# Improving Outcomes for Solid-Organ Transplant Recipients At Risk from Cytomegalovirus Infection: Late-Onset Disease and Indirect Consequences

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**Cytomegalovirus (CMV) is one of the most important pathogens following solid-organ transplantation, and effective prevention of CMV infection is a priority. The long-term control of CMV infection is dependent, in part, on the development of CMV-specific T cells, and controversy exists regarding whether CMV prophylaxis may prevent this. Although preemptive therapy is beneficial for the prevention of CMV disease, monitoring of viral levels in the blood does not always reflect what is occurring in tissues. Persistent low-level CMV infection has been associated with indirect consequences, such as transplant-associated vasculopathy, posttransplantation diabetes, an increased risk of opportunistic infection, and graft rejection. The issues surrounding preventive strategies for CMV disease following solid-organ transplantation are reviewed. We argue that prophylaxis is more effective than preemptive therapy; extending the duration of prophylaxis to the period of less intense immunosuppression could protect patients from late-onset disease, as well as from the indirect effects of CMV infection.**

Despite significant improvements in diagnostic and therapeutic management, cytomegalovirus (CMV) infection continues to influence outcomes of both solid-organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients. CMV has developed a number of strategies to evade the host immune response and can establish a life-long persistent and latent infection in immunocompetent individuals [1, 2]. However, in immunocompromised hosts, as in HIV-infected individuals and transplant recipients, CMV reactivation can occur. In the absence of any preventive therapy, 30%–75% of transplant recipients develop CMV infection (table 1), and the reported incidence of CMV disease is 8%–30% [3]. In addition to the direct consequences of CMV infection [1], it is now accepted that CMV infection is associated with a range of indirect effects [4–8], and recommendations for updating the definitions of CMV infection have been published [9].

Controversy exists concerning how best to protect SOT recipients from the effects of CMV infection. Ultimately, long-term protection from CMV disease correlates with the development of a CMV-specific T cell immune response [10, 11], although the CMV genome encodes a number of gene products that can alter the host's immunological functions [2]. The virus can influence the production of various cytokines and chemokines that can inhibit natural killer and T cell responses, as well as target humoral immune responses [12–16]; in fact, it is these immunomodulatory properties that may be responsible for the indirect consequences of CMV infection [17].

Late-onset CMV disease has emerged as a significant complication for patients (particularly if the donor is seropositive [D+] and the recipient is seronegative [R−]) who receive >3 months of anti-CMV prophylaxis, suggesting that anti-CMV prophylaxis postpones the development of CMV-specific immunity following transplantation [18]. However, interaction between CMV and the host immune system is complex. Viral load in the initial phase of active infection and lower levels of persistent viral replication correlate with CMV disease [19]. Thus, the kinetics of viral replication may impact on the ability of the host to mount a protective immune response, with the situation further complicated in transplantation by the use of immunosuppressive agents.

A main goal when selecting a preventive strategy is to optimize long-term patient outcomes. The 2 main strategies employed by physicians are preemptive therapy or prophylaxis therapy (table 1). The preemptive approach has proven to be beneficial in the prevention of CMV disease and allograft rejection [20, 21]; however, standard monitoring procedures do not

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**Table 1. Definition of key terms.**

| Term | Definition |
|---|---|
| CMV infection | The presence of CMV in body fluid or a tissue specimen as determined by DNA techniques, culture, or antigen tests |
| CMV disease | CMV infection manifesting with signs and symptoms of fever, malaise, leukopenia (CMV syndrome), and/or documented CMV invasive disease into organs (tissue invasive disease) |
| Late-onset disease | CMV disease occurring after the cessation of antiviral prophylaxis |
| Preemptive therapy | Regular monitoring of patients to identify CMV viremia and instigation of therapeutic intervention only when the virus is detected |
| Prophylaxis therapy | The administration of antiviral therapy for a predefined time (usually 3 months) to all patients at risk for CMV infection |

**NOTE.** CMV, cytomegalovirus.

detect local CMV reactivation, risking the development of organ-specific disease if viral load supersedes disease thresholds prior to detection of virus in the blood [21–23]. The development of a CMV-specific immune response is paramount for lifelong protection against CMV infection and disease. Preemptive therapy may allow the development of effective CMV-specific immunity in the early posttransplantation period, thereby reducing the risk of late-onset CMV disease; however, this approach does not offer full protection against all of the indirect effects of CMV infection [20]. It is important to note that persistent low-level viremia can indirectly impact upon long-term transplantation outcomes, such as the development of transplantation-associated cardiovasculopathy [24], opportunistic infection, or posttransplantation diabetes mellitus [6], and thereby decrease overall patient and/or graft survival.

This review will argue that extending the duration of prophylaxis to a period of less intense immunosuppression—yet to be defined—should enable the development of CMV-specific immune responses and, thus, protect against late-onset disease while preventing the indirect effects of CMV infection.

## EFFECTIVE PREVENTION OF CMV INFECTION: CONSIDERATIONS FOR LATE-ONSET DISEASE

Several antiviral agents are available for the prevention and treatment of CMV infection, of which intravenous ganciclovir and the oral prodrug of ganciclovir, valganciclovir, are most commonly prescribed. Although both prophylaxis and preemptive strategies are currently employed, the majority of clinical evidence derives from studies involving prophylaxis, because the number of reported preemptive trials are relatively few [25, 26]. These clinical studies have consistently demonstrated that CMV prophylaxis is beneficial in reducing the risk of CMV disease [27–29] and the indirect sequelae associated with CMV infection [20, 21, 25, 27]. Nevertheless, prophylaxis has also been associated with the development of late-onset CMV disease, the severity of which (at least in renal transplantation) is usually mild.

The PV16000 trial, which assessed the comparative efficacy of 100 days of prophylaxis with valganciclovir versus oral ganciclovir in D+/R− patients, reported an incidence of CMV disease at 12 months after transplantation of 17.2% and 18.4% for valganciclovir and oral ganciclovir, respectively. The vast majority of patients who developed CMV disease did so after the discontinuation of prophylaxis (i.e., they experienced late-onset disease) [28], as has been reported by other investigators [30, 31]. Such studies have led to suggestions that the benefits of prophylaxis are confined to the early posttransplantation period [32].

Evidence suggesting that extending the period of antiviral prophylaxis will avoid the problem of late-onset disease is now accumulating. A recent study compared

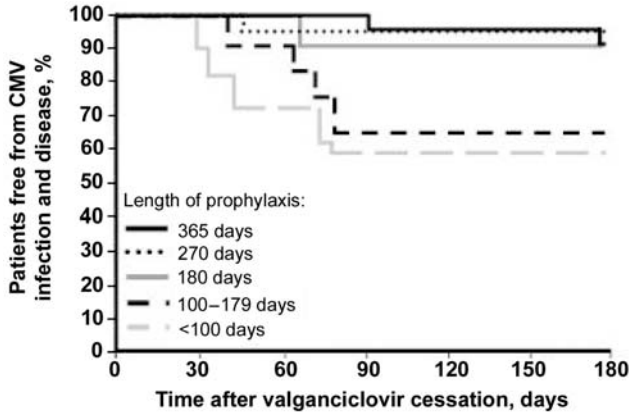


**Figure 1.** Percentage of lung transplant recipients who were free from cytomegalovirus (CMV) infection and disease following prolonged prophylaxis with valganciclovir for <100 days ($n = 18$), 100–179 days ($n = 11$), 180 days ($n = 21$), 270 days ($n = 20$), and 365 days ($n = 20$). $P < .02$ for 180, 270, or 365 days versus <100 or 100–179 days. $P$ value was not significant for 180 days versus 270 days versus 365 days. Reproduced with permission from Zamora et al. [34].

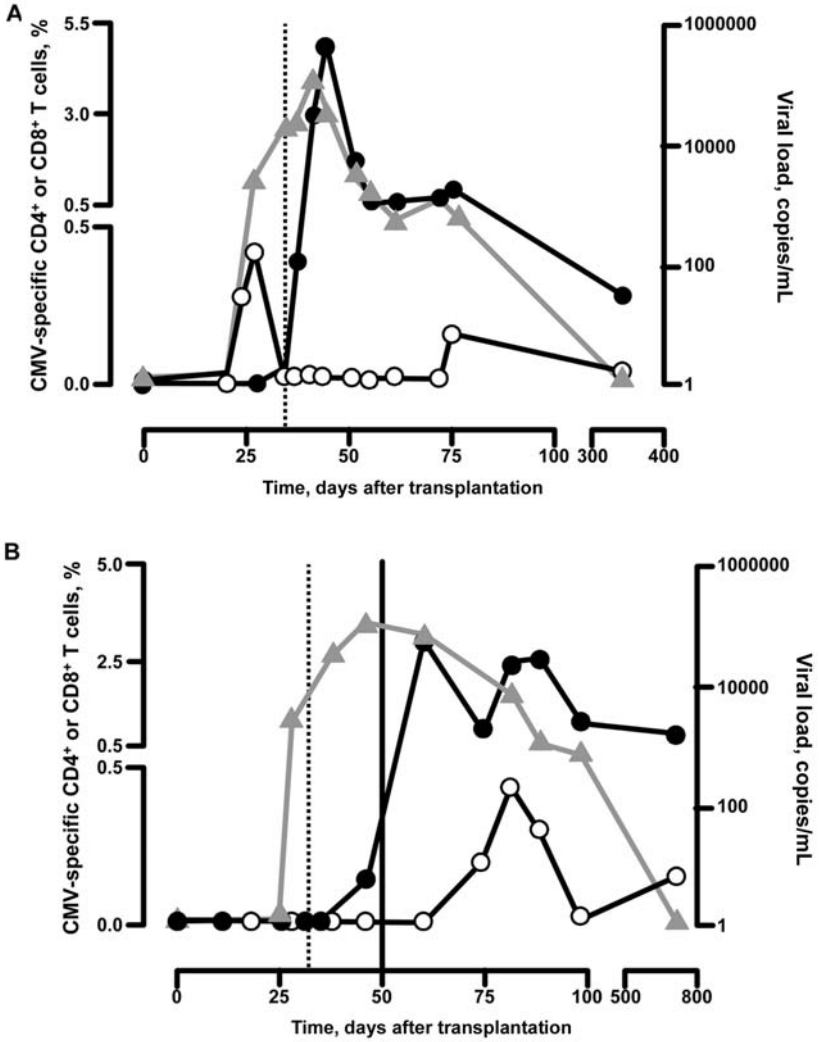


**Figure 2.** Enumeration of cytomegalovirus (CMV)–specific $CD4^+$ and $CD8^+$ T cells in primary CMV infection. Graphs show the frequencies of CMV-specific $CD4^+$ T cells as determined by intracellular staining for CD69 and IFN-$\gamma$ following stimulation *(empty circles),* CMV-specific $CD8^+$ T cells as determined by tetramer binding *(solid circles),* and the first specific antibody appearance *(dotted vertical line)* in relation to CMV load *(triangles)* in 1 asymptomatic representative patient *(A)* and 1 symptomatic patient *(B).* Solid vertical line denotes start of ganciclovir therapy. In this study of donor-seropositive, recipient-seronegative renal transplant recipients, CMV-specific effector memory $CD4^+$ T cell responses (in peripheral blood) appeared prior to CMV-specific antibodies and $CD8^+$ T cell responses, with all responses directed to the clearance of virus. In symptomatic patients, CMV-specific effector memory $CD4^+$ T cell responses were delayed relative to CMV-specific $CD8^+$ T cell and antibody responses and only appeared after the initiation of antiviral therapy. In this study, these CMV-specific $CD4^+$ T cell responses appeared to determine adequate viral clearance. Reproduced with permission from Gamadia et al. [47].

the incidence of CMV disease among high-risk D+/R− kidney transplant recipients receiving a 24-week course of oral ganciclovir prophylaxis with the incidence among those receiving a 12-week course. The proportion of patients who experienced symptomatic CMV infection by the end of the first year after transplantation was considerably lower among those receiving the 24-week course of prophylaxis than among those receiving the 12-week course (7% vs. 31%; $P = .001$). Furthermore, 24 weeks of ganciclovir prophylaxis appeared to be safe and effective [33].

Lung transplant recipients are at high-risk for CMV disease. Zamora et al. [34] investigated the length of prophylaxis required to significantly reduce the incidence of CMV infection and disease. Freedom from CMV infection was determined 180 days after cessation of valganciclovir and was significantly greater ($P < .02$) among patients receiving prophylaxis for 180, 270, or 365 days (90%, 95%, and 90% of patients, respectively) than it was among patients receiving prophylaxis for 100–179 days or <100 days (64% and 59%, respectively) (figure 1) [34]. This study demonstrates that the benefits of extended prophylaxis persist beyond the pe-

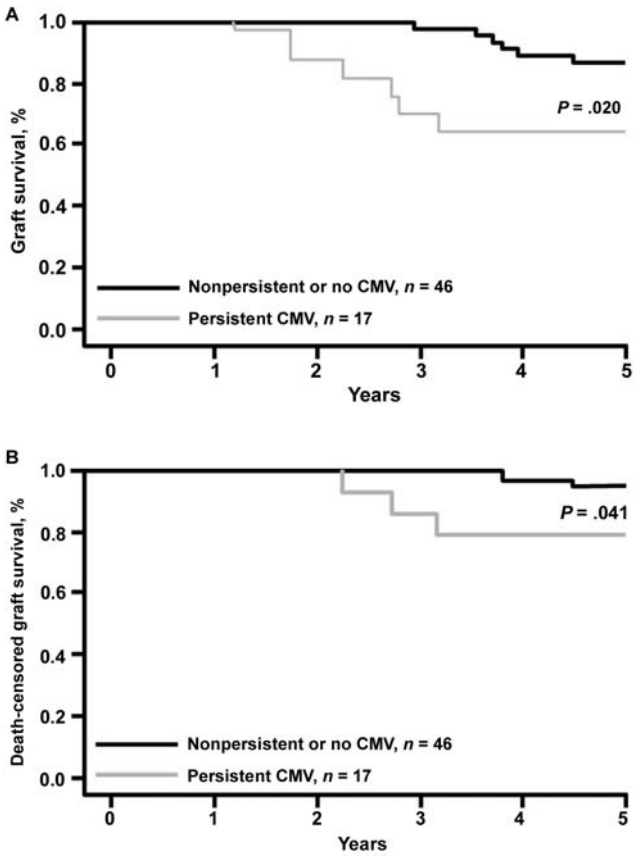


**Figure 3.** Five-year follow-up data for patients with persistent cytomegalovirus (CMV) infection of the graft, showing graft survival uncensored for death (*A;* $P = .020$) and death-censored graft survival (*B;* $P = .041$) in patients with persistent CMV infection, compared with patients with nonpersistent or no CMV infection in the graft. Reproduced with permission from Helantera et al. [67].

riod of administration. A concern with the strategy of extended prophylaxis is that this approach may only postpone the onset of CMV disease. However, Zamora et al. [34] have demonstrated that, by increasing the duration of prophylaxis to at least 180 days, 90% of at-risk lung transplant recipients remained disease-free throughout the first year after transplantation.

A randomized, double-blind, placebo-controlled multicenter trial—the Improved Protection Against Cytomegalovirus in Transplant (IMPACT) study—has recently been initiated. This study will determine the comparative efficacy of 100 days versus 200 days of valganciclovir prophylaxis when given for the prevention of CMV disease in high-risk (i.e., D+/R−) kidney allograft recipients. The primary end point of the study will be the proportion of patients who develop CMV disease within the first 12 months after transplantation. The IMPACT study will also assess allograft rejection, opportunistic infection, patient and/or graft survival, and the occurrence of posttransplantation diabetes mellitus.

The concern regarding the possible development of antiviral resistance is a legitimate one. Risk factors for the emergence of ganciclovir-resistant CMV include treatment of D+/R− patients, prolonged use of oral ganciclovir, and the use of more-potent immunosuppressive therapy [35–37]. However, ganciclovir resistance has also been observed in patients receiving preemptive therapy [36]. Moreover, ganciclovir-resistant CMV strains were not associated with the 3-month course of valganciclovir prophylaxis administered to SOT recipients in the PV16000 study [38–40]. The IMPACT study will also provide appropriate answers.

When making the decision to extend the duration of CMV prophylaxis, the question of cost must be considered. Recent studies assessing the costs of a preemptive strategy relative to a standard prophylaxis course have either shown the overall costs to be similar [41] or shown prophylaxis to be the more cost-effective approach (in renal transplantation) [42]. Although extending the duration of therapy will increase the total drug-associated costs, this additional cost should be offset by a reduction in the costs associated with the management and treatment of late-onset CMV disease and in those associated with the indirect consequences of CMV infection.

## CMV-SPECIFIC IMMUNITY: THE IMPACT OF ANTIVIRAL THERAPY

Preventive strategies have undoubtedly improved the prognosis for transplant recipients who are at risk of developing CMV disease. However, the emergence of late-onset disease emphasizes that the establishment of an effective cellular CMV-specific immune response remains essential for the long-term control of viral replication [10, 43, 44]. Both $CD4^+$ and $CD8^+$ CMV-specific T cells are important for controlling CMV infection (figure 2) [45–47], but $CD8^+$ T cells must be effectively primed upon their first encounter with CMV to prevent a defective response upon a second encounter [45, 46, 48, 49].

The importance of CMV-specific immunity in organ transplantation is highlighted by the correlation between patient exposure to CMV before transplantation and the development of CMV disease. D+/R− transplant recipients are immunologically naive with respect to CMV and are at the greatest risk of developing CMV disease after transplantation. On the other hand, prior exposure to CMV (i.e., being

**Table 2. Meta-analysis of relative risk (RR) for all-cause mortality, allograft rejection, cytomegalovirus (CMV) organ disease, CMV infection, and opportunistic infections for universal prophylaxis, compared with preemptive therapy clinical trials.**

| | RR (95% CI) | | | |
|---|---|---|---|---|
| Variable, study | With prophylaxis | *P* | With preemptive therapy | *P* |
| All-cause mortality | | | | |
| [19] | 0.62 (0.40–0.96) | .032 | 0.94 (0.32–2.76) | .90 |
| [22] | 0.63 (0.43–0.92) | .02 | … | |
| Allograft rejection [19] | 0.74 (0.59–0.94) | .012 | 0.47 (0.24–0.91) | .026 |
| CMV organ disease | | | | |
| [19] | 0.20 (0.13–0.31) | .001 | 0.28 (0.11–0.69) | .003 |
| [22] | 0.42 (0.34–0.52) | .0001 | … | |
| CMV infection [22] | 0.61 (0.48–0.77) | | Not applicable | |
| Opportunistic infections | | | | |
| Bacterial and fungal [19] | 0.49 (0.36–0.67) | | No effect | |
| Bacterial [22] | 0.65 (0.44–0.96) | | … | |
| Fungal [22] | 0.58 (0.19–1.73) | | … | |
| Non-CMV viral [19] | 0.16 (0.12–0.23) | | Not evaluated | |
| Protozoa [22] | 0.31 (0.01–0.99) | | … | |

an R+ patient) is associated with the generation of CMV-specific immune memory [46]; thus, these patients are generally better able to control viral replication than are their D+/R− counterparts. This is reflected by the classification of R+ patients as being at only "moderate risk" of developing CMV disease. However, R+ patients who receive enhanced immunosuppressive therapy, such as polyclonal antilymphocyte antibodies or anti-CD3 monoclonal antibodies, are considered to be at higher risk for CMV disease, because they are less able to mount an effective immune response; therefore, it is recommended that these patients routinely receive CMV prophylaxis [3, 50, 51].

Proponents of the preemptive approach to CMV prevention have argued that, because viral antigens and, therefore, low-level viral replication are required to prime the host immune system, prophylactic strategies may prevent efficient priming of the CMV-specific immune response by completely suppressing viral replication, particularly in D+/R– transplant recipients [18, 52, 53].

Studies, primarily involving HSCT recipients, show that ganciclovir can impair the reconstitution of CMV-specific T cell responses by either preventing in vivo priming or by directly inhibiting T cell proliferation [44, 53]. However, Hakki et al. [54] analyzed factors affecting the recovery of CMV-specific $CD4^+$ and $CD8^+$ T cell immunity 3 months after transplantation in a cohort of 201 HSCT recipients. In their univariate and multivariate analyses, they found that high-dose steroids and $CD4^+$ T cell count $<100 \times 10^9$ cells/L were significantly associated with impaired functional $CD4^+$ T cell recovery and that high-dose steroids, bone marrow as stem cell source, and $CD8^+$ T cell count $<50 \times 10^9$ cells/L were significant predictors of impaired $CD8^+$ T cell function. Notably, this study showed that there was no difference in immune reconstitution between patients who received ganciclovir prophylaxis and those who received preemptive therapy, although patients receiving ganciclovir who had subclinical CMV reactivation had a significantly improved recovery of T cell function [54].

The immune response to viral infections in HSCT recipients is extremely complex, with potential contributions from both the donor and recipient immune systems. In D+/R− SOT recipients, immunity to CMV will require efficient priming of naive T cells; however, HSCT recipients (other than D−/R− recipients) cannot be described as immunologically naive with respect to CMV. In addition, immune recovery following HSCT will be influenced by a number of other factors, including the source of the stem cells, the degree of MHC mismatching, and the type of immunosuppressive or conditioning regimen, which may vary considerably from those used in treating SOT recipients [54–56]. It may not, therefore, be appropriate to extrapolate findings obtained in the context of HSCT to SOT.

A subanalysis of D+/R− SOT recipients who were enrolled in the PV16000 clinical trial sought to identify risk factors for late-onset disease and found that IgG seroconversion occurred in 26.9% of D+/R− SOT recipients by the end of the 100-day prophylactic period and in 63.4% and 75.3% of patients by 6 and 12 months after transplantation, respectively [57]. Although seroconversion by the end of prophylaxis was not predictive of subsequent disease, IgG serostatus became predictive by 6 months and 12 months after transplantation [57]. Importantly, seroconversion occurred while patients were receiving valaciclovir or ganciclovir, demonstrating that prophylaxis permits the development of CMV-specific humoral immunity. Furthermore, of the 26.9% of patients who experienced seroconversion during prophylaxis, only 1% experienced CMV disease during this period [57]. Consistent with these findings, a case study of a kidney and pancreas transplant recipient at high risk for CMV reported the development of anti-CMV IgG and an expansion of activated CMV-specific $CD8^+$ T cells during primary CMV infection in the patient despite ganciclovir prophylaxis [58].

Studies involving SOT recipients, therefore, suggest that the CMV-specific immune response is not suppressed by antiviral prophylaxis, although a full understanding of the impact of antiviral agents on the development of CMV-spe-

cific immunity requires further investigation. A significant advantage of prophylaxis is the reduction in the incidence of primary infection during the prophylactic period. In the absence of prophylaxis, most episodes of CMV DNAemia occur within 100 days after transplantation [41], suggesting that development of the CMV-specific immune response will occur within this time frame. Extending the duration of prophylaxis until the time of less intense immunosuppression will increase the probability that primary infection will occur at a time when the patient is able to mount a more effective CMV-specific response. This should enable a more sustained control of viral replication, thereby inhibiting the development of CMV disease and/or recurrent episodes of CMV infection.

## PROGRESSION TO CMV DISEASE: THE IMPACT OF VIRAL KINETICS

CMV-specific T cells have been shown to mediate protection from CMV disease by effectively limiting the systemic viral load [43]. The relationship between the quantity of CMV detected in blood and the development of symptomatic CMV disease was first reported in 1975 [59]. Since this time, a number of investigators have confirmed viral load to be a significant risk factor for the development of CMV disease [60–62] and a useful prognostic indicator for recipients of SOT [19, 63]. It has been suggested that the high viral load observed in lung transplant recipients, compared with the recipients of other transplants, might explain the classification of lung transplant recipients as being at high risk for developing CMV disease [23, 50].

With the advent of highly sensitive quantitative methods, a more complex picture of the relationship between viral turnover and CMV disease has emerged. The degree of viral replication is strongly associated with progression to CMV disease in liver transplant recipients [64]. Preemptive ganciclovir therapy was associated with the persistence of low-level viral replication in 21% of liver transplant patients, 33% of whom went on to develop CMV disease [64]. Consistent with this, analysis of viral load kinetics in SOT recipients with CMV disease showed that a delay or failure in clearance of the virus were important predictors of disease relapse [65]. Recently, a statistical model incorporating viral load data from 142 liver transplant recipients suggested that peaks in viral load contributed less to disease progression than did phases of low viral load with equal amounts of viral turnover. Of interest, the model accurately predicted the time to onset of CMV disease [66]. These studies suggest that a low level of viral replication—as occurs with preemptive therapy—is not protective against progression to CMV disease but may actually predispose to CMV disease and/or recurrent CMV infection.

## INDIRECT SEQUELAE OF CMV INFECTION

Recent evidence has led to expansion of the definitions needed for the management of CMV infection and disease in SOT recipients [9]. In addition to causing end-organ disease, CMV infection has been associated with considerable allograft pathology (figure 3) [67], including atherosclerosis, bronchiolitis obliterans, vanishing bile duct syndrome, vascular disease, and both acute and chronic graft rejection [5, 8, 17, 67–69]. Animal models have provided important insights into the mechanisms responsible for the deleterious effects of CMV on graft function. For example, prolonged increased expression of intercellular adhesion molecule type 1 and vascular cell adhesion molecule type 1 was observed in rat kidney allograft recipients infected with CMV, with concomitant increased infiltration of inflammatory cells expressing their ligands, and was associated with accelerated chronic allograft nephropathy [70]. An experimental model of liver allograft rejection showed prolonged upregulation of adhesion molecules that mediate lymphocyte adhesion in inflammatory sites, and this was associated with concomitant CMV infection [71].

Clinical studies also demonstrated that antiviral prophylaxis is beneficial in protecting against the indirect effects of CMV infection. A retrospective analysis of CMV-seropositive heart transplant recipients [72] observed a lower incidence of transplant-associated coronary artery disease in patients receiving ganciclovir prophylaxis than in patients receiving placebo (38% vs. 55%) [72]. Furthermore, a lack of ganciclovir prophylaxis was associated with a significantly increased relative risk for transplant-associated coronary artery disease (relative risk, 2.7 vs. 2.9; $P < .01$) [72]. Importantly, a recent study has shown that suppression of subclinical CMV replication during antiviral prophylaxis resulted in a reduced relative risk for acute rejection and a slower progression of cardiac allograft vasculopathy [73]. Valganciclovir prophylaxis has also been shown to lower the incidence of various herpesvirus infections, such as infections due to human herpesvirus 6, human herpesvirus 8, Epstein-Barr virus, and varicella zoster virus, in SOT recipients [74], and studies have demonstrated that improved patient and graft survival and a decreased risk of biopsy-proven rejection, as well as a decrease in the incidence of opportunistic infections, are associated with prophylaxis [20, 75–77]. Recent meta-analysis and systematic reviews of CMV prophylaxis and preemptive therapy have shown that both of these approaches were effective at reducing CMV-associated end-organ disease and allograft rejection, but only CMV prophylaxis was effective in reducing CMV disease, CMV-related mortality, all-cause mortality, and disease caused by opportunistic infections (table 2) [20, 21, 25].

## CONCLUSIONS

CMV infection continues to present a significant challenge to transplant clinicians involved in the care of transplant recipients. Successful long-term prevention of

CMV disease requires the generation of a CMV-specific T cell response. How this might be better achieved is much debated, because it is extremely difficult to dissect the relative contributions of the various factors impacting on the development of CMV-specific immunity.

Both prophylaxis and preemptive strategies have been shown to be effective for disease prevention. Data associating CMV with indirect sequelae and their significant consequences on long-term graft and patient outcomes are accumulating, and the number of studies demonstrating the benefit of CMV prophylaxis in reducing these sequelae is increasing. Systematic reviews and meta-analyses show that prophylaxis is better for protecting against the indirect effects of CMV infection and, thus, may become the preferred preventive strategy. Future studies will indicate whether extending the duration of prophylaxis beyond the period of intense immunosuppression (e.g., up to 6 months) can not only lower the incidence of CMV disease but also have a beneficial effect on graft function and overall outcomes.

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