Demonstration of Resistant or Wild-Type Virus in Recurrent Viremia After Ganciclovir-Resistant Cytomegaloviral Infection

Margaret R. Jorgenson, PharmD¹✉, Jillian L. Descourouez, PharmD¹✉, Robert R. Redfield, MD², Jeannina A. Smith, MD², and Didier A. Mandelbrot, MD²

Abstract

**Background:** Ganciclovir-resistant cytomegalovirus (GR-CMV) is a serious complication of transplantation. Recurrence after primary infection is common. Little is known about CMV drug resistance and latency. **Objective:** Review CMV genotype during episodes of recurrent CMV viremia after prior documentation of ganciclovir resistance to evaluate if resistance is redemonstrated. **Methods:** All adult transplant recipients with history of GR-CMV viremia from January 1, 2011, to December 31, 2015, were screened; those with subsequent laboratory evidence of recurrent CMV viremia and genotyping were included. **Results:** A total of 23 patients had genetically confirmed GR-CMV within the study time period; 14 were excluded due to lack of repeat resistance testing at recurrence and 4 due to lack of negativity between testing, leaving 5 patients with 7 episodes of recurrent viremia to evaluate. At first recurrent viremia, 4 patients (80%) demonstrated resistant genotype; 1 patient had wild type. Two patients went on to have a second viremia recurrence; both demonstrated wild-type genotype, despite the fact that the first recurrence in these patients was resistant genotype. **Conclusion:** In transplant recipients with history of GR-CMV, it appears that there is strain variability in latency; repeat genetic testing in patients with recurrent viremia after GR-CMV should be conducted. In the setting of wild-type repopulation, use of GCV should be considered.

Keywords

transplantation, antivirals, antibiotic resistance, viral infections, herpesvirus

Background

Infection due to cytomegalovirus (CMV), a ubiquitous herpesvirus present in 40% to 70% of the population, is common after solid-organ transplantation and is an independent risk factor for graft loss and mortality. The treatment of choice is ganciclovir (GCV); however, ganciclovir-resistant (GR)-CMV disease is emerging, with an overall incidence of 5% to 12%.¹² Although relatively uncommon, GCV resistance is associated with significant morbidity and mortality: a recently published case series describes a 50% rejection incidence, 27% graft loss, and 20% mortality following GR-CMV disease.³ Activation of GCV requires 3 phosphorylation steps. The first is performed by a viral phosphotransferase encoded by the UL97 gene. After activation, GCV interacts with viral DNA polymerase, encoded by UL54 resulting in premature strand termination. Mutation at either of these genes results in resistance. Recurrence of viremia after primary GR-CMV infection is common, with rates ranging from 20% to 30%.¹² However, little is known about CMV drug resistance and latency.³

Objective

This study reviewed CMV genotype during episodes of recurrent CMV viremia after prior documentation of GCV resistance to evaluate if resistance is redemonstrated.

¹University of Wisconsin Hospital and Clinics, Madison, WI, USA
²University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA

Corresponding Author:
Margaret R. Jorgenson, Department of Pharmacy, University of Wisconsin Hospital and Clinics, 600 Highland Ave, Madison, WI 53792, USA.
Email: Mjorgenson@uwhealth.org
Methods

Design
Data were collected using retrospective analysis of electronic medical records at the University of Wisconsin Hospital. This study was approved by the local institutional review board.

Patients
All adult transplant recipients with history of GR-CMV viremia from January 1, 2011 to December 31, 2015, were screened; those with subsequent laboratory evidence of recurrent CMV viremia were included. Recurrence was defined as CMV detected at a level ≥250 IU/mL by polymerase chain reaction after viral load (VL) clearance to negativity (<50 IU/mL) for ≥7 days. Patients were followed through July 1, 2017; all recurrences were documented.

Outcomes
The primary end point was the presence/absence of resistance during recurrent viremia. Resistance was defined as mutations at UL97 or UL54 genes. Resistance testing was conducted at a reference laboratory (ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT) via Sanger sequencing methods on frozen plasma, where a VL of approximately 1500 IU/mL is required for genotyping.

Statistical Analysis
Descriptive statistics were used to quantitatively describe and summarize aspects of the population.

Results
A total of 23 patients had genetically confirmed GR-CMV within the study time period; 14 were excluded due to lack of repeat resistance testing during episodes of recurrent viremia and 4 due to lack of negativity between testing, leaving 5 patients with 7 episodes of recurrent viremia for evaluation. Mean follow-up time was 505 ± 355 days. Of the patients meeting inclusion criteria, 2 were recipients of renal transplants; 1 received a simultaneous kidney-pancreas transplant, 1 a bilateral lung transplant, and 1 an allogeneic stem cell transplant (Table 1). The mean age at transplant was 48 ± 9 years. All 5 patients were Caucasian, and 60% (n = 3) were male. Patients were transplanted between 2012 and 2015, and 60% (n = 3) were recipients of their first transplant. The majority (80%, n = 4) were considered to be high risk for disease based on donor and recipient CMV serostatus (Table 1). Mean CMV VL at the time of primary detection of GCV resistance was 72 859 ± 105 631 IU/mL (median = 10 814). All included patients had UL97 mutations; none had UL54. All included patients received foscarnet and intravenous immunoglobulin for initial GR-CMV treatment. GCV derivatives were withdrawn and not reintroduced. Duration of negativity (DoN) between clearance of initial viremia and first recurrence was 72 ± 129 days. Four patients (80%) demonstrated the same resistance mutation as in primary infection (Table 1); 1 patient did not redemonstrate resistance. Mean VL at the time of resistance testing is reported in Table 1. Of the 5 patients with viremia recurrence, 2 went on to have a second recurrence. Repeat resistance testing did not identify any mutations at UL97 or UL54 at the second recurrence, despite the fact that the initial recurrence in both these patients was that of the previous GR mutation. Mean DoN and VL at the time of resistance testing can be found in Table 1. Of the 7 recurrences, 86% (n = 6) were symptomatic. Prophylactic suppression with either leflunomide or acyclovir was utilized in 86% (n = 6) of recurrences. The majority received dual-agent immunosuppression at the time of each recurrence, including a calcineurin inhibitor and a corticosteroid (Table 1).

Discussion
This series is the first to describe isolation of drug-resistant CMV and subsequent wild-type repopulation in the setting of multiple recurrent viremias in transplant recipients. A previously published case report describes the phenomenon of wild-type repopulation after documented resistance in a lung transplant recipient. The authors investigated the relationship between wild-type repopulation and exposure to GCV derivatives. The authors concluded that strain variability in latency and recurrence can occur and was hypothesized to be related to antiviral selective pressure; however, the authors could not demonstrate association between time of GCV discontinuation and repopulation with wild-type virus. In reviewing other available case reports not specifically evaluating drug resistance at recurrence, but rather characterizing disease, wild-type repopulation occurred anywhere from 3 to 8 months after cessation of GCV, and in some cases, resistance persisted despite withdrawal. Our series suggests a similar lack of association between withdrawal of GCV derivatives and wild-type repopulation. Additionally, our inclusion of only those patients with distinct viremic episodes separated by clearance to negativity allows us to comment on viral recurrence, rather than strain variation within viremic episodes, as previous studies have done.

We are unaware of any literature describing fitness cost in GR-CMV; however, fitness cost may be the explanation for wild-type repopulation, as is seen in herpes simplex virus. Previous literature has described lower peak CMV VLs in the setting of resistant viremia and have theorized reduced pathogenicity.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Txp No.</th>
<th>Allograft Type</th>
<th>TXP Year</th>
<th>Induction</th>
<th>CMV Status</th>
<th>CMV Status Interpretation</th>
<th>DoN Initial Diagnosis to Recurrence (days)</th>
<th>Antiviral PPX at Detection of Recurrence</th>
<th>CMV VL at Time of GR Test 1 (IU/mL)</th>
<th>DoN Recurrence 1 to Detection of Recurrence 2 (days)</th>
<th>Antiviral PPX at Detection of Recurrence 2</th>
<th>CMV VL at Time of GR Test 2 (IU/mL)</th>
<th>Mutation at Recurrence 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>M</td>
<td>1</td>
<td>K</td>
<td>2013</td>
<td>ALM</td>
<td>D-/R-</td>
<td>Low risk</td>
<td>167</td>
<td>L595S, L595W</td>
<td>2240</td>
<td>L595S</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>F</td>
<td>1</td>
<td>K</td>
<td>2015</td>
<td>ALM</td>
<td>D+/R-</td>
<td>High risk</td>
<td>84</td>
<td>L595S</td>
<td>11 000</td>
<td>None</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>M</td>
<td>2</td>
<td>SPK</td>
<td>2012</td>
<td>BAS</td>
<td>D+/R-</td>
<td>High risk</td>
<td>70</td>
<td>C603W</td>
<td>3550</td>
<td>C603W</td>
<td>70</td>
<td>ACY</td>
<td>Pred, FK</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>F</td>
<td>1</td>
<td>BLTT</td>
<td>2015</td>
<td>BAS</td>
<td>D+/R-</td>
<td>High risk</td>
<td>34</td>
<td>A594V</td>
<td>3720</td>
<td>A594V</td>
<td>55</td>
<td>LEF</td>
<td>Pred, FK, LEF</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>M</td>
<td>1</td>
<td>BMT</td>
<td>2015</td>
<td>--</td>
<td>D-/R+</td>
<td>High risk</td>
<td>417</td>
<td>A594P, C603W</td>
<td>4170</td>
<td>A594P, C603W</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Abbreviations: ACY, acyclovir; ALM, alemtuzumab; BAS, basiliximab; BLTT, bilateral lung transplant; BMT, bone marrow transplant; CsA, cyclosporine; DoN, duration of negativity; FK, tacrolimus; GR, ganciclovir-resistant; IS, immunosuppressive therapy; K, kidney transplant; LEF, leflunomide; MMF, mycophenolate; Pred, prednisone; PPX, prophylaxis; SPK, simultaneous kidney-pancreas transplant; VL, viral load; Txp, transplant.
This study has a number of limitations: most prominently, its seemingly low enrollment. Indeed, with one of the largest described populations of transplant recipients with GR-CMV, the fact that only 5 patients, with 7 recurrences, met our basic inclusion criteria is striking. This is most likely a result of the lack of knowledge regarding the utility of genotypic retesting at each recurrent viremia. Indeed, this is the largest study of its kind, with only a single case report investigating this phenomenon to date, likely because of the misconception that once resistance is documented, recurrences will always redemonstrate resistance. This study takes care to appropriately define recurrence by requiring clearance to negativity. However, it is not overly strict to result in unnecessary exclusion as the DoN was only required to be 7 days, the standard testing interval per international consensus guidelines.

Additionally, we did not limit our inclusion to a single allograft type but rather considered the entire GR-CMV population in the history of our center to improve enrollment. Although this may be considered a limitation because of heterogeneity of the sample, all patients had similar management of primary GR-CMV disease, degree of illness, immunosuppression, and attempted prevention of viral recurrence, creating homogeneity within the group. Finally, molecular diagnostic methodology could be improved with deeper genetic analysis techniques to more definitively determine the presence of mutant populations as clinically used assays require >20% of the viral population to exhibit resistance. However, in the setting of low VL, this assay will typically yield indeterminate rather than susceptible. In all 3 cases of wild-type repopulation, the VL was considered adequate for antiviral drug resistance sequencing per our reference laboratory.

The agents used to treat GR-CMV, such as foscarinet and cidofovir, have significant tolerability concerns and are less efficacious than GCV. Patients who demonstrate historic GR-CMV are typically subjected to these toxic regimens empirically thereafter, and as is evident in our study population with >50% exclusion as a result of lack of repeat testing, repeat resistance testing at each viremic episode is not standardized. However, our finding of wild-type repopulation in 43% of recurrent viremias highlights the fallacy in the common misconception that once resistance is demonstrated, all recurrences will be with a resistant strain. By repeating genetic testing at each recurrence, the clinician could ensure that the most potent antiviral therapy is used.

### Conclusion

In this small case series of transplant recipients, it appears that GCV resistance can be maintained during viral latency. However, we also demonstrate repopulation with wild-type CMV in almost half of viremia recurrences. This finding is clinically important and suggests strain variability in latency: repeat genetic testing in patients with recurrent viremia after GR-CMV should be pursued to select the most efficacious and least toxic drug therapy. In the setting of wild-type repopulation, use of GCV should be considered. Future studies describing fitness costs of GCV resistance in latency and pathogenicity are needed to better elucidate the mechanism behind this observation.

### Acknowledgments

The authors would like to thank Zachary DeGrave for his assistance in data collection, and Christopher Gelbmann for his virological review.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

### ORCID iDs:

Margaret R. Jorgenson [https://orcid.org/0000-0001-6088-9727](https://orcid.org/0000-0001-6088-9727)
Jillian L. Descourouez [https://orcid.org/0000-0002-1196-5110](https://orcid.org/0000-0002-1196-5110)

### References


