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ORIGINAL ARTICLE

Pre-emptive intravenous ganciclovir versus valganciclovir prophylaxis for *de novo* cytomegalovirus-seropositive kidney-transplant recipients

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[Correction added after online publication (04 June 2010): author names were transposed. These are now corrected as follows: Laurence Lavayssiere, David Ribes, Olivier Cointault and Marie-Béatrice Nogier]

Keywords
cytomegalovirus, glomerular-filtration rate, kidney-transplant, neutropenia, prophylaxis, valganciclovir.

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Abstract

This sequential study evaluated two strategies regarding human cytomegalovirus (HCMV) infection/disease in HCMV-seropositive *de novo* kidney-transplant patients. The first cohort of patients (group 1; $n = 132$) was monitored sequentially for HCMV DNAemia; if it was positive (a cut-off at 3 $\log_{10}$copies/ml), the patient was given pre-emptive IV ganciclovir therapy (10 mg/kg/day for 3 weeks). The second cohort consisted of 150 patients (group 2) who were given valganciclovir (VGC) prophylaxis (900 mg/day) for the first 3 months posttransplantation. During the mean follow-up of at least 2 years for both cohorts, VGC prophylaxis resulted in a significant decrease in both CMV infection (68.9% vs. 33.3%; $P < 0.001$) and disease (9.8% vs. 2.68%, $P = 0.021$). Factors associated with HCMV reactivation in multivariate analysis were (i) no HCMV prophylaxis; (ii) recipient's age; (iii) being placed on ciclosporine A and mycophenolic acid from the beginning of transplantation (iv) donor HCMV-seropositivity; and (v) being a male recipient. No cases of ganciclovir resistance were detected in the prophylactic group. HCMV prophylaxis had no impact on 2-year patient/graft survival or on kidney-allograft function. We conclude that VGC-prophylaxis can be reasonably used to treat HCMV-seropositive kidney-transplant recipients.

Introduction

Human cytomegalovirus (HCMV) can cause morbidity in solid-organ transplant recipients. HCMV infection is also an independent risk factor for graft loss and patient mortality/morbidity [1]. It can also influence graft survival in various ways: HCMV can promote both acute [2–4] and chronic allograft rejection [5], and is associated with an increased incidence of graft arteriopathy [6]. Recently, Reischig *et al.* [7] have shown that HCMV infection is associated with increased risk of interstitial fibrosis/tubular atrophy in kidney-transplant recipients. The link between HCMV and chronic allograft dysfunction occurs not only in the context of HCMV disease, but also in asymptomatic HCMV infections [8].

The incidence of HCMV disease is greater in high-risk serogroups, i.e., donor (D) +/recipient (R)−, and in HCMV intermediate-risk groups (R+/D+, R+/D−). The incidence of HCMV infection at 3 months post-transplantation in these three serogroups who do not receive antiviral prophylaxis was found to be about 70% [9].

Many studies show that HCMV prophylaxis can improve HCMV-related morbidity, and patient- and graft-survival in high-risk patients (D+/R−) by decreasing HCMV's 'indirect effects' [4,10,11]. In a registry study, Opelz *et al*. failed to demonstrate any benefit of a prophylaxis regimen in R+/D+ and R+/D− transplant recipients, but this was possibly because anti-HCMV prophylaxis at that time was performed with less-powerful agents as are currently in use [4]. In contrast, Kliem *et al*. have recently shown that prophylaxis with oral ganciclovir versus a pre-emptive therapy was associated with increased 4-year graft-survival rates in D+/R+ recipients [12].

Currently, no data are available concerning valganciclovir (VGC) prophylaxis to prevent HCMV infection in HCMV intermediate-risk patients, i.e., R(+) patients. However, this drug has recently been shown to be as effective as oral ganciclovir (GCV) in preventing HCMV infection in high-risk solid organ-transplant patients, i.e., D+/R− patients [13]. Therefore, we conducted a retrospective, single-center study on HCMV-seropositive kidney-transplant recipients to compare two strategies: either a pre-emptive strategy where i.v. ganciclovir was given when HCMV DNAemia was found to be positive (first cohort), or a prophylaxis strategy where patients were given 3 months of valganciclovir therapy (second cohort). We analyzed (i) the incidence of HCMV infection and disease, and (ii) patient- and graft-survival rates at 1 and 2 years posttransplantation.

Patients and methods

Patients

Kidney-transplant recipients who received a graft at Toulouse Rangueil University Hospital between 01/06/02 and 31/12/07 were included in this retrospective study. Of the 610 kidney transplantations conducted during this period, we only selected HCMV-seropositive patients ($n = 282$). The patients were divided into two groups. The first group, who had received a transplant before 30 November 2005 ($n = 132$: group 1), did not receive any HCMV prophylaxis, but were assessed fortnightly during the first 3 months of posttransplantation for HCMV DNAemia, and thereafter at monthly intervals for a further 3 months. All patients who presented with HCMV-DNAemia above 3 $\log_{10}$copies/ml were given pre-emptive IV ganciclovir therapy adapted to estimated creatinine clearance [eCC] for 2 weeks. This therapy consisted of 5 mg/kg × two/day if eCC was greater than 60 ml/min; 2.5 mg/kg/day if eCC was between 30 and 60 ml/min; 2.5 mg/kg/day if eCC was between 10 and 30 ml/min; 1.25 mg/kg/day if eCC was less than 10 ml/min; and 2.5 mg/kg after each dialysis session if required.

The second group, the valganciclovir prophylactic group, included patients who had received a transplant after 01 December 2005 (group 2; $n = 150$). The patients received valganciclovir prophylaxis starting on day 4 post-transplantation. Valganciclovir was given at 900 mg a day if eCC was greater than 60 ml/min; at 450 mg once a day if eCC was between 40 and 60 ml/min; at 450 mg every other day if eCC was between 25 and 40 ml/min; and at 450 mg twice weekly if eCC was between 10 and 25 ml/min. Valganciclovir was temporarily stopped if hemodialysis was required. The dosage was re-adapted if necessary at each follow-up visit. Valganciclovir treatment was continued for the first 3 months posttransplantation.

During HCMV prophylaxis, HCMV DNAemia was assessed on a fortnightly basis. After the end of prophylaxis, HCMV DNAemia was assessed monthly for 3 months. HCMV reactivation was treated for 2 weeks with IV ganciclovir therapy adapted to estimated creatinine clearance [eCC] (i.e., 5 mg/kg × two/day if eCC was greater than 60 ml/min; 2.5 mg/kg/day if eCC was between 30 and 60 ml/min; 2.5 mg/kg/day if eCC was between 10 and 30 ml/min; and 1.25 mg/kg/day if eCC was less than 10 ml/min). In both groups, any occurrence of HCMV disease was treated with intravenous ganciclovir for three weeks (10 mg/kg/day adapted to eCC).

At years 1 and 2 posttransplantation, and at the end of follow-up, we analyzed (i) HCMV infection and disease, (ii) patient-survival and graft-survival rates, and (iii) kidney-allograft function (creatinine and eCC according to the MDRD formula). We also studied acute allograft-rejection rates as well as the presence of posttransplant *de novo* diabetes mellitus.

Methods

HCMV DNA was extracted from 200 µl of whole blood using a MagNA Pure instrument (Roche Molecular Biochemicals) as previously described [14]. HCMV DNA was detected and quantified using the Light Cycler system (Roche Applied Science, Indianapolis, IN, USA). DNAemia results were conveyed to physicians within 48 h. HCMV serology (IgG and IgM) was assessed with ETI-CYTOK-G Plus and ETI-CYTO-M Reverse Analyzer (DIAsorin, Antony, France). At the same time as HCMV DNAemia was assessed, we also measured the following: alanine (ALT)-, aspartate (AST)- aminotransferase, gamma glutamyl transpeptidase (γGT), alkaline phosphatase (AP), serum creatinine levels, eCC using the simplified MDRD formula, hemoglobin levels, platelet levels, white blood cell (WBC) and polymorphonuclear counts (WBC), and fasting venous glycemia. *De novo* diabetes was defined according to the WHO definition.

Patients were also monitored for symptoms of HCMV disease when HCMV DNAemia was found to be positive. HCMV disease was defined according to agreed criteria [15].

Statistical analyses

An α-level of 5% was used for each endpoint. Data comparisons between the two groups were analyzed using the chi-squared test or Fisher's exact test for qualitative data, and Wilcoxon's test or Student's *t*-test for quantitative data. The endpoints of the follow-up period were also compared using the same methods. A *P*-value of <0.05 was considered statistically significant.

Factors associated with HCMV reactivation, as well as factors predicting kidney-allograft function at 1 and 2 years posttransplantation (<60 ml/min or ≥60 ml/min), were studied by univariate and multivariate analyses. Multivariate analyses used a logistic regression model with the Wald chi-square test. Predictors included in the univariate analysis for HCMV reactivation were age, gender, biological parameters (hemoglobin level, white blood-cell counts, fasting venous glycemia, antiviral prophylaxis, immunosuppressive regimen at baseline, induction therapy, HCMV prophylaxis, number of mismatch HLAs, delayed graft function, immunization for anti-HLA before transplantation, and cold-ischemia time. In addition, age, gender and HCMV serology were assessed from the donors' data.

Factors included in the univariate analysis for prediction of kidney-allograft function at 2 years were the same those used to predict HCMV reactivation, plus the occurrence of acute rejection and HCMV reactivation during the first 2 years, creatinine clearance at 1 and 6 months posttransplantation, and the number of dialysis sessions during the first week posttransplantation. Factors associated by univariate analyses with a significance of $P < 0.2$ with HCMV reactivation or allograft function at 2 years posttransplantation were selected for multivariate analyses. An α-level of 10% in multivariate analysis was chosen to select the predictive factors. The incidence of HCMV reactivation, HCMV disease, acute graft rejection, and *de novo* diabetes mellitus were compared between the two groups using the Kaplan–Meier method and a log-rank test.

Results

Follow-up times were 34.1 ± 6.1 months for the pre-emptive strategy (group 1) and 23 ± 9.2 months for the prophylaxis strategy (group 2). Groups 1 and 2 did not differ significantly except for panel-reactive alloantibodies and donor age, which were significantly lower in group 1 (see Table 1). The patients' immunosuppressive regimens are summarized in Table 2. Patients were given a standard regimen of calcineurin inhibitor (CNI), mycophenolic acid and steroïds. In some cases during the follow-up time, mTOR was used as the inhibitor instead of CNI.

Significantly more patients in group 2 than in group 1 received an induction therapy; this was mainly due to the use of more anti-CD25 monoclonal antibodies (51.5% vs. 64.7%, respectively; $P < 0.001$), whereas the use of polyclonal antilymphocyte preparations was similar in both groups (Table 1). Immunosuppressive medications given at transplantation were similar across the two groups, except for ciclosporine A, which was more frequently used in group 1 (57.7% vs. 36.7%; $P < 0.001$), and belatacept, which was more frequently used in group 2 (0.7% vs. 14.7%; $P < 0.001$; Table 2).

HCMV infection

HCMV reactivation at one year posttransplantation occurred in 67.4% and 28% of pre-emptive and prophylactic groups, respectively ($P < 0.001$). At the end of the follow-up, the respective incidences of HCMV reactivation for the pre-emptive and prophylactic groups were 68.9% and 33.3% ($P < 0.001$; Fig. 1). Prophylaxis treatment resulted in a significantly longer median time until the first HCMV infection: i.e., 211 (10–732) days for the prophylactic group vs. 45 (8–770) days for the pre-emptive group, $P < 0.001$). However, the mean number of HCMV viremia episodes that required antiviral treatment per patient with HCMV infection was not different between the two groups (1.6 ± 1 for the prophylactic group vs. 2 ± 1.3 for the pre-emptive group). In addition, the mean maximal viral load was not significantly different between the two groups (3.82 ± 0.63 $\log_{10}$/ml for the prophylactic group vs. 3.99 ± 1.02 $\log_{10}$/ml for the pre-emptive group). During valganciclovir prophylaxis, only two patients exhibited HCMV reactivation (1.3%). Viral-load data related to these two cases are 3.72 $\log_{10}$/ml and 3.18 $\log_{10}$/ml. In addition, each patient was given 450 mg valganciclovir once a day despite a creatinine clearance of ≥60 ml/min.

Factors associated with HCMV infection in univariate analyses with a significance of $P < 0.2$ were: age at baseline, gender, prophylaxis, donor's age, donor's HCMV-serology, immunosuppressive regimen at baseline (ciclosporine A/mycophenolic acid and tacrolimus/mycophenolic acid regimen). Factors associated with HCMV reactivation in multivariate analyses are summarized in Table 3. These include (i) no HCMV prophylaxis with valganciclovir; (ii) recipient's age; (iii) receiving ciclosporine A and mycophenolic acid since posttransplantation; (iv) donor HCMV-seropositivity and (v) being a male recipient.

HCMV disease

The overall incidence of HCMV disease was significantly greater in the pre-emptive group compared to the prophylactic group (9.8% vs. 2.68%, $P = 0.021$).

Table 1. Demographic data from the recipients and donors within each group.

	Group 1 (pre-emptive treatment) (*n* = 132)	Group 2 (prophylactic) (*n* = 150)	*P*-value
Recipient gender			
Male	74 (56.1%)	85 (56.7%)	ns
Female	58 (43.9%)	65 (43.3%)	
Median age recipient (years)	47.5 (19–74)	50.8 (21–76)	0.052
Anti-HLA immunization (I or II)	24 (18.2%)	54 (36%)	<0.001
Anti-HLA alloantibodies : PRA>80% (anti-class-I and/or -II)	8 (6%)	11 (7%)	ns
Diabetes mellitus prior to transplantation: Yes	6 (4.5%)	13 (8.6%)	ns
Initial nephropathy			
Genetic	35 (26.5%)	36 (29%)	ns
Chronic glomerulonephritis	54 (40.9%)	60 (40%)	
Vascular nephropathy	13 (9.8%)	20 (13.3%)	
Uropathy	12 (9.1%)	15 (10%)	
Mismatch HLA-A			
0	44 (33.3%)	42 (28.0%)	ns
1	66 (50.0%)	91 (60.7%)	
2	22 (16.6%)	17 (11.3%)	
Mismatch HLA-B			
0	62 (47.0%)	71 (47.3%)	ns
1	52 (39.4%)	68 (45.3%)	
2	18 (13.6%)	11 (7.3%)	
Mismatch HLA-DR			
0	32 (24.2%)	46 (30.7%)	ns
1	62 (47.0%)	76 (50.7%)	
2	38 (28.8%)	28 (18.7%)	
Donor median age (years)	43.1 [15–74]	49.1 [7–77]	0.002
Donor gender			
Male	77 (58.3%)	79 (52.7%)	ns
Female	5 (41.7%)	71 (47.3%)	
Donor CMV serology			
Positive	72 (54.5%)	85 (56%)	ns
Negative	60 (45.4%)	65 (44%)	
Median cold-ischemia time (h)	17.6 [0–43]	17.2 [0–37]	ns
Delayed graft function*: yes	45 (34.1%)	68 (45.3%)	0.055
Dialysis session needed the first week after transplantation: yes	29 (22%)	49 (32.7%)	0.045

HLA, human leukocytes antigen; CMV, cytomegalovirus; Mismatch HLA=0 : no mismatch; Mismatch HLA=1 : one mismatch HLA; Mismatch HLA=2 : two mismatches HLA

*Delayed graft function was defined as a creatinine reduction ratio from posttransplant day 1 to day 2 ≤ 30% (Govani MV *et al*. J Am Soc Nephrol 2002; 13: 1645).

The incidence of HCMV disease within the first 100 days posttransplantation was significantly greater in group 1 (8.3%) compared to group 2 (0%; $P = 0.01$). However, after day 100 posttransplantation, 1.65% of group 1 patients presented with HCMV disease compared to 2.68% of group 2 patients (P = ns).

HCMV resistance

HCMV mutations were looked for if viral load was not decreased after 14 days of antiviral therapy. No cases of ganciclovir resistance were detected in the prophylactic group, whereas there was one case within the pre-emptive group. This resistance was related to a mutation of the HCMV UL97 gene.

Effects of HCMV prophylaxis on patient survival and allograft function

The long-term follow-up showed similar mortality rates, i.e., 3% in group 1 compared to 4.7% in group 2. None of these deaths were related to HCMV infection. Likewise,

Table 2. Immunosuppressive treatment used on the day of transplantation for both pre-emptive and prophylactic groups.

Treatment	Group 1 (pre-emptive) (n = 132)	Group 2 (prophylactic) (n = 150)	P-value
Calcineurin inhibitors	131 (99.3%)	127 (84.7%)	ns
Tacrolimus (Tac)	55 (41.7%)	72 (48%)	ns
Cyclosporine A (CsA)	76 (57.6%)	55 (36.7%)	<0.001
CTLA4Ig (Belatacept) + MMF	1 (0.7%)	22 (14.7%)	<0.001
FTY 720 (Fingolimod) + CsA	6 (4.5%)	0 (0%)	0.01
AEB 071 (Sotrastaurin)+ Tac	0 (0%)	3 (2%)	ns
mTOR inhibitors (sirolimus or everolimus)	9 (6.8%) (+ Tac=9)	11 (7.3%) (+ Tac=:9; + AEB071 = 1; + CsA=1)	ns
Mycophenolic acid	116 (88.5%)	137 (91.3%)	ns
Corticosteroids	123 (93.9%)	137 (91.3%)	ns
Induction treatment (Yes; %)	101 (76.5%)	143 (95.3%)	<0.001
ATG	33 (25%)	46 (30.7%)	ns
Anti-CD25 antibodies	68 (51.5%)	97 (64.7%)	0.034

MMF, mycophenolate mofetil; ATG, anti-thymoglobulins.

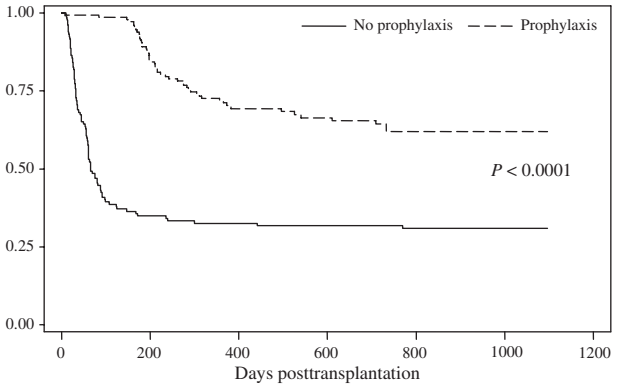

Figure 1 Incidence of CMV reactivation in the two treatment groups: survival curves were obtained using the Kaplan–Meier method.

Table 3. Independent risk factors for CMV reactivation at last follow-up.

	Odds ratio (95% CI)	P-value
No CMV prophylaxis	5.66 (3.21–10)	<0.0001
Age of recipients		
<55 years:	Reference	0.025
55–65 years:	1.99 (1.09–3.66)	0.0004
>65 years:	4.83 (2–11.63)	
Ciclosporine A and mycophenolic acid given from the day of transplantation (No)	0.47 (0.272–0.812)	0.0068
Donor CMV seropositivity (Yes)	2.03 (1.17–3.53)	0.012
Gender = Male	1.8 (1.04–3.12)	0.035

CMV, cytomegalovirus; CI, confidence interval.

the rate of death-censored kidney-allograft survival at last follow-up was similar in the two groups, i.e., 96.2% in group 1 compared to 97.3% in group 2.

The incidences of biopsy-proven acute-rejection episodes (cellular and humoral) at one year and at the end of follow-up were similar between the two groups. At 1 year, 24.2% of patients from the prophylactic group had experienced at least one episode of acute allograft rejection compared to 25.3% of patients from the pre-emptive group (P = 0.941). At the end of follow-up, the incidence of acute allograft rejection was 27.3% in the prophylactic group and 31.1% in the pre-emptive group (P = 0.492; Fig. 2).

Kidney-allograft function, as assessed by estimated creatinine clearance (eCC) at 6 months posttransplantation, was significantly better in group 1 (53.3 ± 17.3 vs. 48.6 ± 16.3 ml/min, P = 0.014). In contrast, at 1 and 2 years posttransplantation, mean eCC was not statistically different between the two groups, i.e., 51.2 ± 16.8 for group 1 compared to 49.2 ± 17 ml/min for group 2 at 1 year, and 50.9 ± 15.5 for group 1 compared to 50.6 ± 18.2 ml/min for group 2 at 2 years.

Because the follow-up periods were different for the two groups, i.e., almost 3 years for group 1 and almost 2 years for group 2, we determined the independent factors that predicted kidney-allograft function at 2-years posttransplantation. Kidney-allograft function was classified as either good, i.e., an eCC rate of ≥60 ml/min, or average, i.e., an eCC rate of <60 ml/min. Univariate analyses revealed 14 parameters that had a significance of $P < 0.2$. For the recipient these were age, ciclosporine A or tacrolimus uptake at baseline, and number of mismatches of HLA-A and HLA-B. For the donor data these were age, gender, HCMV-serology. Acute rejection during the first 2 years, cold-ischemia time, number of dialysis sessions during the first week posttransplantation, HCMV reactivation during the first 2 years, and eCC at 1 and 6 months posttransplantation were also included.

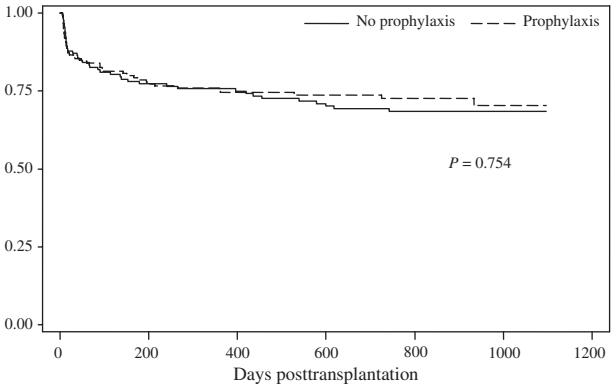

Figure 2 Survival curve of freedom of acute graft rejection according to prophylaxis vs. no prophylaxis (Kaplan–Meier curves).

Multivariate analyses were performed to define the independent factors associated with good allograft function at 2 years posttransplantation: the significant factors were eCC at 1 month posttransplantation, eCC at 6 months posttransplantation, donor HCMV-seronegative, and the absence of HCMV infection within the first 2 years posttransplantation (Table 4).

HCMV prophylaxis and de novo diabetes

The effect of HCMV prophylaxis on the incidence of de novo diabetes was evaluated. At one year, 12.4% patients within the prophylaxis group developed de novo diabetes compared with 18.6% of pre-emptive patients ($P = 0.218$). By the end of follow-up, there was a trend towards less de novo diabetes occurring within the prophylactic group (14.6%) compared to the pre-emptive group (23%; $P = 0.065$).

Tolerability

From transplantation until 2-year posttransplantation, liver-function tests and hematological parameters were not statistically different between the two groups. The only exception was leucocyte count, which was significantly lower in group 2; however, this difference was not clinically relevant.

Neutropenia, as defined by a neutrophil rate of <1000/mm^3, was only recorded in the prophylaxis group; this was observed in 14.6% ($n = 22$) of prophylaxis patients. Prophylaxis was permanently withdrawn before 90 days in four patients for this reason.

Table 4. Independent risk factors associated with creatinine clearance ≥60 ml/min at 24 months posttransplantation.

	Odds ratio (95% CI)	*P*-value
Creatinine clearance at 1 month (10 mL/min increase)	1.27 (0.97–1.65)	0.07
No CMV reactivation in the first 24 months	2.11 (0.97–4.56)	0.057
Creatinine clearance at 6 months (10 mL/min increase)	2.19 (1.57–3.06)	<0.0001
Donor CMV positivity (Yes)	0.50 (0.23–1.09)	0.08

CMV, cytomegalovirus; CI, confidence interval.

Discussion

Our study is the first to demonstrate that using valganciclovir prophylaxis (as compared to a pre-emptive strategy) for HCMV-seropositive kidney-transplants recipients results in significant benefits. This occurs particularly in terms of reduced HCMV infection and disease at the end of follow-up, which was almost of 2 years duration posttransplant. Whether to use universal prophylaxis vs. pre-emptive therapy to prevent HCMV infection/disease in kidney-transplant recipients is still not ascertained in 2009. However, evidence now suggests that universal prophylaxis vs. no prophylaxis is efficient in that population by reducing the incidence of the following events: (i) HCMV infection, HCMV disease, HCMV disease-related mortality, and all-cause mortality, and (ii) Herpes type 1 or 2 infections by 73%, Varicelle Zoster virus by 35%, and protozoal infections by 69%, but not fungal infections [10,11]. Of note, published meta-analysis studies have not included valganciclovir therapy, except for Kalil *et al.*, who reported that valganciclovir had no greater efficacy than other standard therapies [16]. It has also been shown that HCMV prophylaxis with ganciclovir or valganciclovir may modify the natural posttransplant history of some viruses [17].

In kidney-transplant recipients, only one study published so far has compared ganciclovir prophylaxis with pre-emptive ganciclovir-therapy. This randomized study included 138 de novo recipients, though excluded those who were D-/R- [12]. The authors observed that prophylaxis with oral ganciclovir significantly reduced both HCMV infection (by 65%) and HCMV-related re-hospitalizations, and also increased 4-year allograft survival (92.2% vs. 78.3%, $P = 0.04$). Our study, similarly demonstrates that HCMV-seropositive kidney recipients had significantly lower rates of HCMV infection and disease when given valganciclovir prophylaxis compared to a pre-emptive therapy. However, this was not translated into better long-term allograft survival, though the follow-up period of our patients was only 2 years instead of 4.

When we looked at the independent predictive factors for presenting with positive HCMV DNAemia at post-

transplant we found that the strongest factor was the absence of HCMV prophylaxis. This was followed by the recipient's age. Others have found that if the recipient's age is >55 [18] or >60 [19] this acts as an independent risk factor for developing HCMV infection or HCMV disease.

A further predictive factor was the concomitant use of mycophenolic acid and ciclosporine A from the day of transplantation; this resulted in an Odds ratio of 2.12. It has been shown that kidney-transplant recipients receiving mycophenolate mofetil (MMF)-therapy, compared to azathioprine therapy, have an increased risk for HCMV disease [20]. Song *et al.* have written a mini-review on this topic [21]. Similarly, we showed that maintenance immunosuppression with ciclosporine A associated with mycophenolic acid (vs tacrolimus/mycophenolic acid) is an independent risk factor for HCMV infection and/or disease. This is in accordance with previous publications that demonstrated a higher risk of CMV infection or CMV disease with a maintenance immunosuppression containing ciclosporine A [18,19]. Additionally, we have found that donor HCMV-seropositivity is an independent risk factor for HCMV reactivation after kidney transplantation in HCMV-seropositive patients [22].

Development of late-onset HCMV disease is of critical importance when investigating HCMV prophylaxis in organ-transplant patients, particularly after HCMV prophylaxis in high-risk patients, i.e., D+/R− for HCMV [13,23,24]. In order to circumvent the occurrence of late HCMV disease after prophylaxis, attempts have been made to prolong prophylaxis to 6 months [25,26]. In our study, late onset HCMV disease was not increased in patients receiving the prophylactic strategy. In fact, the incidence of HCMV disease after 100 days posttransplantation was similar between the two groups: 2.65% in group 2 compared to 1.65% in group 1. This result suggests that late-onset HCMV disease occurs mainly in high-risk sub-group patients (D+/R−).

The incidence of HCMV disease during preemptive treatment was surprisingly high (i.e., 8.3% in the first 3 months). However, a relatively long delay before initiating antiviral therapy (median time estimated as 14 days) can explain this result.

There was no HCMV reactivation within the prophylactic period except for two cases: this reactivation occurred because these two patients were received insufficient valganciclovir. Thus, we conclude that monitoring HCMV DNAemia during prophylaxis is of no benefit and, therefore, can be stopped.

We found that an absence of HCMV infection was associated with improved allograft function at 2 years posttransplant, as defined by eCC ≥60 ml/min with an OR of 2.11 (95% CI: 0.97–4.56). Kliem *et al.* have shown that serogroup D+/R+ patients exhibited more allograft failures at 4-years posttransplantation when given pre-emptive ganciclovir-therapy compared to ganciclovir prophylaxis [12]. Similar to Kliem's report, in our study we found that having a HCMV-seropositive donor tended to be associated with worse eCC at 2 years posttransplant.

Were not able to demonstrate any long-term renal benefits of valganciclovir prophylaxis as assessed by eCC, but this may be related to the posttransplant follow-up period of only 2 years, and to the higher frequency of delayed graft function within group 2 (data not shown). Thus, HCMV prophylaxis was not a predictive factor for improved renal function at 2 years. Allograft function at 6 months posttransplantation was better in group 1, but this is probably related to the increased incidence of delayed graft function within group 2, and because donors were significantly younger in group 1. However, renal-allograft function was similar between the two groups at 1- and 2-years posttransplant. The transient nature of the improved renal function of group 1 may be because antiviral prophylaxis ameliorates allograft renal function; however this association remains to be demonstrated. The strongest independent predictive factor for an eCC of ≥60 ml/min in our population was eCC at 6 months posttransplant, i.e., this has an OR of 2.19 (1.57–3.06) for each 10 ml/min increase. Hence, this result does agree with previous studies [27,28].

In addition, unlike others studies that report HCMV prophylaxis decreased the risk of acute-rejection episodes, at least in D+/R− patients [4,29], we did not demonstrate a benefit of using prophylaxis with the onset of acute rejection in HCMV-seropositive kidney-transplant patients.

We also sought to determine whether prophylaxis with valganciclovir decreased the incidence of *de novo* diabetes mellitus, as some studies demonstrate that HCMV infection can promote posttransplant diabetes [30]. However, we did not find any significant difference between the prophylactic and pre-emptive treatments regarding the incidence of this disease. Although there was a trend towards a lower incidence of posttransplant diabetes in group 2 patients by the end of follow-up, the mean duration of follow-up was less in this group.

Using valganciclovir may result in an increased rate of neutropenia. But, in our study, only four patients needed valganciclovir withdrawn before 3 months because of neutropenia. Moreover, no severe sepsis was associated with neutropenia. However, other studies have highlighted this toxicity [13,31]. Leuconeutropenia seems to be more frequent with this drug than with other anti-CMV medications (acyclovir, ganciclovir) according to a recent meta-analysis [16].

Our study contains certain limitations. First, the retrospective nature of this study and the relatively short follow-up time are its main weaknesses. In addition, immunosuppressive regimens at baseline and data related to the donor are different between the two groups: thus this could create some discordance when combining the data sets. However, our results show that a 3-month course of valganciclovir prophylaxis reduced both HCMV infection and HCMV disease in intermediate-risk kidney-transplant recipients.

HCMV morbidity is known to increase mortality (1) as well as cost-related recurrent hospitalizations. HCMV infection was significantly associated with poor renal-allograft function in our study. Unfortunately, we were unable to demonstrate the long-term benefits to renal-allograft function in HCMV-seropositive recipients receiving HCMV prophylaxis. Nonetheless, considering the known deleterious effects of HCMV infection, we argue that antiviral prophylaxis given to intermediate-risk patients can be warranted. However, because some of the above results may be due to shortcomings within our study (as discussed above), a prospective study that includes a larger cohort should be performed to test whether valganciclovir prophylaxis is effective in these HCMV-seropositive patients.

Authorship

HW performed the study, wrote the paper, collected data and analyzed data. LE, LL, RD, CO, MBN, and ICD: collected data. JI and CM: contributed important reagents. NK and LR: performed the study and analyzed data. AOM: analyzed data.

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