Absence of Cytomegalovirus-Resistance Mutations after Valganciclovir Prophylaxis, in a Prospective Multicenter Study of Solid-Organ Transplant Recipients

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We investigated the emergence of cytomegalovirus (CMV) ganciclovir-resistance mutations in 301 high-risk solid-organ transplant (SOT) recipients after oral prophylaxis, for 100 days, with either valganciclovir or ganciclovir. For patients treated with ganciclovir, the incidence of CMV UL97 mutations was 1.9% (2/103) at the end of prophylaxis and 6.1% (2/33) for patients with suspected CMV disease up to 1 year after transplantation. No resistance mutations were detected in samples from valganciclovir-treated patients. Dual polymerase (UL54) and UL97 resistance mutations were not seen. Valganciclovir was associated with negligible risk of resistance and thus constitutes a useful alternative to ganciclovir prophylaxis for CMV in high-risk SOT recipients.

Ganciclovir is currently the drug of choice for the prevention and treatment of cytomegalovirus (CMV) disease, and its use has led to a decline in CMV disease and associated morbidity in solid-organ transplant (SOT) recipients [1, 2]. Valganciclovir, a valyl ester prodrug of ganciclovir, was developed to increase the bioavailability of ganciclovir administered orally. The bioavailability of valganciclovir is ~60%, which is ~10 times that of ganciclovir administered orally [3]. A dose of 900 mg administered orally produces exposure similar to that achieved with a 5-mg/kg intravenous dose of ganciclovir [3]. The efficacy and tolerability of valganciclovir prophylaxis has been demonstrated in organ-transplant recipients [4].

The increasingly widespread use of ganciclovir and valganciclovir for prophylaxis of CMV has raised concern that resistant CMV strains may become more prevalent. The aim of our study was to determine the frequency of emergence, the nature, and the clinical consequences of CMV mutations that confer resistance to ganciclovir in high-risk (donor CMV-seropositive [D+] / recipient CMV-seronegative [R−]) SOT recipients who participated in a large randomized, double-blind trial of valganciclovir versus ganciclovir for prophylaxis of CMV disease [4].

Patients and methods. D+/R− patients (age, ≥13 years) who had received a heart (n = 56), liver (n = 175), kidney (n = 120), kidney and pancreas (n = 11), or liver and kidney (n = 2) allograft were randomized (2:1) to receive either 900 mg of valganciclovir once daily or 1000 mg of ganciclovir orally 10 days after transplantation and continued through day 100 after transplantation. Informed consent was obtained from all patients in the study, and human experimentation guidelines of the US Department of Health and Human Services and/or those of the investigators’ institutions were followed in the conduct of the clinical research.

We used the following definitions of CMV disease: CMV syndrome, the presence of both CMV in blood and fever (≥38°C on 2 occasions, ≥24 h apart) and the presence of ≥1 of malaise, leukopenia, atypical lymphocytosis, thrombocytopenia, and elevated hepatic enzymes; and tissue-invasive CMV, biopsy-proven evidence of localized CMV infection and evidence of organ dysfunction. A dual strategy was adopted for the assessment of ganciclovir resistance. First, blood samples were ob-
Table 1. Incidence of cytomegalovirus (CMV) UL97 mutations in solid-organ transplant recipients at the end of study-drug prophylaxis (day 100) and for those patients with suspected CMV disease up to 12 months after transplantation.

<table>
<thead>
<tr>
<th>Category</th>
<th>Mutations at the end of study-drug prophylaxis (day 100) in patients who received</th>
<th>Mutations in patients with suspected CMV disease who received</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valganciclovir ( (n = 239) )</td>
<td>Ganciclovir ( (n = 125) )</td>
</tr>
<tr>
<td>Patients with suspected CMV disease</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>No. of samples available for testing</td>
<td>198</td>
<td>103</td>
</tr>
<tr>
<td>No. of CMV UL97 DNA PCR-positive samples</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>No. of CMV UL97 DNA PCR-positive samples with mutations</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

Mutations present (no.)

- Known wild-type polymorphisms
  - H469Y (1)
  - Q449K (1)
  - A427V (1), M550I (1)
  - A582V (1), A674T (1)
  - P405L (1)

- Novel mutations
  - Y617H (1)
  - A594V (1), C607Y (1)
  - C592G (1), C607Y (1)

- Known resistance mutations
  - A594V (1)
  - C607Y (1)
  - C592G (1), C607Y (1)

Incidence of known UL97 resistance mutations (%)

- Valganciclovir: 0/198 (0)
- Ganciclovir: 2/103 (1.9)
- Valganciclovir: 0/55 (0)
- Ganciclovir: 2/33 (6.1)

**NOTE.** PCR, polymerase chain reaction.

* Samples collected from the same patient.

determined by the Cobas Amplicor Monitor CMV test (Roche Molecular Systems).

**Results.** The results of the study of mutations in the CMV UL97 gene are presented in table 1. At the end of the study-drug prophylaxis regimen (day 100), samples from 301 of 364 enrolled D+/R patients were available for testing. In the valganciclovir arm, no known ganciclovir-resistance mutations were detected. One novel (Y617H) and 1 known (H469Y) polymorphism were observed [9]. In patients who orally received ganciclovir, 2 known ganciclovir-resistance mutations were detected (A594V and C607Y). Additionally, 2 novel (A427V and M550I) and 1 known (Q449K) polymorphisms were detected [9]. The overall incidence of ganciclovir resistance at the end of study-drug prophylaxis was 0% (0/198) and 1.9% (2/103), for patients in the valganciclovir and ganciclovir arms, respectively, when only the known ganciclovir-resistance mutations were considered.

In patients with suspected CMV disease, no known ganciclovir-resistance mutations were detected in the valganciclovir arm (0% [0/55]). Two novel polymorphisms—A582V and A674T—were observed >2 months after the cessation of study-drug prophylaxis, as were 2 established polymorphisms (Q449K and H469Y) [9]. In the ganciclovir arm, 2 known ganciclovir-resistance mutations (C592G and C607Y) were detected (6.1% [2/33]). A novel polymorphism, P405L, was detected 29 days after the cessation of study-drug prophylaxis, as were 2 previously reported polymorphisms, H469Y and D605E [9].

The results of the study of the kinetics of the emergence of known CMV UL97 resistance mutations are presented in table 2. In patient A, the C592G mutation was detected in a suspected CMV-disease sample obtained, on day 91, from a liver-trans-
plant recipient who received ganciclovir. The patient did not meet the protocol definition of CMV disease and was not treated for such. The mutation arose between study days 50 and 64 but was cleared by day 99, without antiviral therapy or a change in immunosuppressive regimen. The peak plasma virus load before and around the time of the event was 1950 copies/mL. The patient did not experience acute graft rejection during the 12 months after transplantation. Thus, there appeared to be no clinical consequence associated with the presence of this drug-resistant virus.

In patient C, the C607Y mutation was observed in both a day 100 sample (actual day, 101) and a suspected CMV disease samples on study day 119 from a kidney transplant recipient who received ganciclovir. The patient did not meet the protocol definition of CMV disease, but intravenous ganciclovir (1000 mg daily) was administered for 2 weeks. The mutation emerged between days 80 and 101 and was present at the time of suspected CMV disease (day 119) but was cleared by the end of treatment (day 133). This patient had acute graft rejection 9 days after transplant, at which time the immunosuppressive regimen was changed. Plasma virus load assays showed a first detectable virus load on day 101 (4680 copies/mL) that rose to 30,200 copies/mL on day 119. The virus load was below the limit of quantification at the next time of testing (day 133).

Thus, although the patient was treated for CMV disease, intravenous ganciclovir treatment was successful in clearing this resistant strain, and there were no further clinical consequences.

Samples that contained known UL97 ganciclovir-resistance mutations (n = 3) or novel mutations (n = 6) were subsequently analyzed for UL54 mutations, by gene sequencing. Of the 9 samples, 8 had, in the UL54 gene, >1 amino-acid substitution relative to AD169. However, these were all known polymorphisms (A885T, P887S, S897L, and N898D), and they were not associated with ganciclovir resistance [10].

**Discussion.** CMV resistance to ganciclovir occurs via mutations in the UL97 kinase gene, alone or in combination with mutations in the UL54 DNA-polymerase gene [11]. Data on the emergence of resistance in organ-transplant recipients who receive valganciclovir are lacking; however, the emergence of ganciclovir-resistant CMV in SOT recipients who receive ganciclovir has been reported [12].

In our study, the incidence of UL97 resistance at the end of the study-drug prophylaxis (valganciclovir, 0%; ganciclovir, 1.9%) and for patients with suspected CMV disease (valganciclovir, 0%; ganciclovir, 6.1%) was low. No resistance mutations were detected in CMV from valganciclovir-treated patients; this may have been due to the fact that valganciclovir increases exposure to ganciclovir. Additionally, the convenience of the once-daily valganciclovir regimen, compared with the 3-times/day ganciclovir regimen, may confer improved patient compliance and therefore reduce the risk of development of resistance.

Resistance data for large-scale prophylaxis and/or treatment studies using ganciclovir in the SOT population are limited. In a study by Limaye et al. [12], 17 (25%) of 67 D+/R+ SOT recipients who orally received ganciclovir prophylaxis developed CMV disease (5 had early disease [before day 100] and 12 had late disease [between days 101 and 365]) after trans-
plantation. Ganciclovir-resistant CMV disease occurred in 5 (29%) of these 17 D+/R− patients (or 7% of all D+/R− patients), and all cases of resistance occurred ≥7 months after transplantation [12]. Our data contrast with those of Limaye et al.’s study, both in the lower frequency of ganciclovir-resistant CMV disease and because ganciclovir prophylaxis did not select for late ganciclovir-resistant CMV disease. Differences in the study design, patient populations, and immunosuppressive regimens in the 2 studies may account for these apparent discrepancies.

In our study, we observed 6 novel UL97 polymorphisms (P405L, A427V, M550I, A582V, Y617H, and A674T). To our knowledge, ours is the first reported observation of these polymorphisms of unknown significance, all of which were considered to be unlikely to confer resistance, because they lie outside the documented regions (codons 460, 520, and 590–607) that are associated with ganciclovir resistance. However, definitive conclusions about the role that such mutations play will require a marker transfer of the mutated UL97 gene in a known susceptible CMV strain. Mutations in both UL97 and UL54 are generally considered to exhibit high-level resistance to ganciclovir [11]. No dual ganciclovir-resistance mutations in the UL97 and UL54 genes were observed for any of the samples tested; therefore, the level of resistance that, in the present study, is associated with ganciclovir administered orally is considered to be modest.

An association between the emergence of ganciclovir-resistant CMV strains and the development of CMV disease has been suggested [13]. However, in our study, none of the patients with proven CMV disease shed ganciclovir-resistant CMV. This suggests that, in our study at least, the presence of ganciclovir-resistant CMV is not necessarily correlated with the development of CMV disease. Other evidence has also indicated that drug-resistant CMV is associated with transplant-organ rejection [14]. In our study, 123 patients (78 of whom received valganciclovir and 45 of whom received ganciclovir) had acute graft rejection during the 12 months after transplantation (data not shown). However, none of these cases were associated with the presence of ganciclovir-resistant CMV.

Our study first evaluated the incidence of resistance for SOT recipients who received valganciclovir prophylaxis. Ganciclovir-resistant CMV did not emerge in patients who received valganciclovir prophylaxis, whereas it did emerge, albeit at a low incidence (1.9%, at the end of the study-drug prophylaxis; and 6.1%, for suspected CMV disease), in patients who orally received ganciclovir. Furthermore, resistance was short-lived and was not associated with any clinical sequelae during the 12 months after transplantation. Therefore, not only is once-daily valganciclovir prophylaxis a more convenient alternative to 3-times-daily ganciclovir, but it may also result in a lower incidence of drug-resistant CMV. Additional studies are necessary to evaluate the rate of drug-resistant CMV after valganciclovir prophylaxis in other organ-transplant populations (e.g., lung and bone-marrow transplant recipients) and when the drug is used as part of preemptive or therapeutic regimens.

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References