analysis $P = .20$ (Gray test).

‖Acute kidney injury as defined by ≥ 2 times baseline creatinine or ≥ 3 times baseline, during the first 100 days.[29]

¶Calculated by log-rank.

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attractive option for prevention, and because high-dose acyclovir/valacyclovir has been shown to decrease CMV reactivation in other HSCT populations,[21,32-34] some centers use this method of prevention as a standard in their UCBT recipients.[35] However, high-dose acyclovir alone may have limited ability to decrease the risk of CMV disease in UCBT and other HSCT recipients.[17,21,32,35] Other antiviral options may offer protection from early CMV disease[19,36] but are known to cause additional toxicities.[37] Primary prophylaxis has also been shown to be associated with a greater rate of late CMV disease in part because of the delayed recovery of CMV-specific T-cell immunity,[13,19]

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,[38] 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[14,39]; therefore, interventions at a lower viral load threshold could also partially explain our decreased rate of CMV disease.

Patients also received ganciclovir before transplantation and late valganciclovir as part of our intensive prevention strategy. Ganciclovir administered before transplantation has been shown to decrease CMV complications in other HSCT populations[23,34,40,41] and is hypothesized to decrease the risk of early posttransplantion CMV reactivation.[41] Perhaps because of this intervention, we found that patients in our intensive strategy were significantly less likely to have preengraftment 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 ganciclovir before transplantation may have contributed to lower rates of invasive disease. In addition, although there are too few cases to evaluate in this study, this intervention before transplantation may also provide some protection against preengraftment disease, which is known to be associated with increased mortality.[42] Because the authors of other studies have shown safety and efficacy of greater dosing before transplantation,[34] an increase to treatment levels (5 mg/kg twice daily) before transplantation may have provided additional benefits. The use of valganciclovir likely led to less late CMV disease events, but because of limited late disease events in either cohort, we were not able to demonstrate a statistically significant benefit to its use.

The incidence of CMV reactivation in our standard cohort is consistent with previous 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%.[15,16,43] However, reactivation rates in our intensive strategy were slightly greater than those reported in CMV-seropositive UCBT recipients who received high dose of acyclovir prophylaxis.[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. The presence of antigenemia detects fewer cases of CMV reactivation,[44,45] may necessitate greater viral loads for detection,[46] and positive results are more likely to be delayed until after the presence of symptoms of disease compared with patients screened by PCR.[47] In fact, even when given identical prophylactic regimens, UCBT recipients tested by PCR for preemptive therapy developed fewer episodes of invasive disease compared with those who were screened with an antigenemia-based strategy.[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.[27] Interestingly, 2 patients who were tested by antigenemia only developed preengraftment CMV, where diagnosis was delayed by more than a week compared with 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 an increased use 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 fewer 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, because standard anti-CMV drugs used in preemptive therapy have toxicities that can in lead to increased mortality,[37] the significant reduction observed 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 because acyclovir has been shown to be associated with nephrotoxicty[48] and neurologic complications.[49] During study follow-up, there were no difference in renal outcomes between the 2 study cohorts, and no reports of drug-associated neurologic complications were noted in those treated with high-dose acyclovir. In addition, there appeared to be no effect on engraftment or nonrelapse mortality comparing the 2 cohorts. Perhaps most importantly, the reductions in intravenous ganciclovir/foscarnet use and CMV disease observed 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 2 populations were not entirely comparable because patients in our more intensive prophylactic strategy were greater-risk transplantation recipients because of age and pretransplantation risk stratification. The most important limitation to our study is the small sample size of our study cohort. The majority of studies in which the authors assess CMV risk focus on entire cohorts of UCBT recipients and often include very low-risk CMV-seronegative patients.[17,18,35,50] Because recipient seropositivity remains the most important risk factor for CMV in HSCT and because others have demonstrated increased rates of disease and reactivation in CMV-seropositive UCBT recipients,[16,19] we limited our analyses to this high-risk population. Although it was a smaller cohort size, it allowed us to assess a greater incidence of adverse CMV end points and demonstrate significant differences between our 2 strategies.

Finally, by implementing multiple components in this intensive approach, we found it was not possible to unravel the benefit of each specific intervention. For example, the protection from early reactivation ($<$ day 30) could be because of pretransplantation ganciclovir, high-dose acyclovir, or a combination of both components. On the basis of these data, we can only recommend this strategy as a combination of therapies, but future prospective

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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 posttransplantation antiviral prophylaxis, increased frequency of preemptive screening, and lowered thresholds for the institution of preemptive therapy, we were able to demonstrate additional protection against CMV disease and the development of preengraftment 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.

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Authorship

Contribution: F.M. and S.A.P. participated in research design, data collection, performed statistical analyses, and wrote the manuscript; H.X. performed statistical analyses under direction of W.M.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; and J.A.G., I.R., M.J.B., and C.D. participated in research design and contributed to the writing and review of the manuscript.

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