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The use of ATG abrogates the antileukemic effect of cytomegalovirus reactivation in patients with acute myeloid leukemia receiving grafts from unrelated donors

Busca A, Passera R, Pini M, Zallio F, Dellacasa C, Audisio E, Giaccone L, Maffini E, Costa C, Cavallo R, Bruno B.

Abstract

Several studies provided evidence of a consistent antileukemic effect induced by cytomegalovirus (CMV) replication in acute myeloid leukemia (AML) patients receiving allogeneic hematopoietic stem cell transplantation (HSCT), however the use of antithymocyte globulin (ATG) as graft-versus-host disease prophylaxis, may potentially abrogate the protective effect of CMV infection. To address this issue, we retrospectively analyzed the risk of relapse in a cohort of 101 patients with AML who received grafts from an unrelated donor after a conditioning regimen including ATG. The cumulative incidence of CMV reactivation, evaluated by RT qPCR, was 59% at 12 months, and 93% of CMV reactivations occurred within the first 100 days post HSCT. The 5-year cumulative incidence of relapse in patients with CMV reactivation was 29% compared with 37% for patients without CMV reactivation, and the only factor associated with a reduced 5-year cumulative incidence of relapse was the disease status at HSCT ($P < 0.001$). In the multivariable model adverse cytogenetics (HR 2.42, 95% CI 1.02-5.72; $P = 0.044$) and acute GVHD (HR 3.36, 95% CI 1.32-8.54; $P = 0.011$) were independent risk factors for reducing overall survival (OS), while the presence of chronic GVHD was associated with a better OS (HR 0.37, 95% CI 0.15-0.89; $P = 0.027$). CMV replication was not an independent risk factor for OS (HR 1.06, 95% CI 0.07-15.75; $P = 0.965$). In Conclusion, the results of present study suggest that relapse prevention in patients with AML receiving T-cell depleted HSCT using ATG do not benefit from CMV reactivation.

Introduction

A consistent number of studies recently published, raised the possibility of an association between cytomegalovirus (CMV) reactivation and a relapse risk reduction in patients with hematologic malignancies receiving hematopoietic stem cell transplantation (HSCT) [1-5]. Several factors were shown to influence the protective effect of CMV reactivation, including diagnosis [3], the intensity of the preparative regimen [6], and the use of T-cell depletion [7]. In this respect, patients with acute myeloid leukemia (AML) receiving myeloablative (MA) conditioning regimens appear to be those who might benefit from the antileukemic effect of CMV reactivation [6]. It is conceivable that the “virus-versus leukemia” effect promoted by CMV reactivation requires a robust T- or NK-cell immune response to elicit a graft-versus leukemia (GVL) effect. According to this observation, the use of in vivo T-cell depletion with alemtuzumab which typically depletes immune cells including NK cells may result in the abrogation of CMV-induced antileukemic effect [7]. On the other hand, very few data have been reported on the potential influence of antithymocyte globulin (ATG) in this context. The study by Manjappa et al. suggests that immune-suppression of ATG might contribute to mitigate the protective effect of CMV-specific T cells, although these data have not been confirmed by others [6]. Given the conflicting results, we aimed to investigate the influence of ATG on antileukemic effect induced by post-HSCT CMV replication.

Methods

Study population

This is a retrospective study conducted in two Italian transplant Centers, including 101 adult patients with AML who received an allogeneic HSCT from an unrelated donor between July 2004 and February 2014 after a conditioning regimen incorporating ATG as part of graft-versus-host disease (GVHD) prophylaxis. The Institutional Review Board of each Center approved the study.

Patients were selected for the analysis according to the following eligibility criteria: (1) all donors and recipients were typed at HLA loci A, B, C, DRB1, and DQB1 through high-resolution genotyping; (2) use of unmanipulated donor stem cells; (3) pharmacologic prophylaxis of GVHD including ATG; and (4) no use of prophylactic donor lymphocyte infusion post-HSCT

Transplant characteristics

A total of 83 patients (82%) received myeloablative preparative regimens consisting of i.v. busulphan (BU) combined with either high-dose cyclophosphamide (Cy) (n = 33), fludarabine (n = 21), or thiotepa and fludarabine (n = 6); high-dose Cy with 12.0 Gy TBI (n = 16); or thiotepa-Cy (n = 7). A reduced conditioning regimen (RIC) was administered to 18 patients (18%), consisting of thiotepa (10 mg/kg) and Cy (100 mg/kg) (n = 6); thiotepa-Cy and melphalan (n = 7); thiotepa and melphalan (n = 3); and fludarabine-melphalan (n = 2).

Prophylaxis of GVHD consisted of cyclosporine (CSA) and short course methotrexate (MTX) in all subjects; ATG (Thymoglobulin) was administered to all patients at a dose of 5–7.5 mg/kg over two or three days (on days -4, -3, -2 or -3, -2).

Transfusion support using leukocyte-filtered blood products was provided to maintain a hemoglobin ≥ 8 g/dL and to prevent severe thrombocytopenia ($\leq 15\text{--}20 \times 10^9/\text{L}$)

CMV monitoring and pre-emptive therapy

CMV reactivation was routinely monitored twice weekly during the inpatient post-transplantation course and weekly in the outpatient setting. All patients received acyclovir as antiviral prophylaxis up to 1 year after transplantation. CMV reactivation was pre-emptively treated with either i.v. ganciclovir or oral valganciclovir. Foscarnet was substituted in case of cytopenias. Antiviral induction therapy was continued for 14 days followed by maintenance treatment for 14 days or until two consecutive negative surveillance results were recorded.

Definitions

Monitoring for CMV infection/reactivation was done on whole blood specimens by molecular methods in 90 patients. In particular, for specimens tested in 2004 and 2005, a home-made quantitative competitive PCR was used, as previously described [8]; any value >100 copies/mL was considered as CMV reactivation. Since 2006, specimens were tested by a commercially available real-time PCR assay (Nanogen Advanced Diagnostics, Elitech group, Milan, Italy; limit of detection, 2,000 copies/mL whole blood), as previously

described [9]; CMV viral load >2,000 copies/mL was considered as CMV reactivation. CMV replication was detected by pp65 antigenemia positivity in 11 patients. pp65 antigenemia assay was performed on peripheral blood leukocytes (PBLs) applied to slides by cyto centrifugation of 200,000 cells each; a CMV reactivation was assumed if one or more cells with characteristic immunofluorescence/2 × 10⁵ PBLs were detectable.

Morphologic relapse of the leukemia was the primary outcome of patients transplanted in complete remission (CR) and progression of disease for patients who received grafts with active disease. Marrow examination was performed after HSCT at 30 days, 100 days, and at 6, 9, and 12 months thereafter. Cytogenetic risk was classified according to Cornelissen et al. [10].

The assessment and grading of acute and chronic GVHD were primarily based on clinical findings and followed the commonly accepted diagnostic criteria [11, 12]. Diagnosis was confirmed histologically whenever indicated and clinically possible.

Study endpoints and statistical analysis

The primary end point was the cumulative incidence of relapse (RI), while the secondary ones were the cumulative incidence of non-relapse mortality (NRM), the overall survival (OS), and the progression-free survival (PFS). The median follow-up for OS from transplantation was 60 months (range 1–118).

The cumulative incidences of RI and NRM were estimated by the cumulative incidence function, comparing the curves of the main event (relapse), in the presence of a competing event (death without previous relapse) by the Gray test [13]. The univariate analyses were performed for the following prognostic factors: recipient age (>50 vs. ≤50 years), gender mismatch (any vs. none), HLA mismatch (≤9/10 vs. 10/10), CD34+ source (PBSC vs. bone marrow), conditioning regimen (myeloablative vs. RIC), disease status at transplantation (less than CR vs. CR2/3 vs. CR1), ATG dose (7–7.5 vs. 5–6 mg/kg), fluorescent in situ hybridization (FISH) abnormalities (high vs. standard risk), donor and recipient pre-transplantation CMV status (positive vs. negative), occurrence of acute GVHD Grade II–IV and/or chronic GVHD (any vs. none), recipient CMV post-transplantation reactivation status (any vs. none). The same covariates were then evaluated by the multivariate Fine and Gray competing risk regression model [14].

The OS and PFS curves were estimated by the Kaplan–Meier method, comparing the two arms by the log-rank test [15]. OS and PFS were also analyzed by the Cox proportional hazards model, comparing the two arms by the Wald test and calculating 95% CIs; the disease status at transplantation, the occurrence of acute GVHD/chronic GVHD, and the CMV reactivation status were treated as time-dependent variables.

Patient characteristics were tested using the Fisher's exact test for categorical variables and the Mann–Whitney test for continuous ones. All reported P-values were two-sided, at the conventional 5% significance level. Data were analyzed as of August 2014 by R 3.1.1 package cmprsk (The R Foundation for Statistical Computing, Vienna-A; www.R-project.org).

Results

Patient characteristics

A total of 60 patients (59%) had a positive q-CMV PCR (n = 49) or pp65 antigenemia (n = 11) at a median of 35 days (range 12–389 days) after allogeneic HSCT, resulting in a cumulative incidence of CMV reactivation of 59% at 12 months. Nine-three percent of CMV reactivations (56/60) occurred within the first 100 days post HSCT. The median number of copies of CMV DNA and the median number of CMV pp65-antigen positive cells at the time of first CMV reactivation were 5012/mL (range 132–126,400 copies/mL) and 4 per 2 × 10⁵ PBLs (range 1–50 positive cells), respectively. Patient and transplant characteristics of the two cohorts with and without CMV reactivations are summarized in Table 1. Patients with and without CMV reactivation were similar relative to age, sex, cytogenetics, disease status at HSCT, HLA matching, preparative regimen, graft source, and onset of acute or chronic GVHD, while patients in the CMV reactivation group were more likely to have a pre-transplantation positive donor/recipient CMV serostatus.

Table 1. Patient, Donor, and Transplant Characteristics According to Post-Transplantation CMV Reactivation

TABLE I. Patient, Donor, and Transplant Characteristics According to Post-Transplantation CMV Reactivation

	All patients	Patients with CMV reactivation	Patients without CMV reactivation	P
No. of patients	101	60	41	
Median patient age, years (range)	50 (17-65)	53 (19-64)	45 (17-65)	0.096
Donor/patient sex mismatch	45 (44%)	29 (48%)	16 (39%)	0.542
CMV serostatus				0.026
D2/R2	14 (14%)	5 (8%)	9 (22%)	
D1/R2	6 (6%)	2 (3%)	4 (10%)	
D2/R1	33 (33%)	23 (39%)	10 (24%)	
D1/R1	47 (46%)	30 (50%)	17 (42%)	
Missing	1 (1%)	0 (0%)	1 (2%)	
Disease risk by cytogenetics				0.825
Low	13 (13%)	9 (15%)	4 (10%)	
Intermediate-1	45 (44%)	27 (45%)	18 (44%)	
Intermediate-2	9 (9%)	7 (12%)	2 (5%)	
Poor	22 (22%)	14 (23%)	8 (19%)	
Missing	12 (12%)	3 (5%)	9 (22%)	
Disease etiology				1.000
De novo	89 (88%)	53 (88%)	36 (87%)	
Secondary	12 (12%)	7 (12%)	5 (13%)	
Disease status at transplant				0.399
CR1	66 (65%)	41 (68%)	25 (61%)	
CR2-CR3	19 (19%)	12 (20%)	7 (17%)	
Relapse/resistant	16 (16%)	7 (12%)	9 (22%)	
Transplantation year				0.501
2004-2006	22 (22%)	14 (23%)	8 (19%)	
2007-2009	29 (29%)	20 (33%)	9 (22%)	
2010-2012	33 (32%)	17 (29%)	16 (40%)	
2013-2014	17 (17%)	9 (15%)	8 (19%)	
HLA matching				0.223
10/10	55 (54%)	36 (60%)	19 (46%)	
9/10	30 (30%)	15 (25%)	15 (37%)	
8/10	12 (12%)	6 (10%)	6 (15%)	
7/10	4 (4%)	3 (5%)	1 (2%)	
Conditioning regimen				0.600
Myeloablative	83 (82%)	48 (80%)	35 (85%)	
Reduced intensity	18 (18%)	12 (20%)	6 (15%)	
Stem cell source				0.225
PBSC	80 (79%)	50 (83%)	30 (73%)	
BM	21 (21%)	10 (17%)	11 (27%)	
ATG regimen				0.685
5 mg/kg	42 (41%)	22 (36%)	20 (49%)	
6 mg/kg	4 (4%)	4 (7%)	0 (0%)	
7 mg/kg	49 (49%)	30 (50%)	19 (46%)	
7.5 mg/kg	6 (6%)	4 (7%)	2 (5%)	
Acute GVHD				0.133
Grades 0-I	64 (63%)	35 (58%)	29 (71%)	
Grades II-IV	35 (35%)	25 (42%)	10 (24%)	
Not applicable	2 (2%)	0 (0%)	2 (5%)	
Chronic GVHD				0.249
No	36 (36%)	25 (42%)	11 (27%)	
Yes	41 (40%)	23 (38%)	18 (44%)	
Not applicable/missing	24 (24%)	12 (20%)	12 (29%)	

CMV indicates cytomegalovirus; D, donor; R, recipient; CR, complete remission; PBSC, peripheral blood stem cell; BM, bone marrow; ATG, antithymocyte globulin; GVHD, graft-versus-host disease.

CMV reactivation and preemptive antiviral therapy

Overall, 53 patients received pre-emptive treatment consisting of valganciclovir in 32 cases, ganciclovir in 13 cases, and foscarnet in 4 cases; 4 patients, who were considered at high risk of CMV disease due to concomitant steroid treatments, received a combination of foscarnet and ganciclovir/valganciclovir. Seven patients with a low or spontaneous decrease of CMV viral load did not receive any specific preemptive treatment.

CMV reactivation and risk of relapse after allogeneic HSCT

Overall, 30 patients (30%) relapsed at a median time of 327 days (range 63–1,229 days) after HSCT, corresponding to a cumulative incidence of relapse of 25%, 32%, and 32% respectively at 1, 3, and 5 years post-transplantation. The cumulative incidence of relapse in patients with CMV reactivation was 19%, 29%, and 29% compared with 34%, 37%, and 37% for patients without CMV reactivation at 1 year, 3 years, and 5 years, respectively (Fig. 1A). The only factor affecting the 5-year cumulative incidence of relapse was an advanced disease phase at HSCT both in univariate (Table 2) and multivariate cumulative incidence analyses (HR 2.12, 95% CI 1.38–3.27; $P < 0.001$). In a subgroup analysis of the 83 patients who received a myeloablative regimen, the cumulative incidence of relapse was 32% in patients with CMV infection compared to 42% in patients without CMV infection ($P = 0.313$). In order to exclude the possibility that patients with active disease were blunting the protective effect of CMV reactivation, we evaluated the risk of relapse in 85 patients who received the graft in CR: the 5-year cumulative incidence of relapse was not statistically different in patients with CMV reactivation as compared to patients without CMV reactivation (23% vs. 30%, $P = 0.395$).

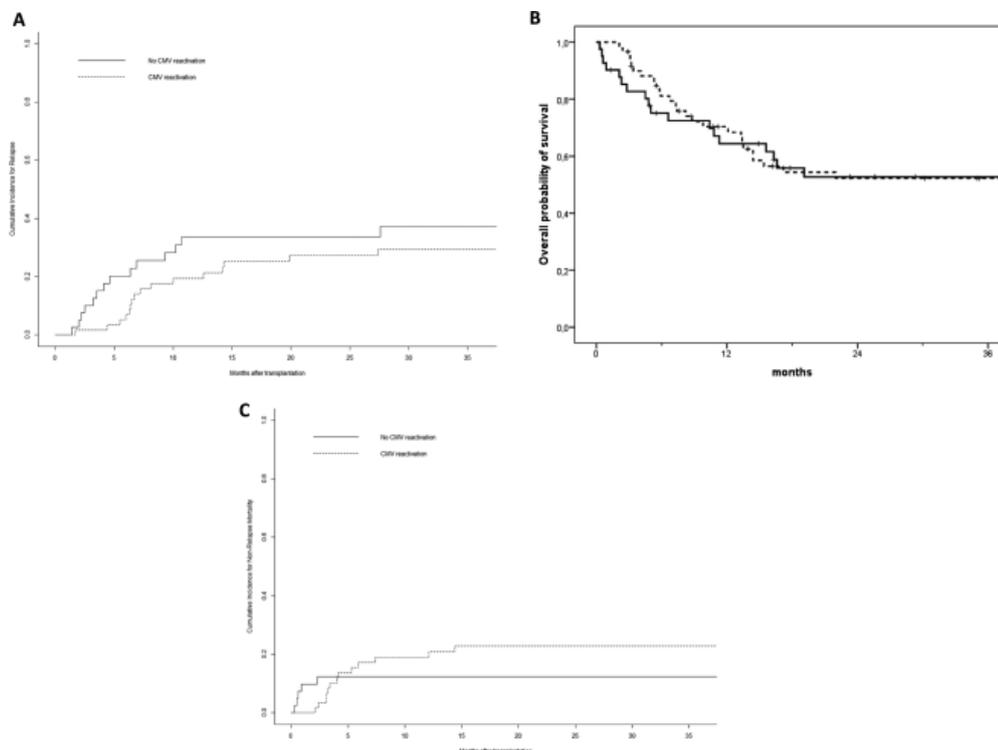


Figure 1 Relapse incidence (A), nonrelapse mortality (B), and overall survival (C) stratified by CMV reactivation.

Table 2

TABLE II. Variables Influencing the Cumulative Incidence of Relapse, Overall Survival, and Non-Relapse Mortality in Univariate Analysis

Characteristic	Cumulative incidence of Relapse at d100	Cumulative incidence of Relapse at d180	Cumulative incidence of Relapse at 5 yrs	P value	5-year OS	P value	1-year NRM	P value
Age				0.078		0.501		0.226
<50 years	9.6%	19.7%	39.3%		48.0%		11.6%	
≥50 years	2.1%	4.2%	25.3%		57.4%		21.0%	
Gender mismatch				0.241		0.729		0.172
Yes	9.6%	19.6%	34.7%		55.1%		7.5%	
No	3.6%	5.5%	27.9%		53.8%		22.0%	
Cytogenetics				0.577		0.615		0.467
Favorable	7.0%	10.7%	30.7%		54.1%		14.3%	
High risk	0.0%	13.2%	38.6%		47.2%		19.6%	
Disease status at HSCT				<0.001		0.004		0.919
CR1	3.1%	6.2%	18.6%		63.2%		15.6%	
CR2-CR3	5.3%	26.3%	47.4%		45.9%		15.8%	
Relapse/resistant	20.2%	20.2%	67.3%		16.2%		19.2%	
HLA matching				0.413		0.174		0.073
10/10	5.5%	13.2%	28.1%		46.8%		22.4%	
7-9/10	6.7%	11.1%	37.2%		60.1%		11.1%	
Stem cell source				0.960		0.911		0.864
BM	5.1%	11.6%	32.8%		53.7%		15.3%	
PBSC	9.5%	14.6%	30.9%		52.2%		20.0%	
Conditioning				0.107		0.209		0.809
MA	6.1%	13.7%	36.1%		49.0%		16.1%	
RIC	5.6%	5.6%	16.7%		66.7%		16.7%	
ATG dose				0.134		0.994		0.432
5-6 mg/kg	6.7%	16.2%	42.4%		53.0%		13.3%	
7-7.5 mg/kg	5.5%	9.1%	25.7%		52.7%		18.4%	
CMV reactivation				0.279		0.849		0.297
Yes	1.7%	6.9%	29.2%		52.3%		18.9%	
No	12.5%	20.1%	37.2%		52.7%		12.3%	
Acute GVHD				0.955		0.011		0.015
Grade 0-I	6.4%	9.6%	34.3%		61.8%		7.9%	
Grade II-IV	5.7%	17.7%	30.5%		38.8%		26.4%	
Chronic GVHD				0.299		0.089		0.117
Yes	-	4.9%	25.6%		74.3%		2.5%	
No	-	8.4%	37.4%		55.9%		8.6%	

OS, overall survival; NRM, non-relapse mortality; CR, complete remission; PBSC peripheral blood stem cell; BM bone marrow; MA, myeloablative; RIC, reduced intensity conditioning; CMV indicates cytomegalovirus.

Effect of CMV reactivation on overall survival and nonrelapse mortality

After a median follow-up of 60 months, 56 patients are alive and 45 died, 27 with progressive disease and 18 because of NRM. The Kaplan–Maier estimate of OS was 68%, 52%, and 52% at 1 year, 3 years, and 5 years, respectively. Figure 1B shows the OS stratified by CMV reactivation. An advanced disease phase at HSCT and the presence of acute Grade II–IV GVHD were the two factors associated with a reduced OS, while there was a non-significant trend toward a better OS for patients developing chronic GVHD (Table 2). The cumulative incidence of NRM at 100 days and 1 year was 10% and 16%, respectively. Figure 1C shows the cumulative incidence of NRM stratified by CMV reactivation. The presence of acute GVHD was significantly associated with an increased NRM rate. CMV reactivation was not associated with different OS or NRM rates (Table 2). In the multivariable model, adverse cytogenetics (HR 2.42, 95% CI 1.02–5.72; $P = 0.044$) and acute GVHD (HR 3.36, 95% CI 1.32–8.54; $P = 0.011$) were independent risk factors for reducing OS, while the presence of chronic GVHD was associated with a better OS (HR 0.37, 95% CI 0.15–0.89; $P = 0.027$). CMV replication was not an independent risk factor for OS (HR 1.06, 95% CI 0.07–15.75; $P = 0.965$)

Discussion

Between 1987 and 2014, five studies were published providing evidence of a consistent antileukemic effect elicited by CMV infection in patients with AML receiving allogeneic HSCT [2, 3, 6, 16, 17]. However, whether in vivo T-cell depletion might potentially mitigate the beneficial effect of CMV reactivation is still a matter of debate. Our retrospective analysis including a large cohort of patients with AML, who received grafts

from a MUD after a conditioning incorporating ATG, showed that pre-transplantation disease status was the only factor affecting the final outcome, whereas CMV replication was not recognized as an independent variable for reduced risk of relapse. Our findings are in line with the results of Manjappa et al. showing that the antileukemic effect of CMV reactivation was not detectable in 58 patients who received a RIC regimen, possibly because the majority (44 out of 58) of these patients received ATG as part of the preparative regimen [6]. On the other hand, we did not observe any effect of the intensity of the preparative regimen on the risk of relapse, although it should be emphasized that given the small number of patients receiving RIC, our finding should be interpreted with caution. It is interesting to note that in our study, patients with CMV reactivation appear to have leukemic relapse later than those who did not have CMV infection. The effect of early CMV replication on leukemic relapse has been recently investigated by others [3, 17]. Since most relapses occur during the early post-HSCT phase when the defect of immune reconstitution may translate into a blunting period of GVL effects, it has been hypothesized that CMV replication may offset the lack of an immune-mediated antileukemic effect [3, 17, 18]. Similarly, Green et al. showed that CMV reactivation in patients with AML marginally decreases the risk of relapse by day 100, but was not associated with a reduced risk of recurrence by 1 year [3].

The mechanism by which CMV exerts the antileukemic effect has not been clarified, and this makes even more difficult to define the role of ATG in this context. It has been demonstrated that $\gamma\delta$ T cells, in particular the subset V δ 2NEG $\gamma\delta$ T cells, are involved in the immune response against CMV and in the final clearance of the virus [19]. Furthermore, expanded $\gamma\delta$ T cells have been shown to be cytotoxic to cancer cells in vitro [20], and more recently Scheper et al. have demonstrated that CMV-induced $\gamma\delta$ T cells are capable of cross-recognizing residual leukemic blasts, supporting the hypothesis that CMV replication might have an unexpected beneficial effect on the risk of leukemic relapse [21]. The use of ATG has been recognized as one of the major risk factors for CMV infection after HSCT, and several studies have analyzed the immune reconstitution kinetics following the administration of ATG [22]. Nevertheless, there are no data evaluating specifically the impact of ATG on $\gamma\delta$ T cells subset. Alternatively, Foley et al. showed that CMV replication may induce the expansion of NK cells expressing NKG2C which were associated with a potent IFN γ production, and this population of NK cells may contribute to the elimination of residual leukemic cells [23]. ATG is known to have a moderate effect on the reconstitution kinetic of NK cells, but we do not have data regarding the possible influence on NKG2C+ NK cells, as documented by the fact that, in the study of Foley et al., only a minority of patients (7 out of 73) received ATG as part of the preparative regimen [23]. The disease status at the time of transplant has emerged as the major determinant for the final clinical outcome of our patients. This is not surprising if we consider that one-third of the patients were in advanced phase of disease (beyond CR1/active disease) at the time of transplant, and this might have obscured the CMV-induced antileukemic effect. In addition, it should be underscored that 14% of our HSCT recipients were CMV seronegative and this might have contributed to alter the impact of CMV reactivation on relapse. In fact, the GVL effect induced by CMV reactivation seems to be maximized in seropositive patients, because donor-derived T cells specifically directed to CMV could be cytotoxic to leukemic blasts harboring CMV [24].

In conclusion, the results of our study suggest that immune suppression with ATG might be able to abrogate the protective effect of CMV infection described in patients with AML, underlining the need for further studies aimed to investigate the mechanisms underpinning the immune-mediated antileukemic effects.

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