

# Prognostic impact of viral reactivations in acute myeloid leukemia patients undergoing allogeneic stem cell transplantation in first complete response

Sarah Guenounou, MD<sup>a,b</sup>, Cécile Borel, MD<sup>a</sup>, Emilie Bérard, MD<sup>c,d</sup>, Edwige Yon, CRA<sup>c</sup>, Marylise Fort, Ph<sup>e</sup>, Catherine Mengelle, MD<sup>f</sup>, Sarah Bertoli, MD<sup>a,b,g</sup>, Audrey Sarry, CRA<sup>a</sup>, Suzanne Tavitian, MD<sup>a</sup>, Françoise Huguet, MD<sup>a</sup>, Michel Attal, MD, PhD<sup>a,b</sup>, Christian Récher, MD, PhD<sup>a,b,g,\*</sup>, Anne Huynh, MD<sup>a</sup>

## Abstract

Cytomegalovirus (CMV) serological status of donor and recipient as well as CMV reactivation have been associated with a lower risk of relapse in acute myeloid leukemia (AML) patients after allogeneic stem cell transplantation (alloSCT). Since immunosuppression following transplant allows resurgence of many other viruses, we retrospectively evaluated the impact of viral reactivations on relapse and survival in a cohort of 136 AML patients undergoing alloSCT in first remission from sibling (68%) or unrelated (32%) donors. Myeloablative and reduced-intensity conditioning regimen were given to 71 and 65 patients, respectively. Including CMV reactivations, at least 1 viral reactivation was recorded in 76 patients. Viral reactivations were associated with a lower risk of relapse (adjusted HR 0.14; 95% CI 0.07–0.30;  $P < 0.01$ ), better disease-free survival (aHR 0.29; 95% CI 0.16–0.54;  $P < 0.01$ ) but higher non relapse mortality. This translated into a better overall survival (aHR 0.44; 95% CI 0.25–0.77;  $P < 0.01$ ) in patients who experienced viral reactivation. Thus, viral reactivations, including but not limited to CMV reactivation, are associated with a better outcome particularly with regard to the risk of relapse in AML patients undergoing alloSCT. New guidelines regarding the choice of donor according to the CMV serostatus are needed.

**Abbreviations:** AlloSCT = allogeneic stem cell transplantation, AML = acute myeloid leukemia, CD = cluster differentiation, CI = cumulative incidence, CIF = cumulative incidence functions, CMV = cytomegalovirus, CR1 = first complete remission, D = donor, DFS = disease-free survival, EBV = Epstein–Barr virus, ELN = European LeukemiaNet, GVHD = graft versus host disease, HR = hazard ratio, MAC = myeloablative conditioning, MMF = mycophenolate mofetil, MTX = methotrexate, NRM = non-relapse mortality, OS = overall survival, PCR = polymerase chain reaction, R = recipient, RIC = reduced intensity conditioning.

**Keywords:** acute myeloid leukemia, allogeneic stem cell transplantation, cytomegalovirus, relapse, viral reactivation

## 1. Introduction

For decade, cytomegalovirus (CMV) infection has been identified as a major risk factor of morbi-mortality after allogeneic stem cell transplantation (alloSCT) for hematologic malignancies.<sup>[1,2]</sup> This implied a careful policy for donor choice with regard to CMV serological status as well as a strict monitoring of CMV during transplant and early introduction of pre-emptive antiviral therapy to limit the risk of CMV disease. Despite these strategies, donor and/or recipient CMV seropositivity is still associated with an adverse prognosis. However, recent studies have reported a beneficial effect of early CMV reactivation after transplant with

respect to relapse prevention, but this effect seems unclear regard to overall survival.<sup>[3–6]</sup> In these studies dealing with various hematologic malignancies, this protective effect appeared to be more specific in acute myeloid leukemia (AML) patients and independent of graft versus host disease (GVHD). However, beside CMV, viral reactivations induced by the profound immunosuppression of alloSCT may also involve many other viruses including polyomavirus, Epstein–Barr virus or herpes viruses. In this study, we evaluated the impact of both viral reactivation and CMV serological status in a cohort of AML patients undergoing alloSCT in first complete remission (CR1).

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CR and AH contributed equally to the study.

**Authorship:** SG conceived and designed the study, performed data collection and wrote the manuscript; EB and EY performed statistical analysis and reviewed the manuscript; MF, CM, SB, and AS contributed to data collection; ST, FH, and MA reviewed the manuscript; CB, AH, and CR designed and supervised the study, analyzed, interpreted data, and wrote the manuscript.

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<sup>a</sup> Service d'Hématologie, Institut Universitaire du Cancer de Toulouse Oncopole,

<sup>b</sup> Université Toulouse III Paul Sabatier, <sup>c</sup> Service d'Epidémiologie, Centre Hospitalier Universitaire de Toulouse, <sup>d</sup> UMR 1027, INSERM-Université de Toulouse III, <sup>e</sup> Service d'Immunologie, Centre Hospitalier de Toulouse Rangueil, <sup>f</sup> Service de Virologie, Centre Hospitalier de Toulouse Purpan, <sup>g</sup> UMR 1037,

Centre de Recherche en Cancérologie de Toulouse, INSERM-Université de Toulouse III, Toulouse, France.

\* Correspondence: Christian Récher, Service d'Hématologie, Institut Universitaire du Cancer de Toulouse Oncopole, 1 avenue Irène Joliot-Curie, 31059, Toulouse Cedex, France (e-mail: recher.christian@iuct-oncopole.fr).

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## 2. Methods

### 2.1. Study population

Between 01/01/2000 and 12/31/2012, 727 AML patients (age 18–70) received intensive chemotherapy and 556 achieved a CR1.<sup>[7,8]</sup> Of them, 150 patients underwent alloSCT. Patients having received umbilical cord blood as stem cell source (n=4) and sequential conditioning regimen (n=10) were excluded leading to a sample of 136 patients for the analysis.

This study was approved by the ethic committee of Toulouse University Hospital (40-0713).

### 2.2. Transplant characteristics

Conditioning regimen were classified according to a consensus definition of conditioning regimen intensity.<sup>[9]</sup> Myeloablative conditioning (MAC) consisted of high-dose cyclophosphamide with total-body irradiation or busulfan. Reduced-intensity and non-myeloablative conditioning were grouped together under the reduced-intensity conditioning (RIC) term. RIC associated busulfan, fludarabine, and antithymocyte globulin or fludarabine and low total-body irradiation.

GVHD prevention consisted in a calcineurine inhibitor (cyclosporine A) alone or in association with either methotrexate (MTX) or mycophenolate mofetil (MMF).

### 2.3. Outcomes and definitions

The primary endpoint was cumulative incidence of relapse.<sup>[10]</sup> Secondary endpoints were overall survival (OS), disease-free survival (DFS), and non relapse mortality (NRM).<sup>[11]</sup>

The end of follow-up of our study was December 31, 2013.

AML risk groups were defined according to the Medical Research Council and the European LeukemiaNet classification.<sup>[11,12]</sup>

Acute and chronic GVHD were diagnosed and graded according to standard consensus criteria.<sup>[13,14]</sup>

### 2.4. Viral monitoring

All patients underwent twice-weekly surveillance of CMV reactivation by pp65 antigenemia ( $\geq 1$  pp65-antigene-positive cell per 2 slides with  $>2.0 \times 10^5$  white blood cells per slide) from 2000 to 2005 or real-time qPCR testing (2 consecutive positive PCR  $\geq 500$  copies/mL) thereafter, from the engraftment until 100 days after alloSCT. History of any other viral infection reported in the medical record and any positive biologic assay (by PCR) were taken into account in this study.

### 2.5. CMV pre-emptive therapy

Pre-emptive anti-CMV treatment consisted of intravenous ganciclovir (5 mg/kg twice a day) or oral valganciclovir (900 mg twice a day) for 14 days. In case of viral load decrease, antiviral therapy was continued on a maintenance dose for at least 14 days and until 2 consecutive monitoring was negative (5 mg/kg for IV ganciclovir and 450 mg twice a day for oral valganciclovir). Thereafter, antiviral treatment was pursued with either acyclovir or valacyclovir as herpes simplex virus type 1 and 2, or varicella–zoster virus prophylaxis.

### 2.6. Statistical analyses

Before doing any analysis, we assessed the power of the study: 41 relapses provide a power  $>80\%$  to detect a hazard ratio (HR) of

relapse  $<0.5$  (for CMV  $-/+$ , CMV  $+/+$ , CMV  $+/-$  vs CMV  $-/-$  or for any viral reactivation versus no viral reactivation) with 2-sided type 1 error rate of 5% ( $\alpha=0.05$ ) for the comparison of 2 exponential survival distributions.<sup>[15]</sup>

Statistical analysis was performed on STATA statistical software, release 11.2 (STATA Corp., College Station, TX).

We described patients' characteristics using number and frequency for categorical data, median and range (minimum–maximum) for continuous data.

Comparisons of patients' characteristics (for CMV  $-/+$ , CMV  $+/+$ , CMV  $+/-$  vs CMV  $-/-$ ) were performed using the  $\chi^2$ -test (or Fisher's exact test in case of small expected numbers) for categorical variables and Student's *t*-test (or Mann–Whitney's test when distribution departed from normality or when homoscedasticity was rejected) for continuous variables.

Survival analyses were then conducted. Cumulative incidence functions (CIF) and Gray's test were used for relapse and NRM, since events of relapse and NRM were treated as competing events. For OS and DFS, Kaplan–Meier survival curves were drawn and differences in survival functions were tested using the Log-Rank test. OS was defined as the time from the first complete remission after intensive induction chemotherapy to death from any cause or last follow-up. The last time of follow-up of our study was December 31, 2013. DFS was defined as the time from first complete remission after intensive therapy to relapse or death without relapse. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were assessed using a standard Cox model, for OS and DFS, and a proportional subdistribution hazard model which is an extension of the Cox model to the situation of competing risks,<sup>[16]</sup> for the cumulative incidence of relapse (multivariate analysis was not conducted for NRM because of small sample of NRM [n=21]). Variables initially introduced in multivariate analyses were all variables associated with relapse, OS or DFS in univariate analyses with a *P*-value  $<0.20$ . Variables assessed in univariate analyses were potential confounding factors (patient characteristics at diagnosis such as cytogenetic and molecular abnormalities and patient, donor and transplant characteristics at allogeneic SCT and after transplant). A backward analysis was then applied until only variables significantly and independently associated with relapse, OS or DFS (*P*-value  $<0.05$ ) remained. The proportional-hazard assumption was tested for each covariate of the Cox model by the “log–log” plot method curves and was always met. When the linearity hypothesis was not respected, continuous variables were transformed into ordered data. Interactions between independent covariates were tested in final models and none were significant. Viral reactivation and GVHD were evaluated as time-dependent covariates. For the assessment of the potential protective effect of CMV serological status, we replaced the viral reactivation by CMV serological status in final multivariate models. Indeed CMV serological status and viral reactivation were known to be involved in a mediation relationship (i.e., CMV serological status promotes viral reactivation). Because the effect of CMV serological status is included in viral reactivation, the effect of viral reactivation and CMV serological status cannot be assessed simultaneously in the same model.

All reported *P*-values were 2-sided and the significance threshold was  $<0.05$ .

## 3. Results

At time of transplant, donor and recipient (D/R) CMV serostatus were as follows: 46 D/R  $-/-$  (CMV– group), and 90 D/R  $+/-$ ,

D/R+/+, or D/R -/+ (CMV+ group). Except for age, stem cell source, and GVHD prophylaxis, both clinical characteristics and GVHD incidence were similar between the 2 groups (Table 1).

At least 1 viral reactivation including CMV reactivation were recorded in 76 (56%) patients, 33% in the CMV- group and 68% in the CMV+ group ( $P < 0.01$ ) (Table 2). There were more CMV (53.3% vs 2.2%;  $P < 0.01$ ) and less EBV reactivations (4.4% vs 15.2%;  $P = 0.044$ ) in the CMV+ group compared with the CMV- group. Among the recipients of RIC, median time to CMV infection and viral reactivations were 35 (Inter-Quartile Range: 21–53) and 44 (24–63) days respectively, compared with

39 (26–55;  $P = 0.47$ ) and 48 (33–91;  $P = 0.15$ ) days among the MAC recipients.

With a median follow-up of 5.6 years, 41 relapses (30%) and 57 deaths (42%) were recorded.

The cumulative incidence of relapse (CIR) at 10 years was 15% (95% CI 7–23) in the group of patients who experienced viral reactivations compared with 54% (95% CI 41–70) in the group of patients without viral reactivation ( $P < 0.01$ ) (Fig. 1A). Ten year-CIR was also significantly lower in the CMV+ group (26%, 95% CI 18–38) compared with the CMV- group (45%, 95% CI 28–58) ( $P = 0.03$ ) (Fig. 2A).

**Table 1**  
Patient, donor, and transplant characteristics according to cytomegalovirus serostatus.

	All patients N = 136	Patients CMV -/- N = 46 (33.8%)	Other patients CMV +/-, +/+, -/+ N = 90 (66.2%)	P
Patient median age, year, range	49.5 (15.4–66.9)	45.4 (17.2–63.2)	50.7 (15.4–66.9)	0.03
Patients' sex, n, %				0.82
Female	58 (42.6)	19 (41.3)	39 (43.3)	
Male	78 (57.4)	27 (58.7)	51 (56.7)	
Cytogenetics classification, n, %				0.56
Good	7 (5.2)	3 (6.5)	4 (4.5)	
Intermediate	95 (70.4)	34 (73.9)	61 (68.5)	
Poor	33 (24.4)	9 (19.6)	24 (27.0)	
ELN classification, n, %				0.44
Good	15 (11.1)	4 (8.7)	11 (12.4)	
Intermediate-I	43 (31.9)	14 (30.4)	29 (32.6)	
Intermediate-II	44 (32.6)	19 (41.3)	25 (28.1)	
Poor	33 (24.4)	9 (19.6)	24 (27.0)	
Donor median age, y, range	43.0 (7.0–67.0)	43.0 (13.0–62.0)	43.5 (7.0–67.0)	0.19
Donor/recipient Sex mismatch, n, %				0.74
F/F	22 (16.2)	6 (13.0)	16 (17.8)	
F/M	22 (16.2)	6 (13.0)	16 (17.8)	
M/F	36 (26.5)	13 (28.3)	23 (25.6)	
M/M	56 (41.2)	21 (45.7)	35 (38.9)	
HLA, n, %				0.34
10/10	135 (99.3)	45 (97.8)	90 (100)	
<10/10	1 (0.7)	1 (2.2)	0 (0.0)	
Donor/recipient CMV status, n, %				–
CMV -/-	46 (33.8)	46 (100)	0 (0.0)	
CMV -/+	26 (19.1)	0 (0.0)	26 (28.9)	
CMV +/-	29 (21.3)	0 (0.0)	29 (32.2)	
CMV +/+	35 (25.7)	0 (0.0)	35 (38.9)	
Transplant type, n, %				0.34
MRD	92 (67.6)	29 (63.0)	63 (70.0)	
MUD	43 (31.6)	16 (34.8)	27 (30.0)	
MMUR	1 (0.7)	1 (2.2)	0 (0.0)	
Conditioning regimen, n, %				0.07
MAC	71 (52.2)	29 (63.0)	42 (46.7)	
RIC	65 (47.8)	17 (37.0)	48 (53.3)	
Stem cell source, n, %				0.04
BM	72 (52.9)	30 (65.2)	42 (46.7)	
PBSC	64 (47.1)	16 (34.8)	48 (53.3)	
GVHD prophylaxis, n, %				–
Cyclosporine A	136 (100)	46 (100)	90 (100)	
MTX	67 (50)	28 (62.2)	39 (43.8)	0.04
MMF	28 (20.9)	6 (13.3)	22 (24.7)	0.13
Acute GVHD, n, %				0.18
Yes	78 (57.4)	30 (65.2)	48 (53.3)	
Grades III-IV	30 (38.5)	13 (43.3)	17 (35.4)	0.48
Chronic GVHD, n, %				0.90
Yes	56 (47.9)	19 (48.7)	37 (47.4)	
Extensive cGVHD	14 (25.0)	3 (15.8)	11 (29.7)	0.34

BM = bone marrow, CMV = cytomegalovirus, ELN = European LeukemiaNet, GVHD = graft versus host disease, HLA = human leucocyte antigen, MAC = myeloablative conditioning, MMF = mycophenolate mofetil, MMUR = mismatch unrelated donor, MRD = match related donor, MTX = methotrexate, MUD = match unrelated donor, PBSC = peripheral blood stem cell, RIC = reduced intensity conditioning.

**Table 2****Viral characteristics according to cytomegalovirus serological status (n[%]).**

	All patients N=136	Patients CMV -/- N=46	Other patients CMV +/-, +/+, -/+ N=90	P
Viral reactivation	76 (56.0)	15 (33.0)	61 (68.0)	<0.01
CMV	49 (36.0)	1 (2.2)	48 (53.3)	<0.01
EBV	11 (8.1)	7 (15.2)	4 (4.4)	0.04
HSV	5 (3.7)	1 (2.2)	4 (4.4)	0.66
VZV	18 (13.2)	5 (10.9)	13 (14.4)	0.56
Polyomavirus	13 (9.6)	2 (4.3)	11 (12.2)	0.22
HHV6	3 (2.2)	2 (4.3)	1 (1.1)	0.26
HBV	2 (1.5)	0 (0.0)	2 (2.2)	0.55
HCV	0 (0.0)	0 (0.0)	0 (0.0)	–
HEV	0 (0.0)	0 (0.0)	0 (0.0)	–
Parvovirus B19	0 (0.0)	0 (0.0)	0 (0.0)	–
Others	6 (4.4)	3 (6.5)	3 (3.3)	0.41

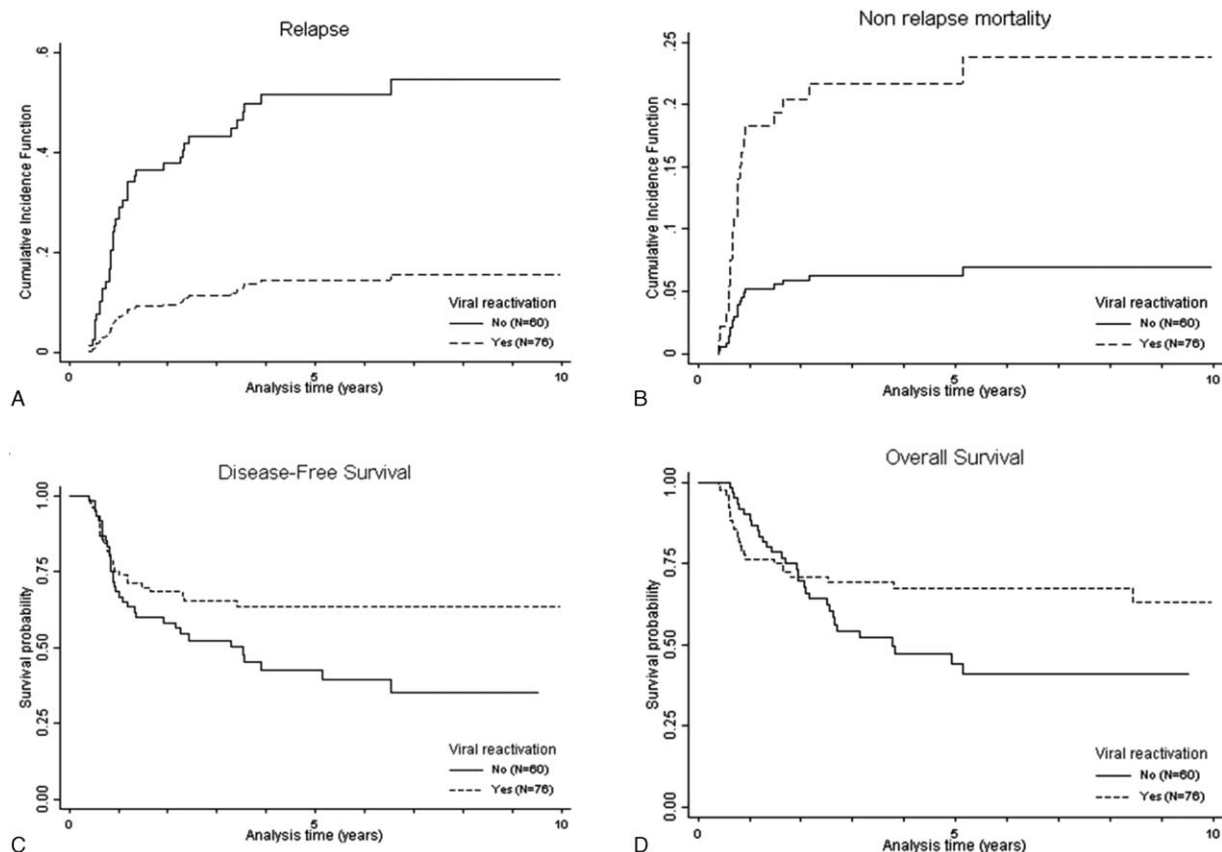
CMV = cytomegalovirus, EBV = Epstein-Barr virus, HBV = hepatitis B virus, HCV = hepatitis C virus, HEV = hepatitis E virus, HHV6 = 6th human herpes virus, HSV = herpes Simplex virus, VZV = varicella-zoster virus.

Other viruses included papilloma virus, adenovirus, respiratory syncytial virus, and parainfluenza virus.

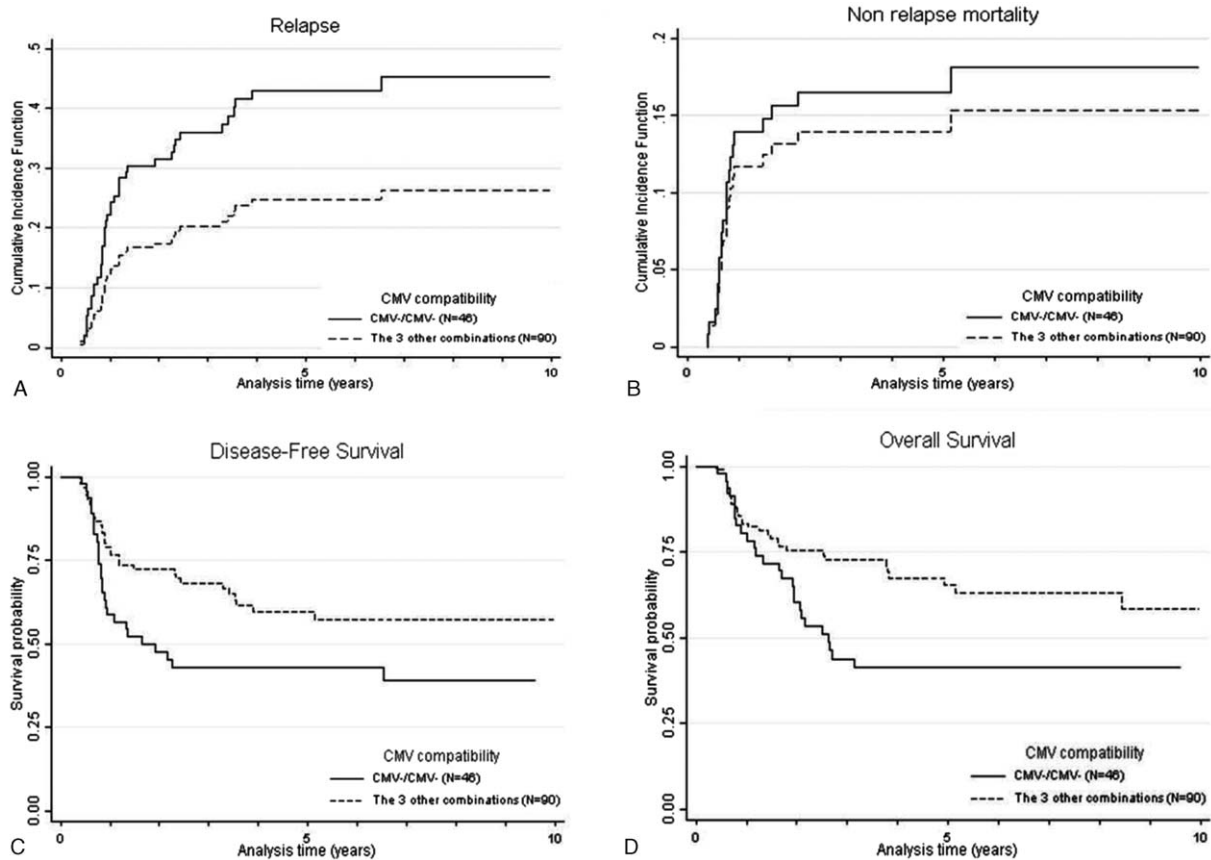
In univariate analysis, viral reactivations and CMV+ serological status were associated with a significant reduced risk of relapse as well as chronic GVHD but not ELN classification, type of donor, conditioning regimen, stem cell source, or acute GVHD (data not shown). To assess the impact of both viral reactivations and CMV serostatus, which are involved in a mediation relationship (i.e., CMV serostatus promotes viral reactivation), 2 multivariate models were built (including viral reactivation for

the first and CMV serostatus for the second). In these multivariate models, viral reactivations and CMV+ serostatus were still significantly associated with a reduced risk of relapse (Table 3).

In the group of patients who experienced viral reactivations, 10 year-NRM was 24% (95% CI 14–32) compared with 7% (95% CI 3–19) in the group of patients without viral reactivation ( $P=0.02$ ) (Fig. 1B). Ten year-NRM was not significantly different between CMV+ (15%, 95% CI 9–25) and CMV- group (18%,



**Figure 1.** Relapse and survival stratified by viral reactivation. (A) Cumulative incidence of relapse ( $P < 0.01$ ), (B) nonrelapse mortality ( $P = 0.02$ ), (C) disease-free survival ( $P = 0.04$ ), and (D) overall survival ( $P = 0.10$ ).



**Figure 2.** Relapse and survival stratified by Cytomegalovirus serological status. (A) Cumulative incidence of relapse ( $P=0.03$ ), (B) nonrelapse mortality ( $P=0.68$ ), (C) disease-free survival ( $P=0.02$ ), and (D) overall survival ( $P=0.02$ ). CMV indicates cytomegalovirus.

95% CI 8–30) ( $P=0.68$ ) (Fig. 2B). However, more patients died following AML relapse in the CMV- group (37%, 95% CI 23–52) compared with the CMV+ group (19%, 95% CI 11–29) ( $P=0.02$ ). There was no difference between both groups, regarding other causes of death (Table 4).

In the group of patients who experienced viral reactivations, 10 year-DFS was 63% (95% CI 51–73) compared with 35% (95% CI 21–50) in the group of patients without reactivation ( $P=0.04$ ) (Fig. 1C). Ten year-DFS was also significantly better in the CMV+ group (57%, 95% CI 45–68) compared with the CMV- group (39%, 95% CI 24–53) ( $P=0.02$ ) (Fig. 2C). In multivariate models (Table 3), viral reactivations and CMV+ serostatus were associated with a significantly better DFS.

In the group of patients who experienced viral reactivations, 10 year-OS was 63% (95% CI 49–74) compared with 41% (95% CI 27–55) in the group of patients without reactivation ( $P=0.10$ )

(Fig. 1D). Ten year-OS was also significantly better in the CMV+ group (59%, 95% CI 44–71) compared with the CMV- group (41%, 95% CI 27–55) ( $P=0.02$ ) (Fig. 2D). In multivariate models, viral reactivations and CMV+ serostatus were still significantly associated with a reduced risk of death (Table 3). Acute grade III-IV GVHD was significantly associated with poorer OS (adjusted HR 3.91; 95%CI 1.83–8.33;  $P < 0.01$ ).

#### 4. Discussion

Our study shows that, in AML patients allografted in first complete response, not only CMV serostatus but also viral reactivations as a whole have a clear impact on relapse incidence and survival independently of conditioning regimen, GVHD or stem-cell source. This finding is in accordance with other studies, although some of them failed to demonstrate an impact on

**Table 3**  
**Multivariable analysis of the impact of viral reactivations and cytomegalovirus serological status on relapse, overall survival, and disease-free survival.**

	Relapse HR (95%CI)	P	OS HR (95%CI)	P	DFS HR (95%CI)	P
Model for viral reactivations, yes vs no	0.14 (0.07–0.30)*	<0.01	0.44 (0.25–0.77)†	<0.01	0.29 (0.16–0.54)‡	<0.01
Model for CMV +/-, +/+, -/+ vs CMV -/-	0.47 (0.25–0.90)*	0.02	0.42 (0.24–0.72)†	<0.01	0.49 (0.28–0.84)‡	0.01

CI = confidence interval, CMV = cytomegalovirus, DFS = disease-free survival, HR = hazard ratio, OS = overall survival.

\* Adjusted for “delay between graft and leucocyte recovery” and “delay between graft and stop of ciclosporin.”

† Adjusted for “delay between graft and leucocyte recovery,” “delay between graft and stop of ciclosporin,” “age of donor,” and “grade III-IV of acute GVHD” (HR = 3.91 (95%CI:1.83–8.33);  $P < 0.01$  and HR = 3.56 (95%CI:1.68–7.56);  $P < 0.01$  in the model for viral reactivation and in the model for CMV, respectively).

‡ Adjusted for “neutrophil level at day 100 after allograft,” “delay between graft and stop of ciclosporin,” and “refractory acute GVHD.”

Table 4

## Causes of death.

	All patients N=136	Patients CMV -/- N=46 (33.8%)	Other patients CMV +/-, +/+, -/+ N=90 (66.2%)	P
Relapse, n, %	34 (25.0)	17 (37.0)	17 (18.9)	0.02
Infection, n, %	17 (12.5)	6 (13.0)	11 (12.2)	0.89
GVHD, n, %	10 (7.4)	4 (8.7)	6 (6.7)	0.73
Hemorrhage, n, %	4 (2.9)	3 (6.5)	1 (1.1)	0.11
Iatrogenic, n, %	2 (1.5)	0 (0.0)	2 (2.2)	0.55
Secondary malignancies, n, %	2 (1.5)	1 (2.2)	1 (1.1)	1.00
Other causes, n, %	5 (3.7)	3 (6.5)	2 (2.2)	0.34

CMV = cytomegalovirus, GVHD = graft versus host disease.

Other causes of death included graft failure, thrombotic microangiopathy, and cirrhosis of the liver.

survival.<sup>[3,4,6]</sup> The mechanisms by which viral reactivations and relapse prevention could be linked remain unclear. The observation of such an effect particularly in AML as compared to other hematological malignancies is also intriguing. Elucidating which immune cell subpopulation is key to control residual disease will be crucial to rationally design immunotherapy against AML. Natural killer, CD8 and gamma/delta T cells have been shown to be stimulated by CMV reactivation and could cross-react with both viruses and leukemic cells.<sup>[17]</sup> Moreover, a direct CMV's cytotoxic effect had recently been shown against blast cells, inducing apoptosis.<sup>[18]</sup> However, viral reactivations are still associated with an increased risk of NRM and thus, choosing a donor according to CMV serostatus has now become a dilemma. Larger prospective studies in well-defined AML populations with respect to disease status, minimal residual disease, conditioning regimen, GVHD prophylaxis, and antiviral therapy are needed to clearly determine the clinical application of these findings.

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