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Brief Article

Cytomegalovirus Prophylaxis versus Pre-emptive Strategy: Different CD4$^+$ and CD8$^+$ T Cell Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Reconstitution of T cells after transplantation is a determinant of the long-term success of the procedure, and the correlation with T cell recovery and cytomegalovirus reactivation and disease is well known. We evaluated 110 patients who underwent transplantation: 55 received pre-emptive antiviral treatment, and in the other 55 patients, prophylaxis with letermovir was employed. A progressive statistically significant difference in T cell reconstitution between the 2 groups was observed, starting from day +60 with faster recovery in the pre-emptive group. Moreover, a higher incidence of cytomegalovirus reactivation was observed in prophylactic group after discontinuation of letermovir, and subsequent antiviral treatment has been necessary. Our findings confirm, as previously reported, that cytomegalovirus reactivation is a potent stimulator of T cell function.

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INTRODUCTION

Viral infections remain important causes of morbidity and mortality after allogeneic stem cell transplantation (SCT), especially infection due to cytomegalovirus (CMV). After SCT, 60% to 70% of patients who are CMV seropositive will experience reactivation and, without any prophylaxis or pre-emptive therapy, 20% to 30% of these will develop end-organ disease [1]. Several studies, from murine model to human, have demonstrated a major role of CD8 CMV-specific cytotoxic T lymphocytes (CTLs) in the control of viral replication [2-4]. Evaluation of CTL function after SCT revealed that 50% of patients, in the absence of pre-emptive therapy, exhibit a detectable CTL response by 3 months after SCT [5]. Parallel evaluation of CD4$^+$ function showed that the proliferative response to CMV antigen was significantly depressed after transplantation and that the restoration of CTL response appeared to be dependent on CD4$^+$ recovery [6,7].

Up to now, no data about CD4$^+$ and CD8$^+$ T cell reconstitution during CMV prophylaxis with letermovir have been reported. As such, we retrospectively compared immune reconstitution of 110 patients submitted to SCT at our institution and undergoing letermovir prophylaxis (55 patients [50%]) or pre-emptive treatment (PET) (55 patients [50%]).

MATERIALS AND METHODS

We retrospectively reviewed data from 110 CMV-seropositive patients, submitted to SCT at the Hematology and Transplant Center Unit, Department of Medical Area, Udine University Hospital from January 2016 to March 2020: patients who died before day +29 post-transplant were excluded.

Fifty-five patients received PET (foscarnet 120 mg/kg i.v. if pre-engraftment period; valganciclovir per os [p.o.] or ganciclovir i.v., according to therapeutic drug monitoring, during postengraftment) only if CMV was detected, while the other 55 patients received prophylactic letermovir from day +3 (range, 1 to 8) until day +100 post-reinfusion.

We tested weekly whole-blood samples for CMV with polymerase chain reaction (PCR): CMV DNAemia was defined as the detection of CMV nucleic acid in whole-blood samples by PCR testing [8].

CMV reactivation was defined as PCR >150 copies/mL once. All the CMV values in the present study are reported in copies/mL. Clearance of CMV DNAemia was defined as documentation of 2 negative consecutive PCR values (ie, below the level of detection) obtained at least 1 week apart. The first of the 2 negative PCR values was considered the date of clearance. Recurrence was defined as any detectable CMV DNAemia after initially achieving clearance.

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Table 1
Characteristics of Patients/Transplants and Outcomes

Characteristic	Pre-emptive (n = 55 Patients)	*P* Value	Prophylactic (n = 55 Patients)
Median age (range), y	56 (21-71)		61 (23-73)
Sex, male/female, No.	29/26		26/29
Diagnosis			
Acute leukemia	39 (71.0)	.09	44 (80.0)
Lymphoma	11 (20.0)		7 (13.0)
Myeloproliferative disease	5 (9.0)		4 (7.0)
Transplant phase			
Early	22 (40.0)	.7	18 (32.5)
Late*	33 (60.0)		37 (67.5)
Donor and HLA matching			
Matched unrelated	5 (9.0)		5 (9.0)
Mismatched unrelated†	26 (47.5)		26 (47.5)
Haploidentical	24 (43.5)		24 (43.5)
Patient/donor CMV			
Positive/negative	20 (36.5)		20 (36.5)
Positive/positive	35 (63.5)		35 (63.5)
Conditioning			
Myeloablative	30 (54.5)	.3	22 (40.0)
Reduced intensity	25 (45.5)		33 (60.0)
GVHD prophylaxis			
CsA + MTX + ATG	5 (9.0)	.2	5 (9.0)
FK + MTX + ATG	28 (51.0)		26 (47.5)
FK + MMF + Cy	22 (40.0)		24 (43.5)
ATG dose			
Tymoglobuline 7.5 mg/kg	19 (57.5)	.2	20 (64.5)
Tymoglobuline 5 mg/kg	14 (42.5)		11 (35.5)
Stem cell source			
Peripheral blood	50 (91.0)	.2	52 (94.5)
Bone marrow	5 (9.0)		3 (5.5)
Acute GVHD	22 (40.0)		24 (43.5)
Grades I-II	17 (31.0)	.7	20 (36.5)
Grade III	5 (9.0)		4 (7.0)
Graft			
Graft failure	1 (2.0)	.5	1 (2.0)
Poor graft function	10 (18.0)		6 (11.0)
CMV reactivation			
No. patients	38 (69.0)	.03	22 (40.0)
CMV reactivation <100 days	33/38 (87.0)		2/22 (9.0)
CMV reactivation >100 days	5/38 (13.0)		20/22 (91.0)
CMV disease			
No. patients	2 (4.0)	.7	1 (2.0)
Peak DNAemia, copies/mL, median (range)	2150 (950-75.000)	.05	1450 (210-59.000)
Other infection			
Viral	4 (7.0)	.6	7 (12.5)
Bacterial	44 (80.0)		40 (72.5)
Fungal	4 (7.0)		8 (14.0)
Days of hospitalizations, median (range)	50 (30-130)	.04	40 (30-60)
No. of deaths	23 (42.0)	.03	14 (25.5)
Infection	6/23 (26.0)		7/14 (50.0)
Disease	13/23 (56.5)		6/14 (43.0)
GVHD	3/23 (13.0)		1/14 (7.0)
CMV disease	1/23 (4.5)		0
Follow-up			
Median (range), mo	24 (12-58)	.09	12 (4-28)
OS log-rank test, %	56.0		70.0

Values are presented as number (%) unless otherwise indicated.
CsA indicates cyclosporine; MTX, methotrexate; FK, tacrolimus; MMF, mycophenolate mofetil; Cy, cyclophosphamide; OS, overall survival.
* Late transplant phase = at least 3 previous therapy lines or second transplant.
† Mismatched donor = at least 1 allelic or antigenic mismatch between donor and recipient in class.

We considered treatment thresholds for PET of >150 copies/mL for high-risk patients and >300 copies/mL for patients at low risk of CMV reactivation and disease.

Patients were considered high risk if they were CMV seropositive, received grafts from mismatched unrelated or haploidentical donors, received anti-thymocyte globulin (ATG), or were being treated with prednisone (at least 1 mg/kg/d).

$CD4^+$ and $CD8^+$ T cell lymphocyte counts were determined by labeling peripheral blood mononuclear cells with specific monoclonal antibodies and subsequent analysis by 3-color flow cytometry [9].

This retrospective study was performed in accordance with the Declaration of Helsinki and was approved by our ethics committee.

The primary endpoint of this study was a comparison of the recovery of $CD4^+$ and $CD8^+$ T cell lymphocyte count at days +30, +60, and +90 between PET and letermovir prophylactic strategy.

Data were collected in an XLS database and imported into Stata/SE15.0 for Windows (StataCorp, College Station, TX). The closeout data for analysis was January 31, 2021. Fisher exact test for categorical data and the Mann-Whitney test for continuous data were used to compare the characteristics of the 2 groups of patients. Outcome probabilities were calculated by the Kaplan-Meier method and compared using the log-rank test. All quoted *P*values are 2sided, and confidence intervals refer (CIs) to 95% boundaries. Overall survival was defined as the time (months) from transplantation to either death or last observation.

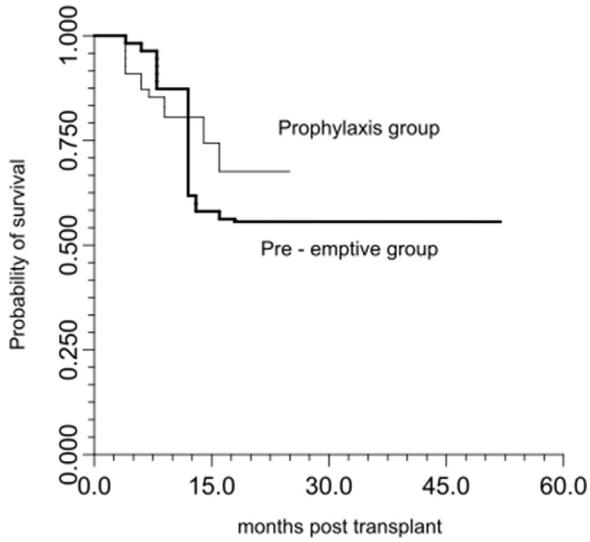

Figure 1. Overall survival post-transplant according to pre-emptive therapy versus prophylaxis treatment.

RESULTS AND DISCUSSION

The clinical characteristics of the study population are shown in Table 1. The 2 groups were well matched with respect to age, sex, CMV status pretransplant, status at transplant, donors, conditioning regimens, graft-versus-host disease (GVHD) prophylaxis, and incidence of GVHD: a direct comparison between the 2 groups could therefore be made. All 110 included patients were at high risk of CMV reactivation and disease.

The proportion of patients with CMV reactivation was 38 (69.0%) in the PET group and 22 (40.0%) in the letermovir prophylactic group, respectively (P = .03). As shown in Table 1, CMV reactivation occurred more frequently within 100 days post-SCT in the PET group (33 [87.0%] versus 2 [9.0%], P< .01), while in prophylactic group, reactivations were observed starting from day +100, after the discontinuation of letermovir (5 [13.0%] versus 20 [91.0%], P < .01). A statistically significant difference in peak of DNAemia was also evident, with a lower copy number in the letermovir prophylactic group (PET: 2150 copies/mL [range, 950 to 75,000] versus prophylactic: 1450 copies/mL [range, 210 to 59,000], P = .05).

There was no difference between the 2 groups in the incidence of graft failure and poor graft function (11 patients [20%] versus 7 patients [13%], respectively). Moreover, no differences in the incidence of CMV disease and other viral, bacterial, or fungal infections between the 2 groups were registered (Table 1).

When the data for incidence and cause of death were analyzed, 23 of 55 (42.0%) deaths in PET group and 14 (25.5%) in the prophylactic group were observed; major cause of death was disease (13 patients [56.5%]) in the PET group and infection (7 patients [50.0%]) in the prophylactic group. In detail, deaths for infections in each group (PET versus prophylaxis) were CMV disease, 1 out of 6 patients (16.5%) versus no death; bacterial, 4 patients (67.0%) versus 6 patients (86.0%); and fungal, 1 (16.5%) versus 1 (14.0%) patient. No deaths within 100 days from transplant was observed in all the 110 patients.

The 1-year overall probabilities of survival were similar in the 2 groups (PET versus prophylactic): 56.0% (CI, 37% to 72%) versus 70.0% (CI, 58% to 84%) with a median follow-up from transplant of 24 months (range, 4 to 58) versus 12 months (4 to 28), respectively (P = .09) (Figure 1).

While no difference in $CD4^+$ and $CD8^+$ T cell recovery was observed in the 2 groups (PET versus prophylactic) at day +30 (CD4: median 38/μL [range, 25 to 105] versus 72/μL [range, 10 to 98], P = .4; CD8: median 35/μL [range, 0 to 92] versus 38/μL [range, 10 to 98], P = .8), a statistically significant difference was detected at day +60 ($CD4^+$: median 270/μL [range, 80 to 350] versus 130/μL [range, 75 to 190], P = .04; $CD8^+$: median 260/μL [range, 90 to 350] versus 100/μL [range, 30 to 190], P = .03) and at day +90 ($CD4^+$: median 430/μL [range, 180 to 630] versus 190/μL [range, 110 to 470], P = .03; $CD8^+$: median 410/μL [range, 170 to 720] versus 270/μL [range, 130 to 490], P = .04) (Figure 2 and Supplementary Table S1).

Starting from day +180, a progressive increase of immune recovery was observed in the prophylactic group, with a difference, but not statistically significant, in the 2 groups at 1 year from transplant (PET versus prophylactic group: CD4: median 650/μL [range, 110 to 920] versus 510/μL [range, 90 to 720], P = .5; CD8: median 310/μL [range, 110 to 750] versus 290/μL [range, 100 to 650], P = .4).

While our 2 cohorts were similar in terms of graft source, ATG use, and donor type, immune recovery would be expected to be quite different in patients who receive cyclophosphamide post-transplant (haploidentical SCT) compared to those who received ATG (unrelated SCT). We compared the 2 cohorts, and no statistically significant difference emerged (Supplementary Table S2).

The 2 groups were comparable for baseline and transplant characteristics, so a correlation between $CD4^+$ and $CD8^+$ T cell quantitative recovery and type of control of CMV reactivation (pre-emptive versus prophylactic strategy) should be postulated. It is well known that CMV reactivation, even if subclinical, is a potent stimulator of T cell function [10,11]. Impaired $CD4^+$ and $CD8^+$ T cell recovery at days +60 and +90 in the prophylactic group should explain the high incidence of CMV reactivation (20 patients [36.5%]) observed in this group after discontinuation of letermovir. Moreover, in our series, infection was the major cause of death in the prophylactic group (7/14 patients [50.0%]).

Our data should be confirmed in a larger series; in addition, as reconstitution of the immune system plays a critical role in the success of the transplant procedure [12-16], it is also mandatory to evaluate (with a longer follow-up) the incidence of overall infections, of deaths from infection, and of disease relapse between the two strategies (PET versus prophylaxis) [17,18].

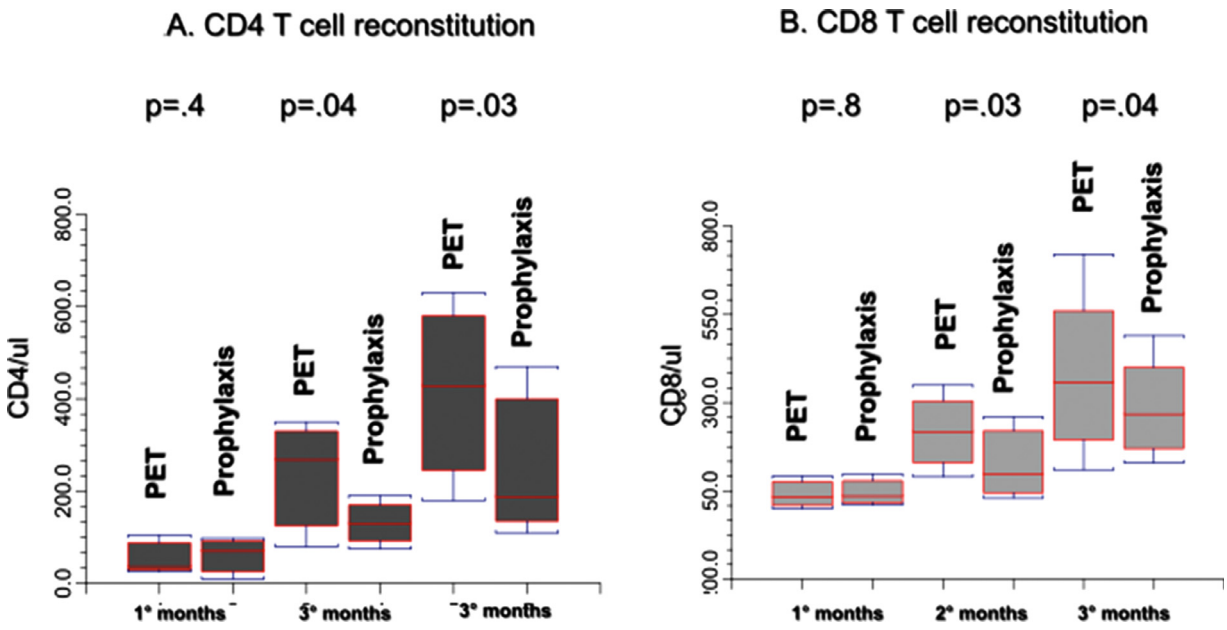

Figure 2. (A) CD4 T cell reconstitution. (B) CD8 T cell reconstitution.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.03.003.

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