A Randomized Trial of Valganciclovir Prophylaxis Versus Preemptive Therapy in Kidney Transplant Recipients

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ABSTRACT

Background The optimal regimen for preventing cytomegalovirus (CMV) infection in kidney transplant recipients, primarily in reducing indirect CMV effects, has not been defined.

Methods This open-label, single-center, randomized clinical trial of valganciclovir prophylaxis versus preemptive therapy included kidney transplant recipients recruited between June 2013 and May 2018. After excluding CMV-seronegative recipients with transplants from seronegative donors, we randomized 140 participants 1:1 to receive valganciclovir prophylaxis (900 mg, daily for 3 or 6 months for CMV-seronegative recipients who received a kidney from a CMV-seropositive donor) or preemptive therapy (valganciclovir, 900 mg, twice daily) that was initiated after detection of CMV DNA in whole blood (≥1000 IU/ml) and stopped after two consecutive negative tests (preemptive therapy patients received weekly CMV PCR tests for 4 months). The primary outcome was the incidence of biopsy-confirmed acute rejection at 12 months. Key secondary outcomes included subclinical rejection, CMV disease and DNAemia, and neutropenia.

Results The incidence of acute rejection was lower with valganciclovir prophylaxis than with preemptive therapy (13%, 9/70 versus 23%, 16/70), but the difference was not statistically significant. Subclinical rejection at 3 months was lower in the prophylaxis group (13% versus 29%, $P = 0.027$). Both regimens prevented CMV disease (in 4% of patients in both groups). Compared with prophylaxis, preemptive therapy resulted in significantly higher rates of CMV DNAemia (44% versus 75%, $P < 0.001$) and a higher proportion of patients experiencing episodes with higher viral load (≥2000 IU/ml), but significantly lower valganciclovir exposure and neutropenia.

Conclusion Among kidney transplant recipients, the use of valganciclovir prophylaxis did not result in a significantly lower incidence of acute rejection compared with the use of preemptive therapy.

Clinical Trial Registry Name and Registration Number Optimizing Valganciclovir Efficacy in Renal Transplantation (OVERT Study), ACTRN12613000554763.

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INTRODUCTION

Cytomegalovirus (CMV) infection belongs to the most common opportunistic infections after organ transplantation. Major advances in the diagnosis and treatment of CMV disease have made it possible to markedly reduce the mortality rates.[1] However, an outstanding issue to be yet tackled is the immunomodulatory properties of CMV associated with a wide range of indirect effects. CMV infection predisposes patients to invasive bacterial or fungal

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infections and reactivation of other viruses.[2] CMV contributes to the higher incidence of cardiovascular disease and thrombotic events.[3,4] Critical for transplant outcomes is its ability to enhance the alloimmune response associated with an increased risk of T-cell–mediated and, possibly, also antibody-mediated allograft rejection, both of which limit long-term graft survival.[5–8] Even in the modern era with routine use of preventive regimens, CMV infection in kidney transplant recipients entails an increase in allograft failure rates and mortality.[9,10]

The two main strategies for CMV prevention include (1) universal prophylaxis with treatment of all at-risk patients with antiviral medication and (2) preemptive therapy on the basis of monitoring for CMV and treatment of only selected patients with significant viral replication.[1,11–17] While prophylaxis with valacyclovir is an option after kidney transplantation, the high pill burden and higher incidence of graft fibrosis makes most centers prefer valganciclovir.[12,13,15] The current International Consensus Guidelines consider both strategies for patients after kidney transplantation equivalent, even so for the high-risk subgroup of seronegative recipients of grafts from seropositive donors (D+R−).[1] Universal prophylaxis and preemptive therapy differ widely by definition. Preemptive therapy allows the development of early asymptomatic CMV viremia with potential occurrence of indirect CMV effects such as acute rejection.[14] At the same time, CMV viremia episodes controlled by preemptive therapy help boost CMV-specific immunity in D+R− patients and prevent the development of CMV disease in the late post-transplant period.[18] On the other hand, late-onset CMV disease and the myelotoxicity associated with universal prophylaxis entail adverse outcomes.[11,19,20] It is unclear whether the two strategies differ in the development of ganciclovir resistance.[21–23]

Only few smaller randomized trials with kidney transplant recipients directly comparing valganciclovir prophylaxis and preemptive therapy have been published to date.[16,17,24] However, two of them used, in their preemptive groups, an insufficiently intensive CMV surveillance monitoring protocol not consistent with current recommendations.[1,16,17] The remaining study, primarily a pharmacoeconomic one, did not focus on comparing indirect CMV effects.[24] Our randomized trial was designed to compare valganciclovir prophylaxis versus preemptive therapy with the primary outcome being the incidence of allograft rejection determined using protocol biopsies and by monitoring of *de novo* donor-specific anti-HLA antibodies (dnDSAs). The trial included a detailed analysis of CMV disease and CMV DNAemia, ganciclovir resistance, CMV-specific T-cell immunity, other infections, and a description of the logistic aspects central to preemptive therapy administration.

Significance Statement

Although cytomegalovirus (CMV) infection is an important factor in the pathogenesis of kidney allograft rejection, previous studies have not determined the optimal CMV prevention strategy to avoid indirect effects of the virus. In this randomized trial involving 140 kidney transplant recipients, incidence of acute rejection at 12 months was not lower with valganciclovir prophylaxis (for at least 3 months) compared with preemptive therapy initiated after detection of CMV DNA in whole blood. However, prophylaxis was associated with a lower risk of subclinical rejection at 3 months. Although both regimens were effective in preventing CMV disease, the incidence of CMV DNAemia (including episodes with higher viral loads) was significantly higher with preemptive therapy. Further research with long-term follow-up is warranted to better compare the two approaches.

METHODS

Study Design

This was an open-label, single-center, randomized clinical trial of valganciclovir prophylaxis versus preemptive therapy in adult kidney transplant recipients at risk of CMV as defined by donor and recipient CMV serologic combinations of D+R−, D+R+, and D−R+ and recruited between June 2013 and May 2018. Exclusion criteria were participation in another clinical trial, D−R− serostatus, allergy to (val)ganciclovir, severe leukopenia or thrombocytopenia, and inability to provide informed consent. The study was approved by the local ethics committee and conducted in compliance with the Declaration of Helsinki and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. Written informed consent was obtained before enrollment. The trial was registered with the Australian New Zealand Clinical Trial Registry (ACTRN12613000554763) on May 16, 2023.

Randomization and Interventions

Eligible patients were randomized before transplantation by the transplant physician in a 1:1 ratio to valganciclovir prophylaxis or preemptive therapy using a random-number table with permuted blocks of four. Randomization was stratified by D/R CMV serostatus. Sequentially numbered sealed envelopes were used for allocation concealment. Patients in the prophylaxis group received valganciclovir (Valcyte, Hoffman-La Roche, Grenzach-Wyhlen, Germany) at a dose of 900 mg daily for 3 months (or 6 months in the case of D+R− status) beginning from day 7 after transplant at the latest. Patients in the preemptive group underwent weekly PCR testing for CMV DNA from whole blood for 4 months after transplantation and monthly until 12 months thereafter. PCR was also performed weekly if CMV DNAemia was detected and during the course of preemptive valganciclovir therapy. On detection of CMV DNAemia of ≥1000 IU/ml, valganciclovir, 900 mg twice daily, was administered within 7 days until two consecutive negative PCR tests a week apart. Recurrent CMV DNAemia was treated similar to the initial episode. In both groups, antiviral drug doses were tapered on the basis of renal function according to the manufacturers' instructions. In patients with antirejection therapy with lymphocyte-depleting antibodies and/or treatment of antibody-mediated rejection after scheduled prophylaxis or weekly PCR testing had been completed, an additional 1-month course of valganciclovir

prophylaxis was administered or weekly PCR testing restarted for 1 month, respectively. In the prophylaxis group, the same schedule for PCR CMV DNA testing was applied including weekly testing for the first 4 months; however, asymptomatic CMV DNAemia episodes were not treated.

Immunosuppression and Other Measures of Antimicrobial Prevention

The standard immunosuppressive protocol included tacrolimus, mycophenolate mofetil, and corticosteroids. Immunologically high-risk patients (retransplantation, panel reactive antibody >60%, and/or preformed DSA) received induction by antithymocyte globulin (Thymoglobuline, Genzyme, Lyon, France). Patients after a previous transplantation were tested for the presence of human histocompatibility complex (HLA) antibodies while on the waiting list, and those with preformed DSA underwent desensitization with plasmapheresis and low-dose intravenous immunoglobulin started immediately before transplantation. Desensitization in HLA-incompatible or AB0-incompatible living donor transplantation involved anti-CD20 monoclonal antibody (rituximab) and immunoadsorption using staphylococcus protein A columns (Immunosorba, Fresenius Medical Care, Bad Homburg, Germany). Low-dose tacrolimus with anti-IL2R monoclonal antibody (basiliximab) induction was given in recipients of grafts from highly marginal donors (donation after circulatory death, 70 years or older, and/or donors with hypertension or diabetes and significant nephrosclerosis on biopsy). Patients received prophylaxis with trimethoprim-sulfamethoxazole for 4 months and oral amphotericin solution for 1 month. Plasma was tested for polyomavirus BK (BKV) DNA every month for the first 6 months and at 9 and 12 months with preemptive immunosuppression reduction at a significant viral load (≥1000 copies/ml).

Outcomes

The primary outcome was the incidence of acute rejection (grade≥IA or antibody-mediated rejection) at 12 months after transplantation diagnosed by "for-cause" biopsy using the Banff classification.[25] Secondary outcomes included CMV DNAemia and CMV disease, CMV ganciclovir resistance, CMV-specific T-cell immunity, subclinical rejection assessed by protocol biopsy at 3 months, development of dnDSA, renal function and graft survival, incidence of other infections, neutropenia, cardiovascular events, malignancy, mortality, and other safety data evaluated by recording adverse events and routine laboratory parameters. CMV DNAemia was defined by the detection of CMV DNA. CMV disease was defined as symptomatic CMV DNAemia and included both CMV syndrome and tissue-invasive disease.[1,26] All patients remained on follow-up for a minimum of 12 months or until death.

Nucleic Acid Testing for Viral Detection

Quantitative real-time PCR for CMV DNA was performed using a commercially available kit (RealStar CMV PCR kit 1.0, Altona Diagnostics, Hamburg, Germany) according to the manufacturer's instructions on QuantStudio5 (Applied Biosystems, Waltham, MA). DNA was isolated from 200 μl of whole blood using a commercially available kit (QuickGene DNA whole blood kit S, Kurabo, Japan). Elution was performed in 100 μl of cell dissociation buffer; the final DNA volume used in the calculation of CMV IU/ml was 100 μl. Quantification was performed using the calibration curve generated from concomitantly amplified quantification standards (IU/μl DNA). The lower detection limit of the investigation was 50 IU/ml of whole blood. Quantitative PCR for BKV DNA in the plasma was performed using a commercially available BK Virus R-GENE kit (bioMérieux, Marcy-l'Étoile, France) on a Rotor Gene Q device, according to the manufacturer's instructions (Qiagen, Hilden, Germany) as described previously.[27,28] The detection limit of BKV DNA was 50 copies/ml. The diagnosis of polyomavirus-associated nephropathy (PVAN) was performed on the basis of both definitive and presumptive PVANs with high-grade BKV viremia (≥10,000 copies/ml) and a negative biopsy. Biopsy-proven definitive PVAN was based on cytopathic changes, confirmed by positive SV40T immunochemistry, and classified according to the new Banff Working Group Classification.[29]

Analysis of Resistance-Associated Mutations in the UL54 and UL97 Genes

Genotypic testing was performed in specimens with viral loads ≥1000 IU/ml as recommended.[30] A predefined group was selected for ganciclovir resistance investigation, which included patients with (1) CMV DNAemia during valganciclovir prophylaxis or after prophylaxis completion, (2) persistent CMV DNAemia after at least 3 weeks of preemptive valganciclovir therapy, (3) recurrent CMV DNAemia after previous preemptive valganciclovir therapy, and (4) CMV disease with persistent DNAemia after at least 3 weeks of (val)ganciclovir therapy. The analysis of resistance-associated mutations of CMV (nomenclature NC_006273) was performed using PCR and Sanger sequencing. Investigated regions of the UL54 gene included codons 340–643 and 660–1010 and of UL97 gene codon 434–630. Briefly, 5–10 μl of isolated DNA was added to a reaction mixture consisting of 12.5 μl of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany), 10 pmol of forward and reverse primers (Supplemental Table 1), and distilled water up to 25 μl. The amplification program comprised denaturation at 95°C/14 min, 43× (95°C/40s, 68°C/50s, 72°C/1 min), 72°C/7 min, 4°C/(UL54), and 95°C/14 min, 43× (95°C/30s, 68°C/30s, 72°C/1 min), 72°C/7 min, 4°C/∞ (UL97). Successfully amplified PCR products selected for sequencing analysis were sequenced on both sides using the Big Dye Terminator Sequencing kit 1.1 (Applied Biosystems, Waltham, MA), according to the manufacturer's protocol. Resistance-associated mutations of CMV were identified using the mutation resistance analyzer database, a web-based search tool that links the sequence to a database containing all published UL97 (protein kinase) and UL54 (DNA polymerase) mutations and corresponding antiviral

drug susceptibility phenotypes (https://www.informatik.uni-ulm.de/ni/mitarbeiter/HKestler/hcmv/).[31]

CMV-Specific T-Cell Immunity

CMV-specific T-cell responses were measured in a cohort of patients (n=95) enrolled until October 2016 before transplantation and at 1, 3, 6, and 12 months thereafter. Quantitation of IFN-γ– and IL-2–producing cells was performed on isolated PBMCs using a modified, dual-color, enzyme-linked immunosorbent spot assay (ELISpot) analysis, as per the manufacturer's instructions (Mabtech, Nacka Strand, Sweden) and as previously described.[32,33] PBMCs were obtained using density gradient centrifugation and stored in liquid nitrogen. Thawed PBMCs at concentrations of 2×10^5 cells per well were pipetted into ethanol-treated polyvinylidene difluoride 96-well microplate precoated with primary monoclonal antibodies specific for human IFN-γ and IL-2 (Mabtech, Nacka Strand, Sweden) and stimulated with antigens; an anti-CD3 monoclonal antibody (Mabtech, Nacka Strand, Sweden) and medium alone were used as positive and negative controls, respectively. Two commercially available sets of CMV peptide pools were used for antigenic stimulation: PepMix human cytomegalovirus antigens (pp65) and PepMix human cytomegalovirus antigens (IE-1) (JPT, Berlin, Germany). All stimulations were performed in duplicate wells. Cell cultures were cultivated in a CO_2 incubator overnight, and thereafter, the plates were washed, dried properly, and incubated with detection antibodies and two fluorescent-labeled conjugates. Fluorescent spots were counted using an automated ELISpot reader equipped with filters for IFN-γ and IL-2 detection (AID, Strassberg, Germany). The mean spot count observed in negative controls was subtracted from the count in the stimulated wells in each run. Results were expressed as the number of spot-forming cells (SFCs) per 2×10^5 PBMCs.

Assessment of Anti-HLA Antibodies

Donor and recipient HLA typing involved HLA class I (A, B) and class II (DRB1, DQB1). Screening for circulating anti-HLA class I and II antibodies was performed in serum samples at transplantation, 3 and 12 months after transplantation, and, if clinically indicated and determined, using single-antigen flow beads assays on a Luminex platform (Luminex 100/200 System, United States) with the use of commercially available kits (Lifecodes LSA Class I; Lifecodes LSA Class II; Immucor GTI Diagnostic, United States). The results were expressed as mean fluorescence intensity for each HLA specificity. All beads showing a normalized mean fluorescence intensity of >1000 were considered positive.

Statistical Analysis

The null hypothesis was that the acute rejection rate would be equal in the two groups. The null hypothesis would be rejected if valganciclovir prophylaxis was superior to preemptive therapy at the 0.05 level. On the basis of our previous studies and

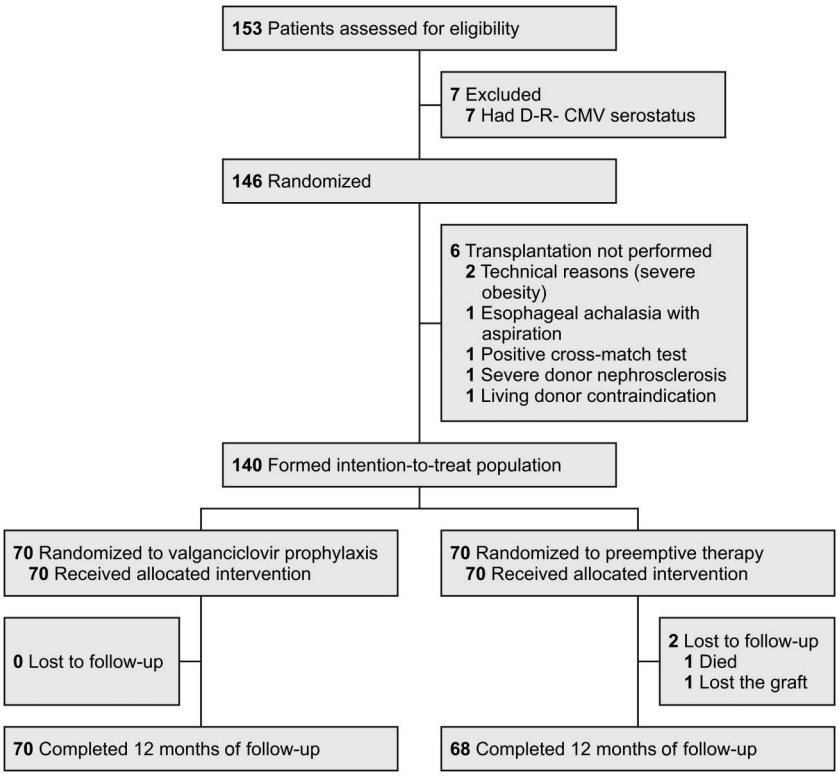

Figure 1. Flow of patients through the study. CMV, cytomegalovirus; D, donor; R, recipient.

given the use of a tacrolimus-based regimen in all patients, it was estimated that acute rejection would occur in 10% of the patients in the prophylaxis group and 25% of the patients in the preemptive therapy group.[12,14] A sample size of 90 patients (45 per group) would detect this difference with 80% power and $\alpha=0.05$. Allowing for dropouts, a minimum of 92 patients were thus planned to enroll. Owing to the clinically important trend toward a lower acute rejection rate in the prophylaxis group (13% versus 27%; $P=0.07$ by the log-rank test) in interim analysis of the planned study population ($n=95$), it was decided to continue recruiting patients. Assuming the same difference in acute rejection, at least 134 patients would have been needed. Finally, 140 subjects were planned to enroll anticipating dropouts. In view of the numerically lower incidence of acute rejection in the prophylaxis group, a *post hoc* analysis according to the type of induction therapy was performed. Quantitative data were compared using the Mann-Whitney *U*-test or Student *t*-test as appropriate. Categorical variables were compared using the χ^2 or Fisher exact test. The incidence of acute rejection, CMV DNAemia and disease, BKV viremia, and patient and graft survival rates were calculated using the Kaplan-Meier curves, with the log-rank test used for comparison. Univariate Cox regression was performed to calculate hazard ratio (HR) and 95% confidence interval (CI) for acute rejection and other variables in the prophylaxis group as compared with the preemptive group. Receiver-operating characteristic curves were used to determine the optimal negative predictive value of various CMV-specific immunity assays for the development of significant (>1000 IU/ml) CMV DNAemia. Statistical calculations were made using SAS software (SAS Institute Inc., Cary, NC). Values of $P < 0.05$ were considered statistically significant.

RESULTS

Participants

Overall, 153 patients had been screened and 146 subsequently randomized; transplantation was not performed in six patients. The intention-to-treat population included 140 patients (valganciclovir prophylaxis, $n=70$; preemptive therapy, $n=70$) (Figure 1). The groups were similar in their basic characteristics (Table 1). Among the 140 randomized patients, 19 (14%) were at risk of

Table 1. Baseline characteristics of the study population

Characteristics	Valganciclovir Prophylaxis ($n=70$)	Preemptive Therapy ($n=70$)	*P* Value
Recipient			
Age (yr)	52±11	50±12	0.257
Sex (male)	50 (71)	46 (66)	0.446
Cause of kidney disease			0.404
Chronic glomerulonephritis	35 (50)	32 (46)	
Nephrosclerosis	9 (13)	12 (17)	
Polycystic kidney disease	10 (14)	12 (17)	
Diabetic nephropathy	5 (7)	3 (4)	
Other	11 (16)	11 (16)	
Previous transplantation	5 (7)	8 (11)	0.382
Preemptive transplantation	3 (8)	4 (12)	0.494
HLA A, B, DR, DQ mismatches (n)	4.2±1.6	4.3±1.5	0.747
Pretransplant PRA>20%	8 (11)	4 (6)	0.227
Preformed DSA[a]	10 (14)	13 (19)	0.494
CMV serostatus			0.382
D+R−	9 (13)	10 (14)	
D+R+	56 (80)	52 (74)	
D−R+	5 (7)	8 (11)	
Donor			
Age (yr)	50±16	49±15	0.571
Deceased donor	66 (94)	66 (94)	1.000
Donation after cardiac death	5 (7)	8 (11)	0.382
Living donor[b]	4 (6)	4 (6)	
Expanded criteria donor[c]	29 (41)	29 (41)	1.000
Cold ischemia time (h)	17.2±5.4	17.6±6.3	0.742
Primary immunosuppression			
Tacrolimus + MMF + steroids	70 (100)	70 (100)	
Thymoglobulin induction	9 (13)	10 (14)	0.973
Basiliximab induction	23 (33)	24 (34)	0.858
Desensitization pretransplant[d]	4 (6)	5 (7)	0.730

Data are number of patients (percentage) or mean±SD. PRA, panel reactive antibody; DSA, donor-specific anti-HLA antibody; CMV, cytomegalovirus; D, donor; R, recipient; MMF, mycophenolate mofetil.

[a]At transplantation, using single-antigen flow beads assays on a Luminex platform with inclusion of weak positive results (mean fluorescence intensity >500).

[b]Including two AB0 incompatible transplantations in valganciclovir prophylaxis and one HLA and AB0 incompatible transplantation in the preemptive prophylaxis group.

[c]According to the UNOS criteria.

[d]Valganciclovir prophylaxis: plasmapheresis plus low-dose intravenous immunoglobulin in 3 and immunoadsorption plus rituximab in 1; preemptive therapy: plasmapheresis plus low-dose intravenous immunoglobulin in 4 and immunoadsorption plus rituximab in 1.

primary CMV infection. Induction therapy was received by 66 (47%) patients. Maintenance immunosuppression did not differ between the groups (Supplemental Table 2).

Rejection and dnDSA

Although the number of episodes was higher in the preemptive therapy group, the incidence of the primary outcome of acute rejection at 12 months did not differ significantly between the groups (13%, 9/70 versus 23%, 16/70, P=0.112 [HR, 0.52, 95% CI, 0.23 to 1.19]) (Figure 2A). Unlike patients with thymoglobulin or basiliximab induction, not showing significant differences, valganciclovir prophylaxis in patients without induction therapy was associated with a significant drop in acute rejection (11% versus 31%, P=0.032 [HR, 0.31, 95% CI, 0.10 to 0.98]) (Figure 2B, Table 2). The incidence of acute rejection was higher in the subgroup with CMV DNAemia compared with those not showing CMV replication (24% versus 9%, P=0.022). In the prophylaxis group, CMV DNAemia preceded only 2 (22%) episodes of acute rejection in contrast to 7 (44%) episodes in patients with preemptive therapy (Supplemental Figure 1).

Protocol biopsy at 3 months was performed in 134 (96%) patients. Subclinical rejection including borderline changes was less frequent in patients treated with valganciclovir prophylaxis compared with the preemptive therapy (13% versus 29%, P=0.027); a similar trend was observed when comparing the incidence of at least grade IA rejection episodes (1% versus 8%, P=0.088) (Figure 3A). The Banff interstitial inflammation and tubulitis score was lower in the valganciclovir group (P=0.007) (Figure 3B). The groups did not differ in chronic histological damage and other outcome measures, with a typically high incidence of vascular nephrosclerosis due to the high number of expanded criteria donors (Supplemental Table 3). In patients without preformed DSA, the development of dnDSA was comparable in both groups with a cumulative incidence of 12% versus 5% (P=0.364); however, their presence was often only transient with dnDSA persisting in 7% versus 4% of patients at month 12 (P=0.723) (Table 2). The incidence of delayed graft function, renal function, and proteinuria were comparable (Supplemental Table 4). Likewise, there were no differences in graft and patient survival. In the preemptive therapy group, one patient died because of severe necrotizing pancreatitis while another lost the graft because of severe T-cell–mediated acute rejection associated with renal vein thrombosis.

CMV Disease and CMV DNAemia

Overall, CMV disease was not a common occurrence; the condition was diagnosed in only three patients each in either group (4% versus 4%, P=0.974 [HR, 0.97, 95% CI, 0.20 to 4.82]) (Figure 4A). In the prophylaxis group, the diagnosis was late-onset CMV disease as a rule (>3 months after transplantation). Likewise, patients in the preemptive therapy group experienced two episodes of late-onset CMV disease during the period when PCR monitoring was performed at 1-month intervals. The response to (val)ganciclovir was good in all patients with no recurrent CMV disease (Table 3).

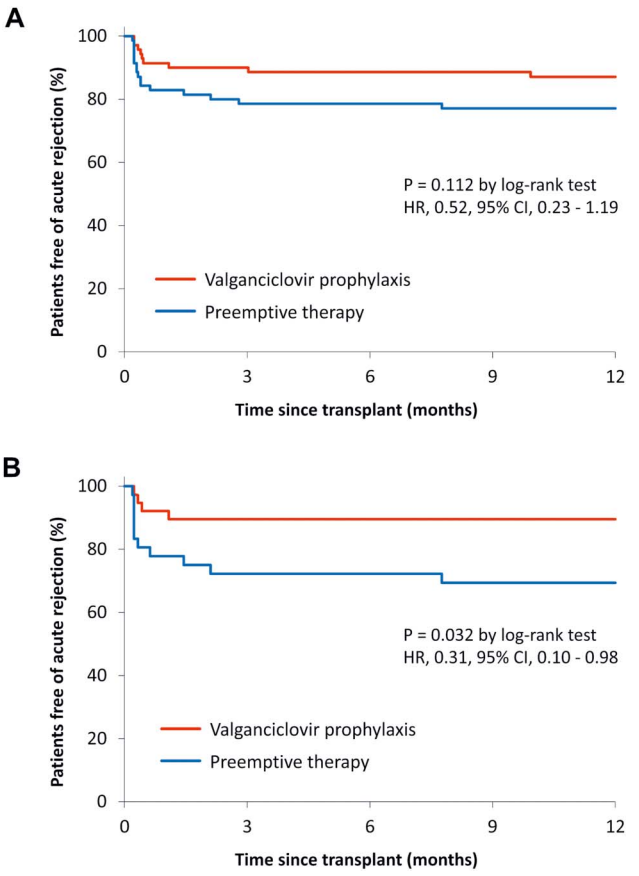

Figure 2. Cumulative incidence of acute rejection. Kaplan-Meier curves for rejection-free survival in (A) the entire study population and (B) patients without induction therapy. CI, confidence interval; HR, hazard ratio.

CMV DNAemia was detected in 31 (44%) and 52 (75%, P<0.001 [HR, 0.32, 95% CI, 0.21 to 0.51]) patients in the prophylaxis and preemptive therapy groups, respectively (Figure 4B). The incidence of CMV DNAemia was significantly lower both in the D+R− group (33% versus 90%, P=0.002) and in R+ patients (46% versus 73%, P<0.001) (Figure 4, C and D). The median time to onset of CMV DNAemia was longer in the valganciclovir prophylaxis group (193 versus 32 days, P<0.001). Consistent with this, late-onset CMV DNAemia was detected more often in the prophylaxis group (36% versus 9%, P<0.001); however, the difference become nonsignificant when counting recurrent episodes also (P = 0.082) (Table 3). Furthermore, valganciclovir prophylaxis resulted in a decrease in the incidence of CMV DNAemia with a significant (≥2000 IU/ml) viral load (21% versus 49%, P<0.001) and total duration of CMV DNAemia (25 versus 42 days, P=0.030) within the first year after transplant. Recurrent CMV DNAemia was common, especially in patients with preemptive therapy (42% versus 77%, P=0.002). Detailed virologic characteristics are presented in Table 3.

Table 2. Incidence and characteristics of acute rejection and *de novo* donor-specific antibodies

Characteristics	Valganciclovir Prophylaxis (*n*=70)	Preemptive Therapy (*n*=70)	*P* Value
Patients with biopsy for a cause	35 (50)	40 (57)	0.397
Acute rejection (grade ≥IA)	9 (13)	16 (23)	0.112[a]
No induction therapy	4 (11)	11 (31)	0.032[b]
Thymoglobulin	1 (11)	2 (20)	0.577
Basiliximab	4 (17)	3 (13)	0.668
Classification according to Banff 2019			
Grade IA	3 (4)	9 (13)	
Grade IB	1 (1)	0 (0)	
Grade IIA	3 (4)	4 (6)	
Grade IIB	0 (0)	1 (1)	
Grade III	1 (1)	0 (0)	
Active antibody-mediated rejection	2 (3)	3 (5)	
Acute rejection (including borderline)	15 (22)	23 (33)	0.117
Depleting ALA for rejection	8 (11)	7 (10)	0.785
Patients without preformed DSA	60	57	
De novo DSA (cumulative incidence)	7 (12)	3 (5)	0.364
Class I	2 (3)	2 (4)	0.648
Class II	5 (8)	2 (4)[c]	0.464
Peak MFI value	2628 (1781–7275)	1822 (1331–7202)	1.000
De novo DSA at month 3	6 (10)	1 (2)	0.136
De novo DSA at month 12	4 (7)	2 (4)	0.723

Data are number of patients (percentage) or median and interquartile range. ALA, antilymphocyte antibody; DSA, donor-specific anti-HLA antibody; MFI, mean fluorescence intensity; HR, hazard ratio; CI, confidence interval.
[a]HR, 0.52; 95% CI, 0.23 to 1.19.
[b]HR, 0.31; 95% CI, 0.10 to 0.98.
[c]*De novo* DSA of both classes I and II were detected in one patient.

On the basis of predefined criteria, genotypic testing was performed in 19 (45 samples) and 29 (73 samples) patients in the valganciclovir prophylaxis and preemptive therapy groups, respectively, without detecting a single mutation associated with ganciclovir resistance. All episodes of CMV DNAemia requiring preemptive therapy were successfully treated with valganciclovir resulting in viral clearance.

Logistics of CMV Prevention, Compliance, and Valganciclovir Exposure

Valganciclovir prophylaxis was administered for an average 104±29 days; this number includes 3 (4%) patients receiving an additional 1-month course of prophylaxis after treatment with lymphocyte-depleting antibodies. Protocol violation occurred in 1 D+R− patient receiving prophylaxis inadvertently for only 3 instead of 6 months as originally scheduled. In the preemptive therapy group, compliance with the CMV surveillance protocol was high, with 1815 of 1825 (99.5%) per protocol PCR tests performed. At least one PCR test was not performed as scheduled in 7 of 70 (10%) patients; none developed CMV disease. Overall, 38 (54%) patients required a preemptive valganciclovir course, a figure consistent with the 73% of patients with detected CMV DNAemia. The median time from detection of CMV DNAemia exceeding a viral load threshold of 1000 IU/ml to preemptive therapy initiation was 1.5 days. An overwhelming majority (29/38, 76%) of patients required additional courses of preemptive valganciclovir because of recurrent CMV DNAemia. Overall, an average of 2.6±1.4 courses per

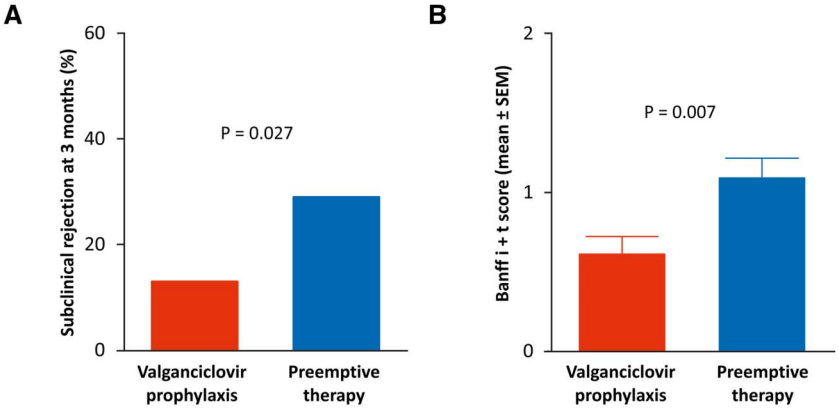

Figure 3. Protocol biopsy findings at 3 months after transplantation. (A) Incidence of subclinical rejection including the Banff borderline category, and (B) Banff interstitial inflammation and tubulitis scores. Data are percentage or mean±SEM.

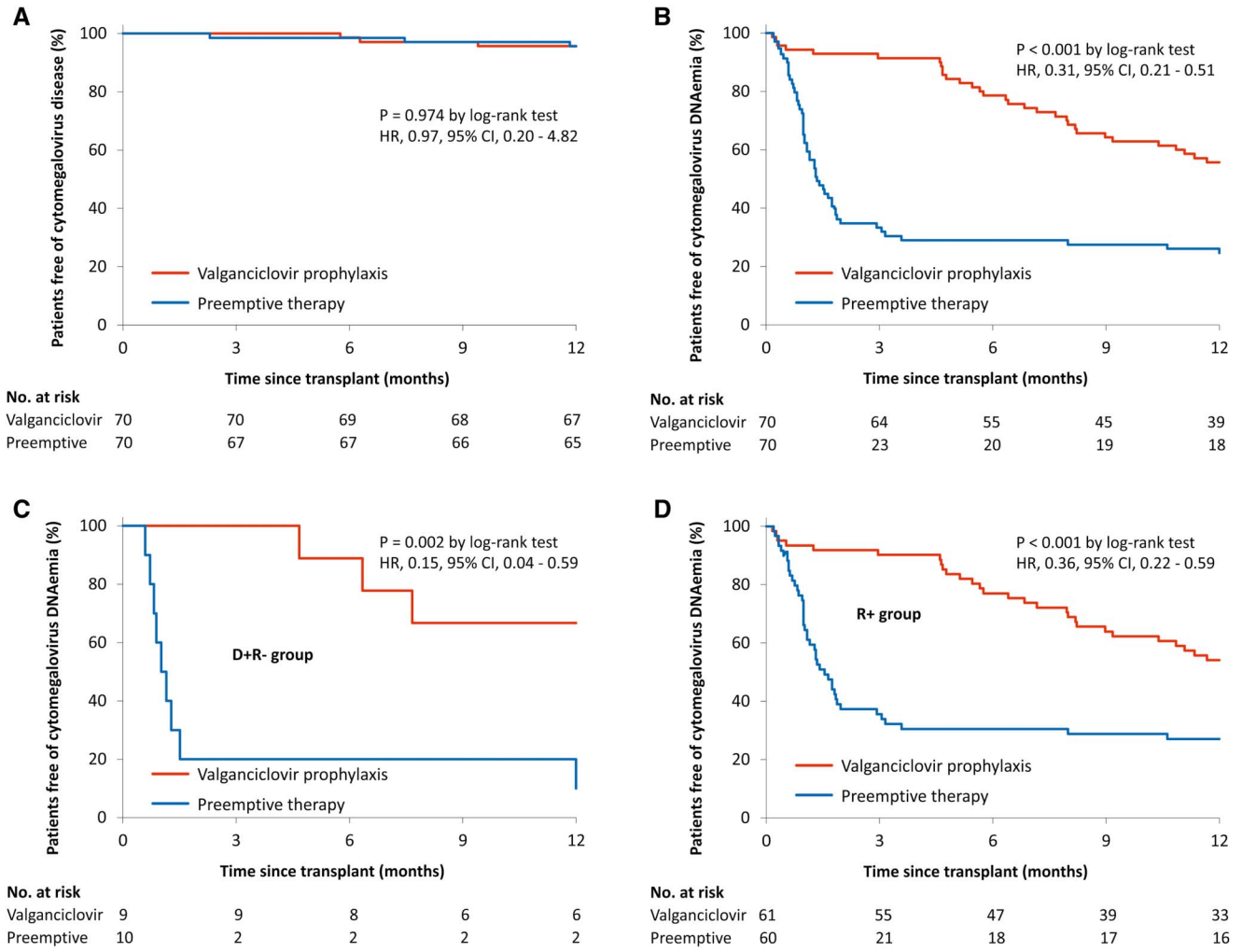

Figure 4. Cumulative incidence of CMV disease and DNAemia. Kaplan-Meier curves for (A) CMV disease-free survival and CMV DNAemia-free survival in (B) the entire study population, (C) D+R− group, and (D) R+ group. CI, confidence interval; CMV, cytomegalovirus; D, donor; HR, hazard ratio; R, recipient.

patient had to be administered; the number was significantly higher in the D+R− group (P=0.012) (Table 4).

Despite the need for multiple courses of preemptive treatment with valganciclovir in a significant proportion of patients, total exposure to valganciclovir used in CMV prevention was significantly higher with prophylaxis because of both longer duration of therapy (104±29 versus 33±42 days, P<0.001) and total cumulative dose (70.3±26.4 versus 50.1±65.8 g, P<0.001). An exception to this was the D+R− subgroup having received a comparable cumulative dose (Figure 5, A and B, Supplemental Table 5).

Polyomavirus BK and Other Infections

The two groups did not differ in the incidence of BKV viremia (20% versus 20%, P=0.955) and PVAN (6% versus 6%, P = 1.000). The incidence of other viral, bacterial, and fungal infections was likewise comparable, except for the higher incidence of pneumonia in the preemptive therapy group (1% versus 10%, P=0.029). However, five of seven patients involved early postoperative pneumonia diagnosed within the first week after transplant (Supplemental Table 6).

Adverse Events

Patients in the valganciclovir prophylaxis group presented more often with neutropenia (41% versus 23%, P=0.019); a similar trend was noticed with leukopenia. However, no significant differences were observed in the incidence of severe neutropenia or use of a granulocyte colony-stimulating factor. A summary of selected adverse events is available in Table 5. Because of its myelotoxicity, valganciclovir doses had to be reduced or temporarily discontinued (median 10 days) in 30% of patients with prophylaxis in contrast to no patient receiving preemptive therapy (P<0.001). Nonetheless, in five of the 38 patients on preemptive valganciclovir therapy, mycophenolate mofetil had to be temporarily withdrawn and/or the granulocyte colony-stimulating factor had to be administered to maintain full valganciclovir dose.

Table 3. Clinical and virologic characteristics of cytomegalovirus disease and DNAemia

Characteristics	Valganciclovir Prophylaxis (*n*=70)	Preemptive Therapy (*n*=70)	*P* Value
CMV disease[a]	3 (4)	3 (4)	0.974[b]
D+R−	1 (11)[c]	2 (20)	0.653
R+	2 (3)	1 (2)	0.597
Late-onset CMV disease[d]	3 (100)	2 (67)	—
Time to onset (d)	191 (179–262)	227 (109–327)	0.986
Time to negative PCR after (val)ganciclovir (d)	22 (12–39)	18 (18–20)	0.609
Recurrent CMV disease	0 (0)	0 (0)	—
CMV DNAemia	31 (44)	52 (75)	<0.001[e]
D+R−	3 (33)	9 (90)	0.002[f]
R+	28 (46)	43 (73)	<0.001[g]
CMV DNAemia≥2000 IU/ml	15 (21)	34 (49)	<0.001
D+R−	2 (22)	9 (90)	<0.001
R+	13 (21)	25 (42)	0.004
Late-onset CMV DNAemia (1st episode)	25 (36)	26 (9)	<0.001
Late-onset CMV DNAemia (including recurrent episodes)	29 (41)	36 (53)	0.082
Time to onset of 1st episode (d)	193 (141–264)	32 (23–52)	<0.001
Peak viral load (IU/ml)	1400 (350–11,350)	4250 (1200–19,500)	0.117
D+R−	4100 (2100–965,800)	40,100 (19,800–52,450)	0.355
R+	1350 (300–11,050)	2800 (950–10,800)	0.323
Duration of CMV DNAemia (d)	25 (9–52)	42 (21–75)	0.030
Recurrent CMV DNAemia	13 (42)	40 (77)	0.002

Data are number of patients (percentage) or median and interquartile range. CMV, cytomegalovirus; D, donor; R, recipient; IU, international unit; HR, hazard ratio; CI, confidence interval.
[a]Valganciclovir prophylaxis: CMV syndrome in 2 and CMV colitis in 1; preemptive therapy: CMV syndrome in 1 and CMV colitis in 2.
[b]HR, 0.97; 95% CI, 0.20 to 4.82.
[c]A D+R− patient nonadherent to study protocol with prophylaxis withdrawal after 3 months instead of 6 months per protocol.
[d]Defined by onset >3 months after transplantation.
[d]A single case of early-onset CMV disease in the preemptive therapy group comprised one patient with rapid development of symptoms of CMV syndrome 2 days after a positive PCR test.
[e]HR, 0.31; 95% CI, 0.21 to 0.51.
[f]HR, 0.15; 95% CI, 0.04 to 0.59.
[g]HR, 0.36; 95% CI, 0.22 to 0.59.

CMV-Specific T-Cell Responses

Pretransplant pp65-specific IFN-γ–producing cells (cutoff of 198 SFC/2×10^5 PBMCs) and both pp65-specific and IE-1–specific IL-2–producing cells (cutoff of 22 SFC/2×10^5 PBMCs) predicted the development of CMV DNAemia with >1000 IU/ml with a sensitivity and specificity in the ranges of 75%–94% and 35%–57%, respectively (Supplemental Figures 2A and 2B). The CMV-specific T-cell response after transplant in patients on valganciclovir prophylaxis and preemptive therapy was comparable both in the R+ group (Figure 6)

Table 4. Details of cytomegalovirus prevention on the basis of donor and recipient serostatus

Characteristics	All Patients	R+ Group	D+R− Group	*P* Value
Valganciclovir prophylaxis	*n*=70	*n*=61	*n*=9	—
Duration (d)	104±29	94±6	176±20	<0.001
Dose (mg/d)	682±189	689±185	630±218	0.451
Preemptive therapy	*n*=70	*n*=60	*n*=10	—
Preemptive valganciclovir				
ITT population	38 (54)	30 (50)	8 (80)	0.097
Patients with CMV DNAemia	38 (73)	30 (70)	8 (89)	0.415
Preemptive valganciclovir courses per treated patient	2.6±1.4	2.3±1.2	3.9±1.5[a]	0.012
Single course	9 (24)	9 (30)	0 (0)	0.153
Two courses	13 (34)	11 (37)	2 (25)	0.689
Three or more courses	16 (42)	10 (33)	6 (75)	0.050
Duration (d)	69±40	50±35	101±28	0.001
Dose (mg/d)	1523±391	1487±413	1659±279	0.216
Time to initiation (d)[b]	1.9±1.6	1.9±1.7	2.0±1.5	0.338
Viral load at start (IU/ml, median, interquartile range)	3200 (1800–7100)	2800 (1750–6850)	4500 (2500–9400)	0.068

Data are number of patients (percentage) or mean±SD unless otherwise stated. D, donor; R, recipient; ITT, intention-to-treat; CMV, cytomegalovirus; IU, international unit.
[a]Maximum of six courses in a single D+R− patient.
[b]Time from detection of CMV DNAemia with a viral load exceeding 1000 IU/ml.

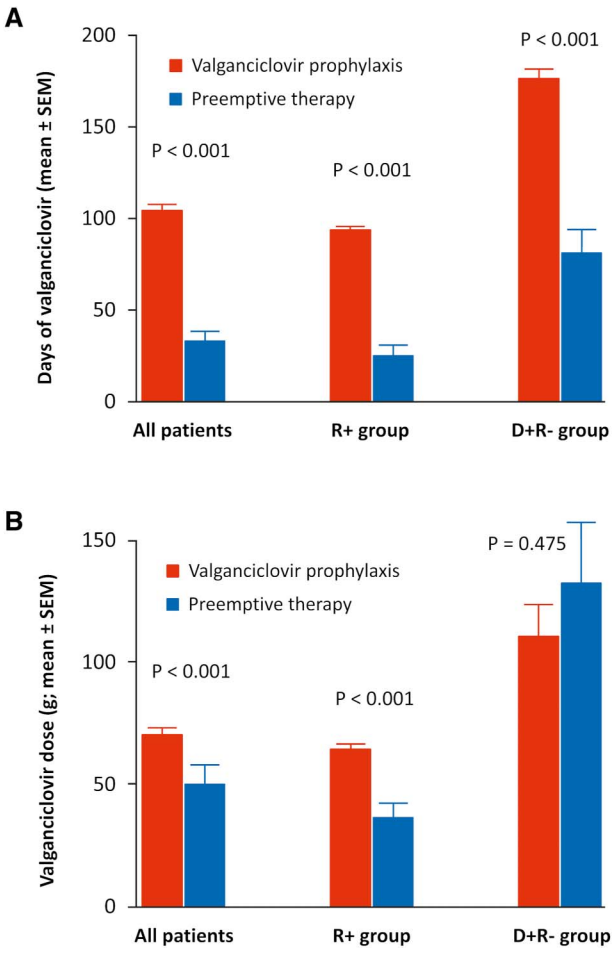

Figure 5. Cumulative exposure to valganciclovir for cytomegalovirus prevention in the intention-to-treat population. (A) Duration of therapy, and (B) cumulative valganciclovir dose per patient. Data are mean±SEM. D, donor; R, recipient.

and the D+R− group; however, the number of tested D+R− patients was small (Supplemental Table 7).

DISCUSSION

In our randomized clinical trial with kidney transplant recipients, valganciclovir prophylaxis—compared with preemptive therapy—led to a nonsignificant reduction in the incidence of acute rejection; however, the decrease in patients with a tacrolimus-based regimen without induction therapy was significant at a clinically relevant difference of 20%; in addition, the prophylaxis group showed a lower incidence of subclinical rejection. Although both strategies were effective in reducing the incidence of CMV disease, preemptive therapy was associated with a higher incidence of not only CMV DNAemia but also CMV DNAemia with a higher viral load. A course of preemptive valganciclovir was necessary in half of the patients, with the overwhelming majority requiring multiple courses because of recurrent CMV DNAemia. On the other hand, prophylaxis was associated with a higher incidence of neutropenia resulting in temporary withdrawal of valganciclovir in almost one in four patients.

The most serious indirect effects of CMV include an increased risk of allograft rejection.[1,5] Regarding the pathogenesis of CMV-associated rejection, several mechanisms have been suggested including stimulation of local inflammation within the allograft with activation of NF-κB induced, in some patients, by intragraft CMV infection.[34] Other hypotheses include heterologous immunity and expansion of natural killer cells, which promote missing self-induced microvascular inflammation, regardless of DSA.[6,35,36] The lower incidence of acute rejection and subclinical rejection in patients with valganciclovir prophylaxis is likely in part due to the reduced incidence of CMV DNAemia. However, only approximately half of the rejection episodes were preceded by CMV DNAemia, and the differences seen in the first 2 weeks after transplantation could not be attributed to CMV replication. Another potential factor to be considered is the effect of valganciclovir on T-cell function with an additive immunosuppressive effect.[37] This mechanism is supported by an earlier study demonstrating a decrease in the incidence of acute rejection in valganciclovir prophylaxis—as compared with valacyclovir—despite a comparable incidence of CMV DNAemia.[12] Our study documented a comparable incidence of acute rejection in patients with induction therapy, implying a minimal effect of the CMV prevention strategy when using very potent immunosuppression. Compared with preemptive therapy, antiviral prophylaxis in an earlier study with predominantly cyclosporine-based immunosuppression resulted in a lower incidence of rejection.[14] Our study is the first to demonstrate a decreased incidence of rejection when the currently preferred tacrolimus/mycophenolic acid-based protocol is used. Furthermore, this was the first time that a randomized trial documented a beneficial effect of (val)ganciclovir prophylaxis on the incidence of subclinical rejection. Some authors did not demonstrate a significant difference between prophylaxis and preemptive therapy in rejection rates, although in some studies, the incidence was numerically lower with prophylaxis.[16,17,24] Compared with our study, earlier, patient populations differed not only in immunosuppressive therapy but also in smaller proportions of high-risk expanded-criteria donors. In the US study, almost all patients received thymoglobulin with a very low incidence of acute rejection, whereas in the other studies, there was no clear distribution between the groups in the use of tacrolimus or cyclosporine. Oral ganciclovir prophylaxis used in one study may lead to lower ganciclovir exposure compared with full-dose valganciclovir regimens with a possibly less pronounced effect on T-cell function.[16] A recent multicenter trial showed similar rejection rates in liver transplant recipients.[38] In addition to the general difficulty in comparing the risk of rejection between different organs, the significantly higher incidence of CMV disease with prophylaxis could modify CMV indirect effects. It should be noted that the reduction

Table 5. Summary of adverse events

Characteristics	Valganciclovir Prophylaxis (*n*=70)	Preemptive Therapy (*n*=70)	*P* Value
Leukopenia[a]	30 (43)	20 (29)	0.078
Neutropenia[a]	29 (41)	16 (23)	0.019
Severe neutropenia[a]	9 (13)	10 (14)	0.805
Granulocyte colony-stimulating factor use	9 (13)	7 (10)	0.595
Thrombocytopenia[a]	11 (16)	8 (11)	0.459
Anemia[a]	6 (9)	12 (17)	0.130
Hallucinations/confusion	4 (6)	5 (7)	0.730
Headache	2 (3)	3 (4)	0.649
Tremor	10 (14)	17 (24)	0.134
Insomnia	9 (13)	8 (11)	0.796
Nausea	20 (29)	22 (31)	0.712
Diarrhea	28 (40)	29 (41)	0.863
New-onset diabetes or IGT[b]	18 (29)	17 (27)	0.799
Hyperlipidemia	59 (84)	53 (76)	0.205
Elevated liver enzymes	14 (20)	22 (31)	0.122
Cardiovascular events	15 (21)	17 (24)	0.687
Malignancy[c]	1 (1)	3 (4)	0.310
Study drug reduction or discontinuation	21 (30)	0 (0)[d]	<0.001
Reduction	10 (14)	0 (0)	0.014
Discontinuation	16 (23)[e]	0 (0)	0.001

Data are number of patients (percentage). IGT, impaired glucose tolerance.
[a]Leukopenia was defined by white blood cell count ≤3.0 cells/μl, neutropenia by absolute neutrophil count ≤1.5 cells/μl with severe neutropenia ≤0.5 cells/μl, thrombocytopenia by ≤100,000 cells/μl, and anemia by hemoglobin level ≤80 g/L.
[b]In patients without diabetes before transplantation.
[c]Including nonmelanoma skin cancer.
[d]Assessed in 38 patients who received at least one course of preemptive treatment with valganciclovir.
[e]Reasons for valganciclovir withdrawal: 15 myelotoxicity and one noncompliance.

was observed only with acute T-cell–mediated rejection, not antibody-mediated rejection. This finding is clinically relevant because, even in the era of modern immunosuppression, T-cell–mediated rejection, including subclinical rejection, is associated with a number of adverse outcomes such as persistent inflammation, progression of fibrosis, development of dnDSA and, finally, increased risk of graft loss.[8,39,40]

In this study and other studies, both strategies of CMV prevention have been shown to be highly effective in preventing the development of CMV disease.[14,24] Late CMV disease predominated in our study, which may be beneficial given that some authors report its easier control.[41] In the case of preemptive therapy, the prerequisites are weekly monitoring of CMV DNAemia with prompt therapy initiation in patients reaching the viral load threshold and, most importantly, high compliance with the protocol achieved in our study because the preemptive approach may completely fail with a less intense surveillance protocol.[16,17] Particularly in the preemptive therapy group, we observed a higher incidence of recurrent CMV DNAemia then reported previously.[42] The finding is likely to be partly influenced by frequent monitoring with high sensitive PCR for CMV. Consistent with earlier studies, no patient with ganciclovir-resistant CMV infection was identified in either group despite systematic genotypic testing[22,24]; however, our patient population included only a small number of patients at risk of developing ganciclovir resistance.[21] An important piece of information for patient management was the need to repeat a course of preemptive valganciclovir in most patients because of recurrent CMV DNAemia, a fact not involving only the at-risk D+R− group (although recurrence was clearly the most common issue in this particular subgroup) and one making the logistics of preemptive therapy more complex and intricate. The need to repeat preemptive valganciclovir was partly because of the relatively low viral load threshold (1000 IU/ml) predefined in our study compared with previous trials.[14,24] However, the low threshold was chosen to enhance patient safety given the well-known correlation between viral load and CMV disease and, also, the risk of indirect CMV effects.[10,43] Because of the rapid viral kinetics, the incidence of CMV DNAemia with a higher viral load was even so higher in the preemptive therapy group. It is debatable whether all episodes of asymptomatic CMV DNAemia reaching threshold levels indeed required therapy. It has been suggested that T-cell–mediated CMV-specific immunity be included in the decision-making algorithm of preemptive therapy.[44]

Despite multiple courses of preemptive valganciclovir, total valganciclovir exposure was markedly lower in patients managed by preemptive therapy with an approximately three times shorter therapy duration and 30% cumulative dose reduction. The lower exposure to valganciclovir is associated with lower costs and, most importantly, lower drug toxicity.[19] Our study documented a higher incidence of leukopenia and neutropenia in the prophylaxis group; valganciclovir had to be temporarily discontinued in 23% of patients. However, no difference was found in the incidence of severe neutropenia or use of the granulocyte colony-stimulating factor. Overall, the safety profile of valganciclovir prophylaxis was a favorable

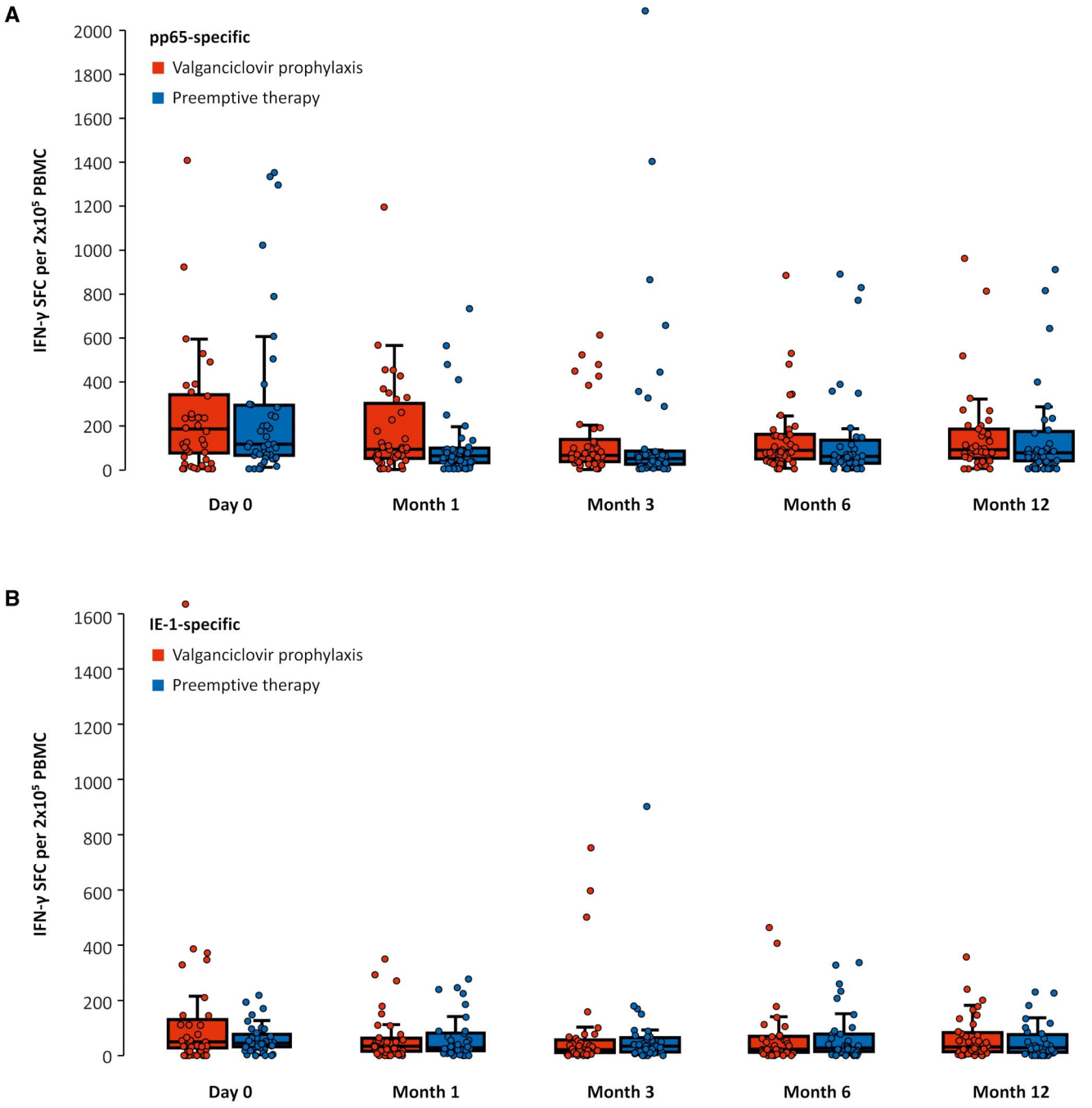

Figure 6. Cytomegalovirus-specific T-cell response by IFN-γ–secreting cells in seropositive recipients. Assessed by the enzyme-linked immunosorbent spot assay after stimulation by (A) pp65 or (B) IE-1 antigen pools and expressed as SFC per 2×10^5 PBMCs. No significant differences were found in comparisons between prophylaxis and preemptive therapy. In both valganciclovir prophylaxis and preemptive therapy groups, there were significant differences between pre-transplant and post-transplant values in both pp65-specific ($P<0.001$) and IE-1–specific ($P=0.001$ and $P=0.004$) response by Friedman analysis of variance on ranks. Minimum and maximum values excluding outliers are represented by whiskers; median and interquartile range are inside boxes. SFC, spot-forming cell.

one. The safety of prophylaxis may even be improved by the use of T-cell–mediated CMV-specific immunity to guide early discontinuation of valganciclovir.[45]

Except for the higher incidence of pneumonia in the preemptive therapy group, which, however, was observed in the early postoperative period and is unlikely to be related to CMV prevention, there were no differences between the two groups in the incidence of other infections. The incidence of BKV viremia and PVAN was also identical, although our previous study documented an increased risk associated with

valganciclovir prophylaxis with potential impairment of T-cell function, a critical factor in the control of BKV replication.[27,46] However, over half of the patients in the preemptive therapy group were also treated with valganciclovir, and the higher incidence of acute rejection and antirejection therapy resulted in accumulation of immunosuppression, possibly affecting the incidence of BKV viremia. It should be noted that the increase in BKV viremia was seen in patients receiving valganciclovir prophylaxis only versus valacyclovir prophylaxis and not preemptive therapy.[12] To be able to draw authoritative conclusions, further studies are warranted comparing valganciclovir prophylaxis with other antiviral drugs not affecting T-cell function such as maribavir.[47]

Similar to previous studies, the level of pretransplant CMV-specific T-cell immunity was predictive of the development of CMV DNAemia.[44,48] CMV immunity after transplantation was comparable in both groups, a surprising observation especially in D+R− patients. When using the preemptive approach, liver transplant recipients have been repeatedly shown to experience more rapid development of CMV-specific T-cell response as a consequence of previous episodes of CMV DNAemia.[18,38] In our study, ELISpot could be performed in only a small number of D+R− patients, a fact making relevant conclusions impossible.

This study has several limitations. First, for reasons of logistics, given the nature of interventions in the preemptive therapy group, it was not a blinded one. Nonetheless, the main outcomes, *i.e.*, rejection and CMV DNAemia, were defined by the biopsy finding or PCR test, and both the pathologists and the physicians assessing the latter were blinded to treatment allocation. Furthermore, the single-center design precludes generalization of our results to another population, a fact applying primarily to D+R− patients whose number was small and making conclusive analyses impossible. The effect on acute rejection or prevention of CMV may differ with other immunosuppressive protocols, not only generally, when using induction therapy, but also in regimens including a mammalian target of rapamycin inhibitors, associated with a lower risk of CMV infection.[49] Currently, most kidney transplant recipients, mostly in the United States, receive induction therapy, which limits the potential benefit of valganciclovir prophylaxis.[50] On the other hand, the reduction in subclinical rejection did not seem to be dependent on induction therapy. Last but not least, a consideration not to be disregarded is the ability of the transplant center to ensure intensive CMV monitoring and patient compliance, as well as a prompt initiation of preemptive valganciclovir therapy, factors critical for the success of preemptive therapy. Annual follow-up and early protocol biopsy do not allow to detect subclinical rejection in the later post-transplant period and are inadequate to assess the real effect of late-onset CMV DNAemia, a condition characteristic for prophylaxis. As a result, short-term outcomes may put the preemptive therapy approach at a disadvantage, as was the case in a previous comparison with valacyclovir prophylaxis.[15] On the other hand, asymptomatic CMV DNAemia after prophylaxis completion was not treated in our study. It cannot be ruled out that possible treatment could improve the prevention of CMV indirect effects. The planned long-term follow-up and protocol biopsy at 3 years should answer the question whether a decrease in the incidence of acute rejection in the early posttransplant period in patients receiving valganciclovir prophylaxis will alleviate the chronic histologic changes and graft function in the long run.

In conclusion, compared with preemptive therapy, valganciclovir prophylaxis in kidney transplant recipients did not result in a significantly lower incidence of acute rejection. Prophylaxis is associated with a lower risk of subclinical rejection at month 3 and reduced incidence of CMV DNAemia. In the presence of intensive CMV monitoring and maintenance of a high compliance rate, preemptive therapy is equally effective in preventing CMV disease at a lower cumulative valganciclovir exposure and a lower incidence of neutropenia. Further research with long-term follow-up is warranted to be able to finally compare both regimens.

DISCLOSURES

D. Lysak reports Consultancy: AbbVie, AstraZeneca, Janssen, and Novartis. J. Machová reports Other Interests or Relationships: Member of the Czech Society of Nephrology and Member of the Czech Transplant Society. All remaining authors have nothing to disclose.

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DATA SHARING STATEMENT

The clinical trial dataset is available through the persistent web link: https://1drv.ms/u/s!AkD3z7X17uL90kV4sp1EOCGSCyO2?e=nEWH8w

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://links.lww.com/JSN/D743.

Supplemental Figure 1. Timing of acute rejection and CMV DNAemia in individual patients.

Supplemental Figure 2A and B. Receiver-operating characteristic curve analysis for prediction of CMV DNAemia.

Supplemental Table 1. PCR and sequencing primers used in analysis of resistance-associated mutations in UL54 and UL97 genes.

Supplemental Table 2. Immunosuppressive therapy during the study.

Supplemental Table 3. Protocol biopsy findings at 3 months after transplantation.

Supplemental Table 4. Renal function and graft and patient survival.

Supplemental Table 5. Cumulative valganciclovir exposure for cytomegalovirus prevention in an intention-to-treat population.

Supplemental Table 6. Incidence of infection other than cytomegalovirus within 12 months.

Supplemental Table 7. Cytomegalovirus-specific T-cell responses assessed by ELISpot.

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