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ORIGINAL ARTICLE

Six months anti-viral prophylaxis significantly decreased cytomegalovirus disease compared with no anti-viral prophylaxis following renal transplantation

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Summary

We followed up 550 primary kidney transplant recipients in an observational retrospective cohort to evaluate the impact of three consecutive cytomegalovirus (CMV) prevention strategies. In period 1 (1996–2000; $n = 190$), no anti-CMV prophylaxis was given; in period 2 (2000–2004; $n = 173$), 6-month valacyclovir was given and in period 3 (>2004; $n = 187$), 6-month valganciclovir was given. Cytomegalovirus disease significantly decreased from 33.2% in period 1 to 13.9% in period 2 and to 8.6% in period 3; onset was significantly prolonged with valganciclovir (228 days) compared with valacyclovir (93 days) and with no prophylaxis (33 days). After Cox regression adjustments, both valganciclovir and valacyclovir were similarly protective factors for CMV disease. Cytomegalovirus diseases encountered in both valacyclovir and valganciclovir groups were primary infections (79.2 and 93.8% respectively) as compared with a significant low number (39.7%) in the nonprophylaxis group. Two cases of valganciclovir resistance were recorded in the valganciclovir group and no resistance was seen with valacyclovir. A significantly reduced incidence of other herpes viruses was only observed with valganciclovir. Valganciclovir was better tolerated than valacyclovir and this long-term prophylaxis was applicable to 85% of patients. Longer follow-up of valganciclovir or valacyclovir prophylaxis is still required to appreciate its impact on graft and patient survivals, as well as other indirect effects, in the mycophenolate mofetil and calcineurin inhibitor immunosuppressive era.

Introduction

Cytomegalovirus (CMV) infection is a common infectious disease among solid organ transplant recipients; more than 50% of patients have laboratory evidence of infection within the first year [1]. Clinical symptoms of CMV infection could be related to direct effects of viral replication, such as fever, leucopaenia, thrombocytopaenia, with or without specific organ dysfunction, and/or indirect effects because of the influence of the virus on the host's immune response, such as acute or chronic rejection of the transplanted organ [2], reduced long-term graft function [3], increased risk of other opportunistic infections and malignancies and reduced patient survival [1,4].

The highest risk of CMV disease is principally observed in CMV sero-negative recipients receiving kidney grafts from CMV sero-positive donors (D+/R−), in recipients treated with polyclonal anti-lymphocyte or anti-thymocyte antibodies and more recently, among patients receiving rituximab therapy [1–6]. *De novo* infection with a new CMV strain can also occur in CMV sero-positive individuals [1–7]. Pre-emptive anti-CMV therapy with ganciclovir or 3-month valganciclovir prophylaxis are currently the gold standard strategies used in CMV donor

positive and recipient negative (D+/R−) transplant recipients [5,7].

Ganciclovir was introduced in the 1980s and early 1990s to treat CMV disease in solid-organ transplant recipients [1] and still remains the standard-of-care for management of overt CMV infection. Ganciclovir is a synthetic analogue of 2′-deoxyguanosine. It is first phosphorylated to a deoxyguanosine triphosphate (dGTP) analogue. This competitively inhibits the incorporation of dGTP by viral DNA polymerase, resulting in the termination of elongation of viral DNA. The impact of pre-emptive therapy for ongoing asymptomatic CMV infection on the direct and indirect effects of CMV is still questionable [5,8]. In contrast, prophylaxis with ganciclovir has proved to be successful in prevention of CMV infection and disease [1–4]. However, because of a low oral bioavailability (about 6–10%), a total of 3 g of ganciclovir administered as 12 capsules/day in a three times a day regimen was needed to deliver plasma exposure of 40–50% of that was achieved with the standard 5 mg/kg dose of i.v. ganciclovir [9,10]. This low bioavailability may limit the degree of viral suppression that can be achieved [11] and may predispose to emergence of viral resistance [12].

Acyclovir was the first antiviral drug developed for systemic use that exhibited activity preferentially against herpes virus-infected cells. It is a 2′-deoxyguanosine analogue and requires activation by the viral-encoded thymidine kinase followed by cellular kinases. The final product, acyclovir triphosphate, irreversibly inhibits viral DNA polymerase via competition with dGTP, preventing further chain elongation. However, acyclovir has poor oral bioavailability and requires either higher dosing or intravenous administration. Valacyclovir (a valine ester of acyclovir) provides a high bioavailability of acyclovir, 3- to 5-fold higher than that obtained with oral acyclovir and it is equivalent to plasma levels achieved with doses of intravenous acyclovir. Valacyclovir was also given for anti-CMV prophylaxis, but its efficacy lacked consistency in several prophylaxis strategies probably because of poor patient's clinical tolerance and compliance [13–17].

Valganciclovir, a prodrug for ganciclovir, is the valine (L-valyl) ester of ganciclovir. The valine ester increases the oral bioavailability 10-fold compared with the original ganciclovir formulation. Valganciclovir was developed to overcome the limitations of oral and i.v. ganciclovir, with a single once-daily 900 mg oral dose providing comparable plasma ganciclovir exposures to those achieved with 5 mg/kg i.v. ganciclovir, with a bioavailability 10-fold higher than that of oral ganciclovir [9]. After oral administration, valganciclovir is rapidly converted to ganciclovir by intestinal and hepatic esterases. Less than 2% of the absorbed valganciclovir dose appears in the plasma as valganciclovir within 3 to 4 h after a dose. Ganciclovir is converted to ganciclovir monophosphate in the viral cell by a viral protein kinase encoded by the UL97 gene. Cellular enzymes phosphorylate ganciclovir monophosphate converting it first to ganciclovir diphosphate and then to ganciclovir triphosphate. Ganciclovir triphosphate inhibits viral DNA polymerase, which interferes with viral DNA synthesis and has a virustatic effect.

Although generally accepted, the precise duration of valganciclovir prophylaxis is not yet determined; 3 months is the standard duration today applied. However, because of the potential recurrence of CMV infection after 3 months of prophylaxis with either ganciclovir or valacyclovir [12,15,18], we have been adopting since the beginning of our prophylaxis approach at the end of 1999, a systematic longer prophylaxis course of 6 months, first with valacyclovir and then with valganciclovir.

The purpose of our study was to compare the incidence of CMV disease (i.e. symptomatic CMV infection or active CMV infection) and its potential after effects in three consecutive periods among the mycophenolate mofetil (MMF) and calcineurin inhibitor (CNI) immunosuppressive era, in which three different strategies of CMV prophylaxis were applied. All patients included in this study were transplanted at the same institution and followed by the same transplant physician and viral laboratory teams throughout time.

Patients and methods

In our centre, i.v. ganciclovir was introduced in 1987 to treat ongoing symptomatic CMV infection (i.e. CMV disease). This strategy persisted until the end of 1999 when 6-month prophylaxis with valacyclovir was introduced for all patients, excepting D−/R−. In the year 2004, 6-month valganciclovir prophylaxis replaced valacyclovir with similar patient population indications. The reasons for this change were mainly valacyclovir side-effects and greater patient compliance expectancy with valganciclovir.

We conducted an observational retrospective cohort analysis of 550 kidney transplant recipients who received CMV prophylaxis with either valacyclovir or valganciclovir and those who did not receive CMV prophylaxis. First-line i.v. ganciclovir was given for overt CMV disease for at least 2 weeks in all cases. We selected all consecutive patients with primary kidney transplants (re-transplants and simultaneous pancreas-kidney transplants were not included) and who received an immunosuppressive regimen based on universal induction, MMF and CNI as follows: induction with either basiliximab (Simulect®; Novartis Pharmaceuticals, Rueil Malmaison, France) or rabbit anti-thymocyte globulin (ATG, Thymoglobulin®; Genzyme Corporation, Saint Germain en Laye, France), and maintenance with CNI (cyclosporin, CyA; Neoral®;

Novartis Pharmaceuticals or Tacrolimus, Tac; Prograf®; Astellas, Levallois-Perret, France) and MMF (Cellcept®; Roche, Neuilly-sur-Seine, France). This standard induction/CNI/MMF-based immunosuppressive regimen varied within time; more patients received ATG induction in periods 1 and 2 and more patients received basiliximab and Tac in period 3 (see below and Table 1). In all three periods, corticosteroids (Cs) were either avoided or given during the first 8–12 weeks following transplantation and subsequently stopped.

From the 550 selected patients, 190 did not receive anti-CMV prophylaxis (period 1; 1996–2000), 173 received valacyclovir (period 2; 2000–2004) and 187 received valganciclovir (period 3; 2004–2009). The valganciclovir and valacyclovir prophylactic doses were adjusted based on serum creatinine and estimated creatinine clearance per manufacturer guidelines. Patients developing CMV disease in all three periods were treated, if no contraindication, with i.v. ganciclovir for at least 2 weeks.

Cytomegalovirus disease was defined as active CMV infection with attributable symptoms either 'CMV syndrome' (fever, malaise, or leucopaenia) or by organ dysfunction in the absence of other documented causes. Active CMV infection was defined as detection of CMV in blood or in other appropriate tissue specimens. The definition of CMV disease used was consistent with the American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation [19]. Cytomegalovirus diagnostic tests for the no prophylaxis and valacyclovir eras were based on CMV-DNA detection loads measured by in

Table 1. Patient's demographic characteristics.

	No prophylaxis (*n* = 190)	Valacyclovir prophylaxis (*n* = 173)	Valganciclovir prophylaxis (*n* = 187)	Valacyclovir versus no prophylaxis (*P*-value)	Valganciclovir versus no prophylaxis (*P*-value)	Valganciclovir versus valacyclovir (*P*-value)
Transplantation year	1996–2000	2000–2004	2004–2008	–	–	–
Recipient						
Age, years (mean ± SD)	51 ± 13	53 ± 14	53 ± 15	0.240	0.333	0.443
Male, *n* (%)	97 (51)	115 (66)	123 (66)	0.003	0.004	0.889
Cause of renal failure, *n* (%)						
Chronic glomerulonephritis	60 (31.5)	57 (32.9)	49 (26.2)			
Chronic tubulo-interstitial nephritis	1 (0.5)	–	–			
Pyelonephritis	24 (12.6)	36 (20.8)	40 (21.4)			
Polycystic kidney	31 (16.3)	14 (8.1)	11 (5.9)			
Diabetic nephropathy	8 (4.2)	15 (8.7)	16 (8.6)			
Hypertensive nephrosclerosis	17 (8.9)	10 (5.8)	22 (11.8)			
Chronic renal insufficiency	23 (12.1)	26 (15)	24 (12.8)			
Others	26 (13.7)	15 (8.7)	25 (13.4)			
No. HLA-A-B-DR mismatches (mean ± SD)	3.5 ± 1.5	3.5 ± 1.5	3.5 ± 1.3	0.826	0.078	0.139
CMV sero-status, *n* (%)						
D+/R–	48 (25.3)	73 (42.2)	67 (35.8)			
Others	142 (74.7)	100 (57.8)	120 (64.2)	<0.001	0.026	0.216
Donor						
Age, years (mean ± SD)	46 ± 17	51 ± 15	53 ± 16	0.001	<0.001	0.816
Male, *n* (%)	119 (62.6)	109 (63)	108 (57.8)	0.941	0.333	0.309
Origin, *n* (%)						
Cadaver	176 (92.6)	154 (89)	166 (88.8)			
Living	14 (7.4)	19 (11)	21 (11.2)	0.232	0.196	0.941
Cold ischaemia time in min (mean ± SD)	1519 ± 684.6	1371 ± 683.2	1230 ± 619.8	0.041	<0.001	0.085
Induction therapy, *n* (%)						
ATG	80 (42)	75 (43)	20 (11)			
Anti-CD25 moAb	67 (35)	93 (54)	166 (89)	0.810	<0.001	<0.001
Maintenance IS, *n* (%)						
CyA	85 (44.7)	22 (12.7)	16 (8.6)	<0.001	<0.001	0.148
Tac	24 (12.6)	69 (39.9)	141 (75.4)	<0.001	<0.001	<0.001

HLA, human leucocyte antigen; CMV, cytomegalovirus; ATG, thymoglobulin; anti-CD25 moAb, basiliximab; CyA, cyclosporin; Tac, tacrolimus; IS, Immunosuppression.

house quantitative methods: a competitive PCR on isolated polymorph nuclear leucocytes [20,21]. A real time quantitative PCR on whole blood samples was utilized since 2003 [22].

Outcome

The main outcome measure was the incidence of CMV disease after transplantation. Other efficacy outcomes assessed included: acute rejection, patient and graft survival, other infections, malignancies, hypertension and *de novo* insulin-dependent diabetes mellitus. Graft loss included all patients who returned to dialysis and those who died with failed graft. Patients who died with functioning graft were censored.

Statistical analyses

Quantitative parametric data were compared between the groups using Student's *t*-test and the Mann–Whitney *U*-test in nonparametric distribution. Cross-tabulated data were analysed by chi-square test or by Fisher test when expected cell count was <5. Patient and graft survival, incidence of CMV disease were drawn using Kaplan–Meier curves, and compared with the log-rank test. The Cox proportional hazard model, which allows time-dependent covariate, was utilized to estimate the risk factors for the development of post-transplant CMV disease and to assess independent covariates on graft survival. All survivals were adjusted to 4-year follow-up from the transplant date to overcome the confounding factor of time of follow-up. The proportionality of hazards was respected for each variable. Data were analysed according to the intention-to-treat principle. SPSS software for windows version 16 (Statistical Product and Services Solutions, version 16, SPSS Inc, Chicago, IL, USA) was utilized. Values of $P < 0.05$ were considered statistically significant.

Results

Patients

The patient's demographic characteristics are summarized in Table 1. Recipients' age was comparable between groups. The proportion of male patients increased significantly in both the valacyclovir and valganciclovir prophylaxis groups compared with the no prophylaxis group. Chronic pyelonephritis and diabetic nephropathy were predominant causes of renal failure among the valacyclovir and valganciclovir prophylaxis groups as compared with the no prophylaxis group. Hypertensive nephrosclerosis as a cause of renal failure was dominant in the valganciclovir prophylaxis group compared with the no prophylaxis and valacyclovir prophylactic groups. The number of human leucocyte antigen (HLA) mismatches was not significantly different among the three groups. Cytomegalovirus sero-status of the recipients was significantly different between the no prophylaxis and valacyclovir prophylaxis group, but not between the no prophylaxis and valganciclovir prophylaxis nor among the valacyclovir and valganciclovir prophylaxis groups. Donor age was significantly lower in the no prophylaxis group (46 ± 17 years) compared with the valacyclovir (51 ± 15 years) and valganciclovir prophylaxis groups (53 ± 16 years). Percentages of living donors and cadaver donors were comparable among groups, with almost 90% of cadaver transplants in all three groups. Cold ischaemia time decreased significantly over time through the no prophylaxis, valacyclovir prophylaxis and valganciclovir prophylaxis groups respectively. Basiliximab was the major induction therapy in the valganciclovir prophylaxis group compared with no prophylaxis and valacyclovir prophylaxis groups in which ATG was more used. A significant higher number of recipients received Tac as primary immunosuppression regimen in the valacyclovir and valganciclovir prophylaxis groups compared with the no prophylaxis group. Transplant recipients were more compliant with the valganciclovir drug intake (85%) compared with the valacyclovir (34%).

CMV disease

The incidence of CMV disease during each treatment period is shown in Table 2; the incidence was the highest in the no prophylaxis group (33.2%) and the lowest in the valganciclovir prophylaxis group (8.6%; $P < 0.001$). Percentages of CMV disease occurring during the first 6 months following transplantation were also significantly lower ($P = 0.018$) in the valganciclovir prophylaxis group (6 from 16; 37.5%) as compared with valacyclovir prophylaxis group (18 from 24; 75%) and with no prophylaxis group (57 from 63; 90.5%). A significant ($P < 0.001$) delayed onset of post-transplant CMV disease was observed in the valganciclovir prophylaxis group in comparison with the no prophylaxis and valacyclovir prophylaxis groups. Cytomegalovirus disease was restricted mainly to the high-risk (D+/R−) patients in the valacyclovir (79.2%) and valganciclovir (93.8%) prophylaxis groups, but was more evenly distributed among both the high-risk and intermediate-risk groups (D+/R+ and D−/R+) in the no prophylaxis group. As previously mentioned, the majority of CMV diseases diagnosed in the valganciclovir prophylaxis group occurred after the end of the anti-viral prophylaxis (10 from 16; 62.5%). All transplant recipients suffering from CMV disease responded to i.v. ganciclovir treatment with the exception of two cases

Table 2. Efficacy outcomes.

	No prophylaxis (n = 190)	Valacyclovir prophylaxis (n = 173)	Valganciclovir prophylaxis (n = 187)	Valacyclovir versus no prophylaxis (P-value)	Valganciclovir versus no prophylaxis (P-value)	Valganciclovir versus valacyclovir (P-value)
Full 6-month prophylaxis (%)	NA	34	85	–	–	–
Post-transplant CMV disease; Total, n (%)	63 (33.2)	24 (13.9)	16 (8.6)	<0.001	<0.001	0.109
No. CMV disease according to donor/recipient CMV sero-status						
D+/R–, n (%)	25 (39.7)	19 (79.2)	15 (93.8)	0.001	<0.001	0.206
Others	38 (60.3)	5 (20.8)	1 (6.2)			
No. CMV cases according to post-transplantation time						
<6 months, n (%)	57 (90.5)	18 (75)	6 (37.5)	0.061	<0.001	0.018
>6 months, n (%)	6 (9.5)	6 (25)	10 (62.5)			
No. CMV cases according to rejection time, n (%)						
No rejection	31 (49.2)	14 (58.3)	4 (25)	0.446	0.082	0.801
Before rejection	21 (33.3)	8 (33.3)	7 (43.8)	NS	0.437	0.145
After rejection	11 (17.5)	2 (8.3)	5 (31.3)	0.286	0.220	0.145
Median onset of CMV disease; days (range)	33 (7–2264)	93 (23–353)	228 (32–748)	0.740	0.164	0.044
Episodes of graft rejection, n (%)						
No rejection	138 (72.6)	127 (73.4)	139 (74.3)	0.867	0.709	0.842
Acute rejection	16 (8.4)	13 (7.5)	22 (11.8)	0.750	0.281	0.174
Chronic rejection	32 (16.8)	30 (17.3)	22 (11.8)	0.900	0.159	0.133

CMV, cytomegalovirus.

of CMV resistance in the 6-month valganciclovir prophylaxis group which required rescue therapy with foscavir for ongoing CMV disease.

Graft rejection

The proportion of patients without graft rejection episodes remained relatively stable over time and within 70% among the three groups (Table 2). Rejection occurring in the no prophylaxis and valacyclovir prophylaxis groups was more often chronic rejection unlike in the valganciclovir group where the incidence of acute and chronic rejections was the same. No difference was observed in the onset of CMV disease in relation to acute rejection episode among all three groups. In the valganciclovir prophylaxis group, five (31.3%) from the observed 16 CMV diseases occurred after an episode of acute rejection. This percentage was higher than that observed in the valacyclovir prophylaxis group (8.3%) and in the no prophylaxis group (17.5%); however, because of the small number of recorded events, no statistical difference was obtained.

Graft and patient survival

Although the absolute number of graft failures was significantly lower ($P < 0.001$) in the valganciclovir prophylaxis group as compared with the other two groups [5 (2.7%) in the valganciclovir group, 26 (15%) in the valacyclovir group and 28 (14.7%) in the no prophylaxis group], actuarial graft survivals did not differ. Main reason of this difference may be the longer follow-up of patients in the valacyclovir and no prophylaxis groups as compared with the valganciclovir prophylaxis one.

Actuarial patient survival did not differ among all groups. However, absolute number of deaths was significantly lower in the valganciclovir group [n = 11 (5.9%); $P = 0.001$] and in the valacyclovir group [n = 12 (6.7%); $P = 0.008$] when compared with the no prophylaxis group [n = 33 (17.4%)]. Interestingly, no death among patients with CMV disease was noted in the in the valganciclovir prophylaxis group. Longer follow-up analysis will determine a possible positive effect of both anti-viral prophylaxis on graft and patient outcomes.

Safety outcomes

Table 3 summarizes the incidence of selected safety outcomes. Of note, infection with other herpes viruses was more common and statistically higher among patients who did not receive CMV prophylaxis, but only statistically lower in the valganciclovir prophylaxis group. Number of other viral complications was very low in all

Table 3. Safety outcomes.

	No prophylaxis (n = 190)	Valacyclovir prophylaxis (n = 173)	Valganciclovir prophylaxis (n = 187)	Valacyclovir versus no prophylaxis (P-value)	Valganciclovir versus no prophylaxis (P-value)	Valganciclovir versus valacyclovir (P-value)
Herpes viral infections, *n* (%)	68 (35.8)	25 (14.5)	10 (5.3)	0.479	<0.001	0.014
Herpes Simplex virus	48 (25.2)	16 (9.2)	6 (3.2)			
Herpes zoster virus	19 (10)	9 (5.2)	3 (1.6)			
Epstein Barr virus	1 (0.5)	–	–			
Human Herpes virus 8	–	–	1 (0.5)			
Other viruses, *n* (%)						
Hepatitis B virus	1 (0.5)	–	–			
Polyoma (BK) virus	1 (0.5)	3 (0.17)	6 (0.32)			
UTI, *n* (%)	86 (45.3)	76 (43.9)	67 (35.8)	0.799	0.074	0.117
Sepsis, *n* (%)	3 (1.6)	06 (3.5)	13 (7)	0.248	0.010	0.140
Hypertension, *n* (%)	92 (48.4)	79 (45.7)	48 (25.7)	0.599	<0.001	<0.001
Malignancy, *n* (%)						
PTLD	3 (1.5)	1 (0.5)	1 (0.5)	0.261	0.261	0.157
Skin cancer	15 (8)	11 (6.3)	6 (3.2)	0.406	0.420	0.256
Other cancers	9 (4.7)	8 (4.6)	3 (1.6)	0.378	0.350	0.527
De novo IDDM, *n* (%)	24 (12.6)	28 (16.1)	39 (20.8)	0.334	0.032	0.255
Immunosuppression regimen among diabetic recipients, *n* (%)						
MMF	14 (7.4)	11(6.4)	6 (3.2)	0.704	0.072	0.159
CyA	5 (2.6)	–	2 (1.1)	–	0.261	–
Tac	5 (2.6)	17 (9.8)	31 (16.6)	0.004	<0.001	0.060

UTI, urinary tract infection; PTLD, post-transplant lymphoproliferative disorder; IDDM, insulin-dependent diabetes mellitus; MMF, mycophenolate mofetil; CyA, cyclosporin; Tac, tacrolimus; Cs, corticosteroids.

groups and more polyoma BK virus infection was observed in the valganciclovir prophylaxis group. Malignancies did not differ among all groups, but again follow-up of the valganciclovir prophylaxis group was shorter. The incidence of *de novo* insulin dependent diabetes mellitus was significantly higher in the valganciclovir prophylaxis group as compared with the no prophylaxis group (P = 0.032). When immunosuppression was analysed among this *de novo* diabetic patient population (91/550; 16.6%), a statistically significant difference was observed in either the valacyclovir and valganciclovir prophylaxis groups when Tac immunosuppression was given, suggesting a more diabetogenic effect of this drug compared with CyA.

Univariate analysis for risk factors for CMV disease is shown in Table 4. From the six factors reaching statistical significance (HLA mismatches, CMV pre-transplant serostatus, cold ischaemia time, induction therapy, Cs therapy and CMV prophylaxis), three were independently confirmed by Cox PH: CMV D+/R−, ATG induction therapy and CMV prophylaxis (Table 5).

Univariate analysis for graft survival revealed five risk factors: HLA mismatches, maintenance immunosuppression based on CNI, acute rejection, chronic rejection and CMV prophylaxis (Table 6). Only two factors had independent negative influence on graft survival: acute rejection and chronic rejection (Table 7).

Discussion

Our 6 months anti-CMV prophylaxis significantly reduced the incidence of CMV disease from 33.2% with no anti-viral prophylaxis to 13.9% with valacyclovir and to 8.6% with valganciclovir (valacyclovir versus valganciclovir, P = NS) following primary kidney transplantation in the MMF/CNI era. Cytomegalovirus diseases observed in the valacyclovir and valganciclovir prophylaxis groups were mainly primary infections, representing an incidence of respectively 26 and 22.4% in this high-risk D+/R− population. This percentage is consistent with observations on the prevention of post-transplant CMV infection and related outcomes with valganciclovir [23,24], but much lower than the 48% recently reported in a small cohort of patients who received 6-month valganciclovir prophylaxis [25].

In a retrospective study assessing the efficacy and safety of various anti-CMV strategies in the high-risk CMV population (D+/R−), a 3-month based strategy of valganciclovir significantly reduced the CMV asymptomatic infection, but not the incidence of CMV disease at 12 months when compared with valacyclovir or i.v. ganciclovir given pre-emptively. Late onset of CMV disease (i.e. after 3 months) remains a problem in this category of patients with these two last treatment's strategies [26]. The results of this study suggest that

Table 4. Significant factors for cytomegalovirus disease (univariate analysis).

	Total no. patients	*P*-value
Recipient age (years)		
<55	291	0.290
>55	259	
Recipient gender		
Female	215	0.308
Male	335	
No. HLA mismatches		
<3	239	0.072
>3	310	
Pre-transplant CMV sero-status		
D+/R−	188	<0.001
Others	362	
Donor age (years)		
<55	328	0.432
>55	222	
Donor gender		
Female	214	0.459
Male	336	
Donor origin		
Cadaver	496	0.420
Living	54	
Cold ischaemia time (min)		
<1000	141	0.090
>1000	408	
Induction therapy		
Basiliximab	326	<0.001
ATG	175	
Maintenance immunosuppression		
Cyclosporin	123	0.273
Tacrolimus	234	
Corticosteroids		
No	90	0.001
Yes	270	
Graft rejection		
No rejection	404	–
Acute rejection	52	0.029
Chronic rejection	83	0.693
CMV prophylaxis		
No prophylaxis	190	–
Valacyclovir	173	<0.001
Valganciclovir	187	<0.001

ATG, thymoglobulin; CMV, cytomegalovirus; HLA, human leucocyte antigen.

although 3-month prophylaxis with valganciclovir was effective regarding viral replication, this period of time was sub-optimal to prevent late onset of CMV disease. In our present study, doubling the prophylaxis period of valganciclovir to 6 months allowed a significantly delayed onset of CMV disease (median 228 days) compared with 93 days with 6 months valacyclovir and 33 days with no prophylaxis at all. Whether delaying the appearance of CMV disease has an impact on graft and patient survival is not yet documented and requires longer follow-up.

The majority of CMV diseases observed in the valganciclovir prophylaxis group was diagnosed after the end of the 6 months prophylaxis (10 from 16; 62.5%), in a significant contrast with the other two groups: 9.5% in the no prophylaxis group and 25% in the valacyclovir prophylaxis group. This finding may suggest that a prolongation of anti-viral prophylaxis or closer viral monitoring could be required, principally in the high-risk D+/R− transplant population. Of interest, we did not find any correlation between acute rejection episode and CMV disease onset. A similar incidence of CMV disease was observed before and after the rejection episode onset in all the three groups. Because of the delayed onset of the CMV disease observed in the valganciclovir prophylaxis group, as well as the low number of recorded events, more episodes of CMV disease following an episode of rejection were noted in this group, although no statistical significance was reached (Table 2).

Our data showed a trend towards better graft survival with 6 months prophylaxis with valganciclovir when compared with 6 months valacyclovir or no prophylaxis. This confounding observation may be clinically relevant if confirmed within time and confirms the trend towards fewer graft losses observed with the reported 3-month universal CMV prophylaxis with valganciclovir [26]. In our study, graft losses, as well as deaths, observed in the valganciclovir prophylaxis group occurred in patients without CMV disease, suggesting that valganciclovir may prevent indirect effects among infected patients, commonly known to develop more chronic allograft failure and death than noninfected patients [27,28]. The fact that the incidence of chronic rejection was lower in the valganciclovir prophylaxis group compared with the no prophylaxis group may support this hypothesis.

Valganciclovir was well tolerated and 85% of treated patients were able to receive the entire 6-month course, compared to only 34% of patients receiving valacyclovir. However, two patients developed an UL97 mutation and valganciclovir resistance requiring rescue therapy for overt CMV disease. These two patients were successfully rescued with foscavir. No patient in the valacyclovir group developed valganciclovir resistance.

It was reported that herpes virus-6 co-infections were a common finding in renal allograft patients with previous CMV infection [29]. The use of ganciclovir and valganciclovir had no clear effect on the beta herpes viruses co-infections [30]. Our results showed that other types of herpes viral complications were significantly lower in the valganciclovir prophylaxis group, if not all herpes viruses (also HHV-6 and HHV-7) were analysed. This observation may indicate a larger anti-viral spectrum of valganciclovir and a possible effect on viral-related post-transplant-induced malignancies. A single case of

Table 5. Cox proportional hazard analysis of risk factors for cytomegalovirus disease.

	Regression estimate (B)	HR Exp(B) (95% CI)	*P*-value
Pre-transplant CMV sero-status			
Others	–	–	–
D+/R−	1.974	7.20 (3.82–16.50)	<0.001
No. HLA mismatches			
<3	–	–	–
>3	0.126	1.13 (0.60–2.15)	0.701
Cold ischaemia time (min)			
<1000	–	–	–
>1000	0.443	1.55 (0.78–3.16)	0.210
Induction therapy			
Basiliximab	–	–	–
ATG	1.429	4.18 (1.16–15.03)	0.029
Maintenance immunosuppression			
Cyclosporin	–	–	–
Tacrolimus	−0.383	0.68 (0.33–1.41)	0.302
Corticosteroids			
No	–	–	–
Yes	−0.457	0.63 (0.38–1.07)	0.085
CMV prophylaxis			
No prophylaxis	–	–	–
Valacyclovir	−1.135	0.32 (0.15–0.71)	0.005
Valganciclovir	−1.694	0.18 (0.08–0.41)	<0.001

ATG, thymoglobulin; CMV, cytomegalovirus; CI, confidence interval; HLA, human leucocyte antigen.

post-transplant lymphoproliferative disorder was observed in the valganciclovir prophylaxis group; however, the shorter follow-up of this group as compared with valacyclovir and no prophylaxis groups impedes any definitive conclusion. Our data also confirm previously published studies [31], supporting ATG as a risk factor for CMV disease as compared with basiliximab induction. However, basiliximab induction was given to all patients (excepted 2) who experienced CMV disease in the valganciclovir prophylaxis group. These observations indicate the importance of systematic use of anti-viral prophylaxis and closer CMV monitoring in ATG-treated and/or D+/R− patients.

Several limitations of our study deserve to be acknowledged. It is an observational study over different time periods and as such the results might be influenced by improvements in transplant patient care, mainly in immunosuppression. To account for this, Cox proportional hazard regression analyses were performed. A second limitation of this study was the relatively small sample size which undoubtedly decreases the power and ability to detect differences in the populations being compared, particularly within the Cox proportional hazard regression analysis [32]. On the contrary, this study has the advantage of a relatively long-term follow-up of patients, which gives the opportunity to diagnose late-onset CMV disease and its possible impact on graft and patient survival.

Table 6. Significant factors for graft survival (univariate analysis).

	Total no. patients	*P*-value
Recipient age (years)		
<55	291	0.327
>55	259	
Recipient sex		
Female	215	0.320
Male	335	
No. HLA mismatches		
<3	239	0.023
>3	310	
Pre-transplant CMV sero-status		
D+/R−	188	0.635
Others	362	
Donor age (years)		
<55 years	328	0.517
>55 years	222	
Donor gender		
Female	214	0.135
Male	336	
Donor origin		
Cadaver	496	0.114
Living	54	
Cold ischaemia time (min)		
<1000	141	0.113
>1000	408	
Induction therapy		
Basiliximb	326	0.405
ATG	175	
Maintenance immunosuppression		
Cyclosporin	123	0.039
Tacrolimus	234	
Corticosteroids		
No	90	0.866
Yes	270	
Graft rejection		
No rejection	404	–
Acute rejection	52	<0.001
Chronic rejection	83	0.004
CMV prophylaxis		
No prophylaxis	190	–
Valacyclovir	173	0.032
Valganciclovir	187	0.234

ATG, thymoglobulin; CMV, cytomegalovirus; HLA, human leucocyte antigen.

In summary, our study demonstrates that anti-CMV prophylaxis during 6 months with valacyclovir or valganciclovir was efficacious in preventing CMV disease in more than 85% of kidney transplant recipients under universal induction/MMF/CNI-based immunosuppression. A better clinical tolerance and efficiency was observed with valganciclovir than valacyclovir. Valganciclovir was also efficient in preventing herpes simplex and

Table 7. Cox proportional hazard analysis of risk factors for graft survival.

	Regression estimate (B)	HR Exp(B) (95% CI)	*P*-value
Donor gender			
Female	–	–	–
Male	−0.16	0.85 (0.60–1.20)	0.354
No. HLA mismatches			
<3	–	–	–
>3	0.41	1.51 (0.72–3.18)	0.282
Cold ischaemia time (min)			
<1000	–	–	–
>1000	−0.165	0.85 (0.35–2.08)	0.718
Maintenance immunosuppression			
Cyclosporin	–	–	–
Tacrolimus	−0.672	0.51 (0.21–1.22)	0.132
Graft rejection			
No rejection	–	–	–
Acute rejection	0.383	1.47 (1.03–2.09)	0.035
Chronic rejection	0.396	1.49 (1.05–2.11)	0.027
CMV prophylaxis			
No prophylaxis	–	–	–
Valacyclovir	−1.003	0.37 (0.11–1.19)	0.095
Valganciclovir	−0.640	0.53 (0.23–1.20)	0.127

CMV, cytomegalovirus; CI, confidence interval; HLA, human leucocyte antigen.

herpes zoster viruses. Graft and patient survivals did not differ among the studied groups, with a cut-off follow-up of 4 years. The 6-month length of anti-viral CMV prophylaxis appeared to be sufficient to prevent CMV disease in almost all low-risk patients, but this efficacy was less evident in the high-risk D+/R− patient population. Whether this high-risk patient population requires longer anti-viral prophylaxis and/or different CMV monitoring remains to be determined.

Authorship

FL: collected data. AA, MG, JD, GB and J-PS: performed research. AA: performed statistical analysis. DC: designed research, performed research and wrote the paper.

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