Cytomegalovirus viremia, disease, and impact on relapse in T-cell replete peripheral blood haploidentical hematopoietic cell transplantation with post-transplant cyclophosphamide

There are multiple recent publications demonstrating an association between the development of post-transplant cytomegalovirus (CMV) infection and a reduced incidence of relapse in patients who receive allogeneic hematopoietic cell transplantation (allo-HCT).[1–3] This was demonstrated in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).[4] Other studies have demonstrated both early and late protective effects, but all have been on matched related or unrelated donors.[5] Studies are focusing on $CD56^{dim}CD57^{+}NKG2C^{+}$ NK cells following CMV reactivation as the potential immunomodulatory mechanism.[6]

Haploidentical hematopoietic cell transplantation (haplo-HCT) has expanded the potential donor pool, and post-transplant cyclophosphamide (PT-Cy) offers effective graft-*versus*-host disease (GvHD) prophylaxis due to the selective depletion of the alloreactive T cell clones,

Table 1. Baseline characteristics stratified by post-transplant CMV viremia.

	No CMV Viremia		CMV Viremia		*P*
Number of patients	58		80		
Patient age - No. (%)					0.04
<60	47	(81.0)	52	(65.0)	
≥60	11	(19.0)	28	(35.0)	
Patient sex - No. (%)					0.04
Female	23	(39.7)	46	(57.5)	
Male	35	(60.3)	34	(42.5)	
Donor sex - No. (%)					0.24
Female	26	(44.8)	28	(35.0)	
Male	32	(55.2)	52	(65.0)	
Race - No. (%)					0.004
White	53	(91.4)	57	(71.3)	
Non-White	5	(8.6)	23	(28.8)	
CMV serostatus - No. (%)					<0.001
D-/R-	35	(60.3)	5	(6.3)	
D-/R+	6	(10.3)	31	(38.8)	
D+/R-	13	(22.4)	5	(6.3)	
D+/R+	4	(6.9)	39	(48.8)	
Diagnosis - No. (%)					0.32
AML	42	(72.4)	51	(63.8)	
MDS	7	(12.1)	8	(10.0)	
Other Diagnoses	9	(15.5)	21	(26.3)	
Disease status at transplant - No. (%)					0.15
Active	23	(39.7)	30	(37.5)	
Remission	35	(60.3)	45	(56.3)	
BM Failure	0	(0.0)	5	(6.3)	
Prior Transplant - No. (%)					0.10
No	36	(62.1)	60	(75.0)	
Yes	22	(37.9)	20	(25.0)	
Conditioning regimen - No. (%)					0.11
Myeloablative	29	(50.0)	29	(36.3)	
Reduced Intensity (RIC)	29	(50.0)	51	(63.8)	
Immune prophylaxis - No. (%)					0.28
Tacro/MMF	54	(93.1)	70	(87.5)	
Other	4	(6.9)	10	(12.5)	
Disease Risk Index					0.92
Low	2	(3.4)	2	(2.5)	
Intermediate	25	(43.1)	34	(42.5)	
High	21	(36.2)	29	(36.3)	
Very High	10	(17.2)	10	(12.5)	
aGvHD - No. (%)					0.51
Grades 0-I	41	(70.7)	50	(62.5)	
Grades II-IV	17	(29.3)	27	(33.8)	
Grades III-IV	6	(10.3)	10	(12.5)	
Not assessable	0		3	(3.8)	
cGvHD - No. (%)					0.57
Yes	17	(29.3)	20	(25.0)	
No	41	(70.7)	60	(75.0)	
Severe	3	(5.2)	2	(2.5)	
Time to Neutrophil Engraftment – Days (SD)	20.2	(7.1)	19.6	(9.5)	0.62
Graft Failure - No. (%)	2	(3.4)	2	(2.5)	1.0

CMV: cytomegalovirus; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; BM: bone marrow; aGHvD: acute graft-versus-host disease; cGHvD: chronic graft-versus-host disease; SD: standard deviation; D: donor; R: recipients.

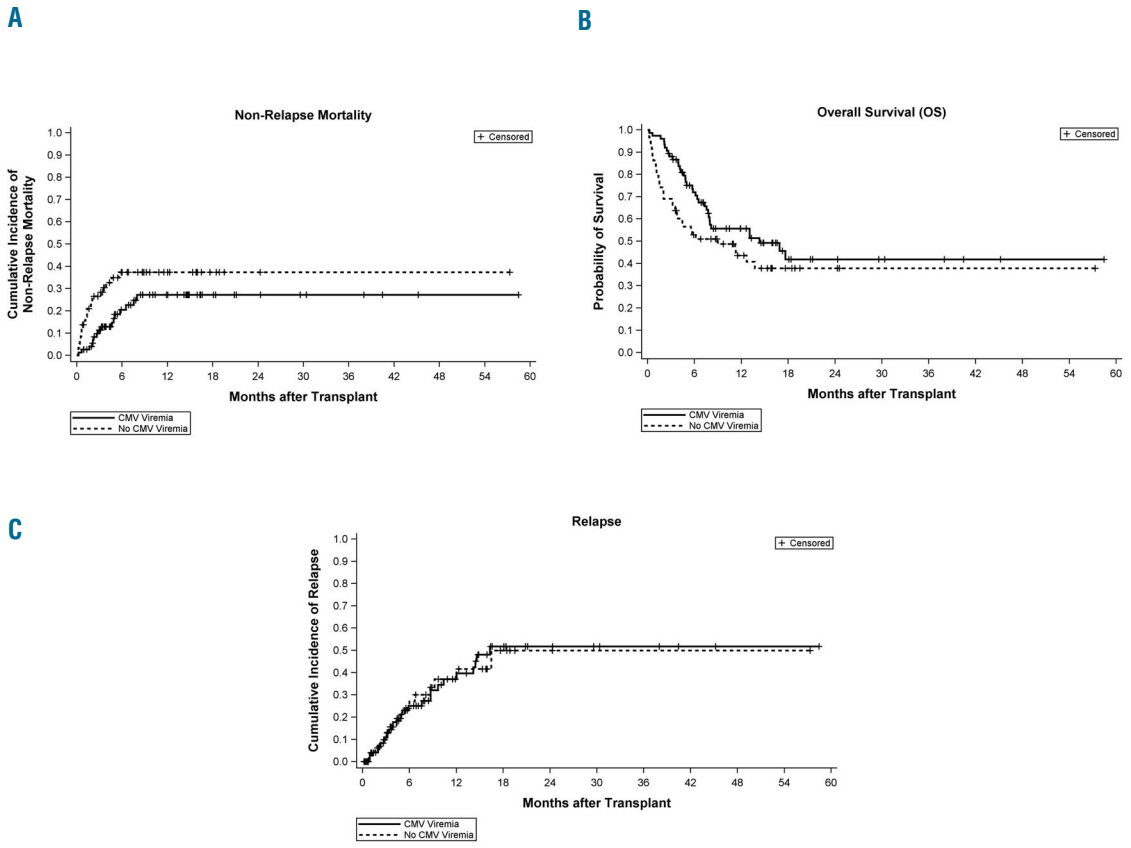

Figure 1. Kaplan-Meier estimates assessing the impact of post-transplant CMV viremia (as a time-dependent covariate) on A) non-relapse mortality; B) Overall Survival; C) Cumulative Incidence of Relapse/Progression.

and is more convenient than labor-intensive *ex vivo* CD34+ cell selection protocols.[7] CMV viremia appears to occur frequently following haplo-HCT, but there is a paucity of CMV-related data in haplo-HCT recipients receiving T-cell replete (TCR) peripheral blood grafts with PT-Cy.[8] We conducted a retrospective study on a large cohort of such haplo-HCT recipients in order to determine the incidence of CMV viremia and disease, and the impact of CMV viremia on the incidence of relapse or progression (CIR), overall survival (OS), non-relapse mortality (NRM), and GvHD.

CMV *viremia* was defined as the detection of CMV DNA in patients' blood, monitored routinely by real-time qPCR. CMV disease was defined by end-organ dysfunction attributable to CMV, as described by published criteria.[9] Acute GvHD (aGvHD) was diagnosed clinically, and graded according to standard critieria.[10] Chronic GvHD (cGvHD) was graded as mild, moderate, or severe.[11] Based on the refined Disease Risk Index (DRI), diseases were labeled as low-, intermediate-, high-, or very high-risk.[12] Disease status at transplant was defined as clinical remission (CR) or active disease based on a morphological assessment of bone marrow conducted 30 days prior to transplant. Bone marrow biopsies were conducted following haplo-HCT at 30 days, 100 days, then every 6 months, or earlier depending on findings concerning for relapse or progression. Relapse and progression were defined per accepted criteria.[13] Descriptive endpoints consisted of incidences of CMV viremia, CMV disease (among those with viremia), aGvHD, and cGvHD. Correlative endpoints included CIR, OS, and NRM with the cohorts stratified by the presence or absence of CMV viremia post-transplant. OS was defined as the time from haplo-HCT to last follow-up or death from any cause. Potential covariates were subjected to univariate proportional hazards Cox regression. CMV viremia was coded as a time-dependent covariate. CIR and NRM were analyzed using Gray's subdistribution method to account for competing risk events, and multivariate Cox models or Gray's subdistribution models were constructed in a stepwise fashion. All tests were two-sided with a *P*-value <0.05 being significant. Subgroup analysis was conducted on patients who had only AML, those with AML who went into transplant in clinical remission (CR), and those patients who survived to 100 days without disease.

One-hundred and thirty-eight patients were identified who had received haplo-HCT with peripheral blood grafts and PT-Cy. Baseline characteristics are presented in Table 1. Median follow-up for all patients was 220 days (range: 5-1777). For those still alive, median follow-up was 441 days (range: 82-1777). Both groups had comparable malignancies, with AML comprising the majority of both cohorts. Patients in the CMV cohort were statistically more likely to be 60 years or older, and belong to a racial minority. CMV serostatus was significantly different between groups (*P*<0.001). Other baseline characteristics were not statistically different. Acute GvHD grades II-IV developed in 27 (33.8%) and 17 (29.3%) patients in the CMV viremia group and non-CMV viremia group, respectively, (*P*=0.51). Ten (12.5%) and 6 (10.3%)

patients developed aGvHD grades III-IV, respectively. Chronic GvHD was diagnosed in 20 (25.0%) and 17 (29.3%) patients, respectively, (*P*=0.57). Three patients from the non-viremia cohort, and 2 from the viremia cohort had severe cGvHD. Time to engraftment and incidences of graft delays or failures were comparable. Information on cytokine release syndrome was available for 75 patients, with no significant difference in incidence or grades.

Of the 138 haplo-HCT recipients analyzed, 80 (58%) had post-transplant CMV viremia. Seventy-five (77%) of the 98 patients with donor and/or recipient seropositivity, those considered to be at higher risk for CMV, had post-transplant viremia. Five (13%) of the 40 D-/R- patients became viremic. All but 2 viremic patients had CMV viremia within 100 days post-transplant, the median time to viremia was 24 days (range: 3-68). Two outliers had viremia at 161 and 240 days post-transplant; both had late aGvHD manifestations and were on prolonged high-dose corticosteroids.

CMV disease occurred in 23 (28.8%) of the 80 patients with CMV viremia. Seven had CMV gastroenterocolitis, 12 developed pneumonitis, and 3 patients had both. One had CMV retinitis, meningitis, and pneumonitis. Four deaths were attributable to CMV pneumonitis. Out of the 18 patients with a D+/R- serostatus combination, 5 developed viremia and none suffered from CMV disease.

In univariate analysis of NRM, there was not a statistical difference between those with CMV viremia and those without (HR: 0.59, 95% CI 0.31-1.13, *P*=0.11, Figure 1A). Among the 133 patients with a hematologic malignancy, post-transplant CMV viremia was not associated with a statistical difference in overall survival (HR: 0.66, 95% CI 0.41-1.06, *P*=0.09, Figure 1B). In a multivariate model for OS adjusting for "very-high" DRI, CMV viremia remained statistically insignificant (HR: 0.68, 95% CI 0.42-1.09, *P*=0.11).

Seventy-three patients with a hematologic malignancy had post-transplant CMV viremia within 100 days post-transplant, and 58 did not have post-transplant CMV viremia. In univariate Cox proportional hazard analysis, the hazard ratio (HR) for CIR in those with post-transplant CMV viremia was 1.05 (95% CI 0.57-1.94, *P*=0.97, Figure 1C). After adjustment for "very-high" DRI, RIC regimen, and D+/R- serostatus combination, the multivariate HR for CIR in patients with post-transplant CMV viremia was 2.46 (95% CI 0.89-6.77, *P*=0.08). D+/R- combination, but not D-/R-, had a significantly higher CIR compared to D-/R+ or D+/R+ (HR 2.68, 95% CI 1.04-6.96, *P*=0.04). We conducted analysis on a subgroup of 88 patients who had survived, relapse-free, to 100 days. Fifty-two (59.1%) had post-transplant CMV viremia within that period, with no significant difference in CIR (HR: 1.03, 95% CI 0.47-2.24, *P*=0.94). We conducted subset analyses using data from 93 patients with AML, of whom 51 (54.8%) experienced CMV viremia within 100 days. In univariate analysis, CMV viremia was not associated with a statistical difference in CIR (HR: 0.84, 95% CI 0.40-1.78, *P*=0.65). Of the 57 AML patients in CR at transplant, 32 had post-transplant CMV viremia. Among them, there was no statistical difference in CIR (HR: 0.67, 95% CI 0.21-2.11, *P*=0.49) or OS (HR: 0.79, 95% CI 0.35-1.77, *P*=0.56).

To the best of our knowledge, this is the first retrospective study of a large cohort of recipients of haplo-HCT with PT-Cy to describe the incidence of CMV viremia and disease, and to analyze its impact on relapse and survival. Our data corroborate that there is a high incidence of CMV viremia following haplo-HCT, especially in those at higher risk.[8] 77% of those at higher risk for CMV infection (R+ or D+/R-) developed CMV viremia in our study. CMV viremia occurred far earlier in our cohort (24 days) than has been reported in the recent large CIBMTR registry study of matched transplantation (41 days).[14] The occurrence of CMV disease in almost one-third of patients with viremia, despite a standardized preemptive treatment protocol, was higher than the expected 5-10%.[9,15] While few in absolute numbers, those who died of CMV and those who developed late CMV viremia tended to have acute or chronic GvHD more frequently, requiring steroids, but there was no obvious association with the conditioning regimen.

Interestingly, while five D+/R- patients developed viremia, none of them manifested CMV disease. While difficult to draw conclusions from such small samples, donor-derived cellular-based immunity to CMV may be able to prevent CMV disease following primary CMV infection, but not following CMV reactivation in seropositive recipients. The high incidence of CMV disease in this patient cohort should promote the development of tailored preemptive treatment protocols for this population.

Unlike the multiple studies of matched unrelated donors and matched related donors, our data of haplo-HCT do not statistically correlate CMV viremia with reduced CIR, either overall or among our subgroups comparable to previous studies. Neither do they demonstrate statistical differences in NRM, aGvHD, or cGvHD. It is unclear as to why relapse protection was not observed as it has been previously in matched transplantation, and it is premature to implicate PT-Cy as mitigating this effect. Interestingly, seronegative recipients receiving seropositive grafts had more than twice the incidence of relapse compared to seropositive recipients, even adjusting for CMV viremia. A large collaborative or registry study of CMV outcomes in haplo-HCT would better elucidate whether conferred immunity or post-transplant infection impact relapse, an option we intend to pursue in the near future.

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