Review of Cytomegalovirus Infection Findings With Mammalian Target of Rapamycin Inhibitor-Based Immunosuppressive Therapy in De Novo Renal Transplant Recipients

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Cytomegalovirus (CMV) infection and disease are major complications in the renal transplant recipient. The occurrence of CMV is associated with acute rejection, allograft dysfunction, significant end-organ disease, and mortality. Several clinical studies have indicated that the use of certain immunosuppressive drugs can delay the reconstitution of CMV-specific cell-mediated immune responses, thereby leading to uncontrolled CMV replication. Accumulating evidence indicates, however, that the use of the mammalian target of rapamycin (mTOR) inhibitors, sirolimus, and everolimus, may decrease the incidence and severity of CMV infection in renal transplant recipients. The purpose of this article is to review CMV infection data from randomized clinical trials that investigated the use of sirolimus- and everolimus-based treatment regimens in de novo renal transplantation. The mTOR inhibitor clinical trials included were primarily identified using biomedical literature database searches, with additional studies added at the authors' discretion. This review will summarize these studies to discuss whether mTOR inhibitor-based immunosuppressive therapy can reduce the magnitude of CMV-related complications in the de novo renal transplantation setting.

Keywords: Cytomegalovirus (CMV), Mammalian target of rapamycin (mTOR) inhibitors, Sirolimus, Everolimus, Anti-CMV mechanism of action.

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Even with recent advances in antiviral therapy, cytomegalovirus (CMV) remains the most important pathogen affecting the immunocompromised host (*1*). CMV infection is usually acquired early in life, and approximately 40% to 70% of the world's population is seropositive and harbors latent virus. Although primary CMV infection is generally self-limiting in immunocompetent individuals, primary infection or reactivation of latent virus in immunocompromised

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patients can have debilitating and even life-threatening consequences (*1, 2*).

CMV infection and disease are commonly observed after solid-organ transplantation, and are leading causes of clinical complications and recipient mortality (*3, 4*). Recipients who are seronegative for CMV, generally as a consequence of lack of previous virus exposure, are at greatest risk of disease after receiving allografts from infected, seropositive donors (donor positive/recipient negative combinations) (*3*). Without adequate preventive therapy to control viral replication, it is estimated that approximately 58% to 80% of solid-organ transplant recipients develop active CMV disease (*5, 6*).

Both invasive disease (''direct effects'') and immunologic phenomena (''indirect effects'') can arise after CMV infection in solid-organ transplant recipients (*3*). The direct effects can be divided into organ-specific manifestations of viral infection (including nephritis, hepatitis, and carditis) and more generalized symptoms termed ''CMV syndrome'' (fever, weakness, and myalgia). Indirect effects reflect altered immune responses associated with CMV infection, including allograft dysfunction, acute and chronic rejection, and opportunistic infections (*1, 3, 7–9*).

It is generally accepted that CMV is best dealt with by prophylactic or preemptive antiviral approaches (*10*). However, recent clinical trials involving immunosuppressants that target the mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus indicate that the agents are not only effective in preventing rejection but also impact on the risk of CMV disease and, therefore, might enable a more novel clinical approach (*11–17*). Mechanistically, mTOR inhibitors act by blocking the serine-threonine kinase activity of the protein complex mTORC1 (*18, 19*) by binding to the intracellular mTOR receptor FKBP12 (*18, 20*). The mTOR inhibitor-FKBP12 complex subsequently binds to mTOR, thereby preventing its association with the essential mTORC1 component Raptor (*18*), leading to inhibition of translational processes dependent on mTORC1 activity (*19*). Hence, blocking the mTOR pathway prevents cell-cycle progression from G1 into S phase (*21*) in cytokine-stimulated T-cells, which largely accounts for the immunosuppressive potency of mTOR inhibitors. Emerging evidence also suggests that mTOR inhibitors may exhibit antiviral effects through interruption of certain mTORC pathways or by invoking immune deviation.

Retrospective analysis of CMV infection and immunosuppressive strategies is difficult because of potential bias and other obstacles such as lack of infection definitions and inhomogeneous prophylaxis. Despite these impediments, findings supporting an important differential impact on CMV disease are accumulating from studies using mTOR inhibitors in comparison with non-mTOR inhibitor-based regimens. This article will summarize a number of these clinical trials, with a focus on whether use of mTOR inhibitors can exert a beneficial impact on CMV infection and disease after kidney transplantation.

Immunosuppressive Therapy as a Risk Factor for CMV Infection and Disease

Immunosuppressive regimens that do not block mTOR can delay CMV-specific immune responses, thereby contributing to the incidence of CMV infection and disease in renal transplant recipients (Table 1). This finding is particularly apparent for lymphocyte-depleting agents, including muromonab-CD3, antithymocyte globulin, and alemtuzumab (*22*). Further studies have suggested that combinations of specific drug therapies may increase the risk of infection and disease after renal transplantation. For example, cyclosporine-based regimens have been shown to increase the incidence of CMV infection. In the ELITE-Symphony study with 1645 renal transplant recipients, 14.3% and 11.0% of recipients who received standard and low-dose cyclosporine regimens, respectively, reported a CMV infection adverse event, whereas a lower incidence (9.7%) was observed with a low-dose tacrolimus-based regimen (*23*). Furthermore, in a Spanish Network for Research on Infection in Transplantation (RESITRA) network study involving 1470 renal transplant recipients, cyclosporine was significantly associated with the occurrence of CMV disease (odds ratio [OR] 1.70; 95% confidence interval [CI] 1.10, 2.90) (*24*). In this study, CMV disease was defined according to predefined RESITRA criteria (for details see Table 1). Using a prospective definition of CMV disease (Table 1), a further study found a higher incidence of CMV disease episodes associated with the use of mycophenolate mofetil (MMF) combination therapies compared with azathioprine-based regimens (*25*). This finding is supported by the results of several clinical trials that demonstrated that tissue-invasive CMV disease was more commonly observed in transplant recipients who received 3 g/day MMF compared with 2 g/day MMF and azathioprine (*26–28*).

In contrast to these studies, a significantly higher incidence of CMV infection has been observed following the administration of a thymoglobulin/azathioprine-based regimen in comparison with two basiliximab/MMF combination treatments (Table 1) (*29*). However, this finding could have been due to the lower cyclosporine dose administered to the recipients who received basiliximab/MMF therapy and/or the potency of depletional induction, emphasizing the complex interactions present in various immunosuppressive regimens.

CMV Findings From Comparative De Novo Studies Evaluating mTOR Inhibitors

CMV findings have been evaluated in several clinical studies comparing the use of sirolimus- or everolimus-based immunosuppressive regimens with non-mTOR inhibitor-based regimens in de novo renal transplantation. The mTOR inhibitor data discussed in this review were obtained from the PubMed biomedical literature database (Tables 2 and 3 for search term and selection criteria details). Only randomized trials were included and no meta-analyses were performed on the publications identified. The studies identified consistently demonstrated low rates of CMV infection with both sirolimus- and everolimus-based regimens in comparison with a number of immunosuppressive regimens (Tables 2 and 3).

Several similarities in study design can be observed across the investigations conducted with both sirolimus and everolimus. In general, the mTOR inhibitors were initially administered within 48 hr of transplantation and CMV prophylaxis was specified in most studies. Various prophylactic treatments were used, including ganciclovir, valacyclovir, acyclovir, valganciclovir, and CMV hyperimmune globulin.

TABLE 1. Risk of CMV infection and disease following the use of non-mTOR inhibitor-based immunosuppressive regimens in renal transplant recipients

Immunosuppressive treatment comparison	N	Anti-viral prophylaxis	CMV definition	CMV results	Reference
Alemtuzumab induction + tacrolimus + steroids vs. tacrolimus + MMF + steroids[a]	131	Oral ganciclovir or valganciclovir for 90 d in D+ recipients	Adverse event	28% vs. 12%; RR 2.28 (1.07–4.88)	Margreiter et al. (*70*)
Basiliximab/daclizumab induction vs. no induction[b]	1398	IV ganciclovir or oral valganciclovir	Definitions provided for CMV viral syndrome, definite tissue-invasive disease, and probable end-organ CMV disease[c]	CMV disease: OR 0.60 (0.33–1.09); $P = 0.097$ (2 yr)	Fortun et al. (*37*)
Thymoglobulin induction vs. no induction[b]	1398	IV ganciclovir or oral valganciclovir	Definitions provided for CMV viral syndrome, definite tissue-invasive disease, and probable end-organ CMV disease[c]	CMV disease: OR 1.53 (0.77–2.96); $P = 0.25$ (2 yr)	Fortun et al. (*37*)
Thymoglobulin induction vs. other immunosuppressive therapies[d]	207	14 d' IV ganciclovir or valganciclovir with prolonged (3 mo) valganciclovir in high-risk (D+/R−) recipients	CMV disease: evidence of CMV infection with attributable symptoms and need for anti-viral treatment	RR 4.19 (0.98–4.80); $P = 0.05$	Kanter et al. (*71*)
OKT3 or ATG induction vs. other immunosuppressive therapies[b]	1470	Performed according to Spanish Study Group of Infection in Transplantation guidelines[e]	According to predefined RESITRA criteria[f]	OR 2.14 (1.10–4.40)[g]; $P = 0.04$[g]	San Juan et al. (*24*)
Cyclosporine vs. other immunosuppressive therapies[b]	1470	Performed according to Spanish Study Group of Infection in Transplantation guidelines[e]	According to predefined RESITRA criteria[f]	OR 1.70 (1.10–2.90)[g]; $P = 0.03$[g]	San Juan et al. (*24*)
Cyclosporine vs. other immunosuppressive therapies[d]	207	14 d' IV ganciclovir or valganciclovir with prolonged (3 mo) valganciclovir in high-risk (D+/R−) recipients	CMV disease: evidence of CMV infection with attributable symptoms and need for anti-viral treatment	RR 2.80 (1.00–7.60); $P = 0.04$	Kanter et al. (*71*)
Standard cyclosporine vs. low cyclosporine vs. low tacrolimus Each with MMF and steroids; low-dose arms also received daclizumab induction[a]	1589 D+/R− for CMV: range 13−16%	Not mentioned	Adverse event; Kaplan-Meier estimate	Adverse event: 14.3% vs. 11.0% vs. 9.7%; Kaplan-Meier estimate: 15.3% vs. 11.5% vs. 10.2% ($P = 0.003$) (All data at 12 mo)	ELITE-Symphony; Ekberg et al. (*23*)

(Continued)

TABLE 1. *(Continued)*

Immunosuppressive treatment comparison	N	Anti-viral prophylaxis	CMV definition	CMV results	Reference
MMF vs. azathioprine Each with steroids with or without cyclosporine[d]	499	Not mentioned	Evidence of CMV antigenemia plus any of the following: decreased leucocytes or platelets, increased transaminases, or worsening of renal function (increase in serum creatinine)	1 episode per 118.1 treatment months vs. 1 episode per 346.5 mo; $P < 0.01$	Basic-Jukic et al. (*25*)
Tacrolimus + MMF vs. cyclosporine + azathioprine. Each with steroids and ATG induction[a]	300	Oral valganciclovir (3 mo)	Evidence of viral replication associated with at least 1 symptom	CMV replication: RR 1.58 (1.05–2.40)[g]; $P = 0.03$[g]; CMV disease: RR 1.31 (0.75–2.28)[g]; $P = 0.35$[g]	Bataille et al. (*72*)
Thymoglobulin + cyclosporine + azathioprine vs. basiliximab + cyclosporine + MMF vs. basiliximab + tacrolimus + MMF Each with steroids[a]	240	IV ganciclovir followed by oral acyclovir (12 wk)	Evidence of CMV infection plus 2 or more of the following: unexplained fever for 2 or more days, leukopenia, thrombocytopenia, arthralgia, a rise in liver enzymes, pneumonitis, gastrointestinal disease	CMV infection: 41% vs. 20% vs. 25%; $P = 0.008$; CMV disease: 9% vs. 4% vs. 11%	Hernández et al. (*29*)

[a] Randomized clinical trial.

[b] Prospective analysis using data from the clinical setting (RESITRA network).

[c] CMV viral syndrome: temperature of >38°C with antigenemia or a positive PCR result with no other cause to account for it, and 1 of the following: leukocyte count <4000 cells/mm^3; atypical lymphocytes ≥3%; platelet count <100,000 platelets/mm^3. Definite tissue-invasive disease: histopathologic evidence of CMV from a tissue sample with or without culture of the virus. Probable end-organ disease: a compatible clinical presentation, evidence of CMV antigenemia or a positive PCR result, and a clinical and/or virologic response to specific treatment with ganciclovir or valganciclovir. CMV infection: a significant positive PCR result (>1000 copies/mL of serum) or pp65 antigenemia (>10 positive cells/200,000 leukocytes).

[d] Retrospective analysis.

[e] Spanish Study Group of Infection in Transplantation guidelines can be found in Torres-Cisneros et al. (*73*).

[f] CMV disease was defined according to predefined RESITRA criteria and included CMV viral syndrome (temperature of >38°C with a positive antigenemia test with no other cause to account for it and 1 of the following findings: leukocyte count, <4000 cells/mm^3; atypical lymphocytes of ≥3%; platelet count, <100,000 platelets/mm^3). Definite tissue-invasive disease: histopathologic evidence of CMV with or without culture of the virus from a tissue sample. Probable end-organ CMV disease: a compatible clinical presentation, evidence of CMV antigenemia, and a clinical and/or virologic response to specific treatment with ganciclovir or valganciclovir.

[g] Multivariate analysis results.

ATG, anti-thymocyte globulin; CMV, cytomegalovirus; D, donor; IV, intravenous; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; OKT3, muromonab-CD3; OR, odds ratio; PCR, polymerase chain reaction; R, recipient; RESITRA, Spanish Network for Research on Infection in Transplantation; RR, relative risk.

TABLE 2. Incidence of CMV infection in de novo renal transplant recipients following the use of sirolimus-based immunosuppressive therapy in randomized clinical trials

Study arms	N	CMV definition	Anti-viral prophylaxis	D+/R– for CMV	CMV results	Reference
Sirolimus vs. cyclosporine. Each with MMF and steroids	78	Adverse event	Standard CMV prophylaxis	Not reported	5% vs. 21%; $P = 0.045$ (12 mo)	Kreis et al. (*15*)
Sirolimus vs. cyclosporine. Each with steroids and azathioprine	83	Adverse event	Standard CMV prophylaxis	Not reported	14% vs. 12% (12 mo); one tissue-invasive CMV in cyclosporine group	SERTS; Charpentier et al. (*74*)
Sirolimus vs. cyclosporine. Each with basiliximab induction, MMF, and steroids	61	Adverse event	Oral acyclovir or ganciclovir (90 d) according to D/R serology	Not reported	6% vs. 10% (5 yr)	Flechner et al. (*75*)
Sirolimus vs. cyclosporine. Each with ATG induction, MMF, and steroids	145	Adverse event	Valacyclovir (16 wk for D+/R− recipients)	22.9% vs. 14.9%; NS	6% vs. 23%; $P = 0.004$ (12 mo)	SPIESSER; Büchler et al. (*67*)
Sirolimus vs. tacrolimus. Each with ATG induction, MMF, and steroids	165	Adverse event	Not mentioned	Not reported	3% vs. 12%; $P = 0.02$, all were D+/R– for CMV (mean follow-up 33 mo); 1 CMV-related graft loss in tacrolimus group	Larson et al. (*35*)
Sirolimus + tacrolimus vs. sirolimus + cyclosporine vs. MMF + tacrolimus Each with daclizumab induction and steroids	150	Adverse event	Not mentioned	Not reported	0% vs. 2% vs. 2% (3 yr)	Ciancio et al. (*76*)
Low sirolimus vs. standard cyclosporine vs. low cyclosporine vs. low tacrolimus Each with MMF and steroids; low-dose arms also received daclizumab induction	1589	Adverse event; Kaplan-Meier estimate	Not mentioned	15.5% vs. 13.6% vs. 13.5% vs. 12.7%	Adverse event: 6.1% vs. 14.3% vs. 11.0% vs. 9.7%; Kaplan-Meier estimate: 6.5% vs. 15.3% vs. 11.5% vs. 10.2% ($P = 0.003$) (All data at 12 mo)	ELITE-Symphony; Ekberg et al. (*23*)
Sirolimus vs. tacrolimus. Each with MMF and steroids; sirolimus arm also received ATG induction	141	Adverse event	Not mentioned	D+: 50.7% vs. 62.9%; R−: 34.3% vs. 43.5%	1.4% vs. 20.0%; $P < 0.001$ (12 mo)	Glotz et al. (*36*)

PubMed search terms used to locate studies: "sirolimus [tiab] AND (renal [ti] OR kidney [ti]) AND random* [tiab] AND cytomegalo*" and "sirolimus [tiab] AND (renal [ti] OR kidney [ti]) AND de novo [tiab]". ATC, ASN, ESOT, WCN, ICTS; years 2007–2010 search terms used "cytomegalovirus OR CMV" in abstract text. Additional significant studies added at authors' discretion.

ATG, anti-thymocyte globulin; CMV, cytomegalovirus; D, donor; MMF, mycophenolate mofetil; NS, not significant; R, recipient.

TABLE 3. Incidence of CMV infection in de novo renal transplant recipients following the use of everolimus-based immunosuppressive therapy in randomized clinical trials

Study arms	N	CMV definition	Anti-viral prophylaxis	D+/R– for CMV	CMV results: mTOR inhibitor vs. control	Reference
Everolimus + low cyclosporine vs. everolimus + standard cyclosporine. Each with basiliximab induction and steroids	111	Adverse event	CMV prophylaxis according to local practice if at increased risk	Not reported	0% vs. 1.9% (36 mo)	Study 156; Nashan et al. (*77*)
Standard everolimus + low cyclosporine vs. high everolimus + very low cyclosporine Each with basiliximab induction and steroids	285	Adverse event	CMV prophylaxis mandatory when D+/R−	8.4% vs. 7.7%	Serious: 1.4% vs. 2.1%; Leading to discontinuation: 0.7% vs. 0% (All data at 12 mo)	EVEREST; Salvadori et al. (*78*)
High everolimus + very low cyclosporine vs. EC-MPS + standard cyclosporine Each with steroids	87	Adverse event	Not mentioned	Not reported	26% vs. 27% (24 mo)	Bertoni et al. (*79*)
Everolimus (1.5 mg) + low cyclosporine vs. everolimus (3 mg) + low cyclosporine vs. MMF + standard cyclosporine Each with steroids	583	Adverse event	CMV prophylaxis mandatory when D+/R− (ganciclovir; CMV hyperimmune globulin or acyclovir); according to local practice for other recipients	Not reported	5.2% vs. 4.1% vs. 6.1% (36 mo)	B251; Lorber et al. (*11*)
Everolimus (1.5 mg) + low cyclosporine vs. everolimus (3 mg) + low cyclosporine vs. MMF + standard cyclosporine Each with steroids	588	Adverse event	CMV prophylaxis mandatory when D+/R− (ganciclovir; CMV hyperimmune globulin or acyclovir); according to local practice for other recipients	19% vs. 22% vs. 20%[a]	5.7% vs. 8.1% vs. 19.9%; $P = 0.0001$ for each everolimus dose vs. MMF (36 mo)	B201; Vítko et al. (*17*)
Everolimus (1.5 mg) + low cyclosporine vs. everolimus (3 mg) + low cyclosporine vs. EC-MPS + standard cyclosporine Each with basiliximab induction ± steroids	833	CMV syndrome and CMV disease prospectively defined	According to local practice (ganciclovir; CMV hyperimmune globulin; acyclovir; valacyclovir) when D+/R−	10.8% vs. 10.0% vs. 15.2%	Adverse event: 0.7% vs. 0.0% vs. 5.9% CMV syndrome: 1.5% vs. 1.4% vs. 4.4% CMV disease: 0.7% vs. 0.7% vs. 2.2% Lower incidence of CMV infection with everolimus: with/without prophylaxis; and in all D/R subgroups (All data at 12 mo)	A2309; Tedesco Silva Jr et al. (*16*)

PubMed search terms used to locate studies: "everolimus [tiab] AND (renal [ti] OR kidney [ti]) AND random* [tiab] AND cytomegalo*" and "everolimus [tiab] AND (renal [ti] OR kidney [ti]) AND de novo [tiab]". ATC, ASN, ESOT, WCN, ICTS; years 2007–2010 search terms used "cytomegalovirus OR CMV" in abstract text. Additional significant studies added at authors' discretion.

[a] Data from Vítko et al. 2004 (*40*).

CMV, cytomegalovirus; D, donor; EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; R, recipient.

Although most of these therapeutics provide effective protection from CMV infection (*30*), a number of studies have now indicated that oral ganciclovir and valganciclovir are more effective than oral acyclovir or valacyclovir (*31–33*). CMV infection was typically reported as an adverse event and not prospectively defined, although one study did include an a priori goal of assessing risk of CMV infection (*16*).

The ELITE-Symphony study compared renal function, efficacy (acute rejection and allograft survival), and relative toxic effects of four immunosuppressive regimens: a standard-dose and a low-dose cyclosporine-based regimen, a low-dose tacrolimus-based regimen, and a low-dose sirolimus-based regimen (*23*). Despite significantly more rejection and anti-rejection treatment, the lowest rate of CMV infection was observed with the sirolimus-based regimen (6.1%), followed by the low-dose tacrolimus, low-dose cyclosporine, and standard-dose cyclosporine regimens (9.7%, 11.0%, and 14.3%, respectively; $P = 0.003$). The tacrolimus results should be interpreted with caution, however, as the standard-dose tacrolimus regimen currently used in many transplantation centers was not included (*34*).

Two clinical trials compared a sirolimus-MMF-corticosteroid regimen with a tacrolimus-MMF-corticosteroid regimen after renal transplantation (*35, 36*). The aim of these studies was to investigate recipient and graft survival, acute rejection, renal function, and adverse events. In both studies, the incidence of CMV infection was higher for tacrolimus-treated patients. In the first study, 10 cases of systemic CMV infection were diagnosed in the tacrolimus group (12%) compared with two cases in the sirolimus group (3%; $P = 0.02$) (*35*). All of the cases developed in CMV-seronegative recipients who received kidneys from CMV-seropositive donors. In the second study, a single recipient (1.4%) was diagnosed with a CMV infection in the sirolimus group compared with 14 recipients (20%) in the tacrolimus group ($P < 0.001$) (*36*).

Two large RESITRA network trials have been analyzed to assess possible risk factors for CMV disease. The results of the study published by San Juan et al. (*24*) showed that the risk of acquiring CMV disease was significantly lower following the administration of sirolimus (OR 0.27; 95% CI 0.10, 0.78). The positive effect observed with sirolimus treatment was supported by the results of a more recent analysis by Fortun et al. (*37*). A maintenance regimen that included sirolimus was independently associated with a lower risk of CMV infection (OR 0.16; 95% CI 0.05, 0.54).

As with sirolimus-based regimens, less CMV infection and disease have also been observed with everolimus-based therapy compared with other non-mTOR inhibitor-based regimens. Study A2309, a 24-month Phase IIIb trial, examined the efficacy and safety of two everolimus-based regimens with reduced-exposure cyclosporine and a mycophenolic acid (MPA)-based regimen with standard-exposure cyclosporine (*16*). The first dose of immunosuppressive therapy was administered within 24 hr posttransplantation. CMV prophylaxis (ganciclovir, CMV hyperimmune globulin, acyclovir, or valacyclovir) was mandatory for all CMV-negative recipients who received a kidney from a CMV-positive donor. The study also aimed to investigate the incidences of CMV syndrome, CMV disease, and CMV infection. At 12 months, lower rates of CMV infection were obtained with both of the everolimus-based regimens compared with the MPA-based regimen: 0.7% and 0.0% for the 1.5 and 3 mg everolimus groups in comparison with 5.9% for the MPA group (Table 3). The incidence of CMV syndrome and CMV disease was also lower for both everolimus arms. Further analysis revealed that the incidence of CMV infection was reduced in recipients with a positive CMV serologic status at baseline, but not in those with a negative CMV status (*38*).

The efficacy and safety of everolimus-based regimens have been compared with an MMF-based regimen in two 36-month trials: B201 and B251 (*11, 17, 39*). In both studies, the recipients were randomized to receive treatment with 1.5 mg everolimus, 3 mg everolimus, or MMF. All recipients received concomitant treatment with cyclosporine and steroids as part of their immunosuppressive regimen. In both studies, all high-risk recipients received CMV prophylactic treatment with ganciclovir, CMV hyperimmune globulin, or acyclovir, and the other recipients received CMV prophylaxis according to local practice. In study B201, 5.7%, 8.1%, and 19.9% of recipients had a CMV infection after immunosuppressive treatment with the 1.5 mg everolimus-, 3 mg everolimus-, and MMF-based regimens, respectively ($P = 0.0001$ for each everolimus regimen in comparison with MMF) (*17*). In study B251, comparatively lower CMV infection rates of 5.2%, 4.1%, and 6.1% were reported following treatment with the 1.5 mg everolimus-, 3 mg everolimus-, and MMF-based regimens, respectively (*11*). The disparity between the two studies may have been due to differing local practice of CMV prophylaxis, which contributed to a higher proportion of recipients receiving prophylactic treatment in study B251 than in study B201 (Novartis Pharma AG, data on file). In study B251, CMV prophylaxis was received by 71% of recipients in both the 1.5 mg everolimus and MMF groups, and 78% of recipients in the 3 mg everolimus group. Comparatively, in study B201, 21% of recipients in the 1.5 mg everolimus group, 20% of the recipients in the 3 mg everolimus group, and 23% of the recipients in the MMF group received prophylaxis (*40*). The variability in CMV infection rates observed in these two studies contributed to CMV infection, CMV disease, and CMV syndrome being prospectively defined in study A2309.

The use of an mTOR inhibitor as part of the immunosuppressive regimen could also be considered in recipients with CMV infection resistant to antiviral therapy. In a study of nine renal transplant recipients who had ganciclovir-resistant CMV infection (*41*), a rapid decrease in antigenemia levels was observed after conversion to sirolimus and ganciclovir administration, and none of the recipients experienced acute rejection or CMV recurrence.

CMV Findings From Comparative Conversion Studies Evaluating a Switch to mTOR Inhibitors

Other investigations have shown that the beneficial findings observed with mTOR inhibitors in the de novo setting may not be apparent in immunosuppression conversion studies following renal transplantation. In the recently completed ZEUS study, rates of CMV infection were similar after immunosuppressive therapy with cyclosporine and delayed everolimus (19% and 18%, respectively) (*42*). Recipients were randomized in ZEUS to continue with cyclosporine or receive an everolimus-based regimen following an initial 4.5 months of immunosuppressive therapy. In the CONCEPT study, similar incidences of CMV infection were reported with a

cyclosporine-based regimen or a delayed sirolimus-based regimen (6% and 4%, respectively) (*43*). In this study, all the recipients initially received immunosuppressive treatment with a cyclosporine-based regimen before being randomized after 3 months to continue with cyclosporine or switch to a sirolimus-based regimen. In the CALLISTO study, when maintenance everolimus was compared with de novo everolimus, CMV infection rates of 6.8% and 1.5%, respectively, were observed for recipients who received everolimus 5 weeks after transplantation after 4 weeks of therapy with MMF and recipients who received everolimus the day after renal transplantation (*44*). A retrospective study also showed that the use of sirolimus-based maintenance immunosuppression was not associated with a reduced incidence of CMV disease (OR 0.76; 95% CI 0.30, 1.90) (*45*).

In contrast, statistically lower CMV infection rates were obtained with a sirolimus-based regimen compared with a cyclosporine-based regimen in the SMART immunosuppression conversion study (7.3% and 28.2%, respectively; $P = 0.0016$) (*46*). This finding may have been due to the fact that the switch to sirolimus-based therapy occurred shortly after transplantation (10–24 days posttransplant).

Anti-CMV Mechanisms of Action of mTOR Inhibition

As only a limited number of preclinical studies have investigated the anti-CMV mechanisms of mTOR inhibitors, several potential molecular mechanisms may account for the anti-CMV potency of mTOR inhibitors at the cellular level (Fig. 1).

Activation of mTOR in host cells is essential for CMV to successfully propagate translation of viral proteins, even under conditions that normally block mTOR activity, such as cellular stress, which is regularly associated with the process of viral entry (*18, 47*). A recent study with the mTOR kinase inhibitor Torin1, which blocks both mTORC1 and mTORC2 (*48*), demonstrated the complex involvement of mTOR during CMV infection (*49*). Torin1 is capable of not only decreasing the accumulation of viral DNA but also dramatically reducing the levels of the pUL99 viral late protein (*18*). Furthermore, it

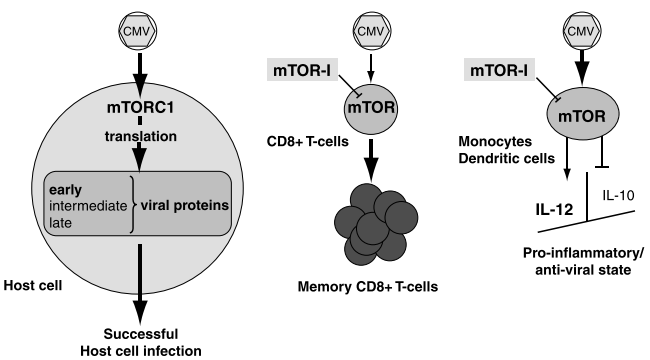

FIGURE 1. Molecular mechanisms of anti-CMV effects of mTOR inhibitors. Mutually non-exclusive roles of mTOR during CMV infection (*left*), the potential of mTOR inhibitors to affect antiviral CD8+ memory T-cell generation (*middle*), and the possibility to deviate CMV-mediated immune evasion by blocking mTORC1 activity in myeloid cells (*right*) are depicted. IL, interleukin; CMV, cytomegalovirus; mTOR, mammalian target of rapamycin.

was demonstrated that inhibition of mTORC1 prevented the accumulation of immediate early, early, and late viral proteins (*49*). However, blocking mTORC1 activity at very early time points after viral infection resulted in the most profound effects on viral translation and overall infection efficiency compared with later time points. These results indicate a dynamic relationship of mTORC1 activation and CMV infection that is also potentially susceptible to the effects of pharmacologic mTOR inhibition.

Further studies on the multifunctional role of mTOR within the immune system suggested that mTOR inhibitors may also exert their antiviral effect by influencing immune-mediated responses. Interestingly, recent studies have demonstrated that mTOR inhibitors regulate CD8 memory T-cell formation, because inhibition of the mTOR pathway enhanced not only the quantity but also the quality of virus-specific CD8+ T-cells (*20, 50*). Using sophisticated models, a recent investigation demonstrated that the environment in which an antigen is presented alters the influence of sirolimus on antigen-specific T-cell expansion (*20, 51*). Following sirolimus monotherapy, the antigen-specific CD8+ T-cell response was inhibited in response to graft transplantation and augmented in response to viral or bacterial pathogens.

Other components of the innate immune system, including γδ T-cells, may also be affected by the administration of mTOR inhibitors. A dramatic expansion in the number of γδ T-cells occurs in the peripheral blood of renal allograft recipients following the development of a CMV infection (*52*). Additionally, γδ T-cells are capable of killing CMV-infected target cells, producing interferon-γ, and limiting CMV propagation in vitro (*52, 53*). Recent investigations have demonstrated that the introduction of sirolimus increased the proliferation of treated and antigen-exposed γδ T-cells in vitro (*54*).

The inhibition of mTOR may affect innate immune cells such as monocytes, macrophages, and dendritic cells (*20, 55*). The production of pro-inflammatory cytokines such as interleukin (IL)-12 and IL-23 is substantially increased, whereas the classical anti-inflammatory cytokine IL-10 is suppressed after sirolimus treatment (*55, 56*). Recent studies investigating the growth of Epstein-Barr virus-positive lymphomas have suggested that sirolimus may exert its effect on IL-10 by inhibiting the phosphorylation of the mTOR substrate p70-S6 kinase (*57, 58*). Several investigations have discovered that early events associated with the invasion of CMV trigger host production of pro-inflammatory cytokines such as IL-12 (*59–61*). This effect is countered by CMV through the production of a viral homolog of IL-10 and the suppression of host IL-12 production, which limits the production of TH1-specific interferon-γ producing T-cells (*55, 62, 63*). Interestingly, a further study also identified a relationship between CMV reactivation after kidney transplantation and a single nucleotide polymorphism in the host IL-12p40 gene (*64*). These findings suggest that the blocking of mTOR through the administration of everolimus and sirolimus might inhibit the potent viral host evasion strategies used by CMV.

DISCUSSION

The occurrence of CMV infection and disease is associated with significant clinical illness, allograft loss, and mortality after renal transplantation (*65*). Despite encouraging results

using candidate CMV vaccines (*66*), antiviral therapy remains the mainstay of patient management for CMV. However, several studies indicate that specific immunosuppressive agents may contribute to the incidence of CMV infection and disease observed in renal transplant recipients.

The evidence summarized above indicates that the mTOR inhibitors may actually decrease the incidence of CMV infection and disease experienced by transplant recipients relative to other protocols. CMV infection in de novo renal transplant recipients was significantly lower following the use of sirolimus-based regimens in comparison with both cyclosporine- and tacrolimus-based regimens (*15, 23, 35, 36, 67*). Likewise, significantly less CMV infection has been observed after immunosuppressive treatment with everolimus in comparison with MMF (*17, 40*).

In light of the relationship between mTOR inhibitors and the reduced risk of CMV infection in de novo renal transplant recipients, large Phase III clinical trials are required to evaluate the relative risk of infection and disease with mTOR inhibitors in comparison with other immunosuppressive therapies (cyclosporine, tacrolimus, and MPA). CMV infection as a clinical end point should be rigorously defined and the prospective incidence of infection should be investigated, along with the prevalence of CMV syndrome and disease.

In keeping with the findings in the renal transplantation setting, mTOR inhibitor-based regimens are also associated with a reduced incidence of CMV infection and disease in cardiac and hepatic transplant recipients. A significantly lower incidence of any CMV event was observed for everolimus plus reduced-exposure cyclosporine in comparison with MMF plus standard-exposure cyclosporine in de novo cardiac transplant recipients (8.8% and 32.5%, respectively; $P < 0.001$) (*68*). Significantly lower rates of CMV infection, CMV syndrome, and organ involvement were also observed. A low incidence of CMV disease (2%) was reported after the use of a prednisone-free, sirolimus-based immunosuppressive regimen in 150 liver transplant recipients (*69*). The incidence of CMV disease was lower than in the other recipient groups who had received conventional CMV prophylactic treatment.

In conclusion, clinical evidence from comparative studies demonstrate that early use of mTOR inhibitor-based regimens can reduce the incidence of CMV infection and so impact on the immediate and long-term clinical sequelae associated with CMV. Further investigation of this observation should include randomized trials with homogeneous antiviral prophylaxis, standardized definitions, and adequate power to confirm or refute the observations.

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