# Impact of Cytomegalovirus Replication and Cytomegalovirus Serostatus on the Outcome of Patients with B Cell Lymphoma after Allogeneic Stem Cell Transplantation

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*Article history:*
Received 5 November 2013
Accepted 18 February 2014

*Key Words:*
Allogeneic hematopoietic stem cell transplantation
Cytomegalovirus (CMV)
Lymphoma

A B S T R A C T

Cytomegalovirus (CMV) replication after allogeneic hematopoietic stem cell transplantation (HSCT) was historically associated with increased nonrelapse mortality (NRM). More recently, different groups have reported an association between CMV replication and reduced risk of acute myeloid leukemia (AML) relapse. Given the conflicting results, we evaluated the impact of CMV replication and other covariates on the outcome of a retrospective cohort of 265 adults with B cell lymphoma receiving allogeneic HSCT from HLA-identical siblings or alternative donors. In time-dependent multivariate analysis, CMV replication, evaluated by pp65 antigenemia, had no independent effect on the risk of relapse (hazard ratio [HR], 1.0; 95% confidence interval [CI], .6 to 1.6; $P = .9$), although it was associated with a reduced overall survival (HR, 2.0; 95% CI, 1.3 to 3.2; $P = .001$) and an increased NRM (HR, 2.5; 95% CI, 1.1 to 5.3; $P = .01$). Consistently, donor and/or recipient CMV seropositivity were not associated with a different outcome relative to CMV double-negative serostatus. In multivariate models, a diagnosis of follicular lymphoma ($P < .0001$) and pretransplantation complete remission status ($P < .0001$) were the main independent predictors for improved relapse-free survival. In summary, contrary to what is observed in patients with AML, this report identifies no independent role for CMV replication or serostatus on the relapse of patients with B cell lymphomas undergoing allogeneic HSCT.


## INTRODUCTION

Patients with lymphoid malignancies who relapse after second-line chemotherapy or autologous transplantation have a dismal prognosis and very few effective options for salvage treatment. Allogeneic hematopoietic stem cell transplantation (HSCT) after myeloablative (MAC) or reduced-intensity conditionings (RIC) is the only potential curative strategy for patients with recurrent indolent or aggressive non-Hodgkin's (NHL) and Hodgkin's lymphomas (HL) [1,2].

In a prospective trial for lymphoma patients [3], we have previously identified lymphoma histotype and pre-transplantation disease status as the main independent variables affecting the risk of disease relapse and post-transplantation outcome. More recently, human cytomegalovirus (CMV) replication was found to be associated not only with higher post-HSCT nonrelapse mortality (NRM) [4], but also with a reduced risk of relapse for patients with acute myeloid leukemia (AML) [5]. This observation, in line with previous reports on the effect of CMV replication and serostatus on the outcome of patients with AML [6-8], was not confirmed in a recent retrospective analysis of subjects with other myeloid and lymphoid malignancies who underwent transplantation [9].

Given the conflicting results, we analyzed a retrospective cohort of 265 B cell lymphoma patients receiving allogeneic HSCT from HLA-identical siblings or alternative donors to investigate the potential role of post-HSCT CMV replication and pre-HSCT CMV serostatus on transplantation outcome.

## PATIENTS AND METHODS

### *Study Design*

This is a retrospective study including 265 consecutive adult B cell lymphoma patients who underwent transplantation in any 1 of 7 Italian institutions between April 1998 and November 2012. The institutional

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1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.02.015

**Table 1**
Patients Characteristics According to Post-transplantation pp65 Antigenemia

| Characteristics | All Patients | Patients with pp65-Antigenemia | Patients without pp65-Antigenemia | *P* Value |
|---|---|---|---|---|
| Patients, n | 265 | 133 (50%) | 132 (50%) | |
| Histologic Subtype | | | | |
| Follicular non-Hodgkin lymphoma | 63 (20%) | 37 (57%) | 27 (43%) | |
| Aggressive non-Hodgkin lymphoma | 94 (31%) | 46 (49%) | 48 (51%) | .3 |
| Chronic lymphocytic leukemia | 22 (7%) | 13 (59%) | 9 (41%) | |
| Hodgkin lymphoma | 84 (27%) | 37 (44%) | 47 (56%) | |
| Patient age, median (range), yr | 45 (18-68) | | | .2 |
| <40 | 101 (38%) | 46 (45%) | 55 (55%) | |
| ≥40 | 164 (62%) | 88 (53%) | 76 (47%) | |
| Donor type | | | | **.003** |
| Sibling | 139 (52%) | 58 (42%) | 81 (58%) | |
| Alternative | 126 (48%) | 76 (60%) | 50 (40%) | |
| Immunosuppression | | | | .03 |
| CSA/MTX | 141 (55%) | 62 (43%) | 79 (57%) | |
| ATG or alemtuzumab | 124 (45%) | 71 (57%) | 53 (43%) | |
| Donor/patient CMV serostatus | | | | **<.0001** |
| Negative/Negative | 28 (12%) | 1 (3%) | 27 (97%) | |
| Others | 237 (88%) | 132 (55%) | 105 (45%) | |
| Disease status at HSCT | | | | .2 |
| CR | 108 (41%) | 61 (56%) | 47 (44%) | |
| PR | 92 (36%) | 43 (47%) | 49 (53%) | |
| Refractory (SD/PD) | 65 (23%) | 30 (46%) | 35 (54%) | |
| Number of lines of CT | | | | 1 |
| ≤2 | 68 (26%) | 34 (50%) | 34 (50%) | |
| >2 | 197 (74%) | 99 (50%) | 98 (50%) | |
| Preparative regimen | | | | .1 |
| MAC | 75 (26%) | 43 (57%) | 32 (43%) | |
| RIC | 189 (74%) | 90 (47%) | 99 (53%) | |
| Graft source | | | | .6 |
| PBSC | 239 (92%) | 122 (51%) | 117 (49%) | |
| BM | 26 (18%) | 12 (46%) | 14 (54%) | |
| aGVHD | | | | |
| No | 134 (51%) | 64 (48%) | 68 (52%) | .5 |
| Yes | 131 (49%) | 69 (52%) | 62 (48%) | |
| Grade I-II | 104 | 51 (49%) | 53 (51%) | .6 |
| Grade III-IV | 27 | 15 (55%) | 12 (45%) | |
| cGVHD | | | | 1 |
| No | 169 (65%) | 66 (51%) | 64 (49%) | |
| Yes | 96 (35%) | 48 (50%) | 48 (50%) | |
| Mild | 36 | 17 (47%) | 19 (53%) | .5 |
| Moderate | 32 | 15 (47%) | 17 (53%) | |
| Severe | 27 | 16 (59%) | 11 (41%) | |

CR indicates complete remission; PR, partial remission; SD/PD, stable disease/progressive disease; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; CT, chemotherapy; CMV, cytomegalovirus; CSA, cyclosporine; MTX, methotrexate; ATG, antithymocyte globulin.
*P* value with Fisher's test.
Bold indicates significant *P* values.

review boards of the 7 hospitals approved the study. The patient population encompasses all subjects with lymphoma who received allogeneic HSCT at the fore-mentioned institutions fulfilling the following criteria: (1) graft source represented by an HLA-identical sibling or an alternative (matched unrelated donor or unmatched sibling, excluding haploidentical relatives) donor; (2) availability of complete information about HLA matching between donor and recipient at the HLA loci A, B, C, DRB1, and DQB1, through high-resolution genotyping (previously described [10]); (3) availability of pretransplantation–CMV antibody serological status of both donor and recipient; (4) availability of CMV-seronegative donors of blood product substitutions for patients and donors pairs with pretransplantation-negative CMV status; (5) regularly monitored, once or twice weekly, CMV replication by pp65-antigenemia until at least week 16 after transplantation; (6) no use of prophylactic donor lymphocyte infusion; and (7) confirmed histologic diagnosis of B cell NHL or HL. Patients with T cell lymphomas were not included, given their different outcome.

### *Transplantation Characteristics*

The pretransplantation conditioning regimen consisted of the combination of thiotepa, either at 15 mg/kg (myeloablative) or 10 mg/kg (reduced intensity) and cyclophosphamide 60 mg/kg plus fludarabine 60 mg/m$^2$ in most patients (n = 159), as previously described [11]; thiotepa-cyclophosphamide plus melphalan 70 mg/m$^2$ in 11 subjects; and thiotepa-cyclophosphamide in 30 patients [12]. Other conditioning regimens were melphalan 140 mg/m$^2$ plus fludarabine 60 mg/m$^2$ in 13 subjects and the combination of low-dose total body irradiation with chemotherapy in the remaining 51 subjects [13]. Pharmacological prophylaxis of acute graft-versus-host disease (GVHD) consisted of oral cyclosporine and short-course methotrexate for the 139 patients who underwent transplantation from an identical sibling. One-hundred twenty-six patients received a graft from an alternative donor (8 from a mismatched sibling, 50 from an HLA-matched unrelated donor (MUD), and 68 from a mismatched MUD): these patients received further immunosuppression, either with antithymocyte globulin (ATG, Thymoglobulin, Genzyme, Europe BV, Naarden, The Netherlands) 7 mg/kg total dose on days -4 and -3 before transplantation (92 subjects) or with alemtuzumab 15 to 30 mg/m$^2$ (30 subjects), as previously published [13-15]. Mismatched donors comprised both antigen- or allele-mismatched transplantations at loci HLA-A, -B, -C, -DR, or -DP.

Pretransplantation disease status and response were evaluated by computed tomography or positron emission tomography and assessed according to criteria established by Cheson et al. [16]. Acute and late acute GVHD [17] and chronic or early chronic GVHD were diagnosed as previously described [18].

### *CMV Antigenemia and Pre-Emptive Therapy*

CMV replication was detected by pp65-antigenemia positivity. Monitoring started when a white blood cell count of 500 per microliter was reached after HSCT and continued until week 16 [19]. CMV replication was

**Table 2**
Variables Influencing Five-Year Cumulative Incidence of Relapse, Overall Survival, and Nonrelapse Mortality in Univariate Analysis*

| Characteristics | Cumulative Incidence of Relapse (95% CI) | *P* Value† | *P* Adjusted§ | OS (%) (95% CI) | *P* Value‡ | *P* Adjusted§ | NRM (%) (95% CI) | *P* Value‡ | P Adjusted§ |
|---|---|---|---|---|---|---|---|---|---|
| Histologic Subtype | | | | | | | | .2 | 1.0000 |
| Follicular non-Hodgkin lymphoma | 14 (6-24) | **<.001** | .0014 | 66 (54-88) | **.02** | .2800 | 28 (16-40) | | |
| Aggressive non-Hodgkin lymphoma | 48 (37-58) | | | 41 (31-51) | | | 25 (15-36) | | |
| Chronic lymphocytic leukemia | 48 (25-69) | | | 52 (31-74) | | | 37 (15-59) | | |
| Hodgkin lymphoma | 52 (41-63) | | | 53 (40-66) | | | 18 (8-28) | | |
| Age | | | | | | | | .3 | 1.0000 |
| <40 | 54 (44-63) | **.0008** | .0112 | 42 (31-53) | .2 | 1.0000 | 21 (12-30) | | |
| ≥40 | 34 (27-42) | | | 57 (50-65) | | | 27 (20-35) | | |
| Donor type | | | | | | | | .2 | 1.0000 |
| HLA-identical Sibling | 42 (34-50) | .7 | 1.0000 | 57 (49-66) | **.03** | .4200 | 21 (14-28) | | |
| Alternative | 42 (32-51) | | | 44 (34-54) | | | 28 (19-38) | | |
| Immunosuppression | | | | | | | | **.04** | .5600 |
| CSA/MTX | 43 (35-51) | .5 | 1.0000 | 57 (49-66) | **.03** | .4200 | 19 (12-27) | | |
| ATG or alemtuzumab | 41 (31-50) | | | 45 (35-55) | | | 31 (22-40) | | |
| Donor/patient CMV serostatus | | | | | | | | .1 | 1.0000 |
| Negative/negative | 55 (34-72) | .1 | 1.0000 | 56 (23-79) | .2 | 1.0000 | 12 (0-23) | | |
| Others | 40 (34-47) | | | 51 (44-58) | | | 26 (20-32) | | |
| CMV reactivation | | | | | | | | .2 | 1.0000 |
| Yes | 34 (25-42) | .03 | .4200 | 48 (39-57) | .2 | 1.0000 | 30 (21-39) | | |
| No | 50 (41-58) | | | 56 (47-65) | | | 19 (12-26) | | |
| Disease status at HSCT | | | | | | | | .3 | 1.0000 |
| CR | 30 (21-40) | **≤.0001** | .0014 | 65 (55-75) | **<.0001** | .0014 | 21 (13-29) | | |
| PR | 43 (33-54) | | | 54 (43-65) | | | 25 (16-35) | | |
| Refractory (SD/PD) | 59 (46-70) | | | 25 (14-36) | | | 34 (17-51) | | |
| aGVHD | | | | | | | | | |
| No | 45 (36-54) | .2 | 1.0000 | 59 (50-68) | .06 | .8400 | 18 (11-26) | **.03** | .4200 |
| Yes | 38 (29-47) | | | 45 (36-54) | | | 31 (22-40) | | |
| Grade I-II | 45 (34-55) | .01 | .1400 | 52 (42-62) | **≤.0001** | .0014 | 23 (14-32) | **≤.0001** | .0014 |
| Grade III-IV | 18 (6-34) | | | 20 (5-35) | | | 60 (51-79) | | |
| cGVHD | | | | | | | | | |
| No | 45 (37-53) | .2 | 1.0000 | 48 (40-56) | **.01** | .1400 | 25 (18-32) | .1 | 1.0000 |
| Yes | 37 (27-47) | | | 59 (49-69) | | | 21 (12-30) | | |
| Mild | 35 (20-50) | .9 | 1.0000 | 75 (62-89) | **.01** | .1400 | 5 (0-12) | **.0008** | .0112 |
| Moderate | 42 (23-60) | | | 59 (39-79) | | | 15 (0-32) | | |
| Severe | 36 (18-53) | | | 38 (20-56) | | | 48 (28-68) | | |

OS indicates overall survival; NRM, nonrelapse mortality; CR, complete remission; PR, partial remission; SD/PD, stable disease/progressive disease; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplant; CT, chemotherapy; CI, confident interval; CMV, cytomegalovirus; CSA, cyclosporine, MTX, methotrexate.
Bold indicates significant *P* values.
* Only variables with a significant *P* values are shown, for variables analyzed see material and methods.
† *P* value with Gray test.
‡ *P* value with log-rank test.
§ *P* adjusted value with Bonferroni method.

assumed if at least 5 CMV pp65 antigen-positive cells per $5 \times 10^5$ white blood cells were detected at 2 consecutive time points. All patients received acyclovir prophylaxis up to 1 year after transplantation. In all patients, CMV pre-emptive treatment consisted of ganciclovir 5 mg/kg twice each day or foscarnet 90 mg/kg twice each day [20], until the first negative antigenemia sample, followed by maintenance treatment for the subsequent 2 weeks.

### *Statistical Analysis*

Univariate overall survival (OS) and nonrelapse mortality (NRM) were calculated with Kaplan-Meier method and groups were compared with log-rank test. Cumulative incidence method and groups were compared with Gray's test to evaluate the variables for relapse, treating death in remission as a competing event. Multiple univariate comparisons were adjusted with the Bonferroni method. The multivariate analysis was done with the Cox model to evaluate risk factors for relapse, NRM, and mortality. The covariates were chosen by the clinicians, based on clinical relevance on the prognosis after transplantation. All the covariates were entered in the model in 1 single step ("enter" method). CMV replication after transplantation, acute GVHD, and chronic GVHD were considered as time-dependent covariates. The other variables included, both for univariate and multivariate analysis, were pretransplantation disease status (complete remission [CR], partial remission, and refractory disease, including stable and progressive disease), lymphoma histology (follicular and aggressive NHL, chronic lymphocytic leukemia, and HL), ex vivo T cell depletion, graft source (peripheral blood versus bone marrow), intensity of the conditioning regimen (RIC versus MAC), and pretransplantation donor-recipient CMV serostatus (donor-recipient double negative or positivity of the donor and/or recipient). The analysis was done with R software version (Ultima, Wien, Austria) (package survival).

## RESULTS

### *Patient Characteristics*

The cohort included a total of 265 patients with B cell lymphoma, 181 with NHL, and 84 with HL. Patient characteristics relative to CMV replication are summarized in Table 1. Pp65-antigenemia positivity occurred in 133 patients (50%) at a median of 34 days after allogeneic HSCT, with 90% of reactivation diagnosed before day 100. Patients with and without CMV replication were similar relative to histologic subtype, graft source, age, number of pretransplantation chemotherapy lines, pretransplantation disease status, and onset of acute or chronic GVHD. As predicted, CMV replication occurred more frequently in patients who had a positive donor or recipient CMV serostatus ($P < .0001$), and in those who underwent transplantation from an alternative donor ($P = .003$), receiving a more potent immunosuppression with in vivo T cell depletion ($P = .03$).

### *Risk of Relapse*

With a median follow-up of 814 days (range, 6 to 5727), 107 of 265 (40%) patients experienced disease relapse or progression at a median of 185 days (range, 21 to 1980) after transplantation. The 5-year cumulative incidence of relapse was 42%. The main variables associated with a reduced 5-year

cumulative incidence of relapse were the following: having follicular lymphoma, being in CR before transplantation, having post-HSCT CMV replication, and being older than 40 years (Table 2). This last variable was related to the fact that follicular lymphoma was more frequent after 40 years of age ($P < .0001$). Of note, when multiple univariate comparisons were adjusted with the Bonferroni method, CMV replication was no longer associated with a reduced incidence of relapse (Table 2). In the time-dependent multivariate analysis, neither CMV replication nor CMV seropositivity were independent predictors for the risk of relapse (hazard ratio [HR], 1.0; 95% confidence interval [CI], .6 to 1.6; $P = .9$ and HR, .8, 95% CI, .4 to 1.6; $P = .6$, respectively) (Table 3). On the contrary, lymphoma subtype and pretransplantation disease status remained independent variables affecting disease relapse: HL, aggressive NHL, and chronic lymphocytic leukemia had higher relapse rates relative to follicular lymphomas and, similarly, patients with persistent disease relative to those in CR (Table 3).

### *Overall Survival and Nonrelapse Mortality*

At the last follow-up, 125 patients had died, 65 with active disease and 60 because of NRM. Five-year OS and NRM were 52% and 25%, respectively. In univariate analysis, CMV replication and CMV serostatus were not associated with different OS or NRM rates. Of note, CR status, follicular NHL, and the occurrence of chronic GVHD were associated with a higher chance of survival (Table 2). In addition, patients receiving in vivo T cell depletion with ATG or alemtuzumab, those who underwent transplantation from an alternative donor, and those developing grade III to IV acute GVHD and severe chronic GVHD had a lower OS because of a higher NRM rate (Table 2). When multiple univariate comparisons were adjusted with the Bonferroni method, only pretransplantation disease status, grade III to IV acute GHVD, and severe chronic GVHD were associated with different OS and NRM rates (Table 2). In time-dependent multivariate analysis, pretransplantation disease status was the main variable affecting mortality, as patients with refractory disease had roughly a 4-fold risk of death compared with those in CR (Table 3). Moreover, CMV replication was associated with a lower chance of survival (HR, 2.0; 95% CI, 1.3 to 3.2; $P = .001$) and an increased NRM rate (HR, 2.5; 95% CI, 1.1 to 5.3; $P = .01$) (Table 3).

### *Effect of pp65-antigenemia on Specific Subtypes*

Recent reports suggested that the antitumor effect associated with CMV replication might be mediated by the donor immune system activated by the viral infection [21,22]. We, therefore, performed subsequent analysis in a subgroup of patients who were more likely to benefit from the immune-mediated antitumor effect—those receiving transplants from an HLA-identical sibling, and, therefore, not treated with in vivo T cell depletion.

We found that CMV replication, together with follicular lymphoma histology, pretransplantation CR status, acute and chronic GVHD, was associated with a lower 5-year cumulative incidence of relapse (Supplementary Table 1). Disease status and histologic subtype were the main factors affecting OS, whereas acute GVHD and severe chronic GVHD were the only variables associated with a higher NRM (Supplementary Table 2). Again, when multiple univariate comparisons were adjusted with the Bonferroni method, only lymphoma histology, pretransplantation disease status, and grade III to IV acute GVHD retained a significant effect on outcome (Supplementary Table 1). In the time-dependent multivariate analysis, CMV replication and serostatus were not independent factors for the risk of relapse (HR, .5 and 2.3, respectively) (Supplementary Table 2). A diagnosis of follicular NHL was an independent predictor of reduced relapse risk, whereas pretransplantation CR status and a peripheral blood donor graft were both associated with reduced chance of relapse and mortality.

## DISCUSSION

In this retrospective study, we reported that CMV replication after allogeneic HSCT is not an independent variable for reduced risk of relapse of patients with B cell lymphomas, but it is associated with lower overall survival and increased nonrelapse mortality. Pretransplantation disease status and lymphoma histotype represented the main variables affecting transplantation outcome, both in terms of relapse risk and mortality rate.

We confirmed the previous findings that the main variables associated with relapse risk after HSCT are lymphoma histotype, with patients with HL and aggressive NHL having a higher

**Table 3**
Variables Influencing the Risk of Relapse, Mortality and Nonrelapse Mortality in Multivariate Analysis

| Covariate | Risk of Relapse | | Mortality | | NRM | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value |
| Diagnosis | | | | | | |
| Follicular non-Hodgkin lymphoma | 1 | | 1 | | 1 | |
| Aggressive B cell lymphoma | 6.0 (2.5-14.3) | **<.0001** | 1.4 (.8-2.4) | .2 | .3 (.1-.8) | **.01** |
| Hodgkin lymphoma | 5.0 (2.0-12.2) | **.0003** | .7 (.4-1.3) | .4 | .2 (.1-.6) | **.003** |
| CLL | 4.7 (1.7-13.1) | **.002** | .9 (.4-1.9) | .8 | .6 (.2-1.8) | .4 |
| Pretransplantation Disease Status | | | | | | |
| CR | 1 | | 1 | | 1 | |
| PR | 2.4 (1.4-4.1) | **.006** | 1.5 (.9-2.6) | .08 | .8 (.3-1.8) | .7 |
| Refractory | | | | | | |
| SD | 2.1 (.9-4.8) | **.07** | 3.1 (1.5-6.5) | **.001** | 2.3 (.7-7.1) | .1 |
| PD | 5.2 (2.8-9.5) | **<.0001** | 4.9 (2.8-8.6) | **<.0001** | 2.2 (.8-5.5) | .1 |
| CMV replication after HSCT | 1.0 (.6-1.6) | .9 | 2.0 (1.3-3.2) | **.001** | 2.5 (1.1-5.3) | **.01** |
| CMV serostatus (D/R positive versus DN) | .8 (.4-1.6) | .6 | .8 (.3-1.7) | .6 | 1.2 (.2-6.1) | .7 |
| Acute GVHD | .6 (.4-1.0) | .1 | 1.0 (.7-1.6) | .6 | 2.3 (1.1-4.6) | **.01** |
| Chronic GVHD | 1.1 (.6-1.9) | .6 | 1.6 (1.0-2.6) | **.03** | 2.9 (1.1-7.1) | **.01** |

CLL indicates chronic lymphocytic leukemia; CR, complete remission; PR, partial remission; PD, progressive disease; HSCT, hematopoietic stem cell transplantation; D/R, donor and/or recipient; DN, double negative; ATG, antithymocyte globulin; GVHD, graft-versus-host disease; PB, peripheral blood; BM, bone marrow; HR, hazard ratio; CI, confidence interval; NRM, nonrelapse mortality.
Only variables with a significant *P* values are shown; for variables analyzed, see material and methods. *P* value with Cox regression analysis.
Bold indicates significant *P* values.

relapse rate compared with those patients with follicular lymphoma [23,24], and pretransplantation disease status [25].

This report did not confirm previous encouraging findings [7-9] that demonstrated a correlation between CMV replication and CMV positive serostatus with an anti-AML effect. On the contrary, the results of our analysis are in line with a recent publication from Green et al. [9] that did not find any association between CMV replication and relapse risk for patients with lymphoma or other hematologic malignancies. Our results extend the findings of Green et al. [9] to a slightly larger cohort (265 versus 254), composed only of patients with B cell lymphomas. Of note, patients with high-risk disease, such as those with Hodgkin lymphoma, were much more numerous in our cohort (32%) relative to the report of the Seattle group (7%). Moreover, our analysis differs from the one of Green et al. [9] because the variables in our multivariate models, such as lymphoma histology and disease status at transplantation, are generally recognized to be those more strictly associated with the outcome of lymphoma patients. Nevertheless, even in this context, we were not able to observe an independent advantage from CMV replication on B cell lymphoma relapse. The absence of a so-called CMV antilymphoma effect is further corroborated by our other observation that any positive pretransplantation CMV serostatus was not associated with a lower incidence of relapse. These data are again in agreement with Green et al. [9] and with a recent retrospective study from the European Bone Marrow Transplant Working Group, comprising 16,628 subjects with AML [26].

A potential explanation for the different antitumor effect observed between AML and lymphoma patients with post-transplantation CMV replication might be the presence of different immunologic mechanisms at play in the hematologic malignancies. Recently, CMV replication was found to induce the expansion of long-lasting memory-like NKG2C$^{+}$ natural killer cells [27,28] and of γδT cells capable of cross-recognizing CMV and AML cells [21]. On the contrary, the antitumor activity of natural killer cells was found to be more prominent in AML than in lymphoma cells [29] and γδT cells were not efficacious against chronic lymphocytic leukemia [30]. Further analyses are warranted to test whether the B cell lymphoma cells are less suitable to an immune-mediated effect.

It is important to underscore that, in our study, CMV replication was confirmed as an independent variable negatively affecting NRM and OS, as previously described [31]. In this sense, our findings suggest to maintain the current strategies aimed at limiting CMV replication through pre-emptive therapy. Besides CMV replication, severe acute GVHD and chronic GVHD were both associated with higher NRM, as formerly described.

In conclusion, our findings confirm lymphoma histotype and pretransplantation disease status as the main variables affecting transplantation outcome, whereas post-transplantation CMV replication and pretransplantation CMV serostatus are not independent variables affecting the risk of relapse. Further preclinical studies are required to better understand whether an immune-mediated effect may be elicited against B cell lymphoma cells.

## ACKNOWLEDGMENTS

*Conflict of interest statement:* There are no conflicts of interest to report.

*Authorship statement:* J.M. and F.M. collected and analyzed data and wrote the paper; F.S. analyzed data; L.R., A.D., L.F., V.M., B.S., F.P., A.R., F.O., F.Z., and A.O. collected data; C.C. wrote the paper; and P.C. designed research and wrote the paper.

*Financial disclosure:* The authors have nothing to disclose.

## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.02.015.

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# Peripheral Blood Hematopoietic Stem Cells for Transplantation of Hematological Diseases from Related, Haploidentical Donors after Reduced-Intensity Conditioning

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*Article history:*
Received 27 December 2013
Accepted 1 March 2014

*Key Words:*
Haploidentical
Peripheral blood stem cell
Stem cell transplantation

A B S T R A C T

In a multicenter collaboration, we carried out T cell–replete, peripheral blood stem cell (PBSC) transplantations from related, HLA-haploidentical donors with reduced-intensity conditioning (RIC) and post-transplantation cyclophosphamide (Cy) as graft-versus-host disease (GVHD) prophylaxis in 55 patients with high-risk hematologic disorders. Patients received 2 doses of Cy 50 mg/kg i.v. on days 3 and 4 after infusion of PBSC (mean, 6.4 × $10^6$/kg $CD34^+$ cells; mean, 2.0 × $10^8$/kg $CD3^+$ cells). The median times to neutrophil (500/μL) and platelet (>20,000/μL) recovery were 17 and 21 days respectively. All but 2 of the patients achieved full engraftment. The 1-year cumulative incidences of grade II and grade III acute GVHD were 53% and 8%, respectively. There were no cases of grade IV GVHD. The 2-year cumulative incidence of chronic GHVD was 18%. With a median follow-up of 509 days, overall survival and event-free survival at 2 years were 48% and 51%, respectively. The 2-year cumulative incidences of nonrelapse mortality and relapse were 23% and 28%, respectively. Our results suggest that PBSC can be substituted safely and effectively for bone marrow as the graft source for haploidentical transplantation after RIC.



## INTRODUCTION

Allogeneic hemopoietic stem cell transplantation from HLA-matched donors is curative in a proportion of patients with hematologic malignancies, as well as in those with inherited diseases, such as hemoglobinopathies and bone marrow failure syndromes. A suitable HLA-identical sibling donor will be available for about 30% to 35% of patients. For

*Financial disclosure:* See Acknowledgments on page 895.

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1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.03.003