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Polymerase Chain Reaction–Based Ganciclovir Resistance Testing of Ocular Fluids for Cytomegalovirus Retinitis

Cytomegalovirus (CMV) retinitis typically presents as a hemorrhagic, full-thickness retinitis in immunosuppressed individuals, often in the setting of human immunodeficiency virus (HIV) infection. The management of CMV retinitis includes systemic and locally administered intravitreal antiviral agents (ie, foscarnet sodium and ganciclovir sodium) and the surgical intravitreal ganciclovir implant. In chronically immunosuppressed patients (ie, transplant recipients and patients undergoing cancer chemotherapy) and in patients with HIV/AIDS who do not immune reconstitute, long-term CMV prophylaxis with valganciclovir hydrochloride may lead to ganciclovir- and foscarnet-resistant CMV strains. Moreover, the identification of drug-resistant CMV may affect the choice or dosing of antiviral medication. Ganciclovir resistance is classified into genotypic resistance, defined as CMV DNA harboring a mutation known to confer antiviral resistance, or phenotypic resistance, meaning that ganciclovir at a therapeutic dose does not exceed the concentration required to inhibit 50% of CMV growth on viral culture media. We characterize a series of patients with CMV retinitis who were evaluated for genotypic ganciclovir resistance using polymerase chain reaction (PCR)–based analysis of ocular fluids and describe its effect on management.

Methods. Patients diagnosed as having CMV retinitis who underwent ocular PCR analysis for ganciclovir resistance from 2 tertiary referral institutions (National Eye Institute, National Institutes of Health; and Casey Eye Institute, Oregon Health and Science University) between June 1, 2007, and December 31, 2008, were reviewed. Institutional review board approval for medical record review was obtained from each institution.

Medical Record Review, Ocular Fluid Sampling, and Clinical Management. Demographic information and medical history, including long-term immunosuppression, were reviewed. Patients were treated with systemic and intravitreal antivirals at the discretion of the physician. All patients received intravitreal antiviral therapy after ocular fluid sampling. Anterior chamber paracentesis typically provided 50 to 100 µL of fluid; a vitreous tap yielded 200 to 300 µL of fluid, which was sufficient for qualitative and quantitative PCR and genotypic antiviral resistance testing. Clinical management was altered depending on whether the patient showed genotypic antiviral resistance.

PCR Analysis and Direct CMV Genome Sequencing for Antiviral Drug Resistance. The Department of Laboratory Medicine, National Institutes of Health, performed all PCR analyses of ocular fluid specimens for CMV, herpes simplex virus, varicella zoster virus, and toxoplasmosis. Patients who developed CMV reactivation while receiving prophylactic valganciclovir therapy were evaluated for ganciclovir resistance. After PCR amplification of CMV DNA from aqueous fluid, direct genome sequencing of the UL97 gene (GenBank EF999921) encoding phosphotransferase and the UL54 gene (GenBank EF999921) encoding DNA polymerase was performed (Appendix and eTables 1 and 2; http://www.archophthalmol.com). The gene sequences were compared with a database of polymorphisms previously defined to confer genotypic antiviral resistance. 

Report of Cases. Direct CMV UL97 and UL54 gene sequencing was performed from DNA extracted from ocular fluid from 6 patients after a reactivation of CMV retinitis. The median patient age was 56.5 years (age range, 10 months to 73 years), and only 2 of 6 patients were HIV positive (Table 1). An aqueous humor specimen was obtained from 5 patients and a vitreous specimen from 1 patient; all samples were sufficient for PCR analyses. Cytomegalovirus DNA from 2 patients was found to contain mutations in the UL97 and UL54 genes, prompting changes in oral and intravitreal therapy. Both patients had been receiving valganciclovir therapy for more than 12 months before CMV retinitis reactivation (Table 2). One patient (patient 4) with disease reactivation who was negative for ganciclovir-resistant CMV later reported nonadherence to antiviral medications owing to tolerability issues. His retinitis eventually re-
perotemporally in the left eye with nasal pigment epithelium atrophy. Dilated funduscopic examination revealed mild anterior chamber inflammation in the right eye. Slitlamp examination revealed floaters in both eyes while reviewing prophylactic valganciclovir (450 mg twice daily). Visual acuities were 20/40 OD and 20/32 OS. Slitlamp examination revealed floaters and a CMV retinitis recurrence (data not shown). Herpes simplex virus, varicella zoster virus, and toxoplasmosis DNA of ocular fluids were negative for all patients. Aqueous humor specimens were obtained in patients 1 to 5, and a vitreous specimen was obtained in patient 6. Reactivation of CMV was confirmed by positive findings on CMV polymerase chain reaction with qualitative and real-time quantitative polymerase chain reaction (data not shown). Herpes simplex virus, varicella zoster virus, and toxoplasmosis DNA of ocular fluids were negative for all patients. Aqueous humor and vitreous specimens were obtained in patients 1 to 5, and a vitreous specimen was obtained in patient 6.

### Patient 1
A 73-year-old man with large granular leukemia with a CD4 cell count of 10/μL, disseminated *Mycobacterium kansasii*, and previously treated CMV retinitis noted floaters in both eyes while receiving prophylactic valganciclovir (450 mg twice daily). Visual acuities were 20/40 OD and 20/32 OS. Slitlamp examination revealed mild anterior chamber inflammation in the right eye. Dilated funduscopic examination showed diffuse peripheral retinal pigment epithelium atrophy superotemporally in the left eye with subtle retinal whitening and hemorrhage at the atrophic retinal pigment epithelium border. Aqueous fluid PCR confirmed 1200 genome equivalents per milliliter of CMV DNA. Findings from direct genome sequencing for ganciclovir resistance were negative. Induction therapy with valganciclovir, 900 mg twice daily, was begun with resolution of the retinitis.

Eight months later, a CMV retinitis recurrence was observed, and repeated ganciclovir resistance testing showed point mutations in the UL54 and UL97 genes, which conferred high-level genotypic resistance to ganciclovir and cidofovir (Table 2). Intravenous and intravitreal foscarnet administration was initiated, and improvement in the retinitis was observed at 3-week follow-up. However, the patient died secondary to systemic complications related to large granular leukemia.

### Patient 2
A 67-year-old woman status post lung transplantation from an H11022 donor in 2005 for end-stage interstitial lung disease secondary to Sjögren syndrome was taking tacrolimus, mycophenolate mofetil, and prednisone. Intravenous ganciclovir administered for CMV viremia was discontinued after severe neutropenia developed. Oral prophylactic valganciclovir, 450 mg twice daily, was eventually initiated, and filgrastim was administered.

In August 2007, she sought care for floaters and a CMV retinitis reactivation posterior to a zone of peripheral retinal pigment epithelium atrophy. Aqueous fluid was positive for CMV DNA, and intravitreal foscarnet was administered. Antiviral resistance testing showed UL54 and UL97 gene mutations conferring high-level ganciclovir and cidofovir resistance. Other UL54 gene polymorphisms were identified but were not associated with ganciclovir resistance (Table 2). Based on the detection of these mutations, intravitreal foscarnet injections were continued until disease resolution.

### Table 2. Clinical Details of 6 Patients Undergoing Ganciclovir Resistance Evaluation Using Ocular Fluid

<table>
<thead>
<tr>
<th>Patient No./Sex/Age</th>
<th>Associated Medical Conditions</th>
<th>Valganclovir Use Before CMV Reactivation, mo a</th>
<th>Presence of Point Mutations in CMV UL97 or UL54 Genes Associated With Ganciclovir Resistance</th>
<th>CMV Retinitis Treatment Before Ganciclovir Resistance Testing</th>
<th>Change in Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/73 y</td>
<td>Natural killer cell leukemia</td>
<td>&gt;12</td>
<td>Yes (9/2008)—UL97: C592S b; UL54: T503I b; No (1/2008)</td>
<td>Valganclovir</td>
<td>Yes, intravitreal foscarnet</td>
</tr>
<tr>
<td>2/F/67 y</td>
<td>Lung transplant, mycophenolate mofetil, prednisone, tacrolimus</td>
<td>&gt;12</td>
<td>Yes—UL97: M460I b; UL54: A987T b; UL54 mutations identified but not associated with ganciclovir resistance: S655L, N685S, A885T, N898D</td>
<td>Valganclovir</td>
<td>Yes, intravitreal and intravenous foscarnet</td>
</tr>
<tr>
<td>3/M/32 y</td>
<td>HIV</td>
<td>1</td>
<td>No</td>
<td>Valganclovir, intravitreal foscarnet</td>
<td>No</td>
</tr>
<tr>
<td>4/M/41 y</td>
<td>HIV</td>
<td>2, Poorly compliant with valganciclovir</td>
<td>No c</td>
<td>Valganclovir, intravitreal foscarnet</td>
<td>No</td>
</tr>
<tr>
<td>5/M/10 mo</td>
<td>Acute lymphocytic leukemia</td>
<td>2</td>
<td>No</td>
<td>Intravenous foscarnet/ganciclovir, intravitreal foscarnet</td>
<td>No</td>
</tr>
<tr>
<td>6/F/72 y</td>
<td>Diabetes mellitus</td>
<td>6</td>
<td>No</td>
<td>Valganclovir, intravitreal foscarnet</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; HIV, human immunodeficiency virus.

a Reactivation of CMV was confirmed by positive findings on CMV polymerase chain reaction with qualitative and real-time quantitative polymerase chain reaction (data not shown). Herpes simplex virus, varicella zoster virus, and toxoplasmosis DNA of ocular fluids were negative for all patients. Aqueous humor and vitreous specimens were obtained in patients 1 to 5, and a vitreous specimen was obtained in patient 6.

b UL97 and UL54 point mutations associated with ganciclovir resistance.

c No UL97 mutations conferring resistance; UL54 base changes identified but none known to confer phenotypic resistance.

<table>
<thead>
<tr>
<th>No./Sex/Age</th>
<th>Condition</th>
<th>UL97 Gene Mutations</th>
<th>UL54 Gene Mutations</th>
<th>Change in Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/73 y</td>
<td>Natural killer cell leukemia</td>
<td>UL97: C592S b; UL54: T503I b; No (1/2008)</td>
<td>UL54 mutations identified but not associated with ganciclovir resistance: S655L, N685S, A885T, N898D</td>
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</tr>
</tbody>
</table>
Ganciclovir implantation was performed, with no evidence of disease recurrence at final follow-up.

Comment. In this series, we used direct CMV genome sequencing to assay for the presence of resistance mutations and demonstrated UL97 and UL54 mutations in 2 patients treated with more than 12 months of prophylactic valganciclovir. The identification of drug-resistant strains of CMV led to a therapeutic change via initiation of intravitreal foscanet administration, with subsequent disease resolution.

Previous studies\textsuperscript{1,2,3} have shown that ganciclovir-resistant CMV identified using peripheral blood may be correlated with a poorer visual outcome and increased mortality risk. Because of the effectiveness of highly active antiretroviral therapy, it is possible that an increasing proportion of patients who develop CMV infections will be chronically immunosuppressed for reasons other than HIV, as observed in this series. Some of these patients may also be receiving long-term prophylactic valganciclovir for nonocular CMV, potentially applying selection pressure for the emergence of resistant CMV strains. Because ganciclovir resistance is a relative and not an absolute phenomenon, options when ganciclovir resistance is identified include increasing the ganciclovir dosage, implanting a sustained-release ganciclovir intravitreal device, switching medications, and initiating combination antiviral therapy.

Ganciclovir resistance is not assessed in every patient with CMV retinitis because of the intensive labor and cost associated with testing. However, in selected clinical circumstances, particularly in patients with recurrent CMV retinitis while undergoing long-term valganciclovir therapy or in refractory cases of CMV retinitis, direct gene sequencing may be helpful. Detection of mutations in the CMV genome conferring antiviral resistance is commercially available through Clinical Laboratory Improvement Act–compliant reference laboratories with molecular diagnostic capabilities, sometimes within 48 to 72 hours. However, because of the small volumes typically obtained from ocular fluids and the greater degree of familiarity of reference laboratories with plasma, serum, and cerebrospinal fluid specimens, close communication with the reference laboratory and confirmation of their ability to process ocular specimens is recommended before ordering CMV drug resistance testing.

Limitations of this study include the retrospective nature of data collection and the potential selection bias in the limited number of patients chosen for antiviral drug resistance testing. However, using small ocular fluid volumes, we assessed qualitative and quantitative CMV PCR and identified genomic mutations that confer genotypic resistance. Although the utility of qualitative and real-time quantitative PCR for the assessment of viral retinitis has been established,\textsuperscript{6,8} antiviral resistance testing using ocular fluid samples may also prove to be extremely valuable in the management of ganciclovir-resistant CMV retinitis.

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