Cytomegalovirus Infections in Everolimus-Based Treatment

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Abstract

Cytomegalovirus infection is a leading cause of morbidity and mortality after renal transplantation and it is associated with increased overall costs. Strategies available to prevent cytomegalovirus show different efficacy and safety profiles. Mammalian target of rapamycin inhibitors have been associated with reduced incidence of cytomegalovirus infection in several clinical trials and meta-analysis. This review addresses the existing evidences regarding the incidence of cytomegalovirus infection in patients receiving everolimus. Because of the known mammalian target of rapamycin inhibitor adverse event profile, patient selection, optimized dosing, and anticipation and proper management of adverse events are critical to fully benefit from the effects of this drug on cytomegalovirus viral replication in recipients of kidney transplants. **(Trends in Transplant. 2013;7:3-10)**

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Key words

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Introduction

Cytomegalovirus (CMV) is the most common opportunistic virus infection after solid organ transplantation, affecting 20-60% of all solid organ transplant recipients. Cytomegalovirus infection is associated with significant increases in morbidity, mortality, and overall costs related to the transplantation procedure[1].

In recipients of solid organ transplants, the clinical manifestations of CMV infection range from mononucleosis-like syndrome to severe clinical presentations of tissue-invasive diseases[2]. In the absence of chemoprophylaxis, CMV infection occurs between the first and third months after transplantation, often as a consequence of the use of induction therapy, higher doses of immunosuppressive drugs, and treatment for acute rejection episodes[3]. The infection may also cause "indirect effects" that are associated with other adverse outcomes including bacterial, viral, and fungal infections, new-onset diabetes mellitus, posttransplant lymphoproliferative disease, atherosclerosis, and acute and chronic rejection[1,2]. Cytomegalovirus infection also appears to be and independent risk factor for worse patient[4] and graft survival unless universal oral chemoprophylaxis is used[5]. The indirect effects are probably consequences of the immunosuppressive and inflammatory

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activities attributed to this viral infection[1,4,6]. Finally, and perhaps underestimated, is the observation that the detrimental effect of CMV infection on patient and graft survivals is comparable to that of acute rejection in kidney transplant recipients[7].

Two main strategies, universal prophylaxis and preemptive therapy, are used to prevent CMV infection. To date there is no evidence demonstrating clear superiority of one strategy over the other. While universal chemoprophylaxis is associated with hematological adverse events, with higher incidence of late and severe disease, preemptive therapy is logistically more complex and may not reduce the burden of the CMV indirect effects[2,8]. Other aspects that have not yet been fully clarified are the ideal time to maintain chemoprophylaxis, the ideal interval between tests for detection of viremia in the preemptive approach, and the role of chemoprophylaxis and preemptive approach in the emergence of ganciclovir-resistant viruses. Finally, both strategies are associated with increased costs[2].

The incidence of CMV in the kidney transplant population is estimated to be 8-32%[9]. The main risk factors for CMV infection and disease after kidney transplantation are donor CMV seropositivity in the absence of prior recipient infection (D^+R^-), treatment for acute rejection episodes, advanced recipient age, and poor kidney transplant graft function. Certain immunosuppressive agents, such as mycophenolate, muromonab anti-CD3, and thymoglobulin are associated with higher risk for CMV infection. Interestingly, the use of mammalian target of rapamycin inhibitors (mTORi) has been associated with lower incidences of CMV infection[2,10,11].

Considering the inferior transplant-related outcomes associated with CMV infection and the difficulties of establishing an ideal preventive therapy, new strategies have been investigated to reduce the burden of CMV infection. The use of immunosuppressive regimens that are both effective for the prevention of acute rejection and associated with lower incidences of CMV infection is a quite obvious and simple alternative, as long as the adverse event profile of this particular immunosuppressive regimen is acceptable. Here we summarize the data on CMV infection obtained from key clinical trials in kidney transplant recipients receiving the mTORi everolimus. We also review data investigating potential mechanisms that might account for the lower incidence of CMV infection observed in these patients.

Cytomegalovirus infection in mammalian target of rapamycin inhibitor-based treatment

The first studies demonstrating reduced incidence of CMV infection in kidney transplant recipients receiving mTORi (sirolimus) were published almost a decade ago[12]. A meta-analysis of 33 trials, 27 using sirolimus, five using everolimus, and one of head-to-head comparison, including 9,097 patients, evaluated the influence of mTORi on several kidney transplant outcomes. In seven studies including 3,094 patients receiving calcineurin inhibitors (CNI), those receiving mTORi showed a 51% reduction in the risk of developing CMV infection compared to those receiving mycophenolate or azathioprine[13]. Recently, a study including 1,470 kidney and kidney/pancreas transplant recipients of Spanish Network on Infection in Transplantation (RESITRA) showed that immunosuppressive regimens containing sirolimus were independently associated with 73% lower risk of CMV disease compared to other regimens without sirolimus[11]. Finally, a more recent meta-analysis showed that patients treated with CNI-based regimens (10 trials, 3,100 patients) had a 2.27-fold risk for a CMV event compared with patients receiving mTORi-based regimens. Interestingly, in patients receiving CNI (15 trials, 7,100 patients),

concomitant use of antimetabolites was associated with a 2.45-fold risk for a CMV event compared to mTORi. These findings suggest that patients receiving mTORi may not need any type of CMV prophylaxis[14].

Cytomegalovirus infection in everolimus-based treatment

The first study associating the use of everolimus with reduced incidence of CMV infection in kidney transplant recipients was the B156 study. This was a 36 month multicenter, randomized, open-label, phase III clinical trial comparing the use of full- or reduced-dose of cyclosporine micro-emulsion in *de novo* kidney transplant recipients receiving basiliximab, everolimus (1.5 mg twice daily), and steroids. In this study, patients at increased risk of CMV infection received prophylaxis according to local practice. Of the 111 patients included, only one patient, in the high-dose cyclosporine group, developed CMV infection[15].

During the same period, two registry studies, B201 and B251, compared the efficacy and safety of two doses of everolimus (0.75 or 1.5 mg twice daily) or mycophenolate mofetil 2 g/day in *de novo* kidney transplant recipients receiving cyclosporine micro-emulsion and steroids. The first study (B201) included 588 kidney transplant recipients and only patients at high risk (D^+R^-) received CMV chemoprophylaxis. CMV prophylaxis was used in 23% of patients receiving mycophenolate mofetil, 21% of those receiving everolimus 0.75 mg twice daily, and 20% of those receiving everolimus 1.5 mg twice daily. The incidence of CMV infection was significantly higher among patients receiving mycophenolate mofetil compared to those receiving everolimus 0.75 or 1.5 mg twice daily (19.9 vs. 5.7 vs. 8.1%, respectively; $p = 0.0001$). In the B251 study, which included 583 patients, CMV prophylaxis was used in 71% of patients receiving mycophenolate mofetil, 71% of those receiving everolimus 0.75 mg twice daily, and 78% of those receiving everolimus 1.5 mg twice daily. The incidence of CMV infection was similar in patients receiving everolimus 0.75 or 1.5 mg twice daily or the mycophenolate mofetil groups (5.2, 4.1, and 6.1%, respectively)[16]. In all these studies there was no protocol-defined and standardized method to report CMV-related events. The CMV events were reported as infection or adverse event at the discretion of the investigators[17].

The A2309 study was a 24-month multicenter, open-label, phase IIIb trial that enrolled 833 *de novo* kidney transplant recipients to compare the efficacy and safety of everolimus 0.75 or 1.5 mg twice daily and reduced concentrations of cyclosporine with mycophenolate sodium (720 mg twice daily) and standard concentrations of cyclosporine. All patients received basiliximab induction and prednisone[18]. The CMV prophylaxis was used according to local practice, except for D^+R^- transplants where CMV prophylaxis was mandatory. The CMV events were predefined as CMV viremia, syndrome, or organ-invasive disease and were obtained prospectively. There were no differences between groups regarding the CMV serostatus of the donor or recipient, or the type of prophylaxis employed. The overall incidence of CMV infection was lower among patients receiving everolimus 0.75 or 1.5 mg twice daily compared to those receiving mycophenolate (0.7 vs. 0 vs. 5.9%, respectively). Similarly, the incidence of CMV syndrome (1.5 vs. 1.4 vs. 4.4%, respectively) and CMV disease (0.7 vs. 0.7 vs. 2.2%, respectively) were lower among patients receiving everolimus[18]. The authors also reported that the incidence of CMV infection was lower in the everolimus groups regardless of serostatus of donor and recipient, as well as the use of prophylaxis.

A pooled analysis of data from the B251, B201, and A2309 studies was carried out

recently[19]. Overall, both everolimus doses (0.75 or 1.5 mg twice daily) were associated with a significantly longer mean time to first CMV event compared to mycophenolate (194 vs. 190 vs. 124 days, respectively). Among patients who did not receive CMV prophylaxis, those receiving everolimus 0.75 or 1.5 mg twice daily compared to patients receiving mycophenolate showed a lower incidence of CMV viremia (3.1 vs. 3.1 vs. 9.1%, respectively; $p \leq 0.0016$) or CMV syndrome (5.0 vs. 4.3 vs. 13.8%, respectively; $p < 0.0001$), but no differences in CMV organ-invasive disease (2.5 vs. 0.9 vs. 1.3%, respectively; $p > 0.05$). Among patients who received CMV prophylaxis, those receiving everolimus 0.75 or 1.5 mg twice daily showed a lower incidence of CMV viremia (5.2 vs. 2.6 vs. 6.6%, respectively; $p < 0.04$), CMV syndrome (6.1 vs. 6.0 vs. 10.5%, respectively; $p < 0.04$) and CMV organ-invasive disease (0.9 vs. 2.0 vs. 3.4%, respectively; $p<0.04$)[19].

Our center is conducting a study aimed to directly assess the incidence of CMV infection as primary endpoint in *de novo* kidney transplant recipients receiving tacrolimus, prednisone, and everolimus or mycophenolate, with induction therapy with thymoglobulin or basiliximab. In this study, low immunological risk kidney transplant recipients are randomized to receive a single 3 mg/kg dose of antithymocyte globulin, reduced tacrolimus exposure (4 ng/ml), everolimus (4-8 ng/ml) and prednisone (Group 1), basiliximab induction, reduced tacrolimus exposure (6 ng/ml), everolimus (4-8 ng/ml) and prednisone (Group 2), or basiliximab induction, reduced tacrolimus exposure (8 ng/ml), mycophenolate, and prednisone (Group 3). None of the patients receives any CMV prophylaxis and CMV infection is monitored weekly by CMV antigenemia and PCR tests. Preliminary data of the first 170 out of 300 patients has shown that in patients receiving everolimus, the incidence of CMV infections was lower than in patients receiving mycophenolate (2 vs. 12 vs. 37%, respectively; $p < 0.0001$). Interestingly, even in patients receiving induction with thymoglobulin, the incidence of CMV infection is lower, probably because of the additional benefit of lower need for the treatment of acute rejection episodes (9 vs. 19 vs. 16%, respectively; $p = 0.383$). Furthermore, 17.5% of patients in Group 3 developed at least one recurrent event of CMV infection. Among the high risk D^+R^- pretransplant CMV serostatus, the incidence of CMV infection was 0, 63 and 100%, respectively (personal communication).

Because CMV infection occurs predominantly during the first three months after transplantation, the influence of everolimus on the incidence of CMV infection has not been observed when the inception of this drug occurs at later times after transplantation. In three large studies where cyclosporine was replaced by everolimus (MECANO, ZEUS, and CENTRAL) there were no significant differences in the incidence of CMV infection[20-22].

MECANO was a multicenter, prospective, open-label, randomized trial that enrolled 264 *de novo* kidney transplant recipients receiving cyclosporine, mycophenolate, and prednisone. Efficacy and safety of withdrawal of cyclosporine, withdrawal of mycophenolate, or withdrawal of cyclosporine and mycophenolate after inception of everolimus six months after transplantation was evaluated only in patients without previous acute rejection. After randomization, only one patient developed CMV disease, in the mycophenolate-steroid group[20].

The ZEUS study was a prospective, multicenter, randomized, controlled, parallel-group trial that enrolled 503 kidney transplant recipients to compare efficacy and safety of maintenance of a regimen containing cyclosporine, steroid, and mycophenolate or conversion from cyclosporine to everolimus 4.5 months after transplantation. There was

no difference in the incidence of CMV infection after randomization between the cyclosporine and everolimus groups (10 vs. 6%; p = 0.3952)[21].

The CENTRAL study was a prospective, multicenter, randomized, open-label, parallel-group trial that enrolled 341 kidney transplant recipients to compare renal function of patients receiving cyclosporine, steroid, and mycophenolate and those converted from cyclosporine to everolimus seven weeks after transplantation. Similar to previous conversion studies, there was no significant difference in CMV infection incidence between the cyclosporine and everolimus groups (13 vs. 8.8%; p = 0.37)[22].

Adverse events associated with mammalian target of rapamycin inhibitors

To fully benefit from the use of mTORi, the reduced burden of CMV infection should not be outweighed by known mTORi-related adverse events. The clinical use of mTORi has been implicated in higher incidence of delayed graft function, wound healing complications, dyslipidemia, proteinuria, and renal dysfunction[23,24].

Evidences have accumulated indicating that the incidences of everolimus-related adverse events are associated with its concentration in the blood[25]. In the A2309 study there were no differences in the incidence of delayed graft function comparing patients receiving everolimus 0.75 mg twice daily (3-8 ng/ml) or 1.5 mg twice daily (6-12 ng/ml) compared to mycophenolate. Higher incidence of wound-healing complications was observed in the 0.75 and 1.5 mg twice daily (6-12 ng/ml) everolimus dose groups compared to mycophenolate (35.0 vs. 38.8 vs. 25.6%, respectively)[18]. A recent study in *de novo* kidney transplant recipients at higher risk to develop delayed graft function did not find any difference comparing immediate versus delayed (four weeks) use of everolimus targeting blood concentration of 3-8 ng/ml on the incidence (24.6 vs. 24.3%) or duration (10.2 ± 5.8 vs. 7.6 ± 8.0 days, respectively; p = 0.746) of delayed graft function. The same study also showed no differences in the incidence of wound healing complications (40 vs. 37.8%; p = 0.86)[26].

The proportion of patients receiving lipid-lowering agents to control dyslipidemia is usually higher compared to patients receiving mycophenolate. Nevertheless, the incidence of everolimus treatment discontinuation due to dyslipidemia is low[27].

In the A2309 study, the incidence of proteinuria ≥ 300 mg/g of creatinine at three months was comparable between everolimus 0.75 mg twice daily (3-8 ng/ml) and mycophenolate (24 vs. 19%; HR: 1.20; p = 0.19), but was higher in patients receiving everolimus 1.5 mg twice daily (6-8 ng/ml) compared to mycophenolate (36 vs. 19%; HR: 1.84; p < 0.001). Everolimus trough blood concentrations > 8 ng/ml were significantly associated with proteinuria compared to concentration of 3-8 ng/ml (HR: 1.86; p < 0.001)[28]. At 12 months, the mean urinary protein/creatinine ratios were higher in the everolimus 1.5 mg twice daily compared to mycophenolate (35.6 ± 66.3 vs. 61.4 ± 165.2 vs. 31.1 ± 68.7 mg/g, respectively)[18].

Regarding kidney graft dysfunction, recent studies show that, with currently recommended concentrations of everolimus and CNI, renal function of patients receiving this regimen is preserved and comparable to that of patients receiving CNI and mycophenolate. In the A2309 trial, there was no difference in creatinine clearance at one year when everolimus 0.75 mg twice daily, everolimus 1.5 mg twice daily, and mycophenolate groups were compared (56.3 ± 20.1 vs. 55.0 ± 19.8 vs.

54.4 ± 26.4 ml/min/1.73 m^2, respectively)[18]. This information was supported by the ASSET study. This was a multicenter, randomized trial that enrolled 228 patients and compared one-year renal function between patients receiving everolimus in combination with tacrolimus in low (4-7 ng/ml) or very low (1.5-3.0 ng/ml) concentration. The study showed that creatinine clearance in the tacrolimus 1.5-3.0 ng/ml group was lower compared to the tacrolimus 4-7 ng/ml group (57.1 ± 19.5 vs. 51.7 ± 20 ml/min/1.73 m^2; treatment difference: 5.3 ml/min/1.73 m^2; 95% CI: –0.2-10.9; p = 0.0299)[29].

Nevertheless, the interpretation and extrapolation of these data are limited by the short-term follow-up of these studies. Scant information still exists regarding long-term tolerability and safety of mTORi. The only study assessing 24-month data was the A2309 study. Mean estimated glomerular filtration rates (Modification of Diet in Renal Disease) were not different in patients receiving everolimus 0.75 mg twice daily (3-8 ng/ml), everolimus 1.5 mg twice daily (6-12 ng/ml), or mycophenolate (52.2 vs. 49.4 vs. 50.5 ml/min/1.73 m^2, respectively). Also, the incidence of proteinuria reported as adverse event (11.3 vs. 13.7 vs. 8.1%, respectively) and mean urinary protein to creatinine ratios (387.5 ± 650.1 vs. 419.2 ± 728.0 vs. 179.2 ± 240.3 mg/g, respectively) was higher in patients maintained with everolimus trough concentrations between 3-8 ng/ml or 6-12 ng/ml, compared to those receiving mycophenolate. These figures are comparable with the 12-month data suggesting stability of renal function and no further increase in the incidence or magnitude of proteinuria within this very limited period of observation[30].

Potential mechanisms for antiviral action

The exact mechanism to explain the association between mTORi and decreased CMV events is not clear. Intuitively, we could speculate that the degree of immunosuppression produced by regimens containing mTORi is inferior to that produced by mycophenolate. Still, in all comparative trials, everolimus and mycophenolate produced comparable efficacy for the prevention of acute rejection, graft loss, or death[16,18,31]. In the A2309 study, the most recent study evaluating these outcomes, the incidence of acute rejection at one year was 16.2% in the everolimus 0.75 mg twice daily group, 13.3% in the everolimus 1.5 mg twice daily group, and 17% in the mycophenolate group (p > 0.05). There was also no difference in the incidences of graft loss (4.3 vs. 4.7 vs. 3.2%, respectively; p > 0.05) and death (2.5 vs. 3.2 vs. 2.2%, respectively; p > 0.05)[18].

Alternatively, inhibition of mTOR may interfere with viral replication or produce immune deviation towards antiviral activity. It is known that viruses are intracellular pathogens that depend on cellular machinery for protein synthesis of their constituents and genomic replication. The mTORi show no inhibitory activity on viral replication[32]. The mTOR is a key regulator of viral protein and viral DNA synthesis. By blocking mTORC1, sirolimus and everolimus inhibit p70S6K, a product of phosphatidylinositol 3-kinase (PI3-K) that is activated during CMV infection. In addition, mTORC1 inactivates the translational repressor 4EBP1, blocking the translation of capped mRNA, which are required for CMV synthesis. Thus, the mTORC1 inhibition leads to inhibition of viral protein synthesis and viral DNA, beyond induction of apoptosis[33].

Another potential mechanism for mTORi antiviral action is the immunostimulatory effect of this drug on the generation of $CD8^+$ memory T-cells, increasing the level and quality of immune response after re-exposure to the virus[34,35]. The mTORi may also interfere with innate immunity. In monocytes and myeloid dendritic cells, inhibition of mTOR enhances

nuclear factor kappa β, IL-12, IL-23, tumor necrosis factor-α, and IL-6, but blocks IL-10 via STAT 3. These effects lead to the reversion of some of the glucocorticoid effects on innate immunity[36].

Conclusion and future directions

Cytomegalovirus infection has significant morbidity and mortality and none of the available prevention strategies is considered ideal. The *de novo* use of mTORi is associated with lower incidence of this infection, regardless of recipient and donor CMV serostatus and the use of prophylaxis. It is possible that *de novo* kidney transplant recipients receiving immunosuppressive regimens containing mTORi may not require universal prophylaxis and that the preemptive approach through monitoring of viral replication might be necessary only in those at higher risk, such as those with negative serology who received kidneys from donors with positive serology (D^+R^-), or after treatment of acute rejection. Whereas CMV infection is a leading cause of re-hospitalization after renal transplantation, and is associated with significant increases in the overall cost of transplantation, a strategy that reduces the incidence of this infection, in addition to impact on morbidity and mortality, would positively impact on the cost[37,38]. Nevertheless, the use of everolimus is associated with drug class-related adverse events that should be taken into account using a risk/benefit mitigation strategy. Future research should investigate risk factors associated with the development of CMV infection in patients receiving mTORi and also the impact of the conversion from mTORi to mycophenolate on the incidence and clinical presentation of CMV infection at various periods after transplantation. Finally, because mTORi have been used primarily in low-to-moderate immunological risk kidney transplant recipients, the data presented here cannot be extrapolated to high immunologic risk patients and also recipients of expanded criteria donor kidney allografts.

Disclaimers

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