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Cytomegalovirus DNAemia Requiring (Val)Ganciclovir Treatment for More Than 8 Weeks Is a Key Factor in the Development of Antiviral Drug Resistance

M. Acquier,[1] B. Taton,[1] S. Alain,[2,3] I. Garrigue,[4] J. Mary,[1] P. Pfirmann,[1] J. Visentin,[5,6] S. Hantz,[2,3] P. Merville,[1,5] H. Kaminski,[1,5,a] and L. Couzi[1,5,a]

[1]Department of Nephrology, Transplantation, Dialysis and Apheresis, Bordeaux University Hospital, Bordeaux, France, [2]National Reference Center for Herpes Viruses, Virology Department, Limoges University Hospital, Limoges France, [3]UMR INSERM U1092, RESINFIT, Limoges University, Limoges France, [4]Laboratory of Virology, Bordeaux University Hospital, Bordeaux, France, [5]CNRS-UMR 5164 ImmunoConcEpT, Bordeaux University, Bordeaux, France, and [6]Laboratory of Immunology and Immunogenetics, Bordeaux University Hospital, Bordeaux, France

Background. Prolonged (val)ganciclovir [(V)GCV] exposure for ≥6 weeks is a known predisposing factor for cytomegalovirus (CMV) drug resistance. However, the selection of this threshold was based on limited data. In this study, we sought to reappraise the risk factors for the development of (V)GCV resistance through a specific focus on kidney transplant recipients (KTRs).

Methods. This single-center retrospective study included 313 consecutive KTRs treated for a first CMV episode. Adjusted Cox multivariate regression analysis was used for identifying independent risk factors.

Results. Antiviral drug resistance was identified in 20 (6%) KTRs. A cumulative (V)GCV exposure for more than 6 weeks (regardless of the viral load) was not associated with antiviral drug resistance (hazard ratio [HR] = 2.45, 95% confidence interval [CI] = 0.33–18.30, $P = .38$). In contrast, persistent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks was the main independent risk factor for antiviral drug resistance (HR = 11.68, 95% CI = 2.62–52.01, $P = .001$). The (V)GCV treatment for more than 8 weeks was given to 9% and 18% of patients who had persistent or recurrent CMV DNAemia, respectively. These scenarios were associated with the occurrence of drug resistance in 39% and 12% of cases, respectively.

Conclusions. Cumulative (V)GCV exposure ≥6 weeks regardless of the viral load is not associated with antiviral drug resistance. In contrast, prolonged exposure to (V)GCV during CMV replication (with a cutoff ³8 weeks) seems to be a key factor.

Keywords. antiviral drug resistance; cytomegalovirus; kidney transplantation; (val)ganciclovir.

Immunocompromised solid organ transplant recipients (SOTRs) are at enhanced risk of opportunistic infections due to cytomegalovirus (CMV) [1]. Based on the results of the VICTOR study [2], oral valganciclovir (VGCV) is recommended for the management of mild-to-moderate disease, whereas intravenous ganciclovir (GCV) should be preferred if the infection is life-threatening [3]. Unfortunately, the emergence of VGCV or GCV [(V)GCV] resistance has been increasingly reported worldwide. The major mechanisms underlying the selection of (V)GCV-resistant CMV are mutations in the viral *UL97* kinase gene and/or the *UL54* DNA polymerase gene [4]. This condition occurs in up to 10% of CMV-infected SOTRs [5–8] and has been associated with longer hospitalization and increased morbidity and mortality [9–11].

Prior studies have identified several risk factors for developing (V)GCV-resistant CMV infections, including high levels of immunosuppression, lack of previous CMV immunity in donor-positive/recipient-negative (D^+/R^-) pairs, high CMV loads, limited drug absorption [8, 9, 12–15], and persistent viral replication [13]. On analyzing a cohort of D^+/R^- kidney transplant recipients (KTRs) treated for CMV infections, we have previously shown that 54% of patients with DNAemia persisting after 7 weeks of anti-CMV treatment ultimately develop antiviral drug resistance [8]. Because prolonged antiviral treatment is a known predisposing factor for drug resistance, current guidelines recommend that patients who have not adequately responded after more than 6 weeks of cumulative (V)GCV exposure should be tested for mutations conferring substantial levels of resistance [3]. However, the selection of this threshold was based on limited data collected in a setting where the drugs were used for both preventive and curative purposes [3]. Therefore, the question as to whether 6 weeks is the optimal cutoff value for KTRs remains unanswered.

In this study, we sought to reappraise the risk factors for the development of (V)GCV resistance through a specific focus on

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[a]H. K. and L. C. share senior authorship.

Correspondence author: Lionel Couzi, MD, PhD, Department of Nephrology, Transplantation, Dialysis and Apheresis, Pellegrin University Hospital, Place Amélie Raba Léon, 33076 Bordeaux, France (lionel.couzi@chu-bordeaux.fr).

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KTRs. In addition, prolonged (V)GCV exposure was analyzed separately in the prophylactic and curative settings.

METHODS

Study Design

This single-center retrospective study was conducted at the Bordeaux University Hospital, (Bordeaux, France) between October 2004 and December 2017. Kidney transplant recipients were eligible if they had been diagnosed with either CMV infection or disease and had undergone CMV treatment. All KTRs were monitored for 2 years after the initial CMV infection requiring treatment. All clinical variables were collected from the R@N database (French data protection authority [CNIL] final agreement, decision 2009–413, number 1357154; July 2, 2009). Written informed consent was obtained from all participants.

Cytomegalovirus Prevention

Different strategies for CMV prevention were implemented throughout the study period. Between January 2004 and November 2006, D^+/R^- KTRs and R + KTRs treated with rabbit antithymocyte globulin received universal prophylaxis with VGCV (900 mg once per day for 3 months), whereas a preemptive strategy was offered to KTRs treated with interleukin-2 receptor antagonists. Between December 2006 and June 2010, all KTRs received a preemptive strategy. With this aim, CMV viral loads were measured by whole-blood, real-time quantitative nucleic acid testing (QNAT) once per week for the first 3 months, twice per month from month 3 to month 6, as well as on months 8, 10, and 12. The criterion for initiation of preemptive therapy was a viral load threshold of 5000 UI/mL. Between July 2010 and December 2017, KTRs received universal prophylaxis for either 6 months (D^+/R^-) or 3 months (R+). After completion of prophylaxis, CMV QNAT was performed at months 4 and 6 for R + KTR and at months 9 and 12, independently of the CMV infection status at transplantation. Thereafter, all KTRs underwent CMV QNAT on an annual basis or when CMV disease was clinically suspected.

Cytomegalovirus Quantitative Nucleic Acid Testing

Different whole-blood CMV QNAT techniques were applied throughout the study. Between October 2004 and June 2012, CMV QNAT was performed using a previously described in-house real-time polymerase chain reaction assay [16, 17] and the results were expressed as copies/mL. With the goal of harmonizing the results with those obtained with more recent QNAT testing techniques, we converted copies/mL to IU/mL using the World Health Organization (WHO) International Standard for human CMV. The thresholds for detection and quantification of CMV DNAemia were both 250 IU/mL. As of June 2012, CMV QNAT was performed using the LightMix Human Cytomegalovirus Kit (TIB MOLBIOL GmbH, Berlin, Germany). The thresholds for detection and quantification of CMV DNAemia were 250 and 1000 IU/mL, respectively. In this study, a CMV QNAT <250 IU/mL was considered as a negative CMV DNAemia (ie, below the detection limit). All of the QNAT assays were performed in our Laboratory of Virology, which is in strict compliance with the standards of the Quality Control for Molecular Diagnostics ([QCMD] Glasgow, Scotland) since 2004.

Definitions of Cytomegalovirus Events

A CMV infection was defined as the presence of a CMV DNAemia ≥1000 IU/mL in the absence of clinical symptoms. In accordance with the recommendations of the American Society of Transplantation and the CMV Drug Development Forum [18], we defined CMV disease as evidence of CMV infection and attributable symptoms, including CMV syndrome and tissue-invasive disease. Late-onset CMV disease was defined as a first episode of CMV disease occurring >3 months (100 days) after transplantation. Conversely, early-onset disease was defined as a first episode of CMV disease occurring within the first 3 months (100 days) after transplantation [19]. Recurrent infection or disease were considered as new infection or disease episodes in patients who had previously undergone treatment with negative CMV DNAemia test results during active posttherapeutic monitoring.

The duration of CMV DNAemia was defined as the time elapsed from the first positive CMV DNAemia to a negative CMV DNAemia test result. When KTRs experienced more than 1 CMV episode, the total duration of CMV DNAemia was calculated as the sum of each episode (Figure 1*A*). The duration of treatment was defined as the period during which a patient received V(GCV) for CMV infection or CMV disease. When KTRs received more than 1 course of (V)GCV for a CMV recurrence, the duration of the (V)GCV treatment was calculated as the sum of all courses (Figure 1*B*).

The cumulative V(GCV) exposure was defined as the sum of the durations of the prophylaxis and all courses of V(GCV) treatment (Figure 1*C*). The duration of CMV DNAemia under (V)GCV treatment was defined as the time elapsed from the day when (V)GCV treatment was started in presence of a positive CMV DNAemia to the day of CMV DNAemia negativization or (V)GCV discontinuation (Figure 1*D*). The sum of all durations was calculated for KTRs who experienced more than 1 episode.

Management of Cytomegalovirus Infection or Disease

Kidney transplant recipients (KTRs) with CMV infection or CMV disease received (val)ganciclovir treatment, either intravenous (IV) GCV (5 mg/kg twice per day) or oral VGCV (900 mg twice per day), with the goal of achieving persistently negative CMV DNAemia [3]. The Cockcroft-Gault equation

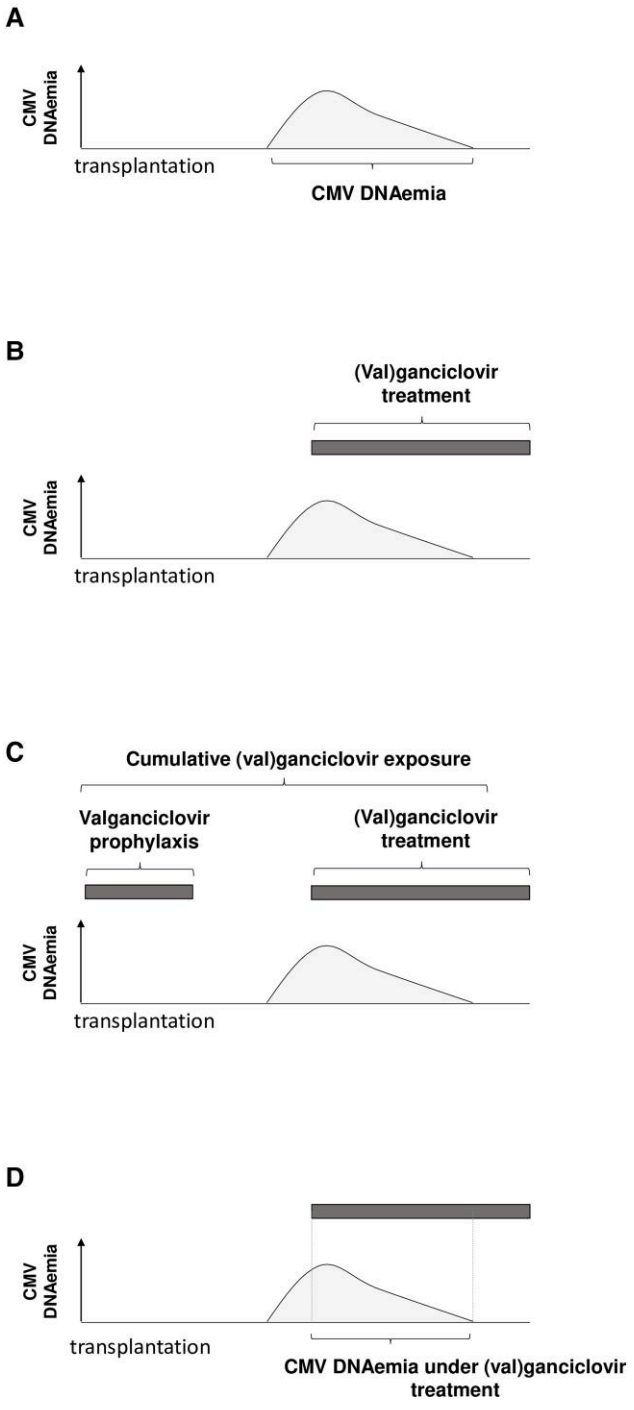

Figure 1. Definition of cytomegalovirus (CMV) DNAemia duration, (val)ganciclovir treatment, cumulative (val)ganciclovir exposure, and duration of CMV DNAemia during (val)ganciclovir treatment. (*A*) Duration of CMV DNAemia is the time elapsed from the first positive CMV DNAemia to a negative CMV DNAemia. (*B*) Duration of (val)ganciclovir treatment is the time interval during which a patient received (val)ganciclovir treatment for either CMV infection or CMV disease. (*C*) Cumulative (val)ganciclovir exposure is the sum of the durations of the prophylaxis and all courses of (val)ganciclovir treatment. (*D*) Duration of CMV DNAemia under (val)ganciclovir treatment is the time elapsed from the day when (val)ganciclovir treatment was started for the presence of a positive CMV DNAemia to either negativization of CMV DNAemia or discontinuation of (val)ganciclovir.

was used for drug-dosing adjustments. Cytomegalovirus QNAT was applied for viral monitoring (once per week until viral eradication followed by monthly assessments for a total of 3 months).

Diagnosis of Antiviral Drug Resistance

The potential presence of anti-CMV drug resistance was investigated at the French National Cytomegalovirus Reference Center (Limoges, France) [20], when a significant increase of CMV load (>1 $\log_{10}$ UI/mL) was observed during treatment with (V)GCV. Resistance was considered to be present when mutations in the viral *UL97* kinase gene and/or the *UL54* DNA polymerase gene were detected. The minimum viral load required for genotypic testing of CMV drug resistance was 3.5 $\log_{10}$ UI/mL.

Statistical Analysis

Receiver operating characteristic (ROC) curve analysis was performed to predict the risk of antiviral drug resistance in relation to the duration of CMV DNAemia during (V)GCV treatment. Univariate Cox regression analysis was initially applied to identify risk factors for antiviral drug resistance. No continuous variable deviated from the assumption of linearity. Covariates with $P < .25$ on univariate analyses were entered into multivariate Cox regression analysis, so 13 were first included, and then with a descending model, only variables with a $P < .05$ were retained. Results were expressed as hazard ratios (HRs) with 95% confidence intervals (95% CIs). Factors associated with the occurrence of a prolonged CMV DNAemia requiring treatment with (V)GCV for more than 8 weeks were identified using logistic regression analyses. Because there were missing data for lymphocyte count at baseline and on day 21, 2 different models were constructed (either with or without the inclusion of this covariate). All analyses were performed using the RStudio statistical software (version 1.1.423; RStudio Inc., Boston, MA, USA).

RESULTS

Study Population

Of the 1792 kidney transplantations performed at our institution between October 2004 and December 2017, we identified 313 (17.5%) KTRs who required antiviral treatment for a first episode of either CMV infection or CMV disease. Table 1 shows the general characteristics of the study participants at the date of CMV detection. The median CMV DNAemia at baseline was 10 900 IU/mL (interquartile range [IQR], 4150–73 850 IU/mL). Antiviral drug resistance was identified in 20 (6%) KTRs after a median of 112 days (IQR, 80–146 IU/mL) from the initiation of antiviral treatment. The median CMV DNAemia at diagnosis of antiviral drug resistance was 39 600 IU/mL (IQR, 15 900–156 000 IU/mL). All cases of antiviral

Table 1. General Characteristics of Kidney Transplant Recipients at the Time of CMV Infection or CMV Disease Requiring treatment

Patient Characteristics	Entire Cohort ($n=313$)
Age at CMV onset, years (median; IQR)	57 (48–65)
Male sex, n (%)	205 (65)
Previous transplant, n (%)	41 (13.1)
Underlying Kidney Disease, n (%)	
Glomerular disease	79 (25)
Tubulointerstitial disease	90 (29)
Vascular disease	35 (11)
Diabetes	20 (6)
Nephrectomy	5 (2)
Congenital disease	31 (10)
Unknown disease	53 (17)
History of dialysis before transplantation, n (%)	262 (84)
Induction of Immunosuppression, n (%)	
Basiliximab	198 (63.3)
Antithymocyte globulin	115 (36.7)
Immunosuppressive Treatment at CMV Onset, n (%)	
Tacrolimus	224 (71.6)
Cyclosporine	79 (25.2)
Mycophenolic acid	286 (91.4)
Azathioprine	14 (4.5)
Steroids	212 (67.7)
mTOR inhibitors	8 (2.6)
Acute Rejection Before CMV, n (%)	
T cell-mediated rejection	28 (8.9)
Antibody-mediated rejection	10 (3.2)
Estimated Glomerular Filtration Rate (mL/min) at CMV Onset, Median, IQR	40 (30–55)
CMV Characteristics	
Donor/Recipient Serostatus at Transplantation, n (%)	
D^+/R^-	143 (45.7)
D^+/R^+	107 (34.2)
D^-/R^+	47 (15)
D^-/R^-	4 (1.3)
Unknown	12 (3.8)
Preventive Strategy Used After Transplantation, n (%)	
Universal prophylaxis	148 (47.3)
Preemptive strategy	148 (47.3)
Unknown	17 (5.4)
Type of CMV Episode at Onset, n (%)	
CMV infection	95 (30.4)
CMV disease	218 (69.6)
Time From Transplant to CMV Onset, Days (Median, IQR)	137 (45–249)
Early-onset CMV, n (%)	142 (45.4)
Late-onset CMV, n (%)	171 (54.6)
Initial anti-CMV therapy, n (%)	
Oral valganciclovir	148 (47.3)
Intravenous ganciclovir	165 (52.7)
Baseline CMV DNAemia, IU/mL (median, IQR)	10 900 (4150–73 850)
Kinetics Characteristics	
Baseline Tacrolimus Blood Concentration, ng/mL (Median, IQR)	8 (7–10)
D 21 tacrolimus blood concentration, ng/mL (median, IQR)	8 (6.8–10.4)
D 49 tacrolimus blood concentration, ng/mL (median, IQR)	8.5 (7–10)
Baseline Cyclosporine Blood Concentration, ng/mL (Median, IQR)	132 (106–165)
D 21 cyclosporine blood concentration, ng/mL (median, IQR)	140 (110–170)
D 49 cyclosporine blood concentration, ng/mL (median, IQR)	120 (100–150)
Baseline Lymphocyte Count, g/L (median, IQR), $n=235$	0.77 (0.38–1.15)
D 21 lymphocyte count, g/L (median, IQR), $n=153$	0.70 (0.42–1.2)
D 49 lymphocyte count, g/L (median, IQR), $n=199$	0.76 (0.42–1.1)

Abbreviations: CMV, cytomegalovirus; D, donor; IQR, interquartile; mTOR, mammalian target of rapamycin; R, recipient.

drug resistance were associated with the presence of at least 1 *UL97* mutation. An additional *UL54* mutation was identified in a single patient (Figure 2). After 2 years of follow up, KTRs who developed antiviral resistance did not differ significantly from those who did not in terms of graft survival (HR = 2.85, 95% CI = 0.68–11.88, $P=.14$).

The Duration of Cytomegalovirus DNAemia During (Val)Ganciclovir Treatment Is the Strongest Risk Factor for Antiviral Drug Resistance

We initially examined whether the occurrence of antiviral drug resistance was associated with the following variables: (1) duration of (V)GCV prophylaxis (categorized as no prophylaxis vs 3 months vs 6 months), (2) duration of CMV DNAemia, (3) duration of (V)GCV treatment, (4) the cumulative V(GCV) exposure (prophylaxis + treatment), and (5) duration of CMV DNAemia during (V)GCV treatment (Figure 1).

No association between the duration of (V)GCV prophylaxis and antiviral drug resistance was observed ($P=.26$) (Figure 3*A*). Kidney transplant recipients who developed antiviral drug resistance had longer median durations of CMV DNAemia (90 days vs 44 days, respectively, $P=.001$) (Figure 3*B*) and (V)GCV treatment (104 days vs 54 days, respectively, $P=.0001$) (Figure 3*C*). No association was observed between the cumulative V(GCV) exposure and antiviral drug resistance ($P=.07$) (Figure 3*D*). Kidney transplant recipients who developed antiviral drug resistance had longer median durations of CMV DNAemia during (V)GCV treatment (87 days [IQR, 69–125 days] vs 33 days [IQ, 20–55 days], respectively; $P<.0001$) (Figure 3*G*) compared with those who did not.

In univariate analyses, a D^+/R^- status, a diagnosis of CMV disease, the administration of IV ganciclovir, the lymphocyte count at baseline and on day 49, and CMV DNAemia during (V)GCV treatment showed significant associations with antiviral drug resistance (Table 2). After adjustment for potential confounders in multivariate model 1, lymphocyte count on day 49 (HR = 0.13, 95% CI = 0.03–0.64, $P=.01$) and a prolonged CMV DNAemia during (V)GCV treatment (HR = 1.06, 95% CI = 1.03–1.09, $P=.001$) continued to show associations with antiviral drug resistance (Table 2).

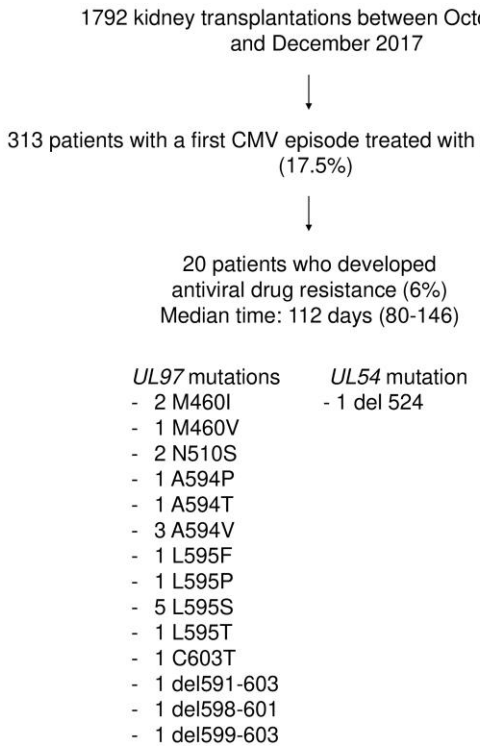

Figure 2. Flow of patients through the study. CMV, cytomegalovirus.

Cytomegalovirus DNAemia Requiring (Val)Ganciclovir Treatment for More Than 8 Weeks Is Associated With an Increased Risk of Antiviral Drug Resistance

Confirming our previous observation, the cumulative (V)GCV exposure was not associated with antiviral drug resistance (area under ROC curve [AUC] = 0.62) (Figure 3*E*). Moreover, antiviral drug resistance was not different between KTRs who experienced a cumulative (V)GCV exposure ≥6 weeks (as proposed in the CMV guidelines) and <6 weeks (6.9% [19 of 276] vs 2.9% [1 of 34], respectively, $P = .38$) (Figure 3*F*).

In contrast, the duration of CMV DNAemia during (V)GCV treatment of more than 57.5 days (ie, 8 weeks) was associated with the occurrence of antiviral drug resistance (sensitivity = 90%, specificity = 76%; AUC = 0.85) (Figure 3*H*). It is notable that antiviral drug resistance occurred more frequently in KTRs who experienced CMV DNAemia requiring (V)GCV treatment for more than 8 weeks (21.2% [18 of 85]) compared with those who did not (0.9% [2 of 228], $P < .001$) (Figure 3*I*). Therefore, 90% (18 of 20) of KTRs who developed antiviral drug resistance showed CMV DNAemia requiring (V)GCV treatment for more than 8 weeks.

We then entered the duration of CMV DNAemia during (V)GCV treatment as a categorical covariate (ie, ≤8 weeks vs >8 weeks) in a second multivariate model. The results revealed that a low lymphocyte count on day 49 (HR = 0.22, 95% CI = 0.05–0.92, $P = .04$) and a persistent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks (HR = 11.68, 95% CI = 2.62–52.01, $P = .001$) were the only independent risk factors for antiviral drug resistance (Table 2).

On analyzing the results of a multivariate model in which lymphocyte counts at different time points were not included, the following parameters were identified as being independently associated with prolonged CMV DNAemia requiring (V)GCV treatment for >8 weeks: history of dialysis before transplantation, a D^+/R^- status at transplantation, the use of a preemptive strategy, and the use of IV GCV as primary treatment for CMV disease. When the lymphocyte count was included in a second multivariate model, independent risk factors associated with prolonged CMV DNAemia requiring (V)GCV treatment for >8 weeks were a D^+/R^- status at transplantation, the use of a preemptive strategy, and a low lymphocyte count on day 21 (Table 3).

Clinical Scenarios Associated With Cytomegalovirus DNAemia Requiring (Val)Ganciclovir Treatment for More Than 8 Weeks

We identified 2 different clinical scenarios associated with prolonged CMV DNAemia requiring (V)GCV treatment for >8 weeks. The first involved KTRs who experienced a single episode of persistent CMV DNAemia treated with (V)GCV for at least 8 weeks ($n = 28$, 9%) (Figure 4*A*). In this patient group, the median viral load measured at 8 weeks after the beginning of (V)GCV treatment was 1000 IU/mL (IQR, 1000–1000 IU/mL), with a median time of CMV DNAemia during (V)GCV treatment of 12.4 weeks (IQR, 9.6–19.8 weeks). Antiviral drug resistance was identified in 39.3% (11 of 28) of these KTRs (Figure 4*D*). The second scenario consisted of patients ($n = 57$, 18%) (Figure 4*B*) who received (V)GCV treatment for at least 8 weeks due to 2 or more CMV episodes (ie, recurrent infection or disease). Of them, 42 KTRs (73.7%) were not receiving any (V)GCV treatment at the time of CMV recurrence, whereas the remaining 15 (26.3%) were still being treated with (V)GCV. In this patient group, the median time of CMV DNAemia during (V)GCV treatment was 11.4 weeks (IQR, 9.9–14.3 weeks) and the prevalence of antiviral drug resistance was 12.3% (7 of 57) (Figure 4*D*).

The remaining 228 KTRs (73%) had CMV DNAemia treated with (V)GCV for less than 8 weeks (Figure 4*C*) and no evidence of CMV recurrences requiring repeated (V)GCV administration. Antiviral drug resistance was extremely rare in this patient group (0.9%; 2 of 228) (Figure 4*D*).

DISCUSSION

Although prolonged exposure to (V)GCV is a well known predisposing factor for the development of CMV antiviral drug resistance, this study provides novel insights into the risk factors and clinical management of this complex condition in KTRs. First, prolonged exposure to (V)GCV for treatment along

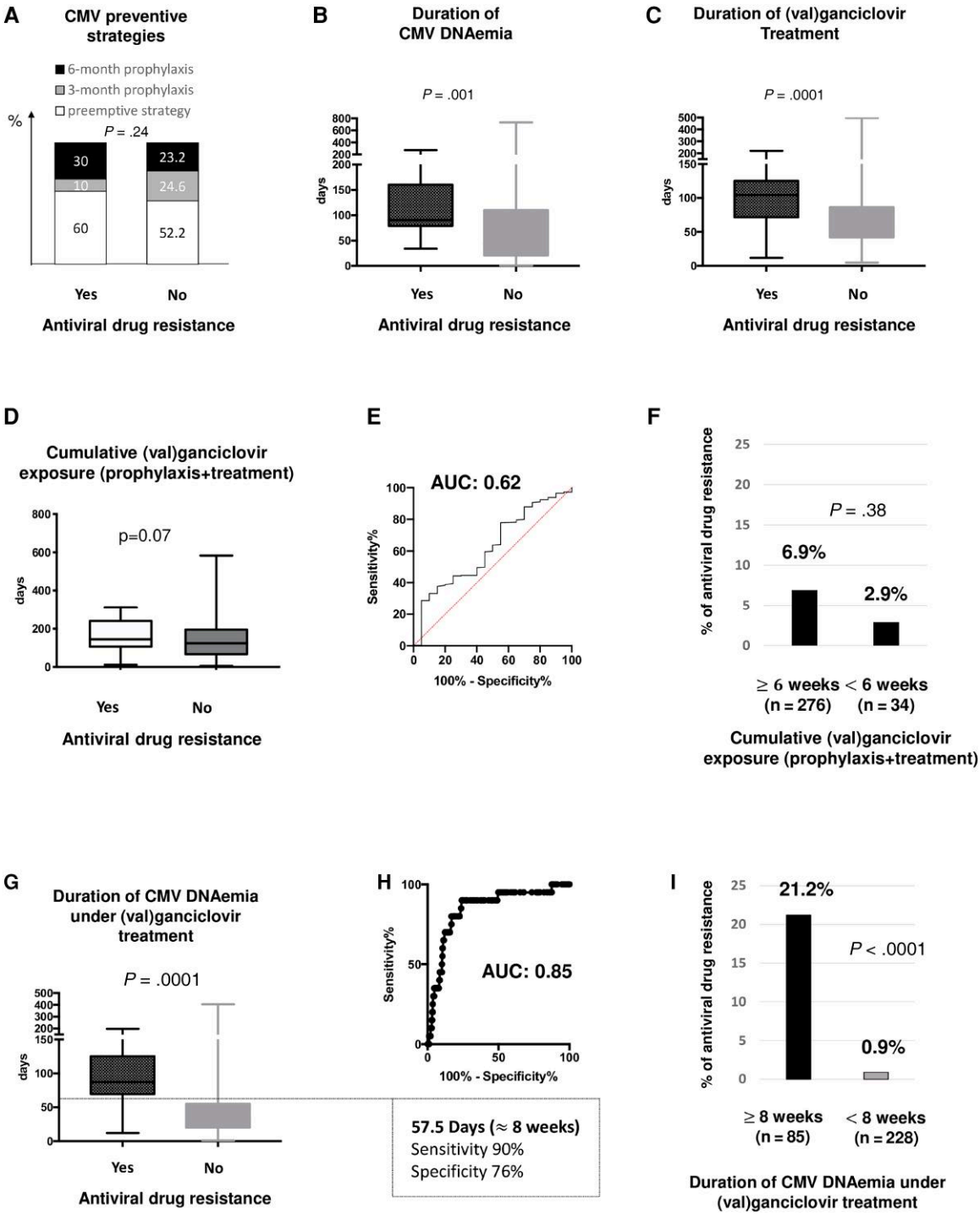

Figure 3. Associations between the emergence of antiviral drug resistance and valganciclovir prophylaxis, duration of cytomegalovirus (CMV) DNAemia, duration of (val) ganciclovir treatment, cumulative (val)ganciclovir exposure, and duration of CMV DNAemia during (val)ganciclovir treatment. (*A*) Association between the emergence of antiviral drug resistance and valganciclovir prophylaxis. (*B*) Association between the emergence of antiviral drug resistance and the duration of CMV DNAemia. (*C*) Association between the emergence of antiviral drug resistance and the duration of (val)ganciclovir treatment. (*D*) Association between the emergence of antiviral drug resistance and cumulative (val)ganciclovir exposure (prophylaxis + treatment). (*E*) Identification of the optimal cutoff for the cumulative (val)ganciclovir exposure predicting the emergence of antiviral drug resistance: results of receiver operating characteristic (ROC) curve analysis. (*F*) Comparison of the emergence of antiviral drug resistance between 2 patient groups, ie, (1) kidney transplant recipients with a cumulative (val)ganciclovir exposure ≥6 weeks and (2) kidney transplant recipients with a cumulative (val)ganciclovir exposure <6 weeks. (*G*) Association between the emergence of antiviral drug resistance and the duration of CMV DNAemia during (val)ganciclovir treatment. (*H*) Identification of the optimal cutoff for the duration of CMV DNAemia during (val)ganciclovir treatment for predicting the emergence of antiviral drug resistance: results of ROC curve analysis. (*I*) Comparison of the emergence of antiviral drug resistance between 2 patient groups, ie, (1) kidney transplant recipients with a duration of CMV DNAemia during (val)ganciclovir treatment ≥8 weeks and (2) kidney transplant recipients with a duration of CMV DNAemia during (val)ganciclovir treatment <8 weeks. AUC, area under ROC curve.

Table 2. Factors Associated With Cytomegalovirus Antiviral Drug Resistance[a]

	Univariate Analysis		Multivariate Analysis Model 1 ($n=199$)		Multivariate Analysis Model 2 ($n=199$)	
Variable	Unadjusted HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Age at CMV onset (years)	1.008 (0.97–1.04)	.65				
Male sex (male vs female)	0.51 (0.21–1.22)	.13				
Previous transplantation (reference = 0)	1.17 (0.34–3.99)	.8				
History of dialysis before transplantation	0.72 (0.24–2.16)	.56				
Antithymocyte globulin	1.64 (0.68–3.93)	.27				
Tacrolimus	3.69 (0.86–15.90)	.08				
Cyclosporine	0.032 (0.07–1.38)	.13				
Mycophenolate mofetil	0.83 (0.19–3.58)	.81				
Azathioprine	2.36 (0.55–10.18	.25				
Steroids	0.88 (0.35–2.21)	.79				
eGFR (per each mL/min)	0.99 (0.97–1.02)	.78				
T cell-mediated rejection	4.54 (0.6–34.48)	.14				
CMV serostatus D^+/R^- (vs other CMV serostatuts)	4.54 (1.54–13.51)	.006				
Valganciclovir prophylaxis (vs preemptive strategy)	1.61 (0.70–3.70)	.26				
Early-onset infection or disease	1.82 (0.75–4.54)	.19				
CMV disease	8.46 (1.13–63.19)	.04				
Intravenous ganciclovir (vs oral valganciclovir) as primary treatment for CMV disease	20.00 (2.38–142.86)	.004				
Baseline CMV DNAemia (IU/mL)	1 (1–1)	.92				
Baseline Lymphocyte Count (per Each 100/mm^2)	0.32 (0.11–0.93)	.04				
D 21 lymphocyte count (per each 100/mm^2)	0.47 (0.12–1.76)	.264				
D 49 lymphocyte count (per each 100/mm^2)	0.13 (0.03–0.59)	.008	0.13 (0.03–0.64)	.01	0.22 (0.05–0.92)	.04
Duration of CMV DNAemia (per each wk of DNAemia)	1.01 (0.99–1.03)	.64				
Duration of (val)ganciclovir treatment (per each wk of treatment)	1.03 (0.99–1.05)	.09				
Cumulative (val)ganciclovir exposure (per each wk of treatment)	1.02 (0.99–1.05)	.15				
Duration of CMV DNAemia during (val)ganciclovir treatment (per each wk of treatment)	1.06 (1.03–1.09)	<.001	1.06 (1.03–1.09)	.001		
Cumulative (val)ganciclovir exposure ≥6 wks	2.45 (0.33–18.30)	.38				
Duration of CMV DNAemia during (val)ganciclovir treatment ≥8 wks	24.84 (5.76–107.10)	<.001			11.68 (2.62–52.01)	.001

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; D, donor; eGFR, estimated glomerular filtration rate; HR, hazard ratio; R, recipient; wk, week.

[a]Covariates with $P < .25$ on univariate analyses were entered into multivariate Cox regression analysis. For enhanced clarity, only factors independently associated with antiviral drug resistance in multivariate analysis are shown in the table.

with an active CMV replication was identified as the strongest risk factor for the development of antiviral drug resistance. Therefore, viral replication during prolonged (V)GCV treatment is paramount for the emergence of this condition. This finding is in accordance with previous studies showing that a persistent CMV DNAemia is associated with an increased risk of developing antiviral drug resistance [7, 8]. Second, the optimal cutoff value for a prolonged (V)GCV treatment in the prediction of drug resistance appears to be 8—and not 6—weeks. Third, although the development of drug-resistant CMV variants has been described during the course of universal prophylaxis with the use of low-dose VGCV [21, 22] or in presence of CMV replication [23], universal prophylaxis was not identified as a risk factor for drug resistance.

In our cohort, antiviral drug resistance was observed in 2 distinct clinical scenarios characterized by the presence of CMV DNAemia requiring (V)GCV treatment for more than 8 weeks. The first scenario consisted of persistent CMV DNAemia occurring during a first episode of CMV infection or CMV disease (9% of all cases). Controversy still exists regarding the clinical management of persistent CMV DNAemia. On the one hand, antiviral treatment should be continued until viral eradication is achieved [3]. On the other hand, the results of our study indicate antiviral drug resistance occurs in 39.3% of KTRs who experienced persistent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks. However, it is noteworthy that the whole-blood CMV QNAT assay used in our study is more sensitive than plasma QNAT [24]. Because the median whole-blood CMV DNAemia at 8 weeks was markedly low (1000 IU/mL), we speculate that a significant number of KTRs would have had a negative plasma QNAT result at this time point. Therefore, the duration of antiviral administration would have been reduced in the event of negative plasma QNAT being used as the criterion for treatment

Table 3. Factors Associated With Prolonged CMV DNAemia Requiring (Val)Ganciclovir Treatment for More Than 8 Weeks[a]

	Univariate Analysis		Multivariate Analysis: Model 1 (Excluding Lymphocyte Count) (294)		Multivariate Analysis: Model 2 (Including Day 21 Lymphocyte Count) ($n = 153$)	
Variable	Unadjusted OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Age at CMV onset (years)	0.99 (0.97–1.01)	.49				
Male (vs female)	1.14 (0.67–1.94)	.63				
Previous transplant (reference = 0)	1.14 (0.53–2.33)	.72				
History of dialysis before transplantation	2.36 (1.06–5.98)	.04	3.21 (1.32–8.85)	.015		
Antithymocyte globulin	0.98 (0.56–1.67)	.95				
Tacrolimus	2.45 (1.32–4.80)	.006				
Cyclosporine	0.35 (0.17–0.68)	.003				
Mycophenolate mofetil	1.35 (0.55–3.80)	.53				
Azathioprine	1.13 (0.30–3.58)	.84				
Steroids	0.87 (0.51–1.50)	.62				
eGFR (per each mL/min)	1.01 (0.99–1.02)	.09				
T cell-mediated rejection	0.72 (0.35–1.39)	.35				
CMV serostatus D^+/R^- (vs other CMV serostatus)	3.57 (2.08–6.25)	<.001	3.84 (2.08–7.69)	<.001	5.56 (2.44–14.29)	<.001
Valganciclovir prophylaxis (vs preemptive strategy)	0.53 (0.32–0.86)	.01	0.24 (0.13–0.44)	<.001	0.20 (0.08–0.48)	<.001
Early-onset infection or disease	0.76 (0.46–1.25)	.28				
CMV disease	2.26 (1.25–4.29)	.009				
Intravenous ganciclovir (vs oral valganciclovir) as primary treatment for CMV infection/disease	3.21 (1.88–5.61)	<.001	2.55 (1.34–4.95)	.005		
Baseline CMV DNAemia (IU/mL)	1.74 (1.33–2.29)	<.001				
Baseline lymphocyte count (per each 100/mm^2)	0.59 (0.35–0.94)	.04	-	-		
Day 21 lymphocyte count (per each 100/mm^2)	0.23 (0.09–0.54)	.001	-	-	0.20 (0.07–0.54)	.002

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; D, donor; eGFR, estimated glomerular filtration rate; OR, odds ratio; R, recipient.

[a]Covariates with $P < .25$ on univariate analyses were entered into multivariate Cox regression analysis. For enhanced clarity, only factors independently associated with prolonged CMV DNAemia requiring (val)ganciclovir treatment for more than 8 weeks in multivariate analysis are shown in the table. Because of missing data for lymphocyte count, this variable was not included in Model 1. In Model 2, we included lymphocyte count on day 21 instead of baseline lymphocyte count because we had more observations for the former rather than the latter.

discontinuation. The second scenario characterized by persistent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks was recurrent infection or disease. This condition was identified in 18% of our KTRs, 12.3% of which developed antiviral drug resistance. Maribavir—a potent orally bioavailable benzimidazole riboside—may represent an option for reducing the duration of (V)GCV treatment in KTRs with recurrent CMV, with the potential to eliminate or minimize the risk of antiviral drug resistance [25].

Another interesting finding from our study is that a lower lymphocyte count was independently associated with antiviral drug resistance. A lack of CMV-specific T-cell immunity has been previously reported in patients with persistent CMV infections [26] or CMV recurrences [27]. Taken together, these data indicate that strategies aimed at boosting immune responses against CMV (eg, adoptive cellular therapy or the use of mTOR inhibitors) may be a viable alternative to continued antiviral treatment in KTRs with persistent or recurrent CMV infections [28, 29].

The question as to whether antiviral drug resistance is the cause or the consequence of persisting CMV DNAemia requiring treatment with (V)GCV for more than 8 weeks remains unanswered. In our study, CMV genotyping was not systematically performed at different time points. Therefore, a disadvantage of our data is that we are unable to precisely date the emergence of CMV mutations. However, the prevalence of (V)GCV-related mutations is typically low at the beginning of antiviral treatment [14]. Because low viral loads (median, 1000 UI/mL) were observed at 8 weeks in patients with persistent CMV [30], we did not screen for CMV mutations at this time point. Using predictive mathematical models that subsequently underwent in vivo validation, Emery and Griffiths [15] showed that resistant CMV strains became the predominant population during prolonged exposure to GCV, ultimately leading to therapeutic failure. In our cohort, screening for mutations conferring (V)GCV resistance was performed at a late stage, ie, after a median of 16 weeks from the beginning of CMV infection. During this time interval, a significant increase of CMV loads occurred while antiviral therapy was being administered. This temporal course clearly suggests that prolonged exposure to (V)GCV leads to selection of resistant CMV strains.

In our study, we identified 5 parameters—including a history of dialysis before transplantation, a D^+/R^- status at transplantation, the use of a preemptive strategy, the use of IV GCV as primary treatment for CMV disease, and a low lymphocyte

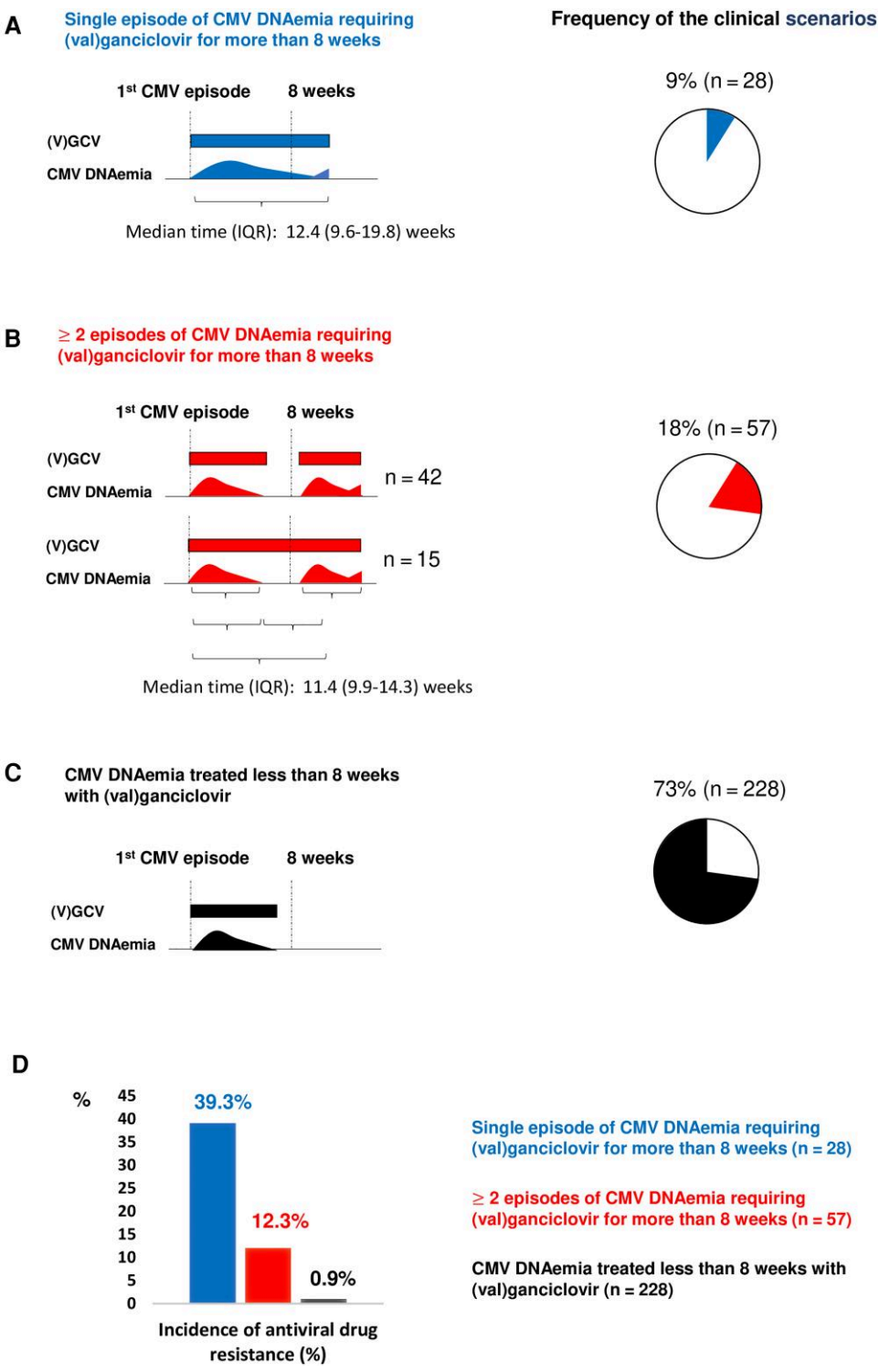

Figure 4. Clinical scenarios associated with persistent cytomegalovirus (CMV) DNAemia requiring (val)ganciclovir treatment for more than 8 weeks. Among the 313 patients with a first CMV episode treated with (val)ganciclovir, 3 distinct clinical scenarios were identified. (*A*) In the first scenario, 28 kidney transplant recipients (9%) experienced a single episode of persistent CMV DNAemia requiring treatment with (val)ganciclovir for at least 8 weeks. (*B*) In the second scenario, 57 kidney transplant recipients (18%) received (val)ganciclovir treatment for at least 8 weeks due to at least 2 different CMV episodes (either infection or disease). (*C*) In the third scenario, 228 kidney transplant recipients (73%) experienced CMV DNAemia requiring treatment with (val)ganciclovir for less than 8 weeks. (*D*) Incidence of antiviral drug resistance in the following 3 patient groups: (1) single episode of persistent CMV DNAemia requiring treatment with (val)ganciclovir for at least 8 weeks (blue), (2) at least 2 different CMV episodes requiring treatment with (val)ganciclovir for at least 8 weeks (red color), and (3) CMV DNAemia CMV DNAemia requiring treatment with (val)ganciclovir for less than 8 weeks (black color). IQR, interquartile range.

count on day 21—as significantly associated with persistent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks. It is notable that most of the variables are related to a defective anti-CMV immune response [19, 26, 31, 32].

Our study has several strengths. First, we included a large population of KTRs with CMV infections showing a wide range of severity, including life-threatening disease. Second, all participants were homogeneously treated according to current CMV guidelines and received regular, long-term follow up. Finally, we specifically focused on KTRs because SOTRs may significantly vary in terms of clinical characteristics [9]. However, our findings should be interpreted in the context of several limitations. Due to the relative rarity of mutations conferring (V)GCV resistance (6%, as previously reported [5–7]), our study had a retrospective design to include a high number of cases. The study encompassed a long period of time, during which the clinical management of KTRs changed considerably. In addition, it is possible that our findings could not be generalizable to other transplantation centers using different immunosuppression regimens or less sensitive CMV QNAT assays. Finally, it would have been interesting to include therapeutic drug monitoring.

CONCLUSIONS

In conclusion, persistent or recurrent CMV DNAemia requiring (V)GCV treatment for more than 8 weeks is independently associated with the occurrence of antiviral drug resistance. Kidney transplant recipients who face the 2 clinical scenarios identified in our study are ideal candidates for the development of new strategies for achieving viral clearance before the onset of CMV drug resistance mutations.

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