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ORIGINAL ARTICLE

Use of letermovir-valganciclovir combination as a step-down treatment after foscarnet for ganciclovir-resistant CMV infection in kidney transplant recipients

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Elena Rho: Present work was conducted as she was working at the University Hospital Zurich.

[Correction added on 22 April 2022, after first online publication: CSAL funding statement has been added.]

Abstract

Background: Letermovir (LTV) might be an alternative treatment to nephrotoxic foscarnet (FOS) in Ganciclovir (GCV) resistant cytomegalovirus (CMV) infection. However, its efficacy in controlling active CMV viremia is unclear, as it is only approved for CMV prophylaxis in hematopoietic stem-cell transplantation.

Methods: This case series describes 14 kidney transplant recipients (KTR) with moderate-level GCV resistant CMV infection, treated by different step-down strategies after initial FOS therapy: (1) Observation without antiviral follow-up or switch to valganciclovir (VGCV) (pre-LTV era), and (2) Switch to LTV±VGCV (LTV era).

Results: One patient died under FOS. Thirteen patients were followed under step-down regimens. All but two patients had ongoing CMV viremia when stopping FOS. In pre-LTV era, 5/9 (56%) experienced a CMV breakthrough > 10 000 IU/ml calling for another course of FOS, as compared to 1/4 (25%) in the LTV era. Addition of VGCV to LTV at low-level viral breakthrough, addressing a possible developing resistance against LTV, prevented viral surge in two patients. In the pre-LTV era, CMV-related death or graft loss occurred in three of nine (33%), compared to no death or graft loss in the LTV era.

Conclusion: A step-down strategy combining LTV+VGCV, might allow to safely stop FOS at ongoing low-level viremia.

KEYWORDS
drug-resistant cytomegalovirus, kidney transplant recipients, letermovir

1 | INTRODUCTION

Cytomegalovirus (CMV) infection is common after kidney transplantation, especially in sero-negative recipients receiving a sero-positive donor kidney (D+/R-) and has negative impact on graft and patient outcome.[1,2] The last decade has seen progress with respect to the development of better diagnostic tools[3,4] and emergence of new drugs.[5] Nevertheless, the control of CMV infection resistant to the currently used antiviral drugs that target DNA polymerase (i.e., ganciclovir (GCV) and valganciclovir (VGCV)) remains a big challenge. In this scenario, very high viral loads are observed and graft as well as patient survival are at risk.[6–10] Resistance to GCV requires a switch to different antiviral drugs, such as foscarnet (FOS) or cidofovir (CDV). However, FOS, the current first line treatment option for GCV-resistant

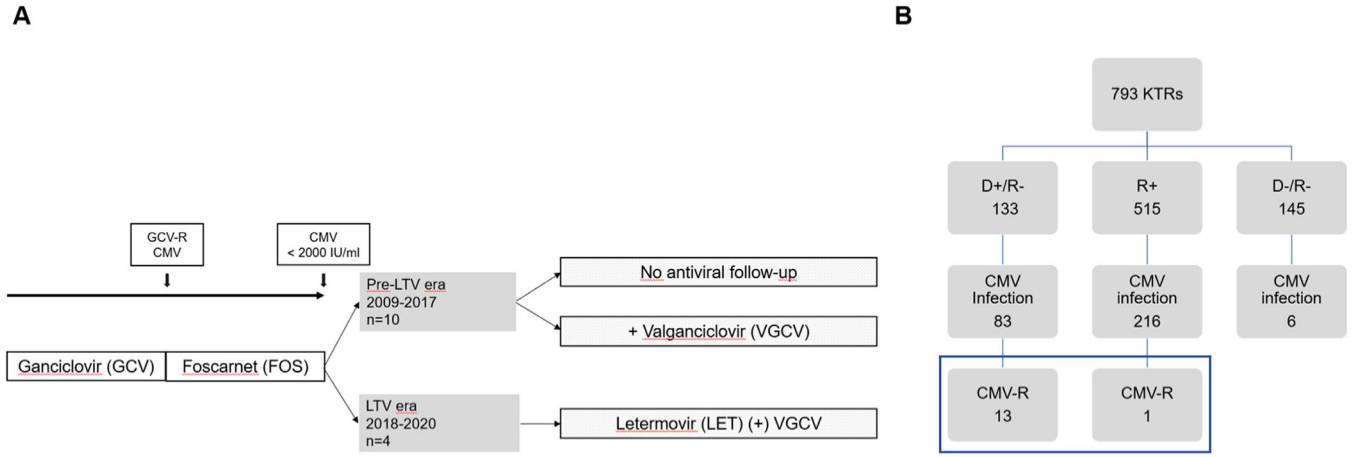

FIGURE 1 Treatment strategies and patient flow chart. (A) Treatment regimen for GCV resistant CMV infection in the pre-LTV era (2009–2017) and LTV era (2018–2020). Pre-LTV era: FOS was either stopped without antiviral follow-up treatment or switched to oral treatment with therapeutic VGCV. LTV era: FOS was either switched to LTV alone or in combination with VGCV. (B) Patient population according to CMV risk classification. Patients analysed in this study are highlighted within the black rectangle

CMV infection, requires intravenous application and in-hospital monitoring due to serious toxic side-effects, including electrolyte imbalances and nephrotoxicity.[11–13]

In the last years, several new drugs with novel antiviral targets have been tested. Among these, maribavir and letermovir (LTV) are of special interest due to their unique mechanism of action and limited toxicity. Maribavir prevents nuclear egress of viral capsids by inhibiting the protein kinase UL97, while LTV inhibits viral terminase complex. While maribavir is still awaiting drug approval, LTV has been approved in the United States and in Europe for prophylaxis of CMV infection in hematopoetic stem cell transplantation (HSCT) in 2017.[14] However, so far, it has not been approved for treating active CMV infection. This is likely due to emerging evidence of rapid development of resistant strains.[15,16] Nevertheless, the literature suggests LTV to be an effective drug for salvage therapy in patients with GCV resistant CMV infection.[17–20] Yet, more information on optimal drug dosage, on use as mono- or combination therapy with other antivirals and on viral load levels at which the antiviral therapy can be safely switched from FOS to LTV, is needed.

In the present study, we describe the clinical course and outcome of 14 GCV resistant CMV infections in KTRs treated initially with FOS and switched thereafter as a step-down strategy either to (1) no antiviral therapy or to VGCV in the pre-LTV era or to, (2) LTV with or without VGCV in the LTV era (**Figure 1(A)**).

2 | METHODS

2.1 | Patient cohort

We report on all cases of laboratory proven GCV-resistant CMV infection observed in adult KTRs transplanted at the University Hospital Zurich, Switzerland between January 1, 2009 and January 1, 2019. The patients were followed until March 31, 2020. All patients had signed general informed consent. The study was approved by the cantonal ethic commission review board of Zurich, Switzerland (KEK-ZH-Number 2019-01274) and has been conducted in compliance with the declaration of Helsinki.

2.2 | Testing of CMV, determination of viral resistance

The real-time PCR assay for CMV is based on the protocol of Yun et al.[21] with a sensitivity of 80 IU/ml and a specificity of > 99%. The analysis has been calibrated against the World Health Organization (WHO) standard in 2015. Before August 27, 2015 viral loads were expressed in copies/ml. According to the manufacturer's suggestion a conversion factor of 0.4 has been applied in order to convert copies/ml into IU/ml. Genotyping for resistance mutations was performed upon clinician request at the Institute for Virology, University Hospital Ulm, Germany. Detection of UL97 and UL54 mutation was performed by direct sequencing.

2.3 | Definitions

Standard definitions were used for CMV infection and CMV disease, whereby "*CMV infection*" refers to a proven CMV replication regardless of symptoms and "*CMV disease*" refers to CMV replication with attributable symptoms.[22] "*Virologic clearance*" was defined as the achievement of two consecutive CMV viral loads below the limit of quantification measured by PCR at least 5 days apart. "*Virologic breakthrough*" refers to a rising CMV viral load despite ongoing antiviral therapy.

2.4 | Immunosuppressive therapy

Immunosuppression protocols at our center are based on immunological risk stratification. Hence, patients at low immunological risk (i.e., without donor specific antibodies (DSA) prior to transplantation) receive basiliximab as induction therapy, while patients at high immunological risk (i.e., sensitized patients with DSA) and recipients of

organs donated after cardiac death receive thymoglobulin as induction therapy. Maintenance immunosuppression consists of a calcineurin inhibitor (cyclosporine or tacrolimus) and mycophenolate mofetil as anti-proliferative drug. Target trough levels up to months 1, 3, 6, 12 and 24 are 200–250 ng/ml, 180–220 ng/ml, 100–160 ng/ml, 80–120 ng/ml, 50–80 ng/ml for cyclosporine, and 10–15 ng/ml, 8–12 ng/ml, 7–10 ng/ml, 6–8 ng/ml, 4–6 ng/ml for tacrolimus respectively. Mycophenolate mofetil is used in a dosage of 1 g every 12 h for patients > 50 kg and 0.75 g every 12 h for patients < 50 kg. Steroids are generally withdrawn 6 months post transplantation. Reduction in CNI, compared to the levels and dosages mentioned above, was recorded at the first viremia, at begin and end of FOS therapy and at rebound of viremia leading to another course of intravenous FOS therapy. Modifications or suspension of the antimetabolite dosages were recorded at the same timepoints (Table 3).

2.5 | CMV prophylaxis and treatment

At our center, a preemptive treatment strategy for CMV is used except for patients who received thymoglobulin as induction therapy. This group receives VGCV prophylaxis for 3–6 months. The threshold for starting therapeutic VGCV is a viral load > 800 IU/ml in low (CMV D-/R-) and intermediate risk patients (D-/R+ and D+/R+), while treatment is initiated immediately at any detectable viral load (i.e. CMV < 80 IU/ml) in high risk patients (D+/R-). In case of failure to control viral replication by oral VGCV or in case of development of CMV disease, the patient is hospitalized and treatment with intravenous GCV (5 mg/kg every 12 h) is started and concomitant CMV hyperimmunoglobulin is administered in the doses of 100 IU per kg every 2 weeks. If CMV viremia levels increase during intravenous GCV treatment, resistance testing is performed and antiviral treatment is switched to intravenous FOS. Decision to stop FOS is made by the transplant team, after risk-benefit evaluation, taking into account infection control, seroconversion, FOS nephrotoxicity and patient wish to switch to oral therapy. After stopping FOS, different step-down strategies were adopted. Hence, in the pre-LTV era until end of 2017 patients were either followed without antiviral treatment or switched to oral treatment with therapeutic doses of VGCV. In 2018 LTV became available. Since then, patients were switched to oral LTV, which was mostly combined with VGCV (LTV era) **Figure 1(A)**. In case of viremia rising > 10 000 IU/ml after such step-down therapy, another course of intravenous FOS treatment was added.

2.6 | Data collection

The following data were collected from the electronic medical record: demographics, immunosuppression, outcomes (death, kidney allograft survival), CMV virology data (peak viral load, viral loads after suspension of FOS, antiviral resistance mutations, virological clearance, seroconversion), CMV treatment (durations of antiviral therapy administered for CMV treatment prior to FOS, durations of FOS and switch to VGCV or LTV±VGCV). Additionally, in order to record drug toxicity, creatinine was recorded at baseline, during FOS therapy (mean of three highest creatinine values) and 3 months after the end of FOS (mean of three lowest creatinine values). Acute Kidney Injury (AKI) was defined according to KDIGO guidelines[23] as an increase of creatinine of at least 50% from baseline. Baseline was defined as mean of three lowest creatinine levels in the 3 months before FOS. In the LTV-era changes in liver parameters and possible side effects (nausea/vomiting, edema, dyspnea, atrial fibrillation, myalgia, transaminase elevation, cough) were additionally collected.

2.7 | Statistical analysis

Descriptive statistics was performed using GraphPad Prism. Comparison of related variables was done by Wilcoxon matched rank test. Comparison of independent variables was computed using one way ANOVA, with Kruskal-Wallis test. Comparison of categorical variables was done by Fisher exact test, using contingency tables. Here subgroups in the pre-LTV era were combined and compared to LTV era due to small patient number. Correlation testing was performed by using Spearman correlation, assuming a non-Gaussian distribution. For survival analysis Kaplan-Meier curves were compared between groups by Log-rank (Mantel-Cox) test.

3 | RESULTS

3.1 | Patient population

A total of 793 KTR with signed general informed consent were transplanted between 2009 and 2019. Of those 38% (305/793) developed CMV infection out of which 5% (14/305) progressed to GCV-resistant CMV infection, that is, 1.8% (14/793) of the total cohort. As expected, risk of developing a CMV infection was highest in the (D+/R-) CMV risk group (83/133, 62%), followed by the (R+) CMV risk group (216/515, 51%) and lowest in the (D-/R-) CMV risk group (6/145, 4%). Thirteen KTRs developing GCV-resistant CMV infection belonged to the high-risk group and one to the intermediate-risk group (**Figure 1(B)**). UL97 resistance was confirmed in all the patients. One patient had an additional mutation in UL54. CMV disease occurred in 10 patients (71%), whereas asymptomatic viremia occurred in four (29%) of 14 patients. Median follow-up was 691 days. Characteristics of the whole cohort are shown in Table 1A.

3.2 | Initial course of CMV viremia

As expected, CMV viral load did not decrease during initial therapy with GCV due to moderate resistance-level mutations in all patients. During this initial treatment phase, immunosuppression was reduced in all patients and CMV hyperimmune globulin was administered in all but one patient (**Table 2**). Yet, only after initiation of FOS therapy, CMV viral loads significantly decreased from a median viral load of 183 029 IU/ml to 432 IU/ml ($P = .0001$), **Figure 2**. One patient lost his graft and subsequently died under FOS therapy. Except for two patients, FOS was discontinued even though CMV viral loads were not cleared. In

TABLE 1A Patient characteristics

	All patients ($n = 14$)
Males, % (*n*)	57% (8)
Age years, median (range)	61 (41–70)
Immunosuppression – Induction	
Basiliximab, % (*n*)	57% (8)
Thymoglobulin, % (*n*)	43% (6)
Immunosuppression – Maintenance	
Ciclosporin A, % (*n*)	21% (3)
Tacrolimus, % (*n*)	79% (11)
CMV-Serostatus	
CMV D+/R−, % (*n*)	92% (13)
CMV D+/R+, % (*n*)	8% (1)
CMV D−/R+, % (*n*)	–
CMV D−/R−, % (*n*)	–
CMV-infection/disease	
Days to CMV viremia, median (range)	35 (4–321)
Days to resistant CMV viremia, median (range)	290 (98–669)
Median peak viral load, IU/ml (range)	183 031 (29 811—10 740 645)
Asymptomatic viremia, % (*n*)	29% (4)
CMV Disease, % (*n*)	71% (10)
CMV-attributable GI symptoms, % (*n*)	50% (7)
CMV-attributable respiratory symptoms, % (*n*)	43% (6)
Mutation UL97, % (*n*)	100% (14)
Mutation UL 54, % (*n*)	8% (1)
Treatment	
Ganciclovir therapy, days median (range)	11 (0–37)
CMV hyperimmune globulins, % (*n*)	92% (13)
Duration of foscarnet days, median (range)	37 (12–153)
Outcome	
Seroconversion %, (*n*)	69% (9)
Virological clearance %, (*n*)	57% (8)
CMV breakthrough > 10 000 IU/ml after 1st cycle of FOS, % (n)	43% (6)
Time to relapse, days median (range)	55 (24–116)
Mortality within 1 year, % (*n*)	14% (2)
Acute Kidney Injury under foscarnet, % (*n*)	57% (8)
Follow up time, median days (range)	691 (228–2530)

all but one patient, viral loads were < 1000 IU/ml. Hence, 13 patients could be evaluated for further course of CMV viremia after cessation of FOS and adoption of different step-down strategies with either (1) no follow-up treatment or VGCV (pre-LTV era), or (2) follow-up treatment with LTV ± VGCV. Comparison of patient characteristics of the different step-down strategies is shown in Table 1B. Course of CMV viremia of the individual patients are summarized in **Table 3** and graphically illustrated in **Figure 3**.

3.3 | Pre-LTV era: No antiviral follow-up treatment after FOS: 6 patients

Here, three out of six patients (50%) experienced a rising viremia requiring a second course of intravenous FOS treatment. Such rebound occurred at a median time of 44 days (range 24–59 days) after FOS discontinuation. At the end of follow-up (median 1611 days, range 228–2530 days), there were two CMV related death with preceding

TABLE 1B Patient characteristics. Groups are divided according to step-down strategy after FOS

	Pre-LTV era ($n = 10$)			
	No antiviral follow-up treatment ($n = 6$)	+ VGCV follow-up treatment ($n = 3$)	LTV era ($n = 4$)	*P*-value
Males, % (*n*)	44% (4)	33% (1)	50% (2)	1
Age years, median (range)	63 (41-70)	54 (53-65)	59 (44-67)	.89
Immunosuppression – Induction				
Basiliximab, % (*n*)	67% (4)	67% (2)	50% (2)	1
Thymoglobulin, % (*n*)	33% (2)	33% (1)	50% (2)	
Immunosuppression – Maintenance				
Ciclosporin A, % (*n*)	33% (2)	0	0	.5
Tacrolimus, % (*n*)	67% (4)	100% (3)	100% (4)	
CMV Serostatus				
CMV D+/R−, % (*n*)	100% (6)	100% (3)	75% (3)	
CMV D+/R+, % (*n*)	–	–	25% (1)	
CMV D−/R+, % (*n*)	–	–	–	
CMV D−/R−, % (*n*)	–	–	–	
CMV-infection/disease				
Median peak viral load, IU/ml (range)	588 538 (32 464-5 170 962)	3 995 321 (215 485-10 740'645)	94 380 (29 811-1 428 885)	.17
Asymptomatic viremia, % (*n*)	33% (2)	33% (1)	25% (1)	1
CMV Disease, % (*n*)	67% (4)	67% (2)	75% (3)	
CMV-attributable GI symptoms, % (*n*)	17% (1)	67% (2)	75% (3)	.55
CMV-attributable respiratory symptoms, % (*n*)	33% (2)	33% (1)	50% (2)	1
Mutation UL97, % (*n*)	100%	100% (3)	100% (4)	1
Mutation UL 54, % (*n*)	0	33% (1)	0 (0)	1
Treatment				
Ganciclovir therapy, days median (range)	13 (5–22)	8 (0–37)	8 (3–11)	.71
CMV hyperimmune globulins, % (*n*)	83% (5)	100% (3)	100% (4)	1
Duration of foscarnet days, median (range)	48 (27–153)	60 (12–138)	27 (13–39)	.19
Outcome				
Seroconversion %, (*n*)	67% (4)	67% (2)	75% (3)	1
Virological clearance %, (*n*)	67% (4)	67% (2)	50% (2)	1
CMV breakthrough > 10 000 IU/ml after 1st cycle of FOS, % (*n*)	50% (3)	67% (2)	25% (1)	.55
Time to rebound > 10 000 IU/ml, days median (range)	44 (24–59)	37.5 (35–40)	55	.13
Mortality within 1 year, % (*n*)	33% (2)	0%	0%	.22
Acute Kidney Injury under foscarnet, % (*n*)	67% (4)	33% (1)	50% (2)	1
Follow up time, median days (range)	1611 (228–2530)	1079 (485–1083)	559 (446–738)	.38

TABLE 2 Additional treatment aspects during CMV viremia

		Patient	CMV-IgG Total dose (IU)	Reduction of Immunosuppression							
				Begin of CMV Viremia		FOS Begin		FOS End		Rebound Viremia	
				CNI	Antimetabolite	CNI	Antimetabolite	CNI	Antimetabolite	CNI	Antimetabolite
† under FOS		**M, 63** (-)	NA	=	↓	↓	=	NA	NA	NA	NA
Pre-LTV era	**no antivirals after FOS**	**M, 65** (G)	2500	=	=	↓	suspended	↓	suspended	NA	NA
		M, 62 (F)	3000	↓	=	↓	↓	=	suspended	↑	AZA
		F, 64 (H)	10000	↓	=	=	suspended	↑	suspended	NA	NA
		M, 41 (E)	4000	↓	=	↓	↓	↓	suspended	=	suspended
		F, 69 (D)	10000	=	=	=	suspended	NA	NA	suspended	suspended
		M, 40 (I)	25000	=	=	↓	suspended	↓	suspended	NA	NA
	VGCV	**F, 65** (C)	4000	↓	↓	↓	↓	↓	↑	=	↓
		F, 52 (B)	115000	↑	↓	↓	↓	↓	suspended	↓	AZA
		M, 53 (A)	15000	=	↓	↓	↓	=	suspended	NA	NA
LTV era		**M, 61** (J)	9000	NA	↓	↓	↓	=	=	NA	NA
		M, 43 (M)	154000	↓	↓	=	=	↓	↑	NA	NA
		F, 57 (L)	10000	↓	↓	↓	↓	=	↑	mTOR	suspended
		F, 66 (K)	3000	↓	=	↓	suspended	↓	↑	NA	NA

NA: used when information regarding immunosuppression was not applicable either in case of patient's death or in case of no relevant viral rebound. CNI ↓/↑: modification of CNI plasma-level respect to recommended target. Antimetabolite ↓/↑: relative modification of antimetabolite dosage compared to previous dosage.

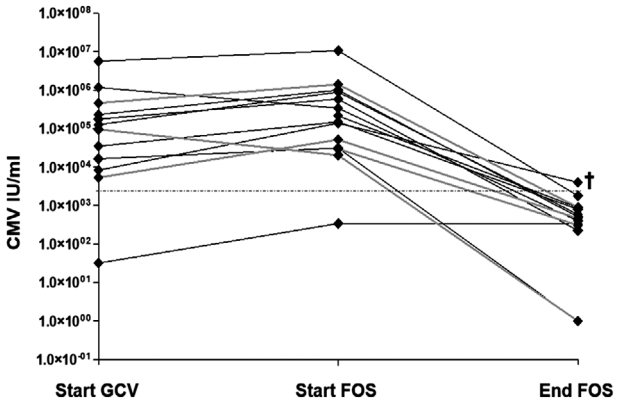

FIGURE 2 CMV viral loads under intravenous antiviral therapy. Each line represents a patient. One patient death under FOS therapy as highlighted. Patients treated in pre-LTV era represented by black lines and in LTV era by grey lines. The dotted line represents the threshold of 2000 IU/ml, below which FOS was stopped

allograft loss and one allograft loss with patient survival. Viral clearance was achieved in four out of six patients.

3.4 | Pre-LTV era: Step-down treatment with VGCV after FOS: 3 patients

In this group, two out of three patients (67%) experienced a breakthrough of CMV-viremia > 10 000 IU/ml. One was treated with FOS and adoptive T-cell transfer and recovered. The other did not receive intravenous FOS therapy (as he was in a tertiary care hospital at the time of breakthrough and therapy was converted to palliative care). Due to rising CMV viremia and concomitant SIRS this death was considered as CMV related. Viral breakthrough occurred at a median of 37.5 days (range 35–40 days) after FOS discontinuation. At the end of follow-up (median 1079 days, range 485–1083 days) viral clearance was observed in two patients.

3.5 | LTV-era: Step down treatment with LTV ± VGCV: 4 patients

All four patients were treated with LTV which was combined with VGCV in three patients. In this group all four patients experienced a viral breakthrough. Yet viral loads increased to > 10 000 IU/ml in only one patient (25%) necessitating another course of intravenous FOS. Of note, in this patient LTV was initially under-dosed with only 240 mg/d. In the other three patients, viral breakthrough could be controlled either by reduction of immunosuppression in one patient or escalation of LTV monotherapy to a combination with VGCV in two patients. Viral breakthrough requiring another course of FOS occurred 55 days after transplantation. At the end of follow-up (median 559 days, range 446–738 days) there were no patient death and no graft loss. Viral clearance was reached in two patients. LTV treatment was well tolerated with only possible minor side effects, namely nausea and cough in two patients, which did not lead to drug discontinuation. The median exposure to LTV was 206 days.

3.6 | Allograft and patient outcome in the whole cohort of GCV resistant CMV infection

In the whole cohort of 14 patients, we observed four graft losses. AKI occurred in 57% (8/14) of the patients (Table 1A). A weak correlation between creatinine and FOS duration could be observed, although statistically not significant in this small cohort ($r = 0.34$, $P = 0.2$) (**Figure 4**). Overall mortality was high with a total of five deaths within the period of observation. Four death were directly related to CMV disease (3 ARDS + 1 SIRS). One patient died of acute myeloid leukemia 2 years after transplantation and with negative CMV viremia. This translates into a 1-year mortality of 14% and a 2 years mortality of 28%. One death occurred during FOS therapy, three death in the pre-LTV group, and none in the LTV group. Patient survival was not related to peak viral load, but mortality was significantly higher when seroconversion was not reached, $P = 0.02$ (**Figure 5**).

4 | DISCUSSION

We describe the course of GCV-resistant CMV infections in KTRs of a large kidney transplant center during a 10 years' observational period with a focus on step-down strategies following an initial course of FOS therapy. The rarity of GCV-resistant CMV infection explains the small number of patients studied for this condition. Nevertheless, its associated high morbidity and mortality as well as the nephrotoxicity of FOS justifiy its clinical relevance.[6–10] Hope is put into emerging novel therapeutic options such as LTV. Several case reports and case series describe LTV as potential salvage therapy for GCV-resistant CMV in HSCT and SOT.[18–21] This option might be of special interest in the setting of kidney transplantation.[24] However, in this field, the literature is scarce, with the present study being the first case series to describe the use of LTV in this setting. Options to shorten FOS treatment are urgently needed in clinical everyday life. This is reflected by our cohort, where FOS, contrary to the guidelines,[22] was not continued until complete viral clearance but stopped at ongoing low-level CMV viremia, because of FOS induced nephrotoxicity. The threshold for choosing an oral step-down follow-up treatment was based on risk-benefit evaluation by the transplantation team in consideration of nephrotoxicity and patient preferences for outpatient treatment.

Here, we describe graft and patient outcome of LTV+VGCV as a step-down treatment after stopping FOS. This therapy is compared to the conventional step-down strategy of the pre-LTV era comprising either no antiviral follow-up treatment or a switch to oral VGCV.

Even though the step-down strategy with LTV lead to viral breakthrough in all four patients, only one patient needed another course of intravenous FOS due to surge of viral loads > 10 000 IU/ml. Impor-

TABLE 3 Synopsis of individual patient data

		Patient (letter in Figure 3)	TPL-Year	CMV-Disease	Viral load & therapy						Outcome				
					Mutation	Peak (IU/ml)	End FOS (IU/ml)	Treatment after FOS	Peak at rebound (IU/ml)	Treatment of rebound	Viral suppr. End FU	Sero-conversion	Survival	AKI stage	Allo-graft
† under FOS		**M, 63** (-)	2010	yes	UL97 (C603W)	138.506	4.013	NA	NA	NA	no	no	death*	3	graft loss
pre-LTV era	no antivirals after FOS	**M, 65**(G)	2010	yes	UL97 (M460I, L595S)	99.602	332	no antivirals	2762	–	yes	yes	death	2	
		M, 62 (F)	2012	yes	UL97 (M460I, L595S)	150.576	borderline	no antivirals	**86.111**	FOS (3 times)	no	no	death*	3	graft loss
		F, 64 (H)	2012	yes	UL97 (A594T, L595F)	32.462	0	no antivirals	867	–	yes	yes	alive	No AKI	
		M, 41 (E)	2013	no	UL97 (L595S)	897.044	584	no antivirals	**92.495**	FOS (2 times)	yes	yes	alive	3	graft loss
		F, 69 (D)	2015	yes	UL97 (599del)	5.170.962	512	no antivirals	**40.609**	FOS (2 times) LEF, CDV	no	no	death*	3	graft loss
		M, 40 (I)	2015	no	UL97 (L595F)	588.538	225	no antivirals	345	–	yes	yes	alive	No AKI	
	VGCV	**F, 65** (C)	2012	yes	UL97 (L595S, L595W)	3.995.321	389	VGCV	**31.857**	NA	no	no	death*	1	
		F, 52 (B)	2016	yes	UL97 (A594V)	1.740.645	1.802	VGCV	**10.920.983**	FOS (2 times) CMV-T cells	yes	yes	alive	No AKI	
		M, 53 (A)	2017	no	UL97,UL54 (A594V) + (N508K, V812L)	215.485	885	VGCV	885	–	yes	yes	alive	No AKI	
LTV era		**M, 61** (J)	2018	yes	UL97 (A594V)	108.803	0	LTV 480 mg	2.500	+ VGCV	yes	NA	alive	No AKI	
		M, 43(M)	2018	yes	UL97 (A594V)	1.428.885	898	VGCV	5.977	+ LTV 480 mg	no	yes	alive	1	
		F, 57 (L)	2018	yes	UL97 (C603W)	79.952	466	LTV 240 mg	**22.443**	FOS (1 time), LTV 480 mg + VGCV	no	yes	alive	2	
		F, 66 (K)	2019	no	UL97 (H520Q)	29.811	308	LTV 480 mg	2.371	LTV continued	yes	yes	alive	No AKI	

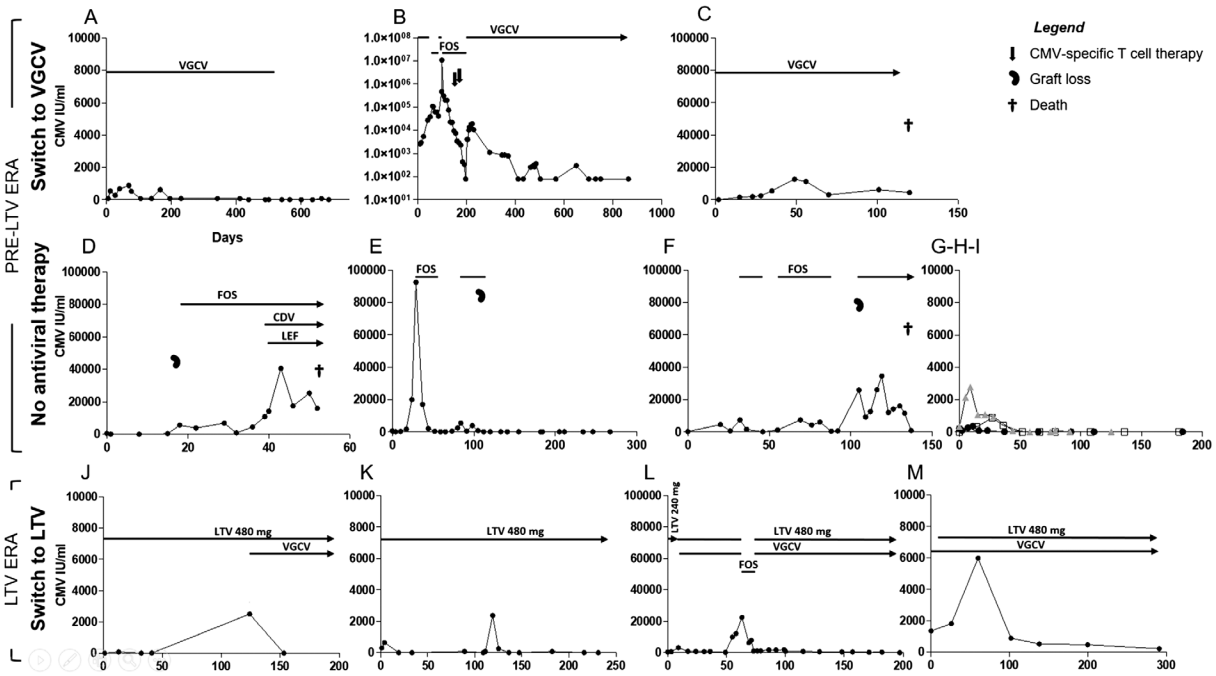

FIGURE 3 CMV viral loads after stopping intravenous therapy with foscarnet. Antiviral therapy, allograft and patient survival are graphically described individually for patients treated in the pre-LTV era (A-I) and the LTV era (J-M). Each graph represents one patient, except for graph G-H-I, where three patients with a favourable course and no interventions are represented together. The patient who died under FOS is not represented

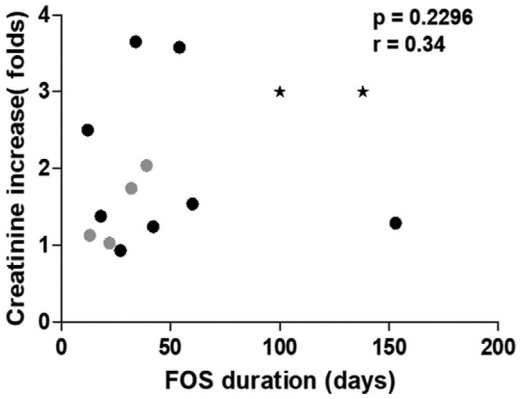

FIGURE 4 Allograft outcome in GCV-resistant CMV infection. Correlation between duration of FOS treatment and kidney function. Patients who received RRT were assigned a three-fold creatinine-increase according to the KDIGO guidelines. These patients are depicted as stars. Patients treated in the pre-LTV era depicted in black and in LTV era in grey

tant to note, that in this patient LTV was initially underdosed. In two patients, viremia could be controlled by a combination of LTV with VGCV. We assumed that LTV resistance, which is well described in the literature,[16,17] was the reason of rising viremia under LTV monotherapy and that we could address the LTV resistant strains by adding VGCV. This is, in our opinion, the pharmacodynamic explanation of the superiority of a combination strategy. Of note, no patient death and no graft loss were observed in the LTV-era. In contrast, a step-down strategy with VGCV monotherapy was associated with high-level viral breakthrough in two out of three patients and similarly in three out of 9 patients with no antiviral follow-up treatment. Such high-level breakthrough led to three CMV-related deaths in the pre-LTV era. In light of the small number of patients, firm conclusions are not possible. Yet, even though FOS is an essential part in the initial treatment of GCV-resistant CMV infection to achieve viral control, we hypothesize that a step-down strategy combining LTV+VGCV might allow to safely stop FOS prior to complete viral clearance. This option opens the possibility to shorten FOS exposure and has the potential to reduce nephrotoxicity. Furthermore, it allows earlier outpatient treatment, which is an important aspect for patients' quality of life. Until now, it is unclear, at which viral threshold a switch from FOS to LTV+VGCV can safely be adopted. In our cohort, all but one patient showed viral loads < 1000 IU/ml at time of step-down therapy. Safety of such threshold is supported by the literature,[25] even though experience with LTV in patients with active CMV viremia is scarce[26] and it remains to be investigated, whether a switch at higher viral load would be feasible. Veit et al.[20] published a case series of four lung transplant recipients who received LTV as salvage therapy in the setting of GCV resistant CMV. In that publication of Veit, LTV was started at viral loads between 2493 and 52 962 IU/ml and in all four patients, infection could be controlled.

There are several limitations to our study: the small patient number does not allow statistical analysis and limits our conclusions. Furthermore, the lack of standardized adjustment of immunosuppression during CMV viremia is another limitation. Additionally, biopsy data of patients with graft loss are missing, hence we can only speculate about the cause. Finally, it would have been desirable to retest genotypic CMV resistance at occurrence of viral breakthrough for improved

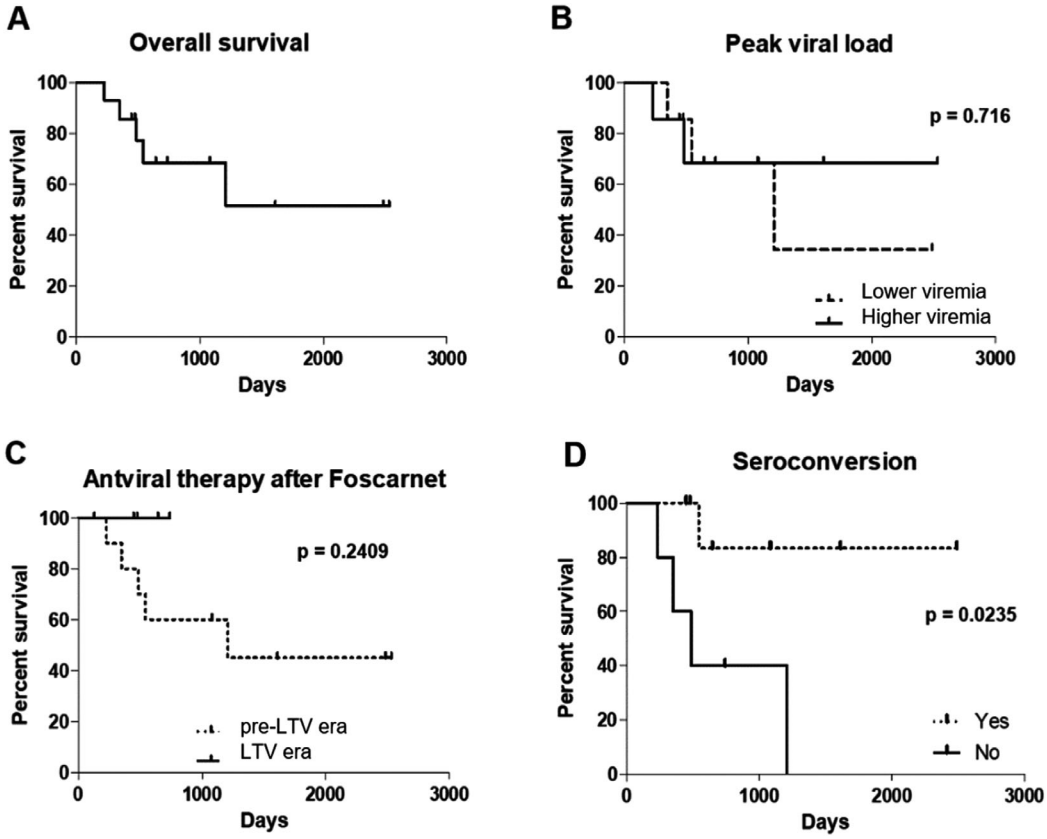

FIGURE 5 Patient outcome in GCV-resistant CMV infection (A) Overall patient survival. Patient survival according to (B) peak viral load (groups separated according to median) (C) treatment strategy and (D) seroconversion

pathophysiologic understanding and support of our hypothesis promoting a combination therapy with LTV+VGCV.

In conclusion, we suggest, that a combination of LTV+ VGCV might be a reasonable step-down treatment strategy in GCV resistant CMV infection in the following situations, provided that initial treatment with FOS significantly reduced CMV viral loads:

1. Signs of nephrotoxicity associated with FOS
2. Unwillingness of a patient to continue hospitalisation with intravenous FOS treatment

According to our observations such combined step-down strategy of LTV+VGCV has the potential to prevent high-level viral breakthrough, which is a relevant concern in case of VGCV monotherapy (due to resistance mutation) or in case of no antiviral follow-up treatment. Furthermore, the combination of LTV with VGCV pharmacologically addresses the problem of low resistance barrier of LTV. However, larger prospective trials are needed to test the safety of such approach and its potential to reduce nephrotoxicity by shortening FOS duration.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTION

Elena Rho was involved in designing the study, data collection, statistical analysis and manuscript preparation. Bettina Näf was involved in data collection. Seraina von Moos was involved in designing the study, data analysis and manuscript finalization. Thomas Schachter was involved in study design and manuscript finalization. R. Wüthrich and T. Müller contributed to manuscript finalization. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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