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MAJOR ARTICLE

Cellular Immunity to Predict the Risk of Cytomegalovirus Infection in Kidney Transplantation: A Prospective, Interventional, Multicenter Clinical Trial

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Background. Improving cytomegalovirus (CMV) immune-risk stratification in kidney transplantation is highly needed to establish guided preventive strategies.

Methods. This prospective, interventional, multicenter clinical trial assessed the value of monitoring pretransplant CMV-specific cell-mediated immunity (CMI) using an interferon-γ release assay to predict CMV infection in kidney transplantation. One hundred sixty donor/recipient CMV-seropositive (D^+/R^+) patients, stratified by their baseline CMV (immediate-early protein 1)–specific CMI risk, were randomized to receive either preemptive or 3-month antiviral prophylaxis. Also, 15-day posttransplant CMI risk stratification and CMI specific to the 65 kDa phosphoprotein (pp65) CMV antigen were investigated. Immunosuppression consisted of basiliximab, tacrolimus, mycophenolate mofetil, and corticosteroids in 80% of patients, whereas 20% received thymoglobulin induction therapy.

Results. Patients at high risk for CMV based on pretransplant CMI developed significantly higher CMV infection rates than those deemed to be at low risk with both preemptive (73.3% vs 44.4%; odds ratio [OR], 3.44 [95% confidence interval {CI}, 1.30–9.08]) and prophylaxis (33.3% vs 4.1%; OR, 11.75 [95% CI, 2.31–59.71]) approaches. The predictive capacity for CMV-specific CMI was only found in basiliximab-treated patients for both preemptive and prophylaxis therapy. Fifteen-day CMI risk stratification better predicted CMV infection (81.3% vs 9.1%; OR, 43.33 [95% CI, 7.89–237.96]).

Conclusions. Pretransplant CMV-specific CMI identifies D+/R+ kidney recipients at high risk of developing CMV infection if not receiving T-cell–depleting antibodies. Monitoring CMV-specific CMI soon after transplantation further defines the CMV infection prediction risk. Monitoring CMV-specific CMI may guide decision making regarding the type of CMV preventive strategy in kidney transplantation.

Clinical Trials Registration. NCT02550639.

Keywords. cytomegalovirus; kidney transplantation; immune monitoring; cell-mediated immunity.

Cytomegalovirus (CMV) infection still remains one of the most common opportunistic infections occurring after transplantation [1], negatively challenging both patient and allograft survival [2, 3]. Despite the significant improvement made in the last decades refining the transplant risk assessment of CMV infection, fundamentally based on the donor (D)/recipient (R)–pair CMV (immunoglobulin G [IgG]) serostatus and the implementation of preventive strategies such as a systematic monitoring of viral replication in peripheral blood (preemptive) or using universal antiviral therapy (prophylaxis), an important number of patients will unpredictably develop CMV infection. An important body of evidence has shown the key role of CMV-specific cell-mediated immunity (CMI) controlling and abrogating CMV viral replication [4–7], thus suggesting that its functional assessment could help identifying at-risk patients of developing CMV infection beyond CMV serostatus. A number of sensitive immune assays measuring CMV-specific CMI have been developed in the last years to interrogate the cellular immune risk of transplant patients to develop CMV infection [8]. Notably, though serologically positive recipients (R^+) are considered to be protected against CMV, a significant number of patients may display weak or even absence of CMV-specific CMI, particularly against the immediate-early protein 1 (IE-1)

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CMV antigen, and have been shown to be at higher risk of CMV infection [9–11].

To validate these previous data, we designed the first prospective, multicenter, interventional clinical trial in which D^+/R^+ kidney transplant patients were stratified according to their pretransplant CMV (IE-1)–specific CMI risk by detecting individual interferon gamma (IFN-γ)–producing cells with a peptide-based enzyme-linked immunosorbent spot (ELISPOT) assay (T-SPOT.CMV), and subsequently randomized to receive either preemptive or 3-month antiviral prophylactic therapy. The main objective of this trial was to demonstrate whether pretransplant assessment of CMV (IE-1)–specific CMI would identify transplant patients at risk of developing CMV infection treated according to the 2 main preventive CMV strategies. Also, 15-day posttransplant CMV-specific CMI and the value of monitoring CMI against the 65 kDa phosphoprotein (pp65) CMV antigen were investigated.

METHODS

Study Design

We carried out a 12-month prospective, multicenter, observational study with an embedded randomized intervention according to antiviral preventive strategy, either preemptive or prophylaxis therapy, in CMV (IgG)–seropositive kidney transplant recipients (R^+) of a seropositive kidney donor (D^+) stratified according to their pretransplant CMV (IE-1)–specific CMI risk (Figure 1). The study was double-blinded with regard to the CMV-specific CMI and open-labeled regarding the type of preventive therapy. According to pretransplant CMI specific to the IE-1 CMV antigen, patients were allocated in 2 groups, group A (low risk) and group B (high risk), and subsequently randomized in a 1:1 ratio to receive either 3-month antiviral prophylaxis (subgroups A1 and B1) or preemptive therapy (subgroups A2 and B2). Additional information about patient eligibility criteria, randomization, and masking procedures is provided in the Supplementary Methods. The study was conducted in compliance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by all respective institutional review boards (AC148/13). All patients provided written informed consent and could withdraw from the study at any time.

Main Endpoints of the Study

The primary endpoint was to evaluate the incidence of CMV infection in patients following preemptive therapy with a pretransplant high-risk CMV (IE-1)–specific CMI (group B2) as compared to those with low-risk CMV (IE-1)–specific CMI (group A2).

As secondary endpoints, the rates of CMV infection requiring antiviral treatment, CMV disease, and late-onset CMV infection after prophylaxis withdrawal; the impact of CMV-specific CMI according to the type of induction therapy; the influence of CMI against the pp65 CMV antigen; and the prediction-risk accuracy of CMV-specific CMI at 15 days posttransplantation were assessed in this study.

Clinical Definitions of CMV Infection

The definition of CMV infection and disease was based on the criteria recommended by the CMV Drug Development Forum [12]. CMV infection was defined as the detection of CMV DNA replication in whole blood or plasma. CMV disease was defined as evidence of CMV DNA replication with compatible symptoms, including both viral syndrome and invasive tissue disease. CMV infection requiring antiviral treatment was defined in this study as the presence of CMV disease or CMV DNA copies >4000 IU/mL in plasma or 10 000 IU/mL in whole blood. These cutoffs were agreed among all investigators and considered as clinically meaningful to initiate antiviral therapy in absence of any symptoms.

CMV Preventive Strategies, CMV Serology, and Microbiological Studies

Detailed information about the type of CMV preventive strategies used, CMV serology, and microbiological studies is provided in the Supplementary Methods.

Immunosuppression

Chronic immunosuppression was homogeneous and based on induction therapy with basiliximab in 80% of patients, whereas 20% received rabbit antithymocyte globulin (rATG) and were stratified to be equally allocated among groups. Maintenance immunosuppression was based on tacrolimus, mycophenolate mofetil, and prednisone (additional information is described in the Supplementary Methods).

Assessment of CMV-Specific Cell-mediated Immunity

The T-SPOT.CMV test (Oxford Immunotec Ltd, Oxford, United Kingdom), was used to assess the CMI against 2 major immunogenic CMV antigens (IE-1 and pp65) using overlapping peptide pools covering the whole antigen length at baseline (n = 160) and at 2 weeks (n = 137) posttransplantation. In accordance with previous works [9, 10], only pretransplant T-SPOT.CMV response against the IE-1 antigen was used to stratify patients into the 2 different risk groups (cutoff of 20 IFN-γ spots/3×10^5 peripheral blood mononuclear cells [PBMCs]). Specific information about the T-SPOT.CMV methodology is provided in the Supplementary Methods.

Main conceptual definitions of CMV monitoring assays are depicted in the Supplementary Methods.

Sample Size and Statistical Analysis

For the primary analysis and according to previous reports [9, 10, 13, 14], assuming a 10% rate of infection in the low-risk group (A2) and a 35% rate in the high-risk group (B2) and a 65:35 allocation ratio between the 2 groups, to achieve

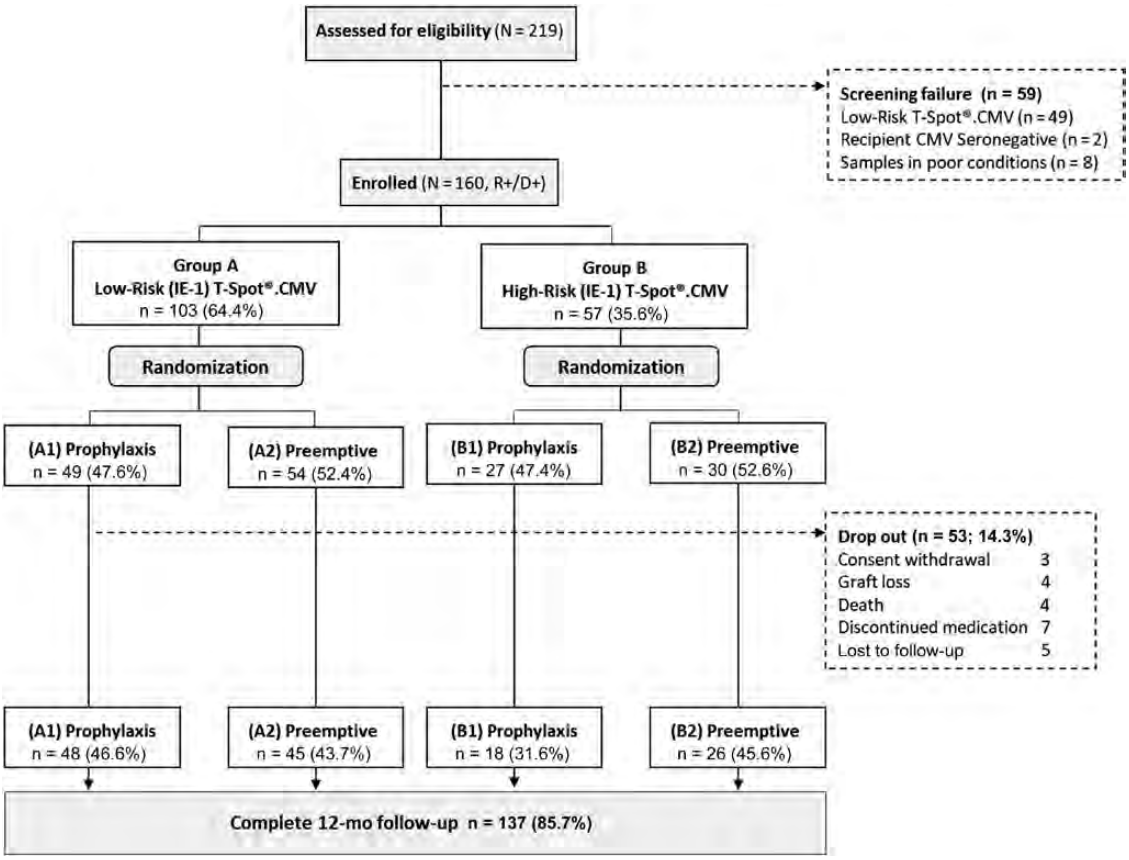

Figure 1. Flowchart of the study. Between 10 June 2014 and 10 September 2017, 219 patients from 5 different transplant centers were screened and 160 underwent randomization. Of the total 219 patients, 59 failed screening; among them, 8 were excluded because of poor blood sample conditions and 2 because of baseline cytomegalovirus (CMV) immunoglobulin G–seronegative status, and 49 patients with low-risk CMV (immediate-early protein 1 [IE-1])–specific cell-mediated immunity (CMI) were excluded as this study arm (group A) was already completed. The prevalence of high-risk and low-risk CMV (IE-1) CMI among the recipient-seropositive (R^+) study population in whom the pretransplant T-SPOT.CMV assay was performed was 27.3% (57/209) and 72.7% (152/209), respectively. Twenty-three (14.3%) patients discontinued the study: 10 of 103 (9.7%) in the low-risk group (1 [0.9%] in the prophylaxis A1 arm and 9 [8.7%] in the preemptive A2 strategy), and 13 of 57 (22.8%) within the high-risk CMI group (9 [15.8%] in the prophylaxis B1 arm and 4 [7%] in the preemptive B2 therapy). Thus, a total of 137 (85.7%) patients completed 12-month follow-up. Abbreviation: CMV, cytomegalovirus; IE-1, immediate-early protein 1; mo, month.

a significant difference between the 2 groups at the 5% significance level and with 80% power would require 52 subjects in the low-risk group and 28 in the high-risk group. This is based further on a prevalence of high-risk CMV (IE-1) CMI at baseline among D^+/R^+ patients and a 83% sensitivity and 65% specificity of the CMV (IE-1)–specific ELISPOT test and a 10% withdrawal rate. A total of 160 subjects were therefore planned for recruitment.

All analyses, unless otherwise specified, were performed on an intention-to-treat basis, and statistical significance was assessed at the 5% level. Receiver operating characteristic (ROC) curve analysis was done to evaluate the optimum cutoffs predicting CMV infection.

For the primary efficacy analysis (high-risk vs low-risk incidence of CMV infection on preemptive therapy), the 2 groups were compared using a 1-sided χ^2 test. The odds ratio (OR) for infection was also calculated and presented with the associated 95% confidence interval (CI). The same analysis was performed for other comparisons of overall risk. The risk of CMV infection over the study period was analyzed using Cox proportional hazards model, and Kaplan-Meier curves of the infection rate over time were produced. The mean time to infection with the associated error was reported, and the hazard ratio calculated with the associated 95% CI. Analyses were performed using SPSS version 23 software, and graphs were generated using GraphPad Prism version 6.0 software (GraphPad Software, San Diego, California).

RESULTS

Study Patients and Main Clinical Outcomes

The study groups were comparable regarding main baseline demographic, clinical, and immunological characteristics (Table 1). The incidence of CMV infection and disease during the 12-month duration of the study was 57 of 160 (35.6%) and 9 of 160 (5.6%), respectively. Four of 9 (44.4%) developed a CMV flulike syndrome, 4 of 9 (44.4%) enteritis, and 1 of 8 (12.5%)

Table 1. Clinical, Demographic, and Immunological Baseline Characteristics (Intent-to-Treat Population)

Characteristic	Group A: Low-Risk (IE-1) T-Spot.CMV (n = 103 [64.4%])			Group B: High-Risk (IE-1) T-Spot.CMV (n = 57 [35.6%])			
	A1: Prophylaxis (n = 49 [47.6%])	A2: Preemptive (n = 54 [52.4%])	*P* Value[a]	B1: Prophylaxis (n = 27 [47.4%])	B2: Preemptive (n = 30 [52.6%])	*P* Value[b]	*P* Value[c]
Clinical and demographic variables							
Donor age, y	59.59 ± 11.80	60.17 ± 14.73	.828	58.52 ± 14.0	64.77 ± 16.55	.149	.437
Recipient age, y	57.43 ± 12.29	58.23 ± 12.61	.746	57.87 ± 12.84	61.08 ± 14.06	.386	.433
Female sex	12 (24.5)	19 (35.2)	.237	13 (48.1)	10 (33.3)	.255	.225
Cause of end-stage renal disease			.383			.545	.165
Glomerulonephritis	9 (18.4)	9 (16.7)		5 (18.5)	2 (6.7)		
Vascular	3 (6.1)	10 (18.5)		3 (11.1)	1 (3.3)		
Diabetes mellitus	6 (12.2)	5 (9.3)		3 (11.1)	4 (13.3)		
Chronic interstitial nephropathy	2 (4.1)	3 (5.6)		4 (14.8)	5 (16.7)		
Polycystic kidney disease	13 (26.5)	8 (14.8)		4 (14.8)	4 (13.3)		
Unknown	16 (32.7)	19 (35.2)		8 (29.6)	14 (46.7)		
Type of donor (deceased)	43 (87.8)	48 (88.9)	.858	22 (81.5)	26 (86.7)	.592	.432
Type of transplant (DBD)	31 (63.3)	41 (75.9)	.162	17 (63)	16 (53.3)	.462	.195
Induction therapy: basiliximab/rATG	39 (79.6)/10 (20.4)	43 (79.6)/11 (20.4)	.996	23 (85.2)/4 (14.8)	23 (76.7)/7 (23.3)	.416	.869
DGF (yes)	16 (32.7)	14 (25.9)	.453	4 (14.8)	7 (23.3)	.416	.235
BPAR (yes)							
TCMR/ABMR	2 (4.1)/0 (0)	4 (7.4)/1 (1.9)	.297	0 (0)/0 (0)	0 (0)/0 (0)	NA	**.044**
Other infections							
Viral	3 (6.1)	3 (5.6)	.902	3 (11.1)	2 (6.7)	.554	.481
Bacterial	25 (51)	23 (42.6)	.392	11 (40.7)	13 (43.3)	.843	.584
Fungal	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA	NA
Kidney graft function (Scr, µmol/L)							
6 mo	142.08 ± 36.84	137.75 ± 34.24	.570	126.81 ± 27.17	139.53 ± 86.38	.575	.517
12 mo	143.79 ± 46.32	135.78 ± 35.61	.388	126.75 ± 28.59	138.42 ± 64.37	.531	.463
Graft loss (yes)	0 (0)	3 (5.6)	.094	0 (0)	1 (3.3)	.339	.653
Patient death (yes)	0 (0)	2 (3.7)	.174	2 (7.4)	0 (0)	.129	.543
Baseline immunological variables							
Calculated PRA, %	0.18 ± 1.29	2.02 ± 10.39	.204	0 ± 0	0 ± 0	NA	.129
No. of HLA mismatches							
Class I mismatch	2.94 ± 1.03	2.80 ± 1.01	.482	2.70 ± 0.77	3.07 ± 0.74	.076	.844
Class II mismatch	0.92 ± 0.61	1.04 ± 0.51	.285	1.11 ± 0.64	1.23 ± 0.68	.489	.062
Mean CMV-sp IgG titers, IU/mL	255.54 ± 255.67	208.73 ± 86.09	.348	220.03 ± 94.61	209.49 ± 95.90	.740	.812
Mean T-lymphocytes							
10^9 cells/L	1.64 ± 0.81	1.62 ± 1.27	.884	1.57 ± 0.71	1.61 ± 0.67	.844	.790
% over total leukocytes	22.53 ± 10.22	21.08 ± 9.81	.465	21.72 ± 9.42	20.61 ± 8.05	.633	.690
Mean CMV-specific CMI (IFN-γ spots/3 × 10^5 PBMCs)							
IE-1 CMV antigen	362.63 ± 287.20	439.24 ± 334.78	.218	9.85 ± 6.26	7.83 ± 6.37	.234	**< .001**
pp65 CMV antigen	444.87 ± 269.68	526.89 ± 281.72	.135	269.13 ± 195.5	158.47 ± 190.55	.095	**< .001**

Data are presented as no. (%) or mean ± standard deviation otherwise indicated. *P* values in bold indicate $P < .05$.

Abbreviations: ABMR, antibody-mediated rejection; BPAR, biopsy-proven acute rejection; CMI, cell-mediated immunity; CMV, cytomegalovirus; DBD, donor after brain death; DGF, delayed graft function; HLA, human leukocyte antigen; IE-1, immediate-early protein 1; IFN-γ, interferon gamma; IgG, immunoglobulin G; NA, not applicable; pp65, 65 kDa phosphoprotein; PBMC, peripheral blood mononuclear cell; PRA, panel reactive antibody; rATG, rabbit antithymocyte globulin; Scr, serum creatinine; TCMR, T-cell–mediated rejection.

[a] *P* value a between the A1 and A2 groups.

[b] *P* value b between the B1 and B2 groups.

[c] *P* value c between CMV CMI groups (A vs B).

Table 2. Efficacy Endpoints at Month 12 (Intent-to-Treat Population)

	Preemptive Therapy						Prophylaxis Therapy					
Endpoints	High-Risk CMI	Low-Risk CMI	Difference, %	OR	(95% CI)	*P* Value	High-Risk CMI	Low-Risk CMI	Difference, %	OR	(95% CI)	*P* Value
Pretransplant CMV (IE-1)–specific CMI (20 IFN-γ spots/3 × 10^5 PBMCs)												
Primary endpoint												
All patients (n = 160)	n = 30	n = 54					n = 27	n = 49				
CMV infection	22 (73.3)	24 (44.4)	28.9	3.44	(1.30–9.08)	**.013**	9 (33.3)	2 (4.1)	29.2	11.75	(2.31–59.71)	**.003**
CMV infection requiring treatment	16 (53.3)	10 (18.5)	34.8	5.03	(1.86–13.57)	**.001**	5 (18.5)	2 (4.1)	14.4	5.34	(.96–29.71)	.056
CMV disease	6 (20)	2 (3.7)	16.3	6.50	(1.22–34.59)	**.028**	1 (3.7)	0 (0)	3.7	NA	NA	NA
Secondary endpoints												
Basiliximab induction (n = 128)	n = 23	n = 43					n = 23	n = 39				
CMV infection	17 (73.9)	16 (37.2)	36.7	4.78	(1.56–14.62)	**.006**	8 (34.8)	2 (5.1)	29.7	9.87	(1.87–51.97)	**.007**
CMV infection requiring treatment	13 (56.5)	7 (16.3)	40.2	6.69	(2.11–21.23)	**.001**	5 (21.7)	2 (5.1)	16.6	5.14	(.91–29.09)	.064
CMV disease	6 (26.1)	2 (4.7)	21.4	7.24	(1.33–39.49)	**.022**	1 (4.3)	0 (0)	4.3	NA	NA	NA
rATG induction (n = 32)	n = 7	n = 11					n = 4	n = 10				
CMV infection	5 (71.4)	8 (72.7)	−1.3	0.94	(.11–7.73)	.952	1 (25)	0 (0)	25	NA	NA	NA
CMV infection requiring treatment	3 (42.9)	3 (27.3)	15.6	2.00	(.27–14.78)	.497	0 (0)	0 (0)	0	NA	NA	NA
CMV disease	0 (0)	0 (0)	0	NA	NA	NA	0 (0)	0 (0)	0	NA	NA	NA
15-day posttransplant CMV (IE-1)–specific CMI (40 IFN-γ spots/3 × 10^5 PBMCs)												
Basiliximab induction (n = 108)	n = 32	n = 22					n = 32	n = 22				
CMV infection	26 (81.3)	2 (9.1)	72.2	43.33	(7.89–237.96)	**< .001**	7 (21.9)	0 (0)	21.9	NA	NA	NA
CMV infection requiring treatment	17 (53.1)	1 (4.5)	48.6	23.80	(2.85–198.85)	**.003**	7 (21.9)	0 (0)	21.9	NA	NA	NA
CMV disease	7 (21.9)	0 (0)	21.9	NA	NA	NA	1 (3.1)	0 (0)	3.1	NA	NA	NA

Data are presented as no. (%) unless otherwise indicated. *P* values are for binary logistic regression. *P* values in bold indicate $P < .05$.

Abbreviations: CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; IE-1, immediate-early protein 1; IFN-γ, interferon gamma; NA, not applicable; OR, odds ratio; PBMC, peripheral blood mononuclear cell; rATG, rabbit antithymocyte globulin.

pneumonitis. CMV infection rates according to each antiviral preventive therapy are depicted in Supplementary Table 1. No major differences were observed between patients developing CMV infection or disease and those who did not, but patients developing CMV infection displayed higher human leukocyte antigen (HLA) class I and II antigen mismatches. Mean time to CMV infection, mean time to disease, and mean time of infection duration in preemptively treated patients were 1.56 ± 0.8 months, 2.84 ± 1.7 months, and 0.84 ± 0.63 months, respectively, compared with 3.87 ± 1.76 months, 3.5 months, and 0.87 ± 0.74 months in patients receiving prophylaxis. Thirty-three (20.6%) patients required antiviral therapy initiation due to either CMV disease (9/33 [27.3%]) or as prespecified per protocol due to CMV DNA >4000 IU/mL in plasma (20/33 [60.6%]) or >10 000 IU/mL in whole blood (4/33 [12.1%]).

Primary Endpoint of the Study

Pretransplant high-risk CMV-specific CMI patients on preemptive therapy (group B2) showed significantly higher incidence of CMV infection than low-risk CMI patients (group A2) (22/30 [73.3%] vs 24/54 [44.4%], respectively; OR, 3.44 [95% CI, 1.30–9.08]). Likewise, the incidences of CMV infection requiring treatment and CMV disease were significantly higher within high-risk than low-risk CMI patients (16/30 [53.3%] vs 10/54 [18.5%], respectively, OR, 5.03 [95% CI, 1.86–13.57] for CMV infection requiring treatment; and 6/30 [20%] vs 2/54 [3.7%], respectively, OR, 6.50 [95% CI, 1.22–34.59] for CMV disease) (Table 2). As illustrated in Figure 2, the cumulative incidences of CMV infection, CMV infection requiring treatment and CMV disease were significantly higher among high-risk CMI than in low-risk CMI patients.

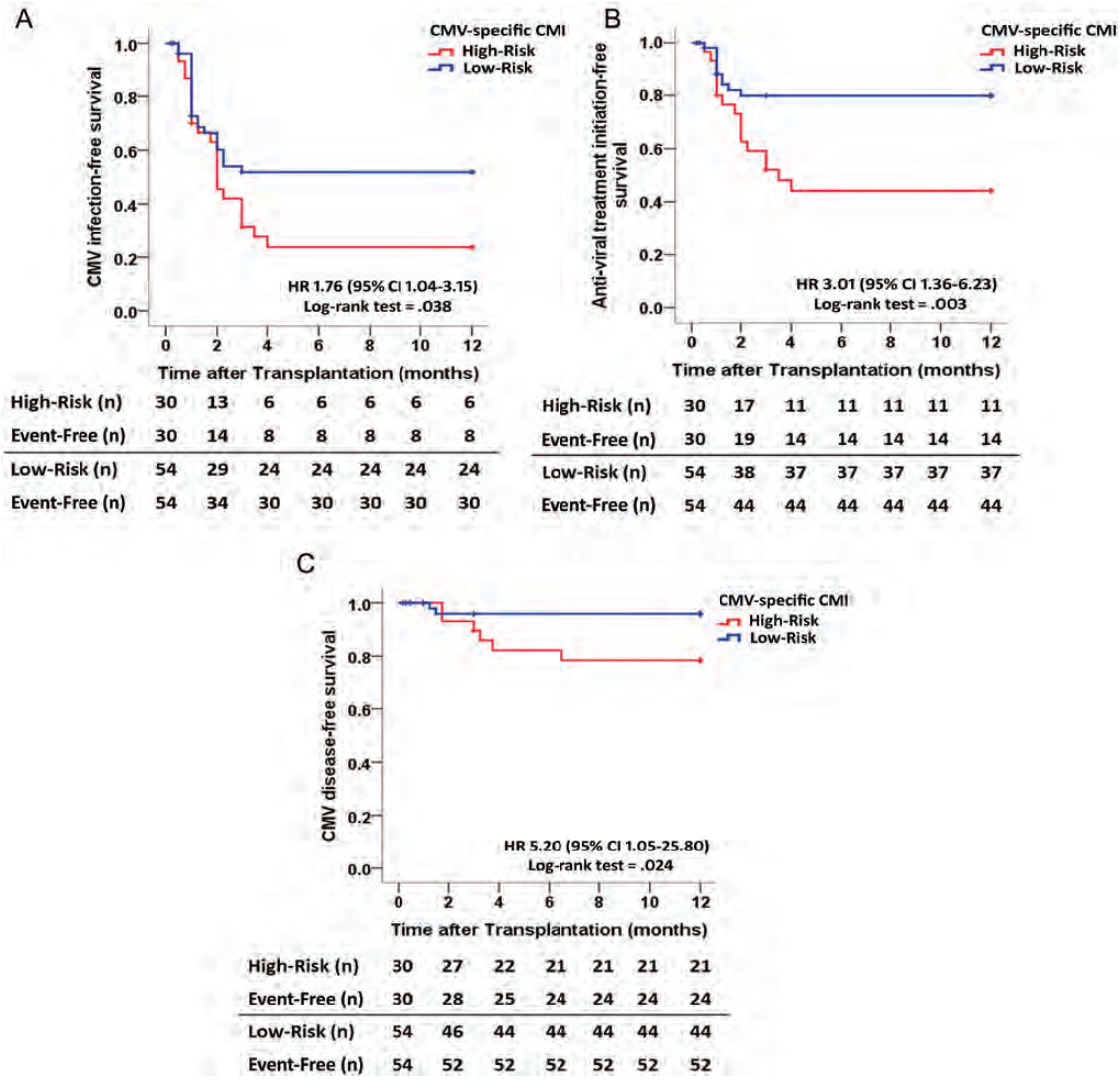

Figure 2. Cytomegalovirus (CMV) infection rates between pretransplant high-risk and low-risk CMV (immediate-early protein 1)–specific cell-mediated immunity in all patients following preemptive therapy. Kaplan-Meier curves for CMV infection-free survival analysis (*A*), CMV infection requiring antiviral treatment-free survival analysis (*B*), and CMV disease-free survival analysis (*C*). Abbreviations: CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; HR, hazard ratio.

Secondary Endpoints of the Study

Effect of Pretransplant CMV (IE-1)–Specific CMI According to Different Induction Therapies in Patients Following Preemptive Therapy

Among patients receiving basiliximab induction therapy, high-risk CMI patients displayed significantly higher incidence of CMV infection, CMV infection requiring treatment, and CMV disease than low-risk CMI recipients (Table 2). Likewise, the cumulative incidences of CMV infection, CMV infection requiring treatment, and CMV disease were significantly higher among high-risk CMI than low-risk CMI patients (Supplementary Figure 1).

Conversely, in rATG-treated patients, no association was observed between pretransplant CMI and incidence of CMV infection or disease.

Similar data were observed when patients on protocol were analyzed (Supplementary Table 2).

Impact of Pretransplant CMV (IE-1)–Specific CMI in Patients Receiving Antiviral Prophylaxis Therapy

Regardless of induction therapy used, pretransplant high-risk CMV-specific CMI patients on prophylaxis showed significantly higher incidence of late-onset CMV infection compared with low-risk CMI patients (9/33 [33.3%] vs 2/49 [4.1%], respectively, OR, 11.75 [95% CI, 2.31–59.71] for CMV infection; and 5/27 [18.5%] vs 2/49 [4.1%], respectively, OR, 5.34 [95% CI, .96–29.71] for CMV infection requiring treatment) (Table 2). The very low incidence of late-onset CMV disease (only 1 event among the high-risk group) precluded performing any analysis (Figure 3). Four high-risk CMI patients randomized to prophylaxis treatment (B1) followed a preemptive strategy and were dropped from the study. Three of these 4 patients developed CMV infection during the first 3 months posttransplantation.

When only basiliximab-treated patients were analyzed, high-risk CMI patients displayed higher rates of late-onset CMV infection and infection requiring treatment compared with low-risk CMI recipients (Supplementary Figure 2 and Table 2).

The very low incidence of late-onset CMV infection among rATG-treated patients did not allow further analysis in this group.

Similar results were observed when patients on protocol were evaluated (Supplementary Table 2).

Pretransplant CMV-Specific T-Cell Frequencies Predicting CMV Infection

Subsequently, we investigated the most accurate CMV-specific CMI threshold against both IE-1 and pp65 CMV antigens predicting CMV infection. Patients developing CMV infection and disease displayed significantly lower IE-1 but not pp65 IFN-γ T-cell frequencies than patients who did not, whereas patients developing CMV infection requiring antiviral treatment showed lower T-cell responses against both IE-1 and pp65 CMV antigens than those who did not (Supplementary Figure 3*A*–*C*). Stronger differences were observed when only basiliximab-treated patients were evaluated (Supplementary Figure 3*D*–*F*). Conversely, no differences were found among rATG-treated patients (Supplementary Figure 3*G* and 3*H*).

Also, no significant differences were observed regarding mean IE-1 and pp65-specific IFN-γ T-cell frequencies between patients with low CMV replication load and those with higher CMV replication requiring therapy (data not shown).

ROC curve analysis in basiliximab-treated patients confirmed 20 CMV (IE-1)–specific IFN-γ spots/3 × 10^5 PBMCs as an accurate cutoff discriminating patients at higher risk of CMV infection (area under the curve [AUC], 0.69 [95% CI, .56–.82]; $P = .007$). Conversely, pretransplant CMV (pp65)–specific IFN-γ

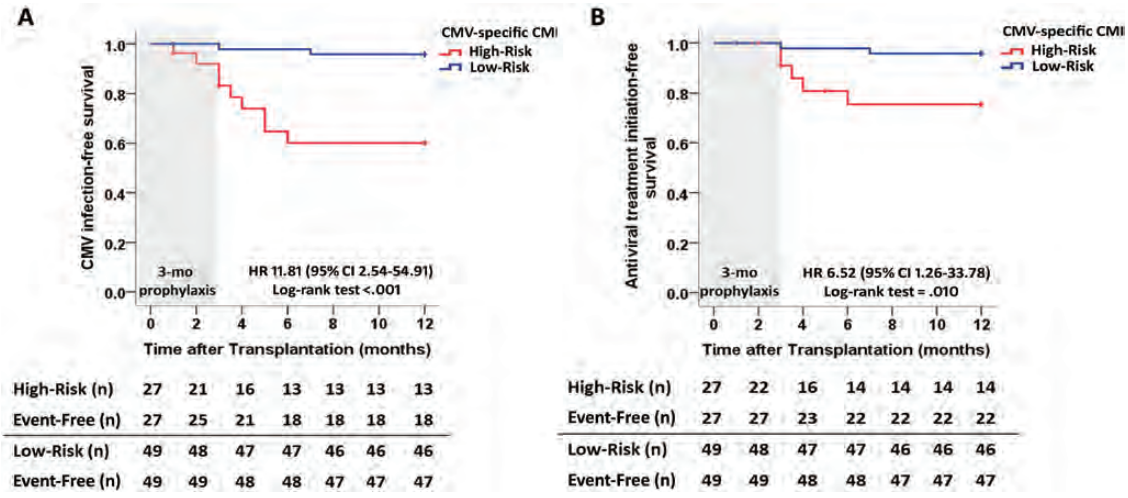

Figure 3. Late-onset cytomegalovirus (CMV) infection rates between pretransplant high-risk and low-risk CMV (immediate-early protein 1)–specific cell-mediated immunity in all patients following prophylaxis therapy. Kaplan-Meier curves for late-onset CMV infection-free survival analysis (*A*) and late-onset CMV infection requiring antiviral treatment-free survival analysis (*B*). Abbreviations: CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; HR, hazard ratio; mo, month.

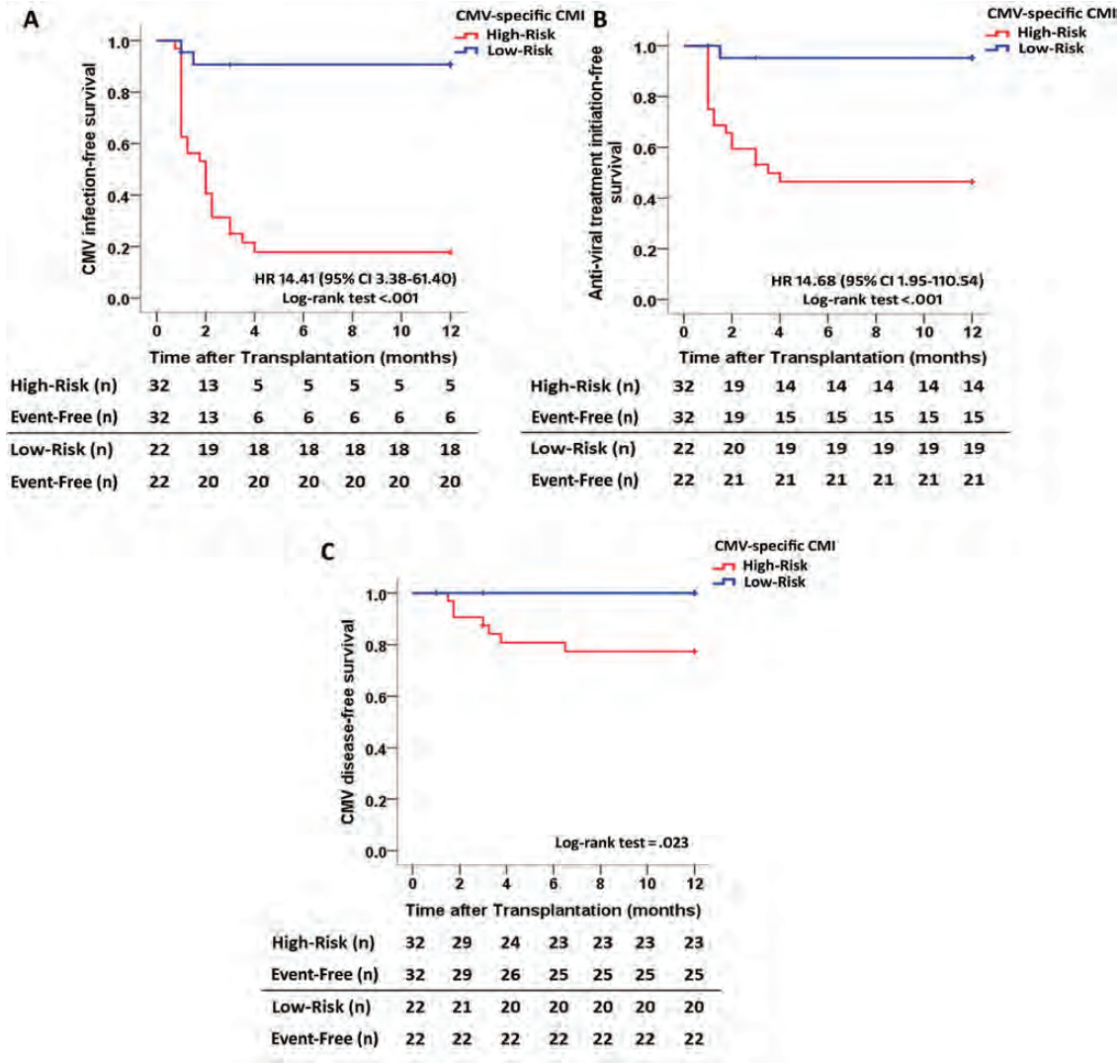

Figure 4. Cytomegalovirus (CMV) infection rates between 15-day posttransplant high-risk and low-risk CMV (immediate-early protein 1)–specific cell-mediated immunity in basiliximab-treated patients following preemptive therapy. Kaplan-Meier curves for CMV infection-free survival analysis (*A*), CMV infection requiring antiviral treatment-free survival analysis (*B*), and CMV disease-free survival analysis (*C*). Abbreviations: CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; HR, hazard ratio.

T-cell frequencies showed a poorer AUC predicting CMV infection (AUC, 0.58 [95% CI, .44–.72]; $P = .276$) (Supplementary Figure 4*A*). The combination of both CMV-specific CMI did not outperform the prediction risk of CMV infection as compared to CMV (IE-1)–specific CMI (Supplementary Figure 5).

Fifteen-Day Posttransplant CMV-Specific CMI and Risk of CMV Infection

At 15 days posttransplantation, a profound abrogation of both total T-lymphocyte counts and CMV-specific CMI was observed in all rATG-treated patients. Conversely, while basiliximab induction therapy did not affect posttransplant total T-lymphocytes counts, a significant, albeit less pronounced abrogation of CMV-specific CMI was also found (Supplementary Figure 6*A*–*C*). The ROC analysis at this time point revealed 40 CMV (IE-1)–specific IFN-γ spots/3 × 10^5 PBMCs as the most accurate cutoff predicting CMV infection (AUC, 0.80 [95% CI, .67–.94]; $P < .001$) (Supplementary Figure 4*B*).

Fifteen-day posttransplant CMV (IE-1)–specific CMI in basiliximab-treated patients outperformed the CMV infection prediction risk of pretransplant CMV-specific CMI (Table 2); indeed, high-risk CMI at 15 days after transplantation in patients on either preemptive or prophylaxis therapy predicted significantly higher risk of CMV infection, CMV infection requiring treatment, and CMV disease as compared to low-risk CMI patients (Figures 4 and 5). Importantly, 32 of 75 (42.7%) pretransplant low-risk CMI patients on basiliximab became high-risk at 15 days, and 14 of 32 (43.8%) of them developed CMV infection. Furthermore, 12 of 15 (80%) of those following preemptive therapy developed CMV infection. Notably, none of the low-risk CMI patients developed late-onset CMV infection after prophylaxis therapy.

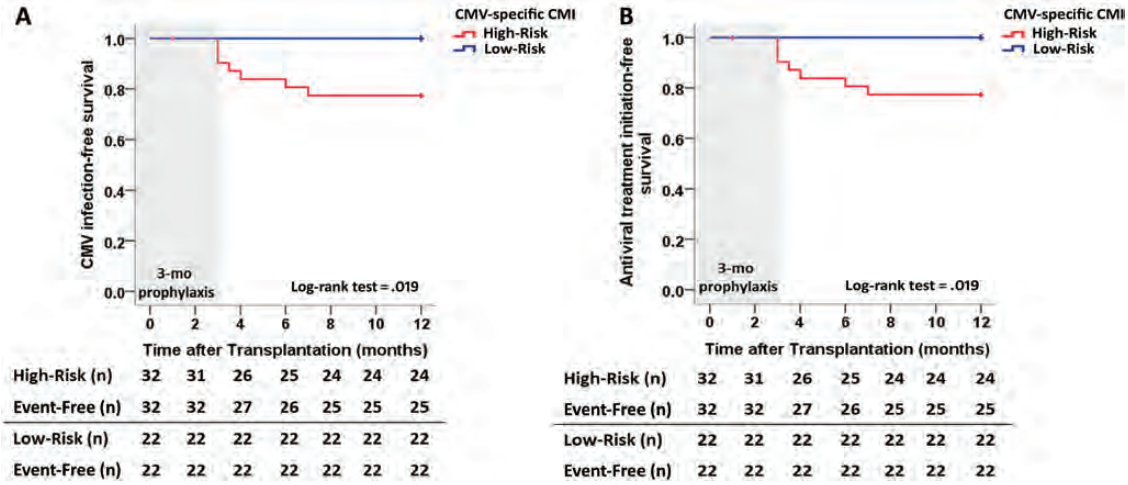

Figure 5. Cytomegalovirus (CMV) infection rates between 15-day posttransplant high-risk and low-risk (immediate-early protein 1) CMV-specific cell-mediated immunity in basiliximab-treated patients following prophylaxis therapy. Kaplan-Meier curves for CMV infection-free survival analysis (*A*) and CMV infection requiring antiviral treatment-free survival analysis (*B*). Abbreviations: CMI, cell-mediated immunity; CMV, cytomegalovirus; mo, month.

The combination of CMV-specific CMI against the 2 main CMV antigens (IE-1, pp65) did not improve the prediction risk of CMV (IE-1)–specific CMI (Supplementary Figure 7). Mean IE-1 and pp65-specific IFN-γ T-cell frequencies at 15 days were numerically lower in patients with high CMV replication load than those with low CMV replication requiring therapy (25 ± 65.8 vs 70.3 ± 175 for IE-1 and 104.7 ± 99.7 vs 185.8 ± 215.8 for pp65).

Protective Effect of the Type of Preventive Therapy According to CMV-Specific CMI

Compared to pretransplant CMV-specific CMI, 15-day posttransplant CMI risk stratification more accurately identified patients that might benefit from a universal prophylaxis vs a preemptive monitoring strategy for CMV prevention (Figure 6 and Supplementary Figures 8 and 9). Among low-risk CMI patients at 15 days on preemptive treatment, only 2 of 22 (9%) developed CMV infection, 1 of 22 (4%) developed CMV infection requiring therapy, and none developed CMV disease.

DISCUSSION

Our study shows that the implementation of the T-SPOT.CMV assay in real-time clinical practice is feasible and safe. Our data demonstrate that CMV immune protection among D^+/R^+ kidney transplantations is largely dependent on preformed CMV-specific CMI, and particularly against the IE-1 CMV antigen, although this protective immune status is dramatically hampered by the use of T-cell depletion and in some patients by basiliximab induction therapy, which may abrogate preformed CMV-specific CMI, thus increasing CMV infection risk. Notably, monitoring CMV-specific CMI at 15 days after transplantation outperformed the prediction accuracy of the test, as it captures the deleterious effect of induction therapy on preformed CMV-specific CMI.

The value of monitoring CMV-specific CMI to improve CMV risk stratification has been suggested by different studies, mainly focusing on D^+/R^- transplants or at later time points after transplantation [11, 15–23]. However, as R^+ patients may have weak CMV-specific CMI despite detectable humoral immunity [8, 9, 24, 25], we thus focused on the pretransplant setting as a clinically relevant timepoint for immune-risk stratification. Indeed, up to 27.3% of R^+ patients showed very low or no CMV-specific CMI at baseline. Importantly, these pretransplant high-risk patients showed significantly higher infection risk than low-risk CMI patients, regardless of induction therapy employed. However, the predictive capacity of the test significantly improved when patients receiving rATG induction were excluded, as T-cell depletion can only affect those patients with preformed CMV-specific CMI [26]. We also found a functional abrogation of pretransplant CMV-specific CMI in some patients receiving basiliximab, which impacted their CMI against CMV. In fact, when the T-SPOT.CMV test was performed at 15 days after transplantation, it improved its prediction risk; only 2 of 22 (9.1%) and no 15-day low-risk CMI patients receiving basiliximab and following a preemptive therapy developed CMV infection and disease, respectively, whereas up to 26 of 32 (81.3%) and 7 of 32 (21.9%) did within the high-risk group. This is in line with findings in the hematopoietic stem cell transplant population wherein dynamic changes in CMV-specific CMI between pretransplantation and 1 month better identified patients at risk of developing CMV infection [27].

The expansion capacity of recipient CMV-specific CMI, which ultimately entails a protective immune state against CMV infection, seems to be driven by D^+ kidney allografts due to optimal donor viral peptide recognition by recipient T cells [9, 28].

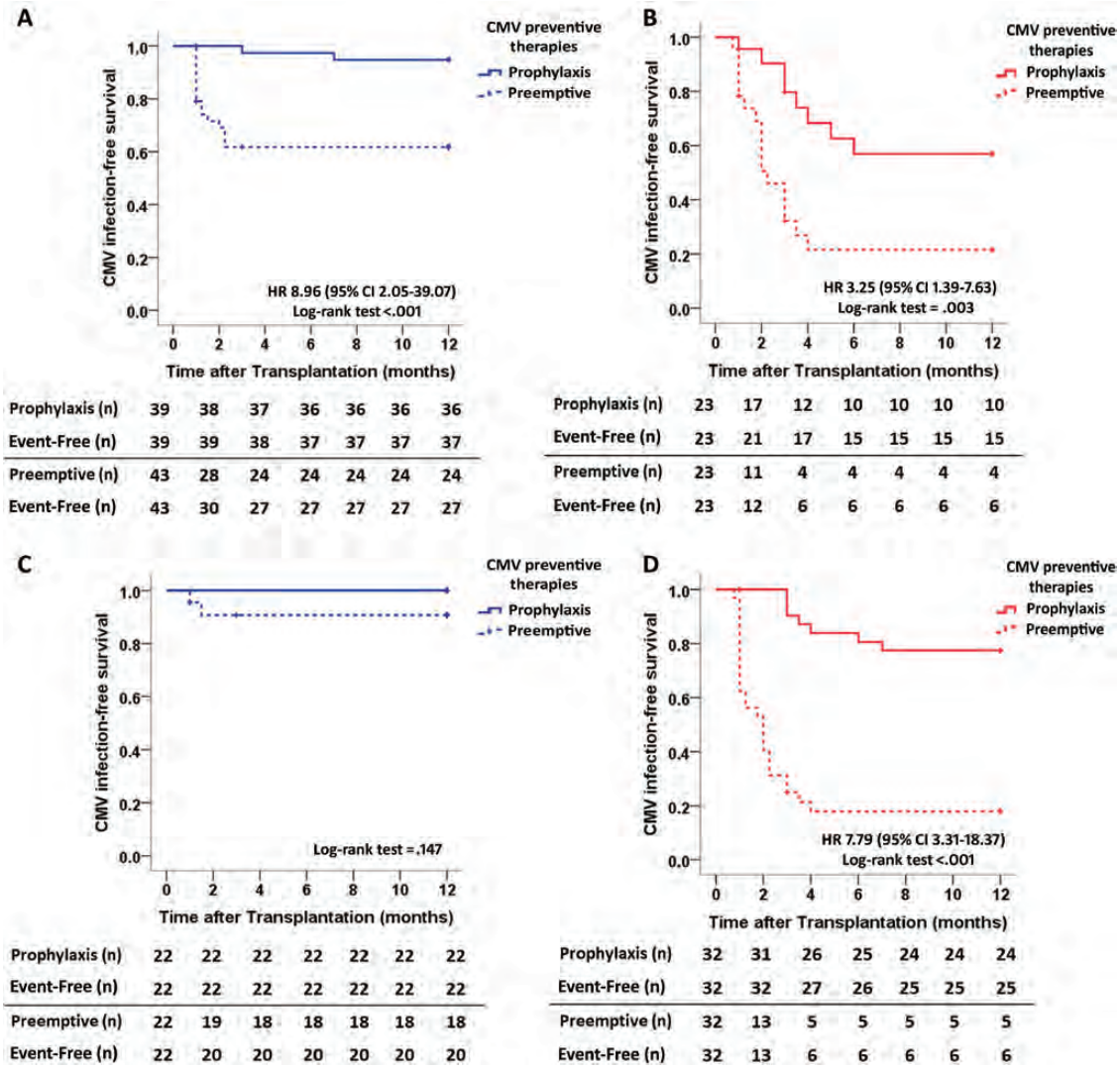

Figure 6. Cytomegalovirus (CMV) infection rates between CMV preventive strategies in basiliximab-treated patients classified as low-risk or high-risk cell-mediated immunity (CMI) according to the pretransplant or 15-day CMI test. Kaplan-Meier curves for CMV infection-free survival analysis among pretransplant low-risk CMI patients (*A*), pretransplant high-risk CMI patients (*B*), 15-day posttransplant low-risk CMI patients (*C*), and 15-day posttransplant high-risk CMI patients (*D*). Abbreviations: CI, confidence interval; CMI, cell-mediated immunity; CMV, cytomegalovirus; HR, hazard ratio.

Interestingly, we found an association between poor HLA class I matching and higher CMV infection rates and, although not statistically different, patients with lower 15-day IE-1–specific CMI displayed numerically higher HLA class I mismatches than patients with higher IE-1-specific CMI, thus highlighting that CMV-specific CMI expansion may be more effective in patients with higher shared HLA class I antigens.

Moreover, the assessment of CMV-specific CMI against the pp65 antigen did not add any additional predictive value in our patients, underscoring a preponderance role of CMV (IE-1)–specific CMI at the early phases of transplantation for CMV replication control [9, 10, 24]. Nevertheless, and as previously reported [29], few transplant recipients developing mild CMV replication showed weak CMI responses against pp65 antigens but moderate against IE-1, thus suggesting that the concomitant evaluation of CMV(pp65)–specific CMI may also be useful to refine CMV risk stratification.

A limitation of this study is the higher number of dropout rates in group B1 due to prophylaxis discontinuation and loss to follow-up. Nevertheless, a relevant number of CMV infections occurred within this group of patients and were therefore analyzed in the intention-to-treat analysis. Importantly, the results did not change the main outcome of the study and were in agreement with those observed in the per-protocol analysis. Despite only 86% of patients being evaluable at 15 days posttransplantation, the patients available for evaluation at this timepoint were well-balanced among the study subgroups.

In conclusion, our study shows that monitoring CMV-specific CMI, particularly at early timepoints after transplantation, accurately predicts the risk of developing CMV infection

in patients not receiving T-cell induction therapy. D^+/R^+ kidney recipients receiving basiliximab with high-risk CMV-specific CMI should preferentially follow a close systematic follow-up or receive antiviral prophylaxis, whereas low-risk CMI patients may require a less stringent preventive approach. The results of this study provide an opportunity for implementing personalized medicine to manage CMV infection in kidney transplantation.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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