Cytomegalovirus disease among donor-positive/recipient-negative lung transplant recipients in the era of valganciclovir prophylaxis

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BACKGROUND: Valganciclovir prophylaxis is advocated for lung transplant recipients, but its efficacy is unknown.

METHODOLOGY: Retrospective review was done of 109 donor-positive/recipient-negative lung transplant patients who received alemtuzumab induction and valganciclovir for cytomegalovirus prophylaxis.

RESULTS: Median duration of follow-up after transplant was 27 months. Valganciclovir dose reductions (< 900 mg/day or renal-equivalent) were required for 18 patients (17%) due to toxicity, most commonly for neutropenia (n = 15) or gastrointestinal symptoms (n = 2). Of the 109 patients, 34 (31%) had no CMV infections, 45 (41%) had asymptomatic viremia, and 30 (27%) had CMV disease. CMV disease developed off prophylaxis in 10 patients (18%) at a median of 8.7 months after transplant and 2 months after valganciclovir discontinuation. Breakthrough disease occurred during prophylaxis in 10 patients (9%) at a median of 6.7 months. Risk factors for CMV disease by univariate analysis were increased age (p = 0.01), single-lung transplant (p = 0.03), chronic obstructive pulmonary disease (p = 0.05), reduced-dose valganciclovir (p = 0.001), and less than 6 months of prophylaxis (p = 0.005). By multivariate analysis, advanced age (p = 0.01) and reduced-dose valganciclovir (p = 0.0006) were independent risk factors for CMV disease. CMV disease developed in 4 patients (4%) due to ganciclovir-resistant viruses. CMV-attributable mortality was 5% (5 of 109), including 100% (4 of 4) with ganciclovir-resistant disease.

CONCLUSIONS: Valganciclovir prophylaxis among donor-positive/recipient-negative lung transplant recipients delayed but did not eliminate CMV disease or CMV-related deaths and was limited by toxicity and ganciclovir-resistance. Our experience suggests that valganciclovir at reduced-doses or for less than 6 months is sub-optimal in preventing CMV disease.

J Heart Lung Transplant 2010;29:1014 –20 Published by Elsevier Inc.

KEYWORDS: cytomegalovirus; valganciclovir; lung transplantation; D+/R-; alemtuzumab; risk factors

Cytomegalovirus (CMV) causes infections in up to 80% of lung transplant recipients in the absence of preventive therapy. Rates of CMV disease are higher after lung transplantation than after other solid organ transplantation (SOT) because the virus has a predilection for lung parenchyma and high latent viral loads. The greatest risk for CMV disease is among seronegative recipients (R–) who receive organs from seropositive donors (D+). Optimal preventive regimens against CMV disease after lung transplantation are undefined. Several regimens have been studied, but there have been no randomized, controlled trials. Most studies have used universal prophylaxis with intravenous or oral ganciclovir, alone or combined with intravenous CMV.
hyper-immunoglobulin G (CMV IVIG). Ganciclovir-based regimens are effective in reducing CMV infections in diverse SOT populations. Intravenous ganciclovir, however, is limited by systemic toxicity and access-related complications and oral ganciclovir is limited by poor bioavailability and resistance. In general, the incidence of CMV disease among lung transplant recipients has remained at 20% to 50%, despite preventive regimens.

Prophylaxis with valganciclovir, an oral pro-drug that achieves serum ganciclovir concentrations comparable to intravenous ganciclovir, has proven effective in heart, kidney, and heart-kidney transplant recipients. There is less experience among lung transplant recipients. Several studies showed that valganciclovir, after initial courses of intravenous ganciclovir, with or without CMV IVIG, was at least as safe and effective as more established regimens.

The optimal duration of valganciclovir prophylaxis remains unknown, however, and recommendations for high-risk patients range from 3 months to indefinitely. Other major unanswered questions surround the effect of valganciclovir on late-onset CMV disease, resistance, and drug toxicity.

The objective of this study was to describe our experience with valganciclovir prophylaxis and CMV disease among D+/R− lung transplant recipients at the University of Pittsburgh Medical Center (UPMC). Universal valganciclovir prophylaxis has been used at UPMC since 2003. During this time, induction therapy with alemtuzumab, an anti-CD52 monoclonal antibody that causes profound and prolonged lymphopenia, has also been standard.

Methods

This study was approved by the University of Pittsburgh’s Institutional Review Board.

Patients

We identified D+/R− patients who underwent lung or heart-lung transplantation from January 2003 to July 2008, received alemtuzumab induction and valganciclovir prophylaxis, and survived 1 month or more. Valganciclovir was initiated immediately after transplant. CMV IVIG or other antiviral agents were not given. All patients received antifungal prophylaxis with voriconazole for 3 months or more. In addition, valganciclovir and voriconazole were given for 3 months or more at the time of acute cellular rejection requiring augmented immunosuppression. Patients were followed-up through July 2009.

Study design

A retrospective review of electronic medical records for each D+/R− case was independently performed by three physician-investigators (D. M., M. H. N., C. J. C.). In the event of disagreement, a consensus was reached.

Definitions

CMV disease included tissue-invasive disease and CMV syndrome.

- **Tissue-invasive disease**: clinical symptoms and histopathologic findings consistent with tissue invasion on biopsy.
- **CMV syndrome**: viremia in the setting of fever >3 days without an alternative etiology and with at least 1 of the following: atypical lymphocytosis (>3%), leukopenia (white blood cell count <4000/ml), thrombocytopenia (platelet count <100,000/ml), and elevated transaminases (alanine aminotransferase >40 IU).
- **Asymptomatic viremia**: viremia in the absence of symptoms.
- **Reduced-dose valganciclovir**: less than 900 mg/day or renally adjusted equivalent.
- **Acute rejection**: International Society of Heart and Lung Transplantation (ISHLT) grade ≥2 treated with augmented immunosuppression.
- **Toxicity**: identified by the treating physician and corroborated by review of pertinent labs.
- **CMV-attributable mortality**: death with ongoing evidence of disease.

Virologic monitoring and ganciclovir resistance testing

Virologic monitoring, resistance testing, and treatment decisions were at the discretion of treating physicians. Virologic surveillance was recommended during illnesses with symptoms suggestive of CMV disease, and at least every other week during valganciclovir prophylaxis, weekly for the first 6 months off valganciclovir, and monthly thereafter. Treatment was recommended for any level of CMV replication, as detected by antigen shell assays (2003–2005) or whole-blood polymerase chain reaction (2006–2009). Resistance was tested by screening for UL97 and UL54 mutations at codons 363–698 and 184–1017, respectively.

Statistics

Instat Software (Graphpad Software Inc, San Diego, CA) was used. Comparison of dichotomous variables was made using chi-square or Fisher’s exact test. Continuous variables were reported as median ± standard deviation, and difference between groups was calculated using Mann-Whitney
U-test. Multivariate analysis was performed on variables with values of \( p < 0.10 \) by univariate analysis, and values of \( p < 0.05 \) were considered significant. The probability of CMV disease-free survival and other outcomes was determined using the Kaplan-Meier product limit method.

**Results**

**D+/R– lung transplant recipients and valganciclovir prophylaxis**

From January 2003 to July 2008, 120 CMV R– patients received D+ lungs, accounting for 25% of 483 lung transplants. The study excluded 11 patients because they died within 1 month of transplant (9) and lack of valganciclovir prophylaxis (1) or alemtuzumab induction (1). The remaining 109 D+/R– recipients resembled the entire lung transplant cohort in demographics, baseline characteristics, types of transplant, and immunosuppression (Table 1).

The 109 patients received valganciclovir for a median of 8 months (range, 1–36 months) after transplantation. Valganciclovir doses were reduced in 18 (17%), due to neutropenia in 15 (14%), gastrointestinal symptoms in 2 (2%), or for unclear indications in 1 (1%). Colony-stimulating factors were administered to 7 of 15 patients (47%) in whom valganciclovir doses were reduced due to neutropenia. Because of drug toxicity, 15 patients (14%) received valganciclovir for less than 6 months. In each instance, the valganciclovir dose was reduced before discontinuation. The median age of patients receiving less than 6 months of valganciclovir did not differ from other patients.

**CMV disease**

Patients were monitored for a median of 27 months after transplantation (range, 3–74 months). CMV infection did not develop in 34 patients (31%), asymptomatic viremia developed in 45 (41%), and CMV disease developed in 30 (28%). Patients without CMV infection and those with asymptomatic viremia did not differ in type of transplant, underlying disease, percentage of patients undergoing repeat transplant, donor age, percentage of patients receiving reduced-dose valganciclovir, duration of prophylaxis, maintenance immunosuppressive regimen, and percentage of patients requiring treatment for rejection (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline Demographics and Patient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
<td>D+/R– cohort (n = 109)</td>
</tr>
<tr>
<td>Male gender, % (n)</td>
<td>49 (53)</td>
</tr>
<tr>
<td>White race (not Hispanic), % (n)</td>
<td>98 (107)</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>54 (17–76)</td>
</tr>
<tr>
<td>Underlying disease, % (n)</td>
<td></td>
</tr>
<tr>
<td>IPF</td>
<td>24 (26)</td>
</tr>
<tr>
<td>COPD</td>
<td>26 (28)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>20 (22)</td>
</tr>
<tr>
<td>Re-transplant</td>
<td>9 (10)</td>
</tr>
<tr>
<td>CTD</td>
<td>6 (7)</td>
</tr>
<tr>
<td>PPH</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Misc</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Type of transplant, % (n)</td>
<td></td>
</tr>
<tr>
<td>Double-lung</td>
<td>64 (70)</td>
</tr>
<tr>
<td>Single-lung</td>
<td>30 (33)</td>
</tr>
<tr>
<td>Heart-lung</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>37 (11–66)</td>
</tr>
<tr>
<td>Reduced dose of VGCV, % (n)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>Prophylaxis duration</td>
<td></td>
</tr>
<tr>
<td>Median (range), months</td>
<td>8 (1–36)</td>
</tr>
<tr>
<td>Maintenance IS, % (n)</td>
<td></td>
</tr>
<tr>
<td>Pred/FK506/MMF</td>
<td>77 (84)</td>
</tr>
<tr>
<td>Pred/FK506</td>
<td>21 (23)</td>
</tr>
<tr>
<td>Pred/CsA/MMF</td>
<td>2</td>
</tr>
<tr>
<td>Rejection, % (n)</td>
<td>21 (23)</td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CTD, connective tissue disease; D, donor; FK506, tacrolimus; IPF, interstitial pulmonary fibrosis; IS, immunosuppression; MMF, mycophenolate mofetil; PPH, primary pulmonary hypertension; Pred, prednisone; R, recipient; VGCV valganciclovir.

*Congenital heart disease, α-1 antitrypsin deficiency, lymphangiomatosis, eosinophilic granuloma, due to radiation for Hodgkin disease, bronchiolitis obliterans, non-re-transplant, sarcoidosis, silicosis.
CMV disease was most common within the first year, but was encountered as late as 25 months after transplantation (Figures 1 and 2). No patients with CMV disease had concomitant infections. Of the 30 patients with CMV disease, 17 (57%) had no previously documented viremia, and 6 (20%) had negative CMV polymerase chain reaction or antigenemia within 2 weeks of diagnosis. Types of disease in the 109 patients included pneumonitis in 22 (20%), colitis in 2 (2%), pneumonitis and colitis in 1 (1%), and CMV syndrome in 5 (5%). Patients who were free of CMV disease received valganciclovir for a median of 8.6 months (range, 2.6–36 months). Duration of prophylaxis did not differ for patients with asymptomatic viremia (median, 8.6 months) and without CMV infection (median, 8.7 months).

Significant risk factors for CMV disease by univariate analysis were increased age ($p = 0.01$), single-lung transplant ($p = 0.03$), chronic obstructive pulmonary disease as the underlying disease ($p = 0.05$), reduced-dose valganciclovir ($p = 0.04$), and shorter duration (< 6 months) of prophylaxis ($p = 0.0005$; Table 2). By multivariate analysis, age ($p = 0.01$) and reduced-dose valganciclovir ($p = 0.0006$) were independently associated with CMV disease (Table 2). CMV disease developed in 11 of the 18 patients (61%) who received reduced-dose valganciclovir vs 9 of 91 patients (21%) of patients who received full doses.

**Ganciclovir resistance**

Genotype testing for ganciclovir resistance was performed in 12 of the 30 patients (40%) with CMV disease. Among these, resistance mutations were detected in 4 (33%), comprising UL97 mutation in 3, and both UL97 and UL54 in 1. Genotype testing was performed in 4 of 11 patients (36%) with CMV disease who received reduced-dose valganciclovir, and resistance mutations were detected in 1 (25%). Overall, 4 of 109 D+/R− recipients (4%) and 4 of 30 patients (13%) with CMV disease were infected with ganciclovir-resistant viruses. Resistance was similar for patients with CMV disease off prophylaxis and breakthrough disease (10% [2 of 20] and 20% [2 of 10], respectively). There was no association between valganciclovir dose, duration of prophylaxis, and drug-resistant CMV disease.
Table 2  Risk Factors for Cytomegalovirus Disease

<table>
<thead>
<tr>
<th>Factor</th>
<th>CMV disease</th>
<th>No CMV diseasea</th>
<th>p-value</th>
<th>Univariate</th>
<th>Multivariateb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median years</td>
<td>57</td>
<td>51</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Single-lung transplant, % (n)</td>
<td>46 (14)</td>
<td>24 (19)</td>
<td>0.03</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Underlying COPD, % (n)</td>
<td>40 (12)</td>
<td>20 (16)</td>
<td>0.05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Reduced-dose valganciclovir, % (n)</td>
<td>37 (11)</td>
<td>9 (7)</td>
<td>0.001</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis duration, median months</td>
<td>6.7</td>
<td>8.6</td>
<td>0.005</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease.
*aIncludes patients with asymptomatic viremia and no CMV infection.
*bIncludes variables with p < 0.1 by univariate analysis.

Treatment and outcomes of CMV disease

The 20 patients with CMV disease that occurred after valganciclovir prophylaxis were treated with intravenous ganciclovir. Two patients harboring the UL97 resistance mutation who were infected with CMV were subsequently treated with foscarnet combined with CMV IVIG or cidofovir. Among the 10 patients with breakthrough CMV disease, 5 (50%) were treated with intravenous ganciclovir alone (n = 3) or combined with CMV IVIG (n = 2). The remaining 50% received foscarnet alone (n = 2), foscarnet combined with CMV IVIG (n = 2, including 1 infected with CMV harboring UL97 and UL54), or foscarnet combined with cidofovir and CMV IVIG (n = 1, infected with CMV harboring UL97).

Of the 109 patients, 5 (5%) died as a direct result of CMV disease (off prophylaxis, n = 3; breakthrough, n = 2), including all 4 patients infected with ganciclovir-resistant virus. All-cause mortality within 6 months of CMV disease was 8 of 30 (27%). For patients with asymptomatic viremia, all-cause mortality within 6 months after the first episode of viremia was 7 of 45 (16%; p = 0.06).

Acute cellular rejection developed in 11 of 30 patients (37%) with CMV disease that required treatment with augmented immunosuppression within the subsequent 6 months, and 10 (33%) had repeated episodes of rejection (range, 2–9 episodes). Among 45 patients with asymptomatic viremia, 11 patients (24%) were treated for rejection within 6 months of viremia, and 10 (22%) with asymptomatic viremia had repeated episodes (range, 2–4).

Bronchiolitis obliterans syndrome (BOS) subsequently developed in 2 of 30 patients (7%) with CMV disease, compared with 1 of 45 (2%) with asymptomatic viremia and 3 of 34 patients (9%) with no CMV infection. A subsequent invasive fungal infection developed in 4 of 30 patients (13%) with CMV disease compared with 10 of 45 (22%) of those with asymptomatic viremia and 6 of 34 (18%) with no CMV infection.

Discussion

In this large study in which valganciclovir was used as the sole preventive agent among D+/R− lung transplant recipients, the incidence of CMV disease was lower than would be anticipated in the absence of preventive therapy. Nevertheless, the efficacy of valganciclovir prophylaxis was limited by the extent and severity of CMV disease, emergence of drug resistance, and toxicity.

CMV developed in 30 of the 109 patients (27%) despite receiving valganciclovir for a median of 6.8 months, and 5 (5%) died as a direct result. CMV disease developed after prophylaxis in 20 patients (18%), most commonly within 2 months of discontinuing valganciclovir, observations that were consistent with prior reports of high-risk lung transplant recipients. More disturbingly, breakthrough disease developed during prophylaxis in 10 of the 109 patients (9%), a rate higher than previously reported. Moreover, 4 of 30 patients (13%) with CMV disease were infected with ganciclovir-resistant virus, and all 4 died (100% mortality). Finally, valganciclovir toxicity developed in 18 patients (17%), most commonly neutropenia (15 of 109 [14%]) and gastrointestinal distress (2 of 109 [2%]). Valganciclovir toxicity was important not only for its direct effect on well-being but also as an indirect risk factor for CMV disease.

Indeed, each patient who experienced toxicity was treated subsequently with a reduced dose of valganciclovir. Valganciclovir dose-reduction, in turn, was the most significant independent risk factor for CMV disease (p = 0.0006). Associations between reduced valganciclovir dosing and CMV disease have not been well studied among lung transplant recipients. As such, this study is notable for demonstrating conclusively the superiority of the recommended 900-mg dose. The feasibility of reduced-dose regimens has been explored in other SOT populations, particularly among low-risk recipients. In general, however, such regimens do not reliably assure adequate systemic ganciclovir exposure.

A theoretic argument for low-dose valganciclovir is that it may lessen toxicity, but we noted that dose reduction was followed shortly by discontinuation of valganciclovir in each patient. The 17% toxicity rate was within the 7.5% to 32% range previously reported for lung transplant recipients receiving valganciclovir prophylaxis, a significant finding because alemtuzumab has potential myelosuppressive effects. Alemtuzumab-associated myelosuppression typically occurs earlier in the period after transplant, however, and our experience suggests that the agents can be used together safely.

The close association between valganciclovir dose-reduction and discontinuation of the agent in response to toxicity limited our ability to draw definitive conclusions about the optimal duration of prophylaxis. Nevertheless,
less than 6 months of prophylaxis was a significant risk factor for CMV disease by univariate analysis ($p = 0.005$), an association that also held if patients with breakthrough disease were excluded ($p = 0.002$). The median duration of valganciclovir prophylaxis among patients who did not develop CMV disease was 8.6 months (range, 2.6–36 months) compared with 6.8 months (range, 1–22 months) for patients with CMV disease. Moreover, CMV disease developed in 9 of 15 patients (60%) who received less than 6 months of prophylaxis vs only 21 of 94 (22%) of patients who received 6 months or more. Taken together, therefore, our data suggest that valganciclovir at reduced doses or for less than 6 months was suboptimal at preventing CMV disease among D+/R– lung transplant recipients.

This conclusion is consistent with a prospective study of 90 consecutive high-risk lung transplant recipients, in whom more than 6 months of valganciclovir was superior to shorter regimens in preventing CMV disease after 30 (R+) or 90 days (D+/R–) of intravenous ganciclovir combined with CMV IVIG.23 In a retrospective study, 12 months of valganciclovir was superior to 3 months among 32 D+/R– recipients who also received CMV IVIG and an initial 2-week course of intravenous ganciclovir.25 Unfortunately, our experience suggests that toxicity and emergence of ganciclovir-resistance may limit the ability to extend prophylaxis.

The other independent risk factor for CMV disease in this study was advanced age ($p = 0.01$). CMV disease developed in D+/R– patients who were a median age of 57 years, compared with 46 for patients without CMV infection and 52 years for those with asymptomatic viremia. Acute rejection requiring augmented immune suppression was not a significant risk factor, likely due to our use of valganciclovir among such patients.37

Our 4% overall rate of ganciclovir resistance was within the 0% to 9% range previously reported for lung transplant recipients.23,26,27,38,39 The findings are consistent with reports linking D+/R– serostatus and prolonged CMV prophylaxis to anti-viral resistance.30 Of note, we cannot ascribe the emergence of resistance to reduced-dose valganciclovir, because all cases except 1 occurred among patients who received full doses.

The 100% mortality among patients with ganciclovir-resistant CMV disease was among the most disturbing findings of this study. Similar associations between CMV disease due to resistant virus and poor outcomes have been noted in some studies,39 but not others.27,40 Of note, our patients died despite the use of aggressive anti-viral regimens that included various combinations of foscarnet, cidofovir and CMV IVIG. These regimens are associated with significant toxicities and expense, facts that further highlight the clinical effect of ganciclovir-resistant CMV.

It is notable that we did not find an association between CMV disease and development of BOS. Indeed, the role of CMV replication in the pathogenesis of BOS remains uncertain, with conflicting results from previous studies.1,5,41–44 Although some have hypothesized that control of CMV replication by anti-viral prophylaxis or treatment might diminish the association between viral reactivation and BOS,6,44–46 our study design precluded us from addressing this issue.

In this retrospective study, we were also unable to determine the extent to which poor compliance or unrecognized pharmacokinetic issues that compromised the efficacy of valganciclovir might have contributed to CMV disease. In addition, we must acknowledge that our results may have been affected by the use of alemtuzumab.47

In conclusion, CMV disease remained a major clinical problem among D+/R– lung transplant recipients despite valganciclovir prophylaxis. Ideally, the number of D+/R– lung transplants should be minimized, especially among the elderly. At present, our policy is to administer ganciclovir for at least 1 year in D+/R– patients, using supportive measures such as use of colony-stimulating factors to allow full dosing. Along these lines, we were surprised that only 7 of 15 patients (47%) in this study who received reduced-dose valganciclovir due to neutropenia were treated with colony-stimulating factors. This observation suggests that our previous efforts to maintain patients on full-dosing regimens were not sufficiently aggressive.

Finally, our experience demonstrates the need for alternative prophylaxis strategies among D+/R– lung transplant recipients. Approaches that merit study include incorporation of CMV IVIG into valganciclovir-based regimens, the development of rigorous preemptive strategies, or the use of CMV-specific immune monitoring to identify patients for whom prophylaxis can be discontinued.

**Disclosure statement**

Dr Nguyen and Dr Clancy are funded by a National Institutes of Health Mycology Research Unit Program Project Award (5P01AI061537-02).

Dr Nguyen has received research funding from Pfizer, Enzon Pharmaceutical, and Merck. Dr Kwak and Dr Silveria have received research funding from Pfizer. Dr Clancy has received research funding from Pfizer, Astellas, Merck, and Ortho-McNeil. None of the other authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

**References**