ORIGINAL ARTICLE

Effect of mammalian target of rapamycin inhibitors on cytomegalovirus infection in kidney transplant recipients receiving polyclonal antilymphocyte globulins: a propensity score-matching analysis

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SUMMARY

Mammalian target of rapamycin inhibitors (mTORi) prevents cytomegalovirus (CMV) infection in kidney transplant (KT) patients. From May 2010 to December 2013, all KT recipients were retrospectively analysed. Maintenance immunosuppression regimen was divided into mTORi or calcineurin inhibitors (CNI)-based regimen. Since June 2011, CMV-seropositive recipients (R+) treated with high-intensity immunosuppression and mTORi did not receive anti-CMV prophylaxis. We analysed 350 consecutive patients, of which 95 (27%) received mTORi and 255 (73%) CNI-based immunosuppression. A Cox-regression multivariate analysis showed that the use of mTORi-based immunosuppression during all follow-up reduced the risk of CMV infection (HR 0.36, 95% CI 0.15–0.89, $P = 0.028$) and confirmed in a propensity score-matched cohort (HR 0.4, 95% CI 0.1–0.9, $P = 0.047$). Early discontinuation of mTORi increased the risk of CMV infection (HR 3.2; 95% CI 1.7–6.0) in univariate analysis. The incidence of CMV infection was not higher among CMV R+ patients on mTORi and requiring high-intensity immunosuppression when CMV prophylaxis was not given. The use of mTORi protected for CMV infection in KT patients, allowing to avoid antiviral prophylaxis for R+ patients receiving high-intensity immunosuppression. The increased risk of CMV infection after early discontinuation of mTORi warrants further research.

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Key words
cytomegalovirus, kidney transplantation, mammalian target of rapamycin inhibitors, polyclonal anti-lymphocyte globulins

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Introduction

Cytomegalovirus (CMV) is the most important opportunistic pathogen in kidney transplant (KT) recipients, causing morbidity and mortality because of direct and indirect effects. Although many advances have been achieved in monitoring, prophylaxis and treatment of CMV infection and disease, the optimal strategy for

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CMV management in KT recipients remains an unresolved issue [1–4].

Although universal antiviral prophylaxis is simple and efficacious for the control of CMV replication, this strategy can lead to adverse effects (neutropenia), and it is expensive and could favour the development of CMV-resistant strains. On the other hand, while pre-emptive therapy may be preferred for low-moderate-risk patients, this strategy increases laboratory workload, is resource-consuming, and it is not exempted of potential adverse effects and development of antiviral resistance [2,3]. The final decision about the best strategy relies on the individual risk, the type of transplantation and the internal policies, many times related to budget availability.

In recent years, many observational studies showed that mammalian target of rapamycin inhibitors (mTORi) has a protective effect for CMV infection after solid organ transplantation, especially after KT [5–16]. This protective effect has also been observed in high-risk solid organ transplantation, such as lung transplantation [17] or ABO-incompatible KT using a desensitization regimen with rituximab [18]. Moreover, there is also some evidence on the efficacy of the conversion from calcineurin inhibitors (CNI) to mTORi to control the replication of ganciclovir-resistant CMV [19,20]. The effect of mTORi on CMV is complex and may be mediated by different mechanisms which include a direct antiviral effect and a modulation on the acquired and innate immunity [5,21,22].

Despite this evidence in observational studies, the most recent guidelines do not take into account the use mTORi to guide the use of antiviral prophylaxis to prevent CMV disease in organ transplant patients [2]. Some authors suggest that antiviral prophylaxis may be dispensable in some groups of KT recipients, although no conclusive clinical data are available to support this recommendation [7].

The aim of this study was to analyse the impact of the use of mTORi-based immunosuppression on the risk of CMV infection in a single-centre cohort of kidney transplant patients. In addition, we analysed the results of avoiding antiviral prophylaxis in CMV moderate-risk patients (R+) receiving mTORi and high-intensity immunosuppression.

Materials and methods

Population

All patients that received a KT at Hospital Clinic, a 800-beds tertiary care institution in Barcelona (Spain), between May 2010 and December 2013 were retrospectively evaluated. Patients were categorized as mTORi-based immunosuppression (mTORi-based group) if the therapy included either sirolimus or everolimus, regardless the need for treatment discontinuation (intention-to-treat analysis) or adjuvant therapy. All other patients not receiving mTORi as maintenance immunosuppression and receiving CNI were categorized as CNI-based immunosuppression (CNI-based group).

Definitions

1. High-intensity immunosuppression: patients were considered to receive high-intensity immunosuppression when they received: (i) treatment with polyclonal anti-lymphocyte globulins as induction or acute rejection therapy (Thymoglobulin®; Genzyme, Cambridge, Mass., USA 5–7 daily doses 1.25 mg/kg or ATG-Fresenius® (Neovii-Biotech, Graefelfing, Germany) 5–7 daily doses 2.5 mg/kg adjusted according to lymphocyte count) or (ii) desensitization therapy (ABO-incompatible living-donor RT or positive flow cytometry cross-match RT or with donor specific antibodies) with plasma exchange (5–7 sessions) plus rituximab (MabThera®: Hoffman-La Roche, Basel, Switzerland 200 mg, 2 doses) and intravenous immunoglobulins (200 mg/kg, Flebogamma IV 5%; Grífols Institute®, Barcelona, Spain) after every second session of plasma exchange. The definition of high-intensity immunosuppression was based on the recommendations by the International Consensus Guidelines for the prevention of low-moderate CMV risk patients under potent immunosuppressive therapy [2].
2. Cytomegalovirus infection and disease: all patients were monitored by means of real-time PCR CMV viral load (PCR CMV Real Time, Nanogen Advanced Diagnostics, Italy). CMV infection or disease was defined according to the definitions proposed by Ljungman *et al.* [23]. A positive CMV viral load was considered to be significant when the number of CMV DNA viral copies was equal or higher than 1000/ml.

CMV prophylaxis protocol

1. Cytomegalovirus-seronegative patients receiving a KT from a CMV-seronegative donor did not receive anti-CMV prophylaxis and were not prospectively monitored for CMV viral load.
2. All CMV-seronegative patients receiving a KT from a CMV-seropositive donor (D+/R−) received 12 weeks of valganciclovir prophylaxis (450 mg twice daily adjusted per renal function). After valganciclovir

discontinuation, CMV viral load was monitored once monthly until month six post-transplantation. In the case of positive viral load, patients were treated with valganciclovir (900 mg twice daily adjusted per renal function) or intravenous ganciclovir in the case of hospitalization.

3. Cytomegalovirus-seropositive recipients (regardless of donor CMV serology) received four-week valganciclovir prophylaxis (450 mg twice daily adjusted per renal function) when they received high-intensity immunosuppression. After that, monthly CMV viral load was performed until month six post-transplantation. CMV-seropositive recipients not receiving high-intensity immunosuppression were monthly monitored by means of quantitative PCR. Patients with viral load higher than 1000 copies/ml and without clinical symptoms related to CMV infection received valganciclovir 900 mg twice daily (adjusted by renal function) for 2 weeks.

4. In June 2011, the CMV prophylaxis protocol for CMV R+ patients was changed according to an internal analysis of our cohort. CMV prophylaxis was avoided in patients receiving high-intensity immunosuppression when maintenance immunosuppression included mTOR inhibitors (mTORi). Those patients were monitored twice monthly for CMV viral load during 6 months and, in the case of positive viral load (>1000 copies/ml), pre-emptive treatment with valganciclovir (see above) for 2 weeks was given.

Immunosuppression protocol

The immunosuppressive regimen was based according to the characteristics of KT. KT from non-high-risk donors included tacrolimus plus mofetil mycophenolate/mycophenolic acid (MMF/MPS) and from January 2013 tacrolimus plus mTORi. High immunological risk recipients received polyclonal antilymphocyte globulins plus tacrolimus plus MMF/MPS and from January 2013 plus tacrolimus plus mTORi. Patients receiving a KT from an expanded criteria donor received polyclonal antilymphocyte globulins plus mycophenolate plus mTORi and from January 2013 basiliximab plus tacrolimus plus mTORi. Patients receiving a KT from a donor after cardiac death received polyclonal antilymphocyte globulins plus mycophenolate plus mTORi and from January 2013 tacrolimus plus mTORi. Patients who received a KT requiring desensitization therapy were treated with rituximab, plasma exchange, intravenous immunoglobulins and polyclonal antilymphocyte globulin and maintenance immunosuppression with tacrolimus plus MMF/MPS. All patients received 0.5 g methylprednisolone before graft revascularization followed by 125 mg the second day, prednisone 0.5 mg/kg the third day and progressive tapering to 5 mg/day by day 90.

Ethical committee review

The Hospital Clinic Ethical Committee reviewed and accepted all the protocols.

Statistical analysis

Categorical variables are expressed as a percentage and were compared using the chi-square or Fisher exact test. Continuous variables are expressed as means or medians (depending on normality) and were compared using the *t*-test or Mann–Whitney test. To analyse the effect of potential risk factors upon the time CMV infection or disease takes to happen, Cox proportional hazard model was used. Cases were censored for competing risk, such as death, re-transplantation or graft loss. The variable maintenance immunosuppression/mTORi treatment was introduced as a time-dependent variable. Propensity score (PS) matching (1:2) was used for balancing the baseline characteristics between kidney transplant patients who received mTORi-based immunosuppression and those who received CNI-based immunosuppression. The PS was the predicted probability of receiving mTORi-based immunosuppression according to a logistic model with the binary outcome variable mTORi-based immunosuppression and the independent variables age, high-intensity immunosuppression, high-risk donor/recipient CMV serology, CMV prophylaxis and type of KT. A calliper restriction of 0.15 standard deviation of the logit of the estimated propensity score on potential matches was imposed [24, 25]. Cases were deleted if not appropriate controls were found. Appropriate controls (109) were properly matched to 65 cases in accordance to this model. A two-tailed P value <0.05 was considered statistically significant. Cumulative incidence curves were constructed for the unmatched cohort and the PS matched cohort to show the confounder-adjusted effect of mTORi on cumulative incidence of CMV infection and performed using R open free software (The R Project for Statistical Computing). The rest of analyses were performed using SPSS software (version 23; SPSS Inc., Chicago, IL, USA).

Table 1. Main characteristics of the patients included in the cohort according to the maintenance immunosuppressive regimen administered.

	mTOR inhibitors n = 95	Calcineurin inhibitors N = 255	P
Mean age (SD)	56.9 (13.5)	51.1 (13.1)	<0.001
Male gender (%)	62 (65%)	163 (64%)	0.816
Aetiology of CRF			0.027
Diabetes	7 (7%)	22 (9%)	
Glomerular	7 (7%)	40 (16%)	
Nephroangiosclerosis	15 (16%)	18 (7%)	
Tubulointerstitial	4 (4%)	11 (4%)	
Polycystic disease	12 (13%)	21 (8%)	
Other	5 (5%)	31 (12%)	
Unknown	45 (48%)	112 (44%)	
Retransplantation	11 (12%)	62 (24%)	0.009
Type of kidney transplantation			<0.001
Living donor	27 (28%)	132 (52%)	
Deceased donor – heart beating	47 (50%)	108 (42%)	
Deceased donor – nonheart beating	21 (22%)	15 (6%)	
Donor/Recipient CMV serology			0.001
D+/R+	61 (64%)	184 (72%)	
D+/R−	22 (23%)	21 (8%)	
D−/R+	8 (9%)	41 (16%)	
D−/R−	4 (4%)	9 (4%)	
High-intensity immunosuppression	76 (80%)	165 (65%)	0.006
Acute rejection	9 (10%)	48 (19%)	0.035
Anti-CMV prophylaxis	53 (56%)	170 (67%)	0.060
Graft failure	4 (4%)	6 (2%)	0.469
Death	5 (5%)	9 (4%)	0.540
Creatinin levels at 12 months (mg/dl) (median, IQR)	1.57 (1.15–1.89)	1.29 (1.04–1.58)	0.005
Incidence of CMV infection			
CMV asymptomatic infection	7 (7%)	16 (6%)	0.713
CMV disease	12 (13%)	23 (9%)	0.317

SD, standard deviation; CRF, chronic renal failure; IQR, interquartile range.

Results

Main characteristics of the cohort

Table 1 shows the main characteristics of the cohort according to the use of CNI or mTORi as primary maintenance immunosuppression. Patients on mTORi were older, received a living-donor kidney transplant less frequently and received a first kidney transplant more frequently. Regarding the risk of CMV infection, patients on mTORi received more frequently high-intensity immunosuppression and had more frequently high-risk donor/recipient CMV serology (D+/R−).

Maintenance immunosuppression

Maintenance immunosuppression consisted of a CNI-based regimen in 255 patients of which seven received cyclosporine A and 248 tacrolimus. mTORi-based regimen was given in 95 patients, of which 48 combined the use of mTORi plus CNI and 47 combined the use of mTORi plus MMF/MPS. Regimens including mTORi consisted of everolimus in 72 (76%) and sirolimus in 23 (24%) patients.

Of 95 patients receiving mTORi, 31 (33%) had to discontinue the drug during the first year because of the development of adverse effects or because of the occurrence of an event that contraindicated the use of mTORi. The causes for mTORi discontinuation were as follows: surgical wound-related complications 8, peripheral oedema 5, acute rejection 4, skin complications 3, pneumonitis 3, dyslipidaemia 2, proteinuria 1 and other reasons 5 cases. mTORi discontinuation occurred at a median of 113 days post-transplant (interquartile range 64.5–204.5 days).

CMV infection and disease according to donor/recipient serostatus

Cytomegalovirus infection (CMV disease and asymptomatic CMV infection) occurred in 58 of 350 patients

(17%): 0/13 of D−/R−, 2/49 (4%) of D−/R+, 40/245 (16%) of D+/R+ and 16/43 (37%) of D+/R− ($P < 0.001$). CMV disease occurred in 35/350 patients (10%): 0/13 of D−/R−, 1/49 (2%) of D−/R+, 22/245 (9%) of D+/R+ and 12/43 (28%) of D+/R−. The remaining 23 patients had asymptomatic CMV infection treated pre-emptively with valganciclovir.

Cytomegalovirus disease occurred at a median of 97 days post-transplantation (interquartile range 63-183). The type of CMV disease was viral syndrome in 21 cases (60%) and biopsy-proven end-organ disease in the rest 14 cases (7 colitis, 5 gastritis, 1 pneumonia and 1 glomerulonephritis). We did not find an association between CMV end-organ disease and CMV donor/recipient serostatus ($P = 0.565$) or the use of CMV prophylaxis ($P = 0.704$).

As CMV D−/R− patients had no risk of CMV infection or disease, we exclude them from the analysis of the impact of mTORi on the risk of CMV infection or disease.

Risk factors for CMV infection or disease

Table 2 shows the results of the Cox-regression analysis of the variables associated with CMV infection or disease. CMV D+/R−, the use of high-intensity immunosuppression and age over 50 years were associated with higher risk of CMV infection. In contrast, the use of antiviral prophylaxis had a protective effect. Taking the use of CNI-based immunosuppressive regimen as the reference, the use of mTORi without discontinuation was protective for the development of CMV infection. However, when mTORi had to be discontinued, the risk of CMV infection was higher compared to that of the CNI-based immunosuppression in univariate analysis (HR 3.2; 95% CI 1.7–6.0). To evaluate this subject in detail, we analysed the impact of antiviral prophylaxis and the type of maintenance immunosuppression. Those patients with mTORi discontinuation not receiving antiviral prophylaxis had a risk of CMV infection around 50% and higher than the risk of the rest of subgroups analysed ($P = 0.005$) (Fig. 1).

Because of the misbalanced groups according to the immunosuppression regimen, a propensity score analysis was used to adjust for confounding variables. Appropriate controls were properly matched to 65 cases in accordance with this model. The incidence of CMV asymptomatic infection or disease was 17% (11 patients) for those who receive an mTORi-based immunosuppressive treatment and 20% (22 patients) for those who receive a CNI-based immunosuppressive

Table 2. Cox-regression analysis of the risk factors associated with cytomegalovirus (CMV) infection or disease.

	Category	*N*	CMV infection/ disease *N* (%)	Univariate analysis		Multivariate analysis	
				HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age more than 50	Yes	208	43 (21%)	1.9 (1.1–3.4)	0.032	1.8 (1.0–3.3)	0.045
	No	129	15 (12%)				
High-intensity immunosuppression	Yes	232	43 (19%)	2.0 (1.0–4.1)	0.053	2.6 (1.2–5.3)	0.011
	No	105	15 (14%)				
CMV donor +/recipient −	Yes	43	16 (37%)	2.7 (1.5–4.8)	0.001	4.4 (2.3–8.4)	<0.001
	No	294	42 (14%)				
Use of anti-CMV prophylaxis	Yes	218	38 (17%)	1.0 (0.6–1.8)	0.948	0.4 (0.2–0.7)	0.006
	No	119	20 (17%)				
Previous acute rejection	Yes	55	11 (20%)	0.9 (0.7–1.3)	0.561	–	–
	No	282	47 (17%)				
mTORi treatment	Time-dependent variable					0.4 (0.2–0.8)	0.008

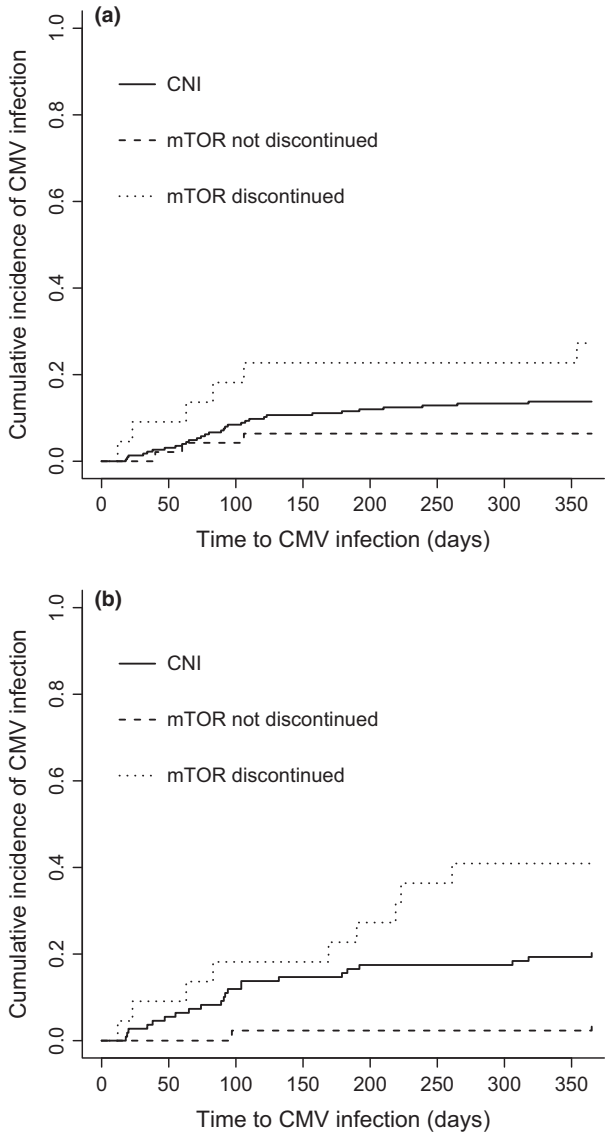

Figure 1 Graph comparing the cumulative incidence of cytomegalovirus (CMV) infection or disease according to the immunosuppressive regimen used in (a) the whole cohort and (b) propensity score-matched cohort. CMV D+/R− and CMV D−/R− are excluded of the analysis. Patients receiving mammalian target of rapamycin (mTOR) inhibitors were subdivided according to the use of anti-CMV prophylaxis and discontinuation of the immunosuppressive drug. The risk of CMV infection or disease was significantly higher among those patient receiving mTOR inhibitors with further discontinuation and without anti-CMV prophylaxis ($P = 0.005$).

treatment. mTORi-based regimen was introduced in the model as a time-dependent variable, and the model was additionally adjusted for age and CMV mismatch serology (D+/R−). The results of the Cox-regression analysis of the PS matching confirmed the protective effect of the use of mTORi for CMV infection and/or disease (HR 0.4, 95% CI 0.1–0.9, $P = 0.047$).

Impact of mTORi-based immunosuppression in patients at moderate risk of CMV infection and disease (R+)

After excluding D+/R− and D−/R−, 294 patients were categorized as having moderate risk for CMV infection (245 D+/R+, 83%; and 49 D−/R+, 17%).

CMV infection in patients on mTORi

Of 294 patients at moderate risk for CMV, 69 received mTORi and 225 CNI-based immunosuppression. CMV infection occurred in 10/69 (15%) of patients receiving mTORi and in 32/225 (14%) of patients receiving CNI-based immunosuppression ($P = 0.955$) and CMV disease in 5/69 (7%) of patients on mTORi and 18/225 (8%) of patients on CNI ($P = 0.838$). However, CMV infection among patients on mTORi occurred more frequently after the discontinuation of this drug during the first post-transplant year (14%, 32% and 8% of patients receiving CNI, mTORi with discontinuation and mTORi without discontinuation, respectively, $P = 0.044$).

Effect of avoiding CMV prophylaxis in R+ patients under high-intensity immunosuppression

Overall, 204 CMV R+ patients received high-intensity immunosuppression, of which 151 received a CNI-based and 53 an mTORi-based regimen. Of this 53 patients on mTORi, 17 (32%) had to discontinue the treatment because of adverse effects. Thus, 36 patients receiving high-intensity immunosuppression received a complete maintenance treatment with mTORi. Of the 151 patients under CNI-based immunosuppression, 139 (92%) received antiviral prophylaxis. Of the 36 patients with complete mTORi maintenance immunosuppression, 17 (47%) received antiviral prophylaxis and 19 (53%) did not (according to the modified internal protocol). Patients on mTORi and high-intensity immunosuppression without antiviral prophylaxis did not have an increased risk of CMV infection compared to those patients that received anti-CMV prophylaxis (Fig. 2).

Impact of mTORi-based immunosuppression on the risk of CMV infection among high-risk patients (CMV D+/R−)

All but one patient at high risk for CMV infection (D+/R−) received 100 days valganciclovir prophylaxis for CMV disease prevention. The patient not receiving

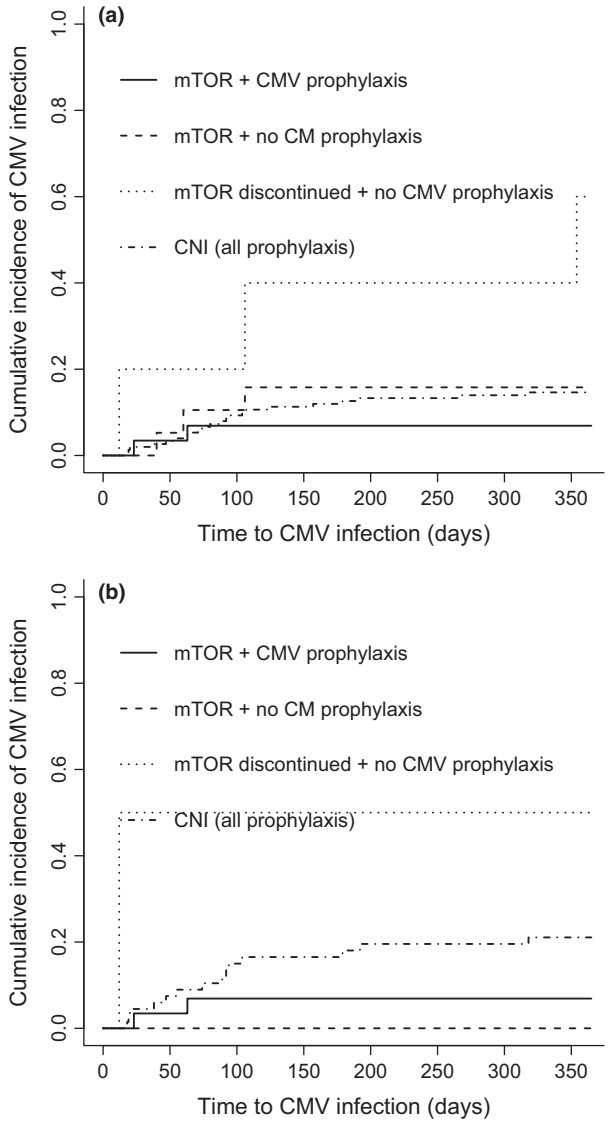

Figure 2 Graph comparing the cumulative incidence of cytomegalovirus (CMV) infection or disease according to the immunosuppressive regimen among patients receiving high-intensity immunosuppression in (a) the whole cohort; and (b) propensity score-matched cohort. CMV D−/R− and CMV D+/R− patients were excluded. All patients on calcineurin inhibitor (CNI) received anti-CMV prophylaxis. Patients on mammalian target of rapamycin (mTOR) inhibitors were subdivided according to the use of anti-CMV prophylaxis. The incidence of CMV infection or disease was equivalent between the three arms.

antiviral prophylaxis was treated with high-intensity immunosuppression for induction and CNI-based immunosuppressive therapy and developed CMV disease 92 days after transplantation. Maintenance immunosuppression was based on mTORi in 22/43 (51%), of which 13 (59%) had to discontinue the drug because of adverse effects. CMV infection appeared in 16/43 (37%) and 12 of this 16 patients with CMV infection (28% of the total and 75% of patients with CMV infection) had CMV disease. The median post-transplant days to CMV infection was 178 (interquartile range 118–242 days). We found no statistical significant difference in the occurrence of CMV infection according to the use of mTORi as initial immunosuppressive maintenance therapy (41% with vs. 33% without mTORi, $P = 0.607$). However, only three patients with CMV infection (1 CMV disease and two asymptomatic infection) occurred during mTORi treatment, while in the rest of cases (11 CMV disease and 2 asymptomatic infections) occurred on CNI treatment (Fig. 3). CMV asymptomatic infection or CMV disease was more frequent in patients discontinuing mTORi compared to patients on CNI or patients who did not discontinue mTORi [CMV infection 64%, 33% and 18% ($P = 0.083$); CMV disease 55%, 24% and 9%, respectively ($P = 0.053$)]. The median days of mTORi exposure in those 22 D+R− patients receiving mTORi as primary regimen was 301.5 (interquartile range 113–365) and 17/22 (77%) received mTORi for 100 days or more after transplantation.

Discussion

Experimental and clinical data support the protective effect of the use of mTORi on the development of CMV infection after KT [5–16,19,20]. In addition, there is also some published evidence on the efficacy of switching immunosuppression to mTORi for the treatment of ganciclovir-resistant CMV strains, although the evidence is limited to case reports [19,20].

In vitro studies have shown a complex and dynamic relationship between CMV and components of mTOR [26,27]. Both the direct antiviral effect and the modulation of the innate and CMV-specific immunity have been proposed as the principal mechanisms of the anti-CMV effect of mTORi. The later stages of the viral life cycle, including production of CMV-specific late-phase proteins such as pp65 and pUL-44, are highly dependent on mTORC1 activity [28–30]. Thus, during the treatment with mTORi, mTORC1-dependent stages are blocked, reducing viral replication [5,21]. mTORi have also the ability to increase the number and quality of CMV-specific memory effector CD4+ and CD8+ T cells [22,31].

In a recent meta-analysis, the impact of the use of mTORi on CMV infection was analysed [7]. In this study, two groups were evaluated: the first one comparing mTORi and CNI-based immunosuppression (10 trials and 3100 patients) and the second one comparing combined mTORi and CNI with CNI-based

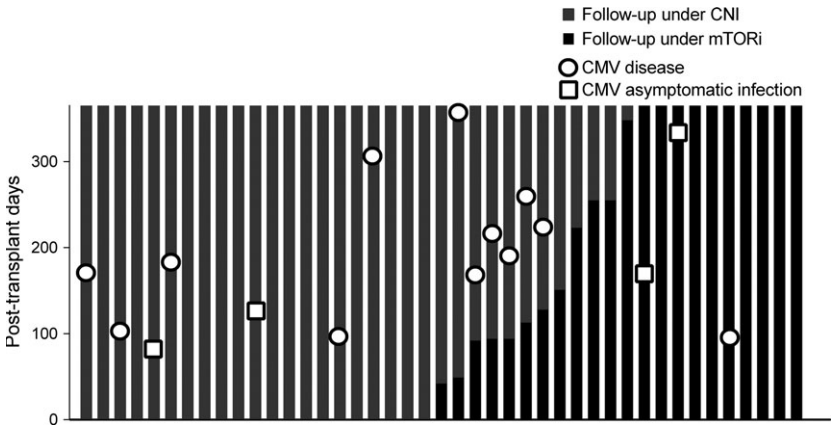

Figure 3 This graph represents the occurrence of cytomegalovirus (CMV) infection or disease among CMV high-risk patients (CMV D+/R−). Each bar correspond to one patient (43 D+/R− patients). The grey portion of the bar represents days on CNI maintenance immunosuppression, while the black bar represents days on mammalian target of rapamycin (mTOR) inhibitors. Twenty-one patients received CNI and 22 mTOR inhibitors as primary regimens. Twelve patients on mTOR inhibitors had to discontinue the drug during the first year. Note that most cases of CMV infection or disease (13/16) occurred on patients receiving calcineurin inhibitor (CNI). Only 1 of 12 cases of CMV disease occurred in a patient receiving mTOR inhibitors.

immunosuppression (15 trials and 7100 patients). In both groups, a higher incidence of CMV infection was found among patients receiving CNI-based maintenance immunosuppression (RR 2.27 and RR 2.45, respectively), which led to conclude that the use of mTORi alone or combined with CNI reduced significantly the incidence of CMV infection after transplantation [7]. Recently, Koch *et al.* [18]. demonstrated that the rate of CMV infection in ABO-incompatible KT patients treated with mTORi-based regimen was very low, despite the intense immunosuppression required. In our cohort, the use of mTORi reduced the risk of CMV infection near threefold when compared to a CNI-based regimen in a Cox-regression multivariable analysis. This protective effect was limited to those patients who successfully completed one-year treatment. Our results are concordant with the metaanalysis and with other nonrandomized studies, with similar rates in the protective associations [7,8].

Although the protective effect of the use of mTORi to control CMV replication is evident, there is still lack of consensus of which is the role of using these drugs for the management of CMV in transplant patients. The most recent published guidelines for the prevention of CMV recommend antiviral prophylaxis for CMV R+ patients receiving lymphocyte-depleting antibodies or rituximab. Although the protective effect of mTORi is mentioned, no recommendation has been given about how these drugs may eventually modify the actual preventive strategies [2]. In June 2011, we changed our internal protocol and we did not use antiviral prophylaxis for R+ recipients on mTORi who received high-intensity immunosuppression. Based on our results, avoiding the use of antiviral prophylaxis in this group of patients is safe if they tolerate mTORi during the first post-transplant year. This may be a relevant finding in order to reduce side effects related to antivirals potential considerable costs saving, although additional studies will be required to prove this effect. In fact, some authors suggest that in patients receiving lymphocyte-depleting antibodies, it should be considered the possibility of replacing mycophenolic acid by everolimus [32]. In a recent article, Tedesco-Silva and colleagues evaluated a group of *de novo* KT recipients treated with a single dose of antithymocyte globulin (ATG) and a reduced dose of tacrolimus and everolimus without CMV prophylaxis. A significant reduction on the incidence of CMV infection/disease was observed in comparison with the standard tacrolimus plus mycophenolate immunosuppressive regimen [33]. This study, as ours, also suggests that antiviral prophylaxis can be avoided in patients on mTORi. However, this study patients only received a single dose of ATG and the effect of this strategy in the high-risk population (D+/R−) was not evaluated.

In our study, we confirmed that the use of mTOR inhibitors is protective for CMV infection (HR 0.4). Interestingly, early discontinuation of mTORi increased the risk of subsequent CMV infection or disease. This effect was much more evident among high-risk patients (D+/R−), in which CMV infection or disease occurred statistically more frequently in patients receiving CNI or early discontinued mTORi. However, we should take into account that confirming these results would require

complex statistical analysis and a larger sample size cohort. Thus, the effect of mTOR inhibitors discontinuation on CMV infection must be addressed in further studies. Early discontinuation of these drugs is common in the clinical practice, as has been shown in previous clinical trials [34]. The pathophysiological mechanisms underlying the increased risk of CMV infection after mTORi discontinuation should be investigated, but it can be hypothesized that either a loss of direct control of CMV replication by these drugs or a depression in the production of CMV-specific CD8+ T cells could be major determinants. Regardless of the underlying mechanisms, an important conclusion of our findings is that we should consider reinitiating or prolonging antiviral prophylaxis for patients with early mTORi discontinuation, especially in the case of CMV D+/R− serology. An optimization in the clinical use of mTORi in KT to avoid early withdrawal of the drug can increase the beneficial effect of mTORi on CMV infection and the outcome of KT recipients.

In conclusion, mTORi were protective for the development of CMV infection in KT patients. Based on our findings, antiviral prophylaxis may be dispensable for moderate-risk patients receiving high-intensity immunosuppression and an mTORi-based regimen. However, early discontinuation of mTORi was followed with an increased risk of CMV infection. Further studies are warranted to confirm our findings.

Authorship

CC, FC and FO: participated in research design, performance of the research (data collection included), data analysis and writing of the article. CH: participated in data analysis and writing of the article. DS, MAM, GS and MB: participated in performance of the research, data analysis and critical review of the manuscript. AM, FD and JMC: participated in research design and critical review of the manuscript.

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Conflict of interest

The authors have declared no conflicts of interest.

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