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Effect of Sirolimus Exposure on the Need for Preemptive Antiviral Therapy for Cytomeglovirus Infection after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The current study evaluates the clinical effect of sirolimus exposure on the occurrence of cytomegalovirus (CMV) DNAemia necessitating preemptive antiviral therapy. A total of 167 consecutive recipients of reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (allo-HSCT) who received sirolimus- and tacrolimus-based graft-versus-host disease (GVHD) prophylaxis and whose CMV serostatus was positive for donors and/or recipients were included in this multicenter retrospective study. A parametric model with consecutive sirolimus blood levels describing the time to CMV DNAemia-RAT was developed using NONMEM version 7.4. Overall, 122 of 167 patients (73%) were allografted from an unrelated donor, and the donor CMV-serostatus was negative in 51 cases (31%). Fifty-six recipients (34%) developed CMV DNAemia necessitating preemptive therapy, with a cumulative incidence of 36% at a median follow-up of 25 months. Time to CMV DNAemia necessitating preemptive therapy was best described using a Gompertz function. CMV DNAemia necessitating preemptive therapy-predicting factors were antithymocyte globulin-based conditioning regimen (hazard ratio [HR], 2.2; 95% confidence interval [CI], 1.1 to 4.1; $P < .01$) and sirolimus concentration (HR, .94; 95% CI, .87 to .99; $P < .01$). The risk of CMV DNAemia-RAT decreased by 6% for each 1 ng/mL increase in sirolimus trough concentration. In conclusion, we provide evidence on the association between sirolimus blood concentration and incidence of CMV DNAemia necessitating preemptive therapy in allo-HSCT recipients. Moreover, this study presents the first predictive model describing the time to CMV DNAemia necessitating preemptive antiviral therapy as a function of sirolimus drug concentration.

INTRODUCTION

Sirolimus has been associated with a significant protective effect against active cytomegalovirus (CMV) infection and disease (CMV-D) in organ transplantation (OT) in several randomized controlled clinical trials [1-4]. In contrast, this beneficial effect remains controversial in the allogeneic hematopoietic stem cell transplantation (allo-HSCT) setting [5-9]. OT and allo-HSCT procedures differ in many aspects, including lymphodepleting conditioning chemotherapy, thymus dysfunction, and dysregulation from the conditioning regimen. In addition, characteristics related to sirolimus exposure and treatment duration, such as lower targeted immunosuppressant drug concentration,

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common concentration fluctuations (eg, variability in absorption, due in part to frequent episodes of diarrhea; drug-drug interactions), and early tapering in allo-HSCT recipients, also vary widely [10]. In combination, these latter factors may contribute to reduced overall exposure to sirolimus, mitigating its potential anti-CMV effect in allo-HSCT compared with OT.

We previously demonstrated that higher sirolimus exposure significantly reduced the risk of detectable CMV DNAemia at any level after allo-HSCT [11]. Our next step was to explore whether higher sirolimus concentrations could also have the clinical benefit of reducing the number of CMV DNAemia events necessitating preemptive antiviral therapy in allo-HSCT recipients. With this aim, we developed a fully parametric time-to-event analysis taking into account the dependency and association between longitudinal data (ie, trough blood concentration of sirolimus) and time-to-event data (ie, occurrence of CMV DNAemia necessitating preemptive therapy). We examined the pharmacokinetic (PK)-pharmacodynamic (PD) relationship between CMV DNAemia necessitating preemptive therapy and sirolimus exposure using consecutive trough blood concentrations of sirolimus. Here we report the clinical effect of sirolimus exposure over time on the risk of developing a first CMV DNAemia episode necessitating preemptive therapy in a multicenter series of consecutive reduced-intensity conditioning (RIC) allo-HSCT recipients who received a sirolimus and tacrolimus combination as graft-versus-host disease (GVHD) prophylaxis.

METHODS

Patients

This multicenter study included 167 consecutive patients who underwent RIC-allo-HSCT between October 23, 2008, and October 29, 2015, and received sirolimus- and tacrolimus-based GVHD prophylaxis and whose CMV serostatus was positive for donors and/or recipients. The Institutional Review Board of each participating institution approved the study, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The study was registered by the Spanish Agency of Medicines and Health Products (reference code PIN-SIR-2016-01).

Conditioning Regimen and GVHD Prophylaxis

Three RIC regimens were used in this study, as described previously [12]: fludarabine combined with melphalan (for lymphoid malignancies), fludarabine combined with oral (p.o.) or i.v. busulfan (for myeloid malignancies), and fludarabine with i.v. busulfan and thiotepa (for both myeloid and lymphoid malignancies). Antithymocyte globulin (ATG) was administered to transplant recipients with 1 HLA-mismatched (considering high-resolution typing of HLA-A, -B, -C, and -DRB1) with either a sibling donor or an unrelated donor (URD) at doses of 6 mg/kg divided over 3 days.

GVHD prophylaxis consisted of sirolimus at a dose of 6 mg/day p.o. on day -6, followed by 4 mg/day p.o. starting on day -5; tacrolimus was started on day -3 at a dose of .02 mg/kg/day as a continuous i.v. infusion or equivalent oral doses. In the absence of GVHD, sirolimus and tacrolimus doses were tapered as described previously [10].

Sirolimus and Tacrolimus Blood Concentration Monitoring, Management, and Technical Considerations

Sirolimus and tacrolimus trough blood concentrations were monitored at least twice weekly for the first 4 weeks after transplantation or until discharge, then once a week until day +100 and thereafter at each outpatient visit. Sirolimus levels were measured by liquid chromatography for separation and tandem mass spectrometry at the Hospital Vall d'Hebron of Barcelona (HVH), by the semiautomated microparticle enzyme immunoassay method (IMx sirolimus assay; Abbott Laboratories, Abbott Park, IL) at the Hospital de la Santa Creu i Sant Pau (HSCSP), and by chemiluminescent magnetic microparticle immunoassay (Architect i-System; Abbott Laboratories) at the Hospital Clínico Universitario of Valencia (HCUV). A cross-validation study comparing sirolimus levels measured by the 2 laboratory methods demonstrated a significant correlation ($r \geq .91$) [13]. Doses were titrated to achieve the target blood concentrations of 5 to 12 ng/mL for sirolimus and 5 to 10 ng/mL for tacrolimus.

CMV DNA Monitoring and Load Quantitation

At 3 centers, all patients at risk were routinely monitored at least twice weekly during hospital admission, and once weekly thereafter during the first 100 days and monthly until 1 month after the withdrawal of immunosuppression. Patients who developed acute or chronic GVHD were monitored at each outpatient visit. At HCUV, CMV DNA load monitoring in plasma was performed using 2 PCR platforms: a Qiagen Real-Time PCR Kit (Qiagen, Hilde, Germany) from October 2008 to May 2012 and an Abbott Real-Time CMV PCR (Abbott Molecular, Des Plaines, IL) thereafter [7,14]. At HVH, CMV DNA load monitoring in plasma was performed using a RealStar CMV PCR system (Altona Diagnostics, Hamburg, Germany). At HSCSP, CMV DNA load monitoring in plasma was done with the Affigene CMV trender (Cepheid, Solna, Sweden) from October 2008 to December 2012 and with the RealStar CMV PCR Kit 1.2 (Altona Diagnostics) thereafter.

Preemptive Anti-CMV Therapy and Definitions

At HCUV and HVH, preemptive therapy was initiated on detection of CMV DNA levels >1500 IU/mL irrespective of the PCR assay used. Beginning in 2014 at HCUV, preemptive antiviral therapy was started either when CMV DNA load doubling time was ≤2 days or when CMV DNAemia reached >1500 IU/mL, whichever occurred first [15]. At HSCSP, preemptive therapy against CMV was started when the CMV DNAemia viral load reached >1000 UI/mL, whereas 3 consecutive samples with CMV DNAemia values ranging from 500 to 1000 UI/mL were required before to prompt the initiation of preemptive therapy.

CMV DNAemia necessitating preemptive therapy was defined when the CMV DNAemia load exceeded the established trigger levels for preemptive antiviral therapy at each center. Preemptive anti-CMV therapy was based on ganciclovir 5 mg/kg i.v. or foscarnet 90 mg/kg i.v. every 12 hours during the admission (reserving foscarnet for patients with neutropenia) or with oral valganciclovir at a usual dose of 900 mg twice daily in the outpatient clinic. CMV-D was divided into pneumonia, gastrointestinal disease, and retinitis based on consensus definitions [16]. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined and graded based on preestablished criteria [17]. Transplantation-associated microangiopathy (TAM) was classified as confirmed or probable according to previously defined international criteria [18-20].

Statistical Methods

The primary objective of this study was to identify risk factors for a first CMV DNAemia necessitating preemptive therapy episode, with special emphasis on the potential effect of estimated exposure to sirolimus on this complication. Secondary objectives were to analyze CMV DNAemia necessitating preemptive therapy and/or CMV-D characteristics and to evaluate the effect of CMV DNAemia necessitating preemptive therapy in nonrelapse mortality (NRM) and overall survival.

Nonparametric and Semiparametric Analyses

The probabilities of post-transplantation events were estimated by the cumulative incidence method. Univariate analysis of the association between clinical risk factors and post-transplantation outcomes were calculated taking competing events into account by using the Gray test [21,22]. Time-dependent covariates were analyzed using univariate and multivariate Cox regression models. Tests of significance were 2-sided, with statistical significance at $P \leq .05$. All nonparametric and semiparametric statistical analyses were performed using SPSS version 20 (IBM, Armonk, NY) and R version 2.12.2 (R Project for Statistical Computing, Vienna, Austria) with survival v2.36-10, Design 2.3-0, prodlim v1.2.1, and cmprsk v2.2-221packages.

Parametric Analysis

A parametric survival model describing time to CMV DNAemia necessitating preemptive therapy after allo-HSCT was developed by means of nonlinear mixed-effects modeling using NONMEM version 7.4 (ICON Development Solutions, Dublin, Ireland) [12,23]. The model was developed in 2 steps: a baseline model without any explanatory factors was developed, and the impact of the study variables was explored and included in the baseline model.

Development of the Baseline Model

The time to CMV DNAemia-RAT was calculated using the following parametric survival function:

$$s(t) = e^{-\int_0^t h(t)dt},$$

where $h(t)$ is the hazard and survival, $S(t)$, is a function of the cumulative hazard within the time interval 0 and the time t describing the probability of not experiencing CMV DNAemia necessitating preemptive therapy within this interval. The baseline model was developed by exploring different functions for the hazard $h(t)$, starting from a simple time-independent constant hazard and gradually progressing to more complex functions, including Weibull and Gompertz. For nested models, decisions about the best choice of model were made based on the likelihood ratio test principle. The difference in objective function value (ΔOFV) between a full and reduced model follows approximately a chi-square distribution, with the number of degrees of freedom corresponding to the difference in number of parameters between the 2 competing models. For a statistical significance level of $P = .05$ and 1 degree of freedom, a ΔOFV of -3.84 is required.

Development of the Covariate Model

Potential covariates explored were patient, donor and transplantation characteristics, transplantation center, sirolimus and tacrolimus trough concentrations, and post-transplantation events tested as time-dependent covariates (ie, several combinations of GVHD; aGVHD grade I-IV, grade II-IV, and grade III-IV; cGVHD; and a combination of moderate-to-severe GVHD) taking competitive events into account. Sirolimus and tacrolimus levels were censored at the time of either occurrence of CMV DNAemia necessitating preemptive therapy or relapse of the baseline disease. Initially, the covariates were tested in a univariate manner; that is, each covariate relationship was evaluated on the baseline hazard separately. Based on the results, covariate inclusion was carried out using a stepwise covariate inclusion procedure. The significance level was set to .05 for the forward inclusion approach and .01 for the backward deletion approach. Covariates were incorporated into the model as shown in the following equation:

$$h(t)_j = h(t) \cdot e^{\theta_{Cov} \cdot Cov},$$

where $h(t)_j$ represents the instantaneous hazard for an individual with covariate value *Cov*, $h(t)$ represents the hazard function of patients with values equal to 0 (for continuous covariates) or in the reference groups (for categorical covariates), and θ represents the regression coefficient describing the influence of the covariate on the instantaneous hazard. HR can be calculated as $e^{\theta_{Cov} \cdot Cov}$. Drug concentration effect on hazard was explored using linear and E_{max} models:

$$h(t)_j = h(t) \cdot exp^{C_{drug} \cdot Eff}$$

and

$$h(t)_j = h(t) \cdot exp^{\frac{E_{max} \cdot C_{drug}}{C_{50} + C_{drug}}},$$

where C_{drug} represents immunosuppressant drug concentration, *Eff* represents the effect of drug concentration on instantaneous hazard, E_{max} represents the maximum drug effect, and C_{50} represents the concentration that obtains one-half the maximum effect.

To ensure that the models described the data adequately, internal validation of the model was performed by visual predictive check (VPC) Kaplan-Meier plots. The plots were based on simulations of 100 replicates of the study dataset. For the final model, a nonparametric bootstrap analysis of the data with 1000 resampled datasets was performed, and the uncertainty (95% confidence interval [CI]) of the parameter estimates was obtained from the distribution of the bootstrap estimates (2.5th and 97.5th percentiles). The final model was then used to perform deterministic simulations of CMV DNAemia necessitating preemptive therapy-free survival curves for increasing sirolimus trough concentrations.

RESULTS

Patient Characteristics

Patients' disease and transplantation characteristics and outcomes are summarized in Table 1. The median age of recipients was 59 years (range, 23 to 72 years), and 60% were male. The median duration of follow-up was 25 months (range, 4 to 85 months). Most recipients (73%) were allografted from an URD. Thirty-two patients (19%) had an HLA mismatch with the donor and received ATG as a part of conditioning. In 51 of 167 cases (31%), CMV serostatus was positive for the recipient and negative for the donor. For the whole cohort, the cumulative incidence was 37% for aGVHD grade II-IV, 17% for aGVHD grade III-IV, 75% for overall cGVHD, and 39% for extensive cGVHD.

CMV-DNAemia-RAT and CMV Disease Characteristics

Fifty-six of 167 patients (34%) developed CMV DNAemia necessitating preemptive therapy at a median of 56 days (range, 12 to 535 days) after stem cell infusion. The 24-week and 2-year cumulative incidences of CMV DNAemia necessitating preemptive therapy were 33% (95% CI, 25% to 40%) and 36% (95% CI, 28% to 44%), respectively. The 2-year cumulative incidence of CMV-D was 2% (95% CI, 0 to 5%). Three patients (2%) developed CMV-D at 120, 200, and 201 days after stem cell infusion.

Table 1
Patient Characteristics and Post-Transplantation Outcomes (n = 167)

Characteristic	Value
Recipients age, yr, median (range)	59 (23-72)
Male recipient, n (%)	100 (60)
Previous lines of therapy, n (range)	3 (0-8)
Previous allo-HSCT, n (%)	58 (35)
HCT-CI, n (%)	
0	33 (20)
1-2	71 (42)
≥3	63 (38)
Diagnosis, n (%)	
Acute leukemia/myelodysplastic syndrome	60 (36)/21 (13)
Non-Hodgkin lymphoma/Hodgkin disease	42 (25)/16 (10)
Multiple myeloma/chronic lymphocytic leukemia	10 (6)/7 (5)
Other	11 (7)
Disease status at allo-HSCT, n (%)	
CR	103 (62)
PR	37 (22)
PROG	27 (16)
Donor/recipient CMV serostatus	
+/+	106 (63)
+/-	10 (6)
-/+	51 (31)
Female donor to male recipient, n (%)	36 (22)
Donor type	
Related	45 (27)
Unrelated	122 (73)
HLA mismatch, n (%)	32 (19)
Conditioning regimen, n (%)	
Flu-Bu	50 (30)
Flu-Mel	73 (44)
Flu-Bu-TT	44 (26)
ATG as a part of conditioning, n (%)	31 (19)
Peripheral blood source, n (%)	164 (98)
$CD34^+$ cell count, $\times 10^6$/kg, median (range)	6 (.5-13.8)
Transplantation outcomes	
Days to myeloid recovery, median (range)	
Neutrophils >.5 $\times 10^9$/L	15 (8-27)
Platelets >20 $\times 10^9$/L	12 (0-386)
aGVHD	
Cumulative incidence of aGVHD II-IV, % (95% CI)	37 (30-45)
Day of onset, median (range)	65 (5-275)
Cumulative incidence of aGVHD III-IV, % (95% CI)	17 (12-23)
Day of onset, median (range)	53 (6-202)
cGVHD in evaluable patients	
Cumulative incidence of cGHVD at 2 yr, % (95% CI)	75 (66-84)
Day of onset, median (range)	188 (77-982)
Cumulative incidence of extensive cGHVD, % (95% CI)	39 (20-50)
Cumulative incidence of NRM, % (95% CI)	
At day +100	9 (4-13)
At day +180	12 (8-18)
At 2 yr	21 (15-28)
Cumulative incidence of TAM (95% CI)	
Proven	9 (3-14)
Probable-proven	17 (8-21)
Cumulative incidence of SOS (95% CI)	4 (2-8)
CMV	
Cumulative incidence of CMV DNAemia-RAT at 24 wk, % (95% CI)	33 (25-40)
Cumulative incidence of DNAemia-RAT at 2 yr, % (95% CI)	36 (29-44)
Cumulative incidence of CMV-D at 2 yr, % (95% CI)	2 (0-5)
Cumulative incidence of relapse at 2 yr, % (95% CI)	18 (12-25)
DFS at 2 yr, % (95% CI)	61 (58-65)
OS at 2 yr, % (95% CI)	68 (64-72)
Follow-up for survivors, mo, median (range)	25 (4-85)

HCT-CI indicates hematopoietic cell transplantation-specific comorbidity index; CR, first complete remission; PR, partial remission; PROG, nonresponder or progression before RIC-allo-HSCT; Flu, fludarabine; Bu, busulfan; Mel, melphalan; TT, thiotepa; SOS, sinusoidal obstruction syndrome of the liver: CMV-D, CMV disease: DFS, disease-free survival.

Risk Factors for CMV DNAemia Necessitating Preemptive Antiviral Therapy

Nonparametric and Semiparametric Analyses

Table 2 shows the univariate and multivariate risk factor analyses for CMV DNAemia necessitating preemptive therapy. In multivariate Cox model analysis, ATG use, presence of a lymphoid malignancy, and undergoing allo-HSCT at the HSCSP transplantation center were associated with a risk of CMV DNAemia necessitating preemptive therapy.

Parametric Analyses

For the parametric time-to-event model, a total of 3289 sirolimus blood samples throughout a median of 140 days (range, 7 to 535 days) after transplantation were available for analysis. Sirolimus withdrawal was done in 98 recipients (58%) at a median of 217 days (range, 1 to 2088 days), whereas tacrolimus was withdrawal in 148 patients (88%) at median of 100 days (range, 1 to 1237 days) after stem cells infusion. Differences over time in median and range values of sirolimus and tacrolimus levels according to transplantation center and baseline disease are illustrated in Figure 1A and B, respectively.

The OFV for the main structural models tested in the nonlinear model building procedure were 860.7 for the exponential survival function with 1 parameter, 837.9 for the Weibull function with 2 parameters, and 809.1 for the Gompertz function with 2 parameters. Finally, the time to CMV DNAemia necessitating preemptive therapy was best described by a Gompertz function with regard to OFV and Kaplan-Meier VPC plots. After the stepwise covariate inclusion procedure, the retained final time to CMV DNAemia necessitating preemptive therapy model included the use of ATG and sirolimus exposure as statistically significant predictors of CMV DNAemia necessitating preemptive therapy. The risk of experiencing CMV DNAemia necessitating preemptive therapy was twice the baseline risk in patients who received ATG as part of conditioning regimen (HR, 2.2; 95% CI, 1.1 to 4.1). With regard to sirolimus exposure, the risk of experiencing CMV DNAemia necessitating preemptive therapy decreased linearly by 6% per each 1 ng/mL increase in sirolimus trough concentrations (HR, .94; 95% CI, .87 to .99). Tacrolimus exposure had no influence on the risk of CMV DNAemia necessitating preemptive therapy.

Regarding model validation, the VPC Kaplan-Meier plots (Figure 2) proved an adequate performance of the model given that the observed survival curve was situated inside the simulation prediction intervals. In addition, the bootstrap results reinforced the robustness of the model because final model parameter estimates were inside the CIs obtained from bootstrap reestimations, as shown in Table 3. Furthermore, the statistical significance of the effect of sirolimus exposure on CMV DNAemia necessitating preemptive therapy-free survival was reinforced because the 95% CI of the reestimated values for this parameter did not include the 0 value (-.135 to -.001). This was also true for the parameter representing ATG influence. The influence of sirolimus exposure on the probability of not experiencing CMV DNAemia necessitating preemptive therapy is represented through deterministic simulations in Figure 3. A subgroup analysis through nonlinear mixed-effects modeling, fitting the final model to data from each center separately and checking the estimated effect

Table 2
Univariate and Multivariate Risk Factor Analyses of CMV DNAemia Necessitating Preemptive Antiviral Therapy at a Median Follow-Up of 25 Months

Variable[Σ]	CMV DNAemia-RAT		Multivariate Analysis, Cox Regression	
	Cumulative Incidence, % (95% CI)	*P* Value	HR (95% CI)	*P* Value
Disease				
Myeloid	26 (16-36)	.01		
Lymphoid	45 (34-56)		1.8 (1.01-3.1)	.05
ATG				
Yes	51 (33-70)	.01	2.2 (1.2-3.99)	.009
No	33 (24-41)			
Conditioning regimen*				
Flu-Bu	24 (12-36)			
Flu-Mel	43 (31-55)	.10	NS	
TBF	37 (22-52)			
Donor/recipient sex mismatch				
Female donor to female recipient	14 (0-29)	.02	NS	
Others	40 (31-48)			
CMV serostatus				
R+/D-	45 (31-59)			
R+/D+	35 (26-45)	.03	NS	
R-/D+	0			
Previous lines of therapy				
<3	31 (21-41)	.10	NS	
≥3	42 (31-54)			
Previous aGVHD grade II-IV†	1.36 (0.91-2)	.13		
Previous aGVHD grade III-IV†	1.37 (0.93-1.9)	.10	NS	
Transplantation center				
HCUV	49 (35-63)		1	
HSCSP	25 (14-35)	.03	0.45 (0.23-0.92)	.03
HVH	39 (23-55)		0.8 (0.4-1.6)	.50

D indicates donor; R, receptor; NS, not significant; TBF, fludarabine with i.v. busulfan and thiotepa.
Other tested covariates that did not achieve statistical significance included donor type (related versus unrelated), recipient age, $CD34^+$ cell count, HCT-CI, and previous autologous HSCT.

* Conditioning was not entered into the multivariate analysis because it showed collinearity with the transplantation center variable, because all recipients at HVH were conditioned with TBF, whereas Flu-Bu was used at HCUV and Flu-Mel was used at HSCSP.

† Analyzed as time-dependent covariates. Results are expressed as HR from a univariate Cox regression model.

of sirolimus on the risk of CMV reactivation was performed (data not shown). All 3 subgroup analyses demonstrated a numerical benefit (risk reduction) for higher exposures to sirolimus, and 2 of them estimated this parameter with sufficient precision to prove statistical significance. Only 1 subgroup (that with the smallest number of patients) did not prove statistical significance, most likely due to reduced sample size, but indicated a numerical benefit (risk reduction). In addition, a subgroup analysis was performed in donor-negative/recipient-positive CMV serostatus pairs that also demonstrated a numerical benefit (risk reduction) for greater exposures to sirolimus. However, the results of this subgroup analysis were not statistically significant, likely due to the small sample size.

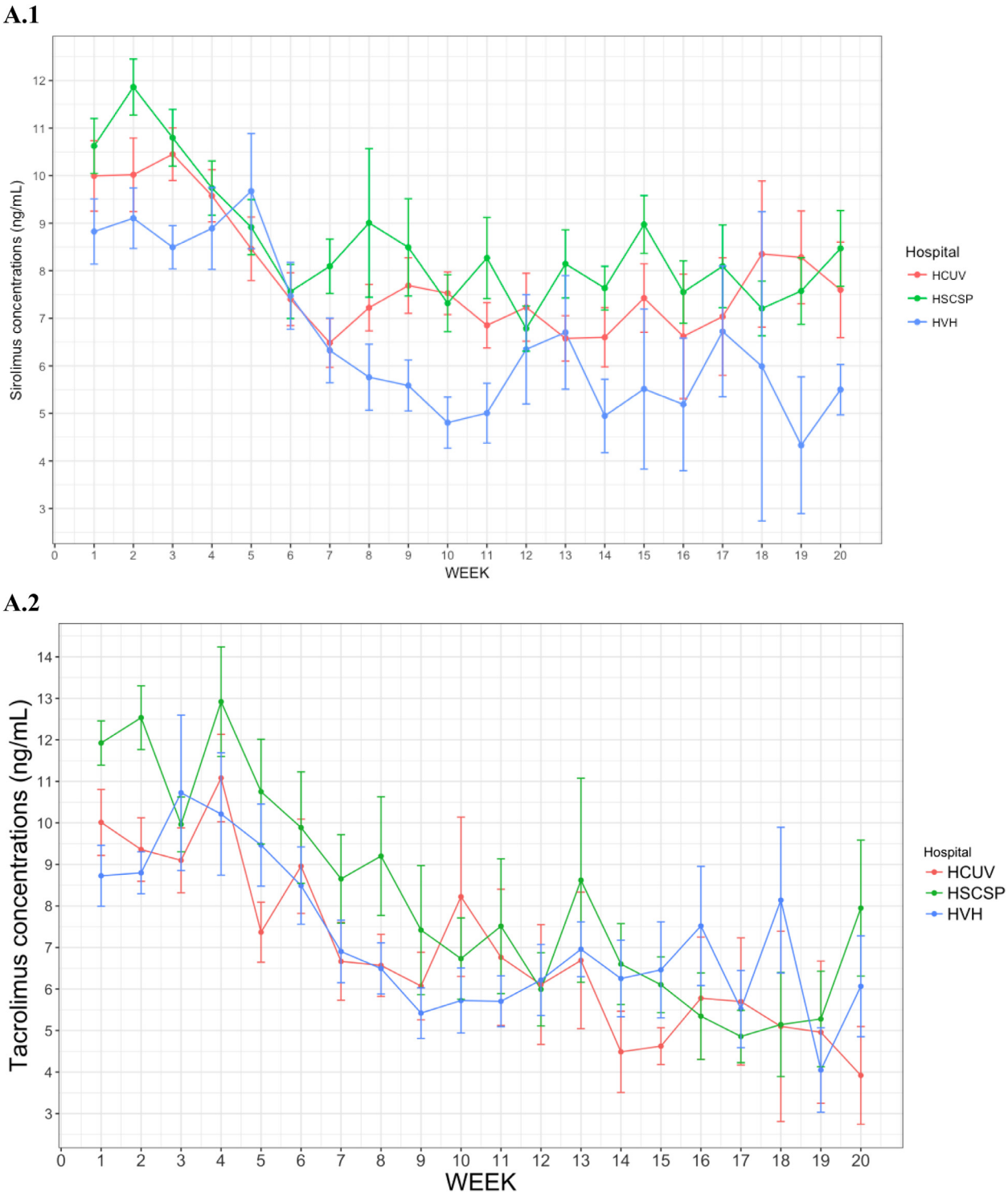

Figure 1. Sirolimus and tacrolimus levels by transplantation center (A) and baseline disease (B). A total of 3289 sirolimus blood samples at a median of 140 days (range, 7 to 535 days) after transplantation were available. Of these, 1033 sirolimus trough concentrations were available after 100 days, corresponding to 107 of the 167 patients included in the analysis.

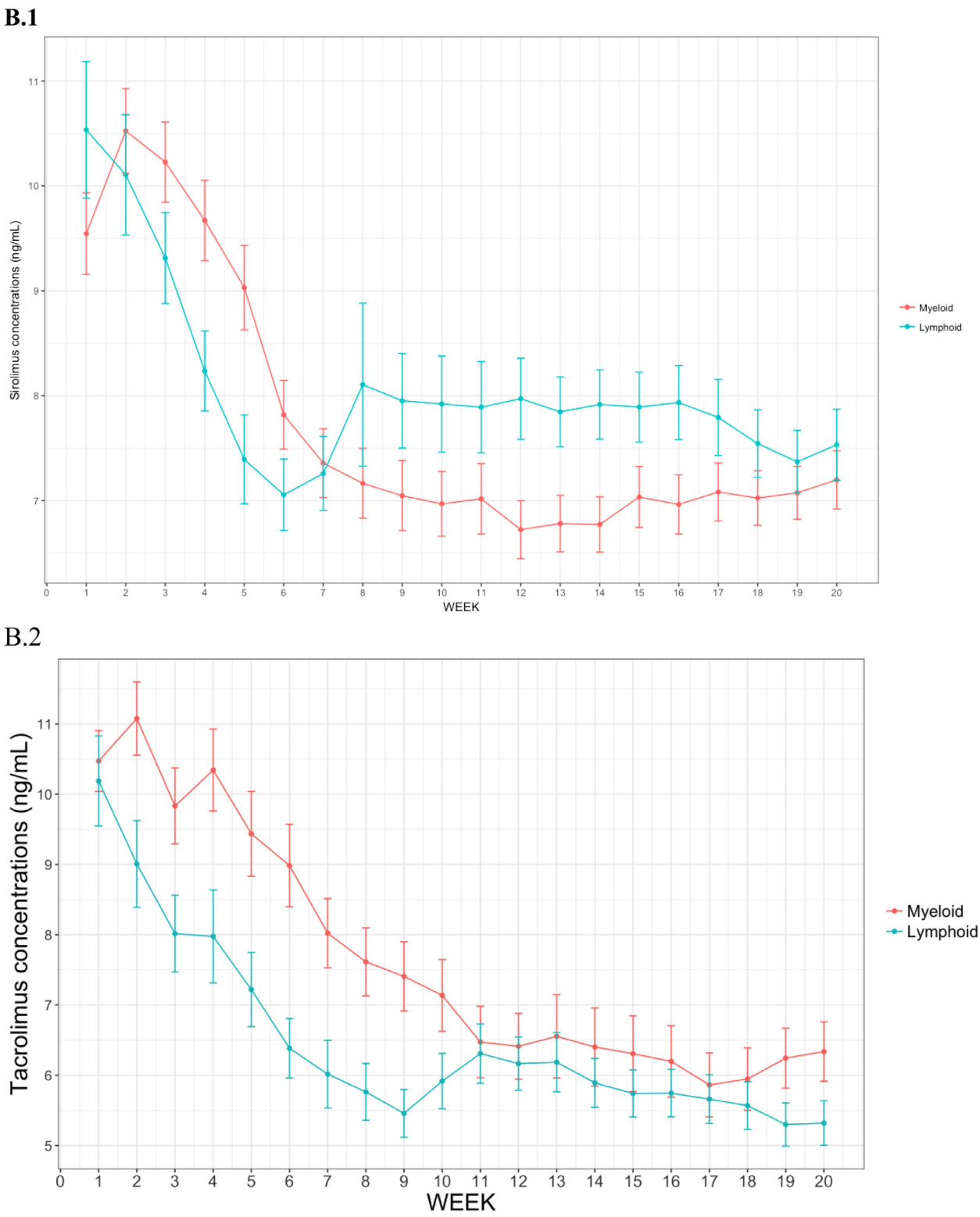

Figure 1 Continued.

NRM, Relapse, and Survival

Thirty-one patients (19%) died due to NRM at a median time of 124 days (range, 13 to 652 days). The +100-day, +180-day, and 2-year cumulative incidence of NRM for the whole group was 9% (95% CI, 4% to 13%), 12% (95% CI, 8% to 18%), and 21% (95% CI, 15% to 28%), respectively. In univariate analyses, the development of CMV DNAemia necessitating preemptive therapy was not associated with NRM (HR, 1.5; 95% CI, .8 to 3.2; $P = .2$) or with lower relapse incidence (HR, .5; 95% CI, .2 to 1.2; $P = .10$). Patients who developed CMV DNAemia necessitating preemptive therapy had a 2-year overall survival of 65%, compared with 70% for those who did not ($P = .20$).

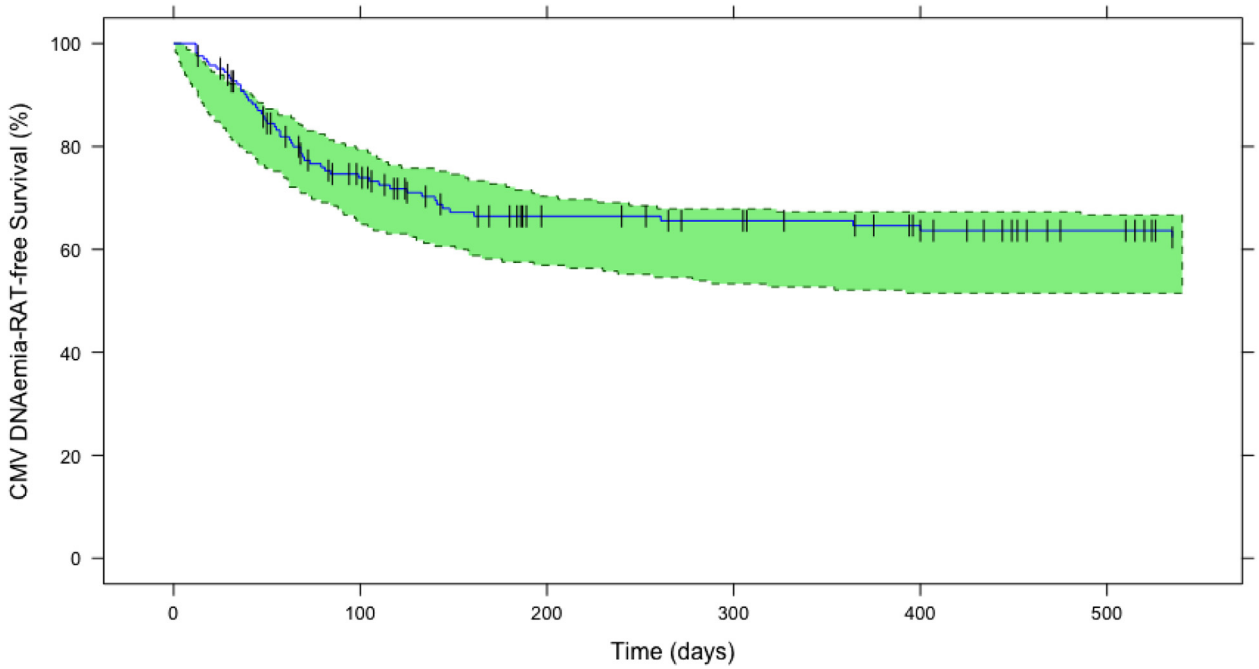

Figure 2. Kaplan-Meier visual predictive check plot. The blue line represents observed CMV DNAemia-RAT-free survival data, and the green area represents the 95% CI of 200 simulated CMV DNAemia-RAT-free survival curves based on the final model.

Table 3
Final Model Parameter Estimates and Bootstrap Results

Parameter	Value	RSE, %	Bootstrap Results	
			Median	95% CI (Percentile)
λ_0	.006	31	.006	.003-.011
β	-.009	19	-.009	(-.013)-(-.006)
θ_{ATG}	.793	39	.805	.110-1.418
θ_{SIRO}	-.063	50	-.067	(-.135)-(-.001)

RSE indicates relative standard error; λ_0, baseline hazard; β, coefficient from Gompertz function; θ_{ATG}, regression coefficient for ATG; θ_{SIRO}, regression coefficient for sirolimus concentration effect.

DISCUSSION

This study found a 2-year incidence of CMV DNAemia necessitating preemptive therapy of 36% in a multicenter cohort of RIC-allo-HSCT recipients at very high risk of CMV infection (73% of URDs and 31% of CMV-seronegative donors) given sirolimus-based GVHD prophylaxis. By means of a fully parametric survival data analysis using a detailed drug concentration dataset, we found evidence of a significant inverse linear relationship between sirolimus concentration over time and the risk of developing CMV DNAemia necessitating preemptive therapy, even when targeting a narrow therapeutic range (5 to 12 ng/mL). We also identified ATG use as a risk factor, associated with a 2-fold increase in the baseline risk of CMV DNAemia necessitating preemptive therapy.

We previously observed that higher sirolimus concentrations had an inhibitory effect on CMV replication by reducing the probability of detectable CMV DNAemia at any level after RIC-allo-HSCT [11]. The present study confirms that higher sirolimus exposure also translates into a clinical benefit by reducing the probability of preemptive anti-CMV therapy in a larger multicenter cohort of RIC-allo-HSCT recipients. Our findings are supported by previous in vitro models demonstrating the role of mechanistic target of rapamycin signaling pathways in CMV protein synthesis [24-29] and the potent inhibition on CMV replication exerted by sirolimus [24,27] in an inverse concentration-dependent manner [27].

The fact that sirolimus exposure could be a key element for anti-CMV activity merits several considerations. First, it may partially help to explain the inconsistencies in the anti-CMV effect observed between OT and allo-HSCT studies [1-9]. In the allo-HSCT setting, sirolimus exposure is lower in terms of targeted blood concentrations and duration. Second, in the context of narrow therapeutic levels, we observed that variability of sirolimus exposure among centers, among diseases (Figure 1), and likely among studies could be common. This fact may have distorted the ability to perceive an anti-CMV effect of sirolimus in allo-HSCT studies [5-9]. In this regard, exposure-response analyses emerge as a valuable tool that could deal with discrepant observations analyzing the effect of exposure variability on the outcome. Variability in exposure could result in different anti-CMV activity patterns, as has occurred with other anti-CMV drugs [30-32]. Third, our data provide a rationale for exploring higher sirolimus targeted levels, similar to those used in OT. Although sirolimus blood concentrations in allo-HSCT should be increased with caution due to increased safety risks, knowledge from OT prospective studies comparing calcineurin inhibitors and sirolimus have shown comparable safety profiles with targeted levels of 15 to 20 ng/mL [33]. To explore the safety of greater sirolimus exposure with regard to the development of TAM, in the present study we also performed an exploratory nonlinear mixed-effects analysis of the impact of sirolimus and tacrolimus concentrations on the incidence of TAM. This exploratory analysis has not found a statistically significant relationship between sirolimus and tacrolimus concentrations and the incidence of overall and proven TAM (data not shown). However, adverse events are common and should be managed with increased awareness and close monitoring of trough blood concentrations [34]. The lack of prospective PK/PD efficacy and safety studies comparing different targeted doses of sirolimus in terms of GVHD prophylaxis and CMV infection limits the potential to optimize its use in the allo-HSCT setting.

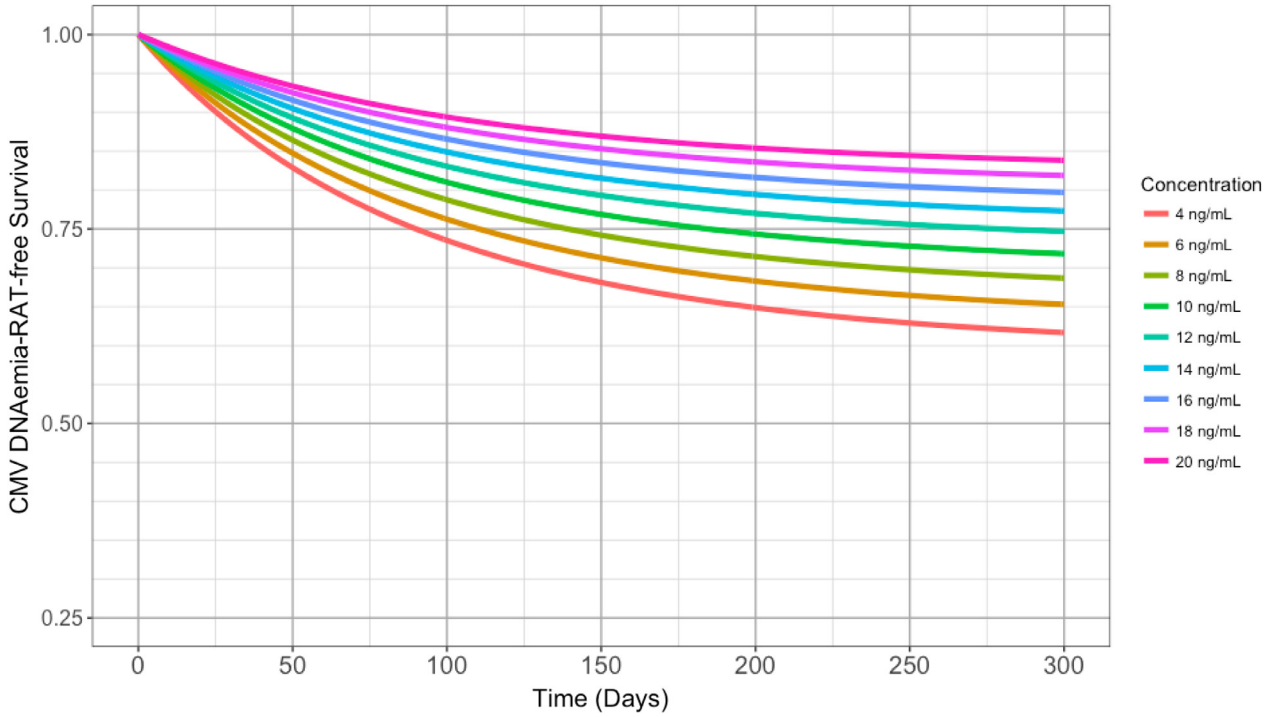

Figure 3. Simulated CMV DNAemia-RAT-free survival curves at various sirolimus exposure levels. Simulations were based on patients who did not receive an ATG-based conditioning regimen and were exposed to sirolimus trough concentrations ranging between 4 and 20 ng/mL. The mixed-effects model was applied across the data as a whole. We also performed a subanalysis for each individual center that confirmed the findings of the primary analysis.

Another relevant issue that arises from our study is the discrepant results observed in the 2 multivariate statistical models. Although ATG use as part of a conditioning regimen was consistently identified as a risk factor for CMV DNAemia necessitating preemptive therapy in both statistical methods (parametric and semiparametric), lymphoid malignancies and transplantation center were identified as risk factors only in the multivariate Cox regression model. ATG is a strong and well-known risk factor for CMV reactivation because it produces a significant delay in T cell immune reconstitution [35,36]. Thus, it is not surprising that regardless of sirolimus exposure, ATG had a strong effect on the development of CMV DNAemia necessitating preemptive therapy. In contrast, lymphoid malignancies and transplantation center showed different sirolimus exposure (Figure 1). These data suggest that, irrespective of the preemptive anti-CMV therapy strategy and transplantation characteristics, sirolimus exposure may outweigh pretransplantation conditions that could have a marginal effect on CMV DNAemia necessitating preemptive therapy in conventional analyses. Although there are no well-defined cutoff and range of sirolimus levels in terms of anti-CMV activity, we previously reported in a sensitivity analysis that sirolimus blood concentration >8 ng/mL significantly reduced the risk of CMV DNAemia, whereas blood concentration <8 ng/mL were not significantly protective [11]. Interestingly, at HSCSP, the median trough blood concentration of sirolimus was ≥8 ng/mL during most of the study period (13 out of 20 weeks; 65%). In contrast, sirolimus blood concentrations were less frequently ≥8 ng/mL in the other 2 centers (HCUV, 7 out of 20 weeks [35%]; HVH, 5 out of 20 weeks [25%]).

A recent in vitro cell culture model also showed that rapamycin at concentration of 8 nM inhibited CMV replication by up to 60%. Remarkably, a much greater inhibition (>90% reduction) was observed when combined with maribavir, making this combination appealing for CMV prophylaxis [37]. The optimization of sirolimus exposure (setting the lower limit of the therapeutic range at 8 ng/mL) could facilitate clinical research on CMV prophylaxis by exploring potential synergisms between sirolimus and the novel anti-CMV drugs in allo-HSCT.

Finally, we acknowledge that our study has several limitations, including its retrospective nature, the use of different PCR tests among centers (all of which were calibrated to the first World Health Organization international standard), differences in CMV DNA loads prompting the initiation of preemptive anti-CMV therapy (although they all used relatively high CMV DNA level cutoffs triggering antiviral inception) and different laboratory methods to estimate sirolimus levels. Nonetheless, this study has some strengths that merit consideration. First, our findings confirm, in a larger and multicenter cohort, our previous observation in a smaller and homogenous (in terms of PCR test and preemptive anti-CMV therapy strategy) series of RIC-allo-HSCT, that sirolimus exposure matters to perceive an anti-CMV effect. In addition, the implementation of fully parametric PK/PD analyses with a large dataset and the homogenous GVHD prophylaxis offer certain advantages, such as more efficient parameter estimates compared with the Cox regression model [38].

In conclusion, our pharmacometric analysis supports the idea that sirolimus may have a clinically relevant exposure-dependent anti-CMV effect in the allo-HSCT setting. The identification of sirolimus concentration as a key element of anti-CMV activity should facilitate further prospective PK/PD studies exploring the value of increasing sirolimus blood concentration levels in allo-HSCT.

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