Mutations Conferring Foscarnet Resistance in A Cohort of Patients with Acquired Immunodeficiency Syndrome and Cytomegalovirus Retinitis

Adriana Weinberg, Douglas A. Jabs, Sunwen Chou, Barbara K. Martin, Nell S. Lurain, Michael S. Forman, and Clyde Crumpacker, for the Cytomegalovirus Retinitis and Viral Resistance Study Group and the Adult AIDS Clinical Trials Group Cytomegalovirus Laboratories

The clinical significance of cytomegalovirus (CMV) foscarnet resistance was studied in patients with acquired immunodeficiency syndrome and CMV retinitis. Sequencing of the CMV \( \text{pol} \) gene was performed in 30 isolates. Phenotypic resistance was characterized by the DNA hybridization assay (DHA) in 30 isolates and by plaque-reduction assay (PRA) in 18 isolates. Nine isolates had foscarnet resistance mutations, including V787L and E756Q that were confirmed by marker transfer experiments. Seven of 9 isolates with a 50% inhibitory concentration (IC\(_{50}\)) of \( >600 \mu M \) by DHA had genotypic resistance, compared with 2 of 21 with an IC\(_{50}\) of \( \leq 600 \mu M \) (\( P = .0005 \)). By PRA, 5 isolates had an IC\(_{50}\) of \( >400 \mu M \) and genotypic resistance, whereas only 1 of 13 susceptible isolates had genotypic resistance (\( P = .0007 \)). Sixteen of 18 isolates had concordant PRA and DHA phenotypes. Among 44 patients treated with foscarnet, drug resistance increased the risk of retinitis progression (odds ratio, 14; \( P < .001 \)). The incidence of foscarnet resistance after 6, 9, and 12 months of therapy was 13%, 24%, and 37%, respectively.

Cytomegalovirus (CMV) retinitis is one of the most common infections in patients with AIDS [1]. Before the era of highly active antiretroviral therapy (HAART), CMV retinitis was estimated to affect 30% of patients with AIDS [2]. Unless immune reconstitution occurs as a consequence of HAART, long-term suppressive anti-CMV therapy is required to prevent relapse of the disease, because untreated CMV retinitis results in total retinal destruction and blindness, and discontinuation of treatment in patients without immune reconstitution results in prompt relapse of the disease [1, 3].

Patients receiving long-term suppressive anti-CMV therapy may develop antiviral resistance. The Cytomegalovirus Retinitis and Viral Resistance (CRVR) Study, which prospectively follows patients with AIDS and CMV retinitis [4], has previously reported an association between ganciclovir-resistant CMV isolates and retinitis progression [5]. The clinical significance of in vitro resistance to other antivirals licensed for the treat-
ment of CMV, such as foscarnet, cidofovir, and fomivirsen, is less well established.

Antiviral resistance can be characterized by phenotypic or genotypic methods. The original description of CMV resistance was done phenotypically by use of the plaque-reduction assay (PRA) [6] and defined susceptibility as an IC_{50} \leq 6 \mu M for ganciclovir [7], 400 \mu M for foscarnet [8], and 4 \mu M for cidofovir [9]. PRA is a lengthy and cumbersome procedure which led to the development of alternative phenotypic assays, such as DNA hybridization assay (DHA) [10] and antigen-reduction assay [11–13]. These methods have an objective readout and somewhat faster turnaround time, but their resistance-defining IC_{50} values are less well defined. Previous studies showed that DHA had excellent agreement with PRA for ganciclovir and good agreement for foscarnet [14–16]. Overall, the clinical utility of phenotypic assays is limited by the requirement for large volumes of replication-competent virus, which may not be attainable or may require up to 4 weeks of in vitro culture. In contrast, genotypic assays, which detect resistance-conferring mutations in the viral genome, require only small amounts of viral nucleic acids, can use nonviable virus, and provide faster results [17, 18].

Genotype interpretation presupposes knowledge of the clinical significance of mutations, which is usually established by phenotypic characterization of mutant viruses [19–21]. The phosphotransferase gene (UL97), which is critical for ganciclovir antiviral activity, has been relatively well characterized with respect to both clinically significant mutations [21–24] and irrelevant polymorphisms [25]. Clinically significant mutations [22, 23, 26] and common polymorphisms [27] of the DNA polymerase gene (UL54), the target of ganciclovir, foscarnet, and cidofovir antiviral activity, also have been defined, but there are multiple areas in the conserved regions of UL54 that need further characterization. For ganciclovir, good-to-excellent agreement between resistance phenotypes and genotypes has been demonstrated on clinical isolates [28, 29].

Another area of investigation has been the agreement between CMV resistance mutations detected in virus in the blood, urine, or oropharynx and those in virus present in the eye or other affected organs. Although some reports identified discrepancies between CMV strains from different sites [30–32], previous data from the CRVR Study Group have shown good-to-excellent agreement between mutations in the UL97 gene that confer ganciclovir resistance from virus detected in the blood and in the eye [33], as well as a strong association between ganciclovir resistance in virus detected in the blood or urine and clinical outcomes of CMV retinitis [5]. Nevertheless, there are few data on the clinical import of foscarnet resistance detection of virus in the blood or urine.

In this report, the CRVR Study Group and the Adult AIDS Clinical Trials Group (AARC) Cytomegalovirus Resistance Laboratories present data on the phenotype-genotype agreement for foscarnet resistance and on the association of foscarnet resistance detected in the blood or urine with clinical outcomes, characterize 2 newly described pol mutations using marker transfer experiments, and refine the CRVR Study Group’s estimate of the incidence of foscarnet resistance.

PATIENTS, MATERIALS, AND METHODS

Patients. The CRVR Study enrolled patients with AIDS and previously untreated CMV retinitis. Patients received standard treatment regimens [4], which for foscarnet consisted of induction at 90 mg/kg intravenously twice daily for 14 days, followed by maintenance at 90–120 mg daily as a single intravenous infusion. Patients were seen monthly for ophthalmological examinations, at which time fundus photographs were taken, using the standard 9 60° fields. Photographs were sent to the CRVR Study Fundus Photograph Reading Center (University of Wisconsin, Madison), where the evaluation of retinitis was performed by graders masked to the results of laboratory tests and treatment. Retinitis progression was defined as the movement of the border of a CMV lesion >750 \mu m along a >750-\mu m front or the occurrence of a new lesion one-fourth disc area in size, as per the standard definition for clinical trials of anti-CMV drugs [4, 5].

Before the initiation of therapy, patients underwent phlebotomy and collection of a urine specimen for CMV isolation. Follow-up cultures were performed at 1 and 3 months after enrollment and every 3 months thereafter [32, 34].

Phenotypic susceptibility. All CMV isolates were tested for foscarnet susceptibility by DHA at the CRVR CMV Laboratory at the Johns Hopkins Hospital (Baltimore). This assay used the Hybriwix Probe System–CMV Susceptibility Test Kit (Diagnostic Hybrids), as described elsewhere [10, 14, 16]. Susceptibility testing was performed without knowledge of treatment or other clinical characteristics. A subset of isolates were tested by PRA at the Johns Hopkins Laboratory as part of ongoing quality control comparison between the DHA and the PRA [14, 16, 35]. An additional subset was forwarded to the AACTG Cytomegalovirus Laboratories for PRA, which was performed by a consensus protocol [36]. This latter subset included the isolates with foscarnet IC_{50} values >400 \mu M by DHA and companion isolates obtained at other time points from the same patients who generated the index isolates.

Genotypic testing. Thirty isolates from 14 patients were selected for genotyping. All isolates with an IC_{50} >400 \mu M by DHA were selected, and companion isolates with an IC_{50} <400 \mu M obtained at other times were selected. Additional isolates were selected randomly for PRA and genotyping to provide an adequate number of susceptible and resistant samples. Frozen isolates from the original cultures were thawed, regrown, and
used for CMV DNA polymerase coding region (pol) sequencing. The entire 3.7-kb CMV pol coding region of isolates was amplified by polymerase chain reaction (PCR) from DNA extracts of infected cell cultures. If the thawed isolate could not be regrown, an attempt was made to directly amplify the DNA by use of PCR. A full-length PCR product was obtained with the Gene AMP XL PCR kit (PE Applied Biosystems) and the following primers that flank the pol coding region: forward primer, 5’-GTCAGGCTTCTACCGTGCGTGAT-3’; reverse primer, 5’-CCTCAGTCTACCGAGCATCATAC-3’. The PCR products were sequenced without cloning, with primers internal to the amplified segment that produced overlapping coverage of the pol sequence. Sequencing reactions were performed by dye primer chain termination chemistry, with an automated sequencer (ABI 373 or 377) and fluorescent dye terminators (Applied Biosystems). Derived sequences of each isolate were aligned with the strain AD169 reference sequence with a sequence text editor. Amino acid differences were tabulated and compared with previously published pol resistance mutations [18, 21–23, 25, 27].

**Marker transfer.** The CMV pol (UL54) gene mutations E756Q and V787L that had not been shown previously to confer foscarnet resistance were individually transferred to a laboratory strain of CMV to determine the effect of these mutations on drug resistance. The chosen laboratory strain T1472 was derived from Towne strain (ATCC VR977) and contains unique Pme restriction sites within pol (S.C., unpublished observations). T1472 has the same pol amino acid sequence as does the Towne strain, except for S897L and D898N, which are common polymorphisms in CMV isolates [27]. A bluescript-cloned DNA segment of Towne strain (nt 74828–82550 based on the standard AD169 numbering scheme), modified with the same Pme and other changes as in T1472, was further mutagenized by PCR to contain either mutation E756Q (plasmid SC112) or mutation V787L (plasmid SC115). Each plasmid (2 μg) containing E756Q or V787L was linearized by restriction digestion (XbaI and EcoRI) and was cotransfected into fibroblast cultures with PmeI-digested genomic T1472 DNA (15 μg) by the calcium phosphate coprecipitation method, as described elsewhere [24]. Because of the targeted cleavage of the viral genomic DNA at the PmeI restriction sites, no drug selection was needed or used to recover infectious recombinant CMV containing the desired pol mutations from the transfected cultures. Extracellular virus from the transfected cultures was assessed by DNA sequencing, to confirm the presence of the expected pol mutation, and then was plaque purified. The final viruses were resequenced in their entire pol sequences, to exclude changes other than those intended, and were tested for drug susceptibility by PRA [36].

**Statistical analysis** Comparisons between phenotypic and genotypic results were performed by use of the 2-tailed Fisher’s exact test. In addition, agreement among resistance phenotype by DHA, resistance phenotype by PRA, and resistance genotype was measured using the k statistic, which adjusts for agreement expected by chance alone [37, 38]. k > 0.6 is considered to be “substantial,” and k > 0.8 is considered to be an “almost perfect” agreement [38]. The incidence of foscarnet-resistant CMV was calculated by use of the Kaplan-Meier method [39]. For the association of resistance with retinitis progression, follow-up of patients while they were receiving foscarnet was divided into 3-month blocks of time centered around the collection of cultures for CMV, and the occurrence of resistance to foscarnet was compared with the occurrence of retinitis progression, as described elsewhere [5]. The Fisher’s exact value for the association was calculated. Because of the small number of specimens and “zero cells,” generalized estimating equations regression analyses to obtain P values corrected for the possible correlation of repeated measures were not performed.

**RESULTS**

**Study population.** The present report includes data collected between November 1993 and December 1998 from 176 patients with CMV retinitis, of whom 44 patients were treated with foscarnet. The 44 patients who received foscarnet therapy received a minimum of at least 1 month of therapy. An additional 5 patients received <1 month of therapy, typically only a few days. This data set is similar to the one in the previous report from the CRVR Study Group on foscarnet therapy [9]; the same 44 patients are included, but the follow-up period in the present report is longer (median, 11 months) than in the previous report (median, 6 months). All the 44 patients received foscarnet therapy at some time during their course, and 21 patients (48%) received foscarnet therapy as initial treatment. Of the remaining 23 patients, the initial treatment was a ganciclovir implant in 1, cidofovir in 3, and ganciclovir systemically in 19. Among these 23 patients, the median duration of other therapy before foscarnet therapy was 6 months (range, 2–18 months). The 30 isolates selected for CMV pol genotyping (using the criteria described in Patients, Materials, and Methods) were obtained from 14 patients with the following characteristics: 86% were men; mean age was 37 years; 64% were white; 50% received foscarnet therapy; 57% had received HAART at any time; median CD4+ T cell count at baseline was 7 cells/μL; median follow-up was 15 months; and median duration of foscarnet therapy among those who received foscarnet as therapy for CMV retinitis was 13.5 months. The demographic characteristics were similar to those of the 44 patients followed for the incidence of foscarnet resistance, except that 25% of this latter group received HAART [16]. The demographic characteristics also were similar to those of the 176 patients with CMV retinitis enrolled in the CRVR Study during the study period, except that 40% of the larger group was white and 28% received HAART. Among the 44 patients who received foscarnet resistance
Table 1. Foscarnet resistance–conferring mutations in isolates from patients with cytomegalovirus (CMV) retinitis who were treated with foscarnet.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visit, months</th>
<th>Duration of foscarnet therapy, months</th>
<th>Source</th>
<th>Foscarnet-resistance phenotype IC₅₀, µM</th>
<th>Mutation(s)</th>
<th>CMV pol genotypeᵃ</th>
<th>Polymorphisms</th>
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<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>21</td>
<td>Blood</td>
<td>690</td>
<td>V715M</td>
<td>A885T, N898D</td>
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<tr>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Blood</td>
<td>646</td>
<td></td>
<td>A688T, S897L, N898D, A1122T</td>
<td></td>
</tr>
<tr>
<td>3ᵃ</td>
<td>Dx</td>
<td>6</td>
<td>Blood</td>
<td>1055</td>
<td>E756Q</td>
<td>S655L, N685S, F669L, A885T, N898D</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Dx</td>
<td>—</td>
<td>Blood</td>
<td>229</td>
<td>220</td>
<td>P116S, A619T, N685S, A885T</td>
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<tr>
<td>3</td>
<td>—</td>
<td>0</td>
<td>Blood</td>
<td>510</td>
<td></td>
<td>P116S, A619T, N685S, A885T</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>—</td>
<td>Blood</td>
<td>174</td>
<td></td>
<td>S655L, N685S, A885T, N898D</td>
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</tr>
<tr>
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<td>1</td>
<td>—</td>
<td>Blood</td>
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<td>—</td>
<td>6</td>
<td>Blood</td>
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<td></td>
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<tr>
<td>7</td>
<td>Dx</td>
<td>—</td>
<td>Urine</td>
<td>177</td>
<td>37</td>
<td>G629E, S655L, N685S, A885T, N898D, N1116H</td>
<td></td>
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<tr>
<td>13</td>
<td>7</td>
<td>11</td>
<td>Urine</td>
<td>545</td>
<td>162</td>
<td>G629E, S655L, N685S, A885T, N898D, N1116H</td>
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<tr>
<td>14</td>
<td>8</td>
<td>5</td>
<td>Urine</td>
<td>635</td>
<td>672</td>
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<tr>
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<td>12</td>
<td>8</td>
<td>Urine</td>
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<td>—</td>
<td>Blood</td>
<td>249</td>
<td>179</td>
<td>A885T, S897L, A1122T</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>1</td>
<td>Blood</td>
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<td>152</td>
<td>S676G, A885T, S897L, N898D</td>
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<tr>
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<td>5</td>
<td>6</td>
<td>Blood</td>
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</tr>
<tr>
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<td>6</td>
<td>6</td>
<td>Blood</td>
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<td>507</td>
<td>S676G, A885T, S897L, N898D</td>
<td></td>
</tr>
<tr>
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<td>Dx</td>
<td>—</td>
<td>Blood</td>
<td>135</td>
<td></td>
<td>S676G, A885T, N898D, A1122T</td>
<td></td>
</tr>
<tr>
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<td>—</td>
<td>5</td>
<td>Blood</td>
<td>538</td>
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<td>S676G, A885T, N898D, A1122T</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Dx</td>
<td>—</td>
<td>Urine</td>
<td>223</td>
<td></td>
<td>S655L, N685S, A885T, N898D</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>4</td>
<td>Blood</td>
<td>426</td>
<td></td>
<td>S655L, N685S, A885T, N898D</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Dx, at diagnosis of CMV retinitis before treatment; DHA, DNA hybridization assay; PRA, plaque-reduction assay; —, a patient who did not receive foscarnet therapy or a specimen that was taken before a patient received foscarnet therapy.

ᵃ “Mutation” refers to a sequence change known to confer a resistance to phenotype and “polymorphism” to a sequence change that does not alter phenotype.

ᵇ Length of follow-up between diagnosis of CMV retinitis and time at which isolate was obtained.

ᶜ Patient 3 received 6 weeks of foscarnet therapy for varicella-zoster virus retinitis before developing CMV retinitis.

d The P522S mutation confers resistance to ganciclovir and cidofovir but not to foscarnet. Resistance to ganciclovir was confirmed in the 29-month isolate from patient 10, which had a ganciclovir IC₅₀ of 40 µM.

Net, the median time to first progression of the retinitis was 65 days, and the rate of progressions was 3.31 events/year.

**UL54 mutations.** Thirty isolates from 14 patients receiving foscarnet were available for sequencing of the UL54 gene (table 1). Five different mutations in the conserved pol region were detected in 9 isolates from 5 patients. Other polymorphisms were common. Of these 5 patients, 1 patient (patient 3) had a foscarnet resistance mutation detected at the time of diagnosis of CMV retinitis, before anti-CMV therapy was begun. However, the patient had received 6 weeks of foscarnet therapy for presumed varicella-zoster virus retinitis, which was discontinued 1 week before the diagnosis of CMV retinitis. Hence, this mutation also appeared to occur in the context of the selective pressure of foscarnet therapy.
**Phenotypic characterization of UL54 mutations.** Among the foscarnet resistance mutations identified in this patient population, V715M, V781I, and P522S have been characterized elsewhere [18, 21–23, 25, 27], E756Q was identified for the first time in the present study, and V787L was described elsewhere [9] but without phenotypic characterization by marker transfer experiments. PRA, performed with cell-associated virus, as described elsewhere [36], showed that E756Q recombinants had IC_{50} values of 477 μM for foscarnet, 3.43 μM for ganciclovir, and 3.63 μM for cidofovir; the V787L recombinants had IC_{50} values of 460 μM for foscarnet, 4.8 μM for ganciclovir, and 3.58 μM for cidofovir. In the same runs, the T1472 wild-type CMV parent to the recombinant viruses showed IC_{50} values of 111 μM to foscarnet, 2.03 μM to ganciclovir, and 3.66 μM to cidofovir. These data indicate that both E756Q and V787L are foscarnet resistance mutations, because they increase the IC_{50} values of 477 μM to foscarnet, 2.03 μM to ganciclovir, and 3.66 μM to cidofovir. Of note, the week 13 urine isolate obtained from patient 7 with discrepant genotypic and phenotypic results for cidofovir; the V787L recombinants had IC_{50} of 162 μM for foscarnet when the foscarnet resistance genotype was detected. The resistance phenotype (IC_{50} ≥600 μM by the DHA assay) was detected in 6 of these 7 episodes, and, in the seventh episode, the IC_{50} was 545 μM. Retinitis progression occurred during each of the 7 episodes in which foscarnet resistant virus was isolated, compared to its occurrence during 52% of the 114 blocks of time in patients receiving foscarnet who had no evidence of resistance. Because progression occurred in all time blocks when resistance was detected, the odds ratio (OR) for the association of resistance with progression is infinite. Although this does not account for the correlation of repeated measures, the lower confidence limit is 1.27, and Fisher’s exact P value is .016. If the OR is estimated by the typical method of adding 0.5 to all the counts, the result is an OR of 14.0.

**Comparison of genotypic and DHA- and PRA-measured CMV resistance to foscarnet.** Foscarnet-resistant CMV isolates have generally been defined as those with an IC_{50} >400 μM in a phenotypic assay. In addition, a 4-fold increase in the IC_{50} compared with that in a laboratory strain control, has sometimes been required. Of the 18 isolates tested by PRA and genotyped in this study, 5 had an IC_{50} >400 μM, and all had at least 1 foscarnet resistance mutation, whereas only 13 isolates with an IC_{50} ≤400 μM contained foscarnet resistance mutations (P = .0007; χ = 0.87). Of note, the week 13 urine isolate obtained from patient 7 with discrepant genotypic and PRA results (since it contained the V781I mutation and had an IC_{50} of 162 μM) was run in an assay in which the CMV AD169 control had a foscarnet IC_{50} of 37 μM. Although this urine isolate did not meet the 400 μM IC_{50} threshold resistance criterion, its reduced susceptibility to foscarnet was indicated by the 4.4-fold higher foscarnet IC_{50} compared with the that of the laboratory control. For the 30 isolates tested by DHA and genotyped, 9 of 17 with an IC_{50} >400 μM by DHA and none of 13 with an IC_{50} ≤400 μM contained foscarnet resistance mutations (P = .003; χ = 0.49). When DHA was compared with PRA, 5 of 9 isolates with an IC_{50} >400 μM by DHA and 0 of 9 with an IC_{50} ≤400 μM by DHA had an IC_{50} >400 μM by PRA (P = .03; χ = 0.56). A better agreement for DHA with foscarnet resistance as defined by genotyping and PRA was obtained by changing the threshold for DHA-measured foscarnet resistance to 600 μM. Seven of 9 isolates with an IC_{50} >600 μM and 2 of 21 with an IC_{50} ≤600 μM by DHA had foscarnet resistance mutations (P = .0005; χ = 0.68). In addition, 4 of 5 isolates with an IC_{50} >600 μM and 1 of 13 isolates with an IC_{50} ≤600 μM by DHA had an IC_{50} >400 μM by PRA (P = .008; χ = 0.72). That is, using the 600 μM threshold for DHA and the 400 μM threshold for PRA, there was agreement on phenotype resistance in 16 of 18 simultaneous specimens.

**Incidence of foscarnet resistance.** The original publication on the incidence of the foscarnet resistance phenotype among patients receiving foscarnet therapy for CMV retinitis from the CRVR Study Group calculated this incidence by using the 400 μM IC_{50} threshold measured by the DHA assay and a median follow-up of 6 months [16]. Therefore, we recalculated the incidence of foscarnet resistance in this cohort by using the 600 μM threshold DHA assay and a median follow-up of 11 months (figure 1). The 1 patient with foscarnet resistance at the diagnosis of CMV retinitis, who had received foscarnet therapy for varicella-zoster virus retinitis, is not included in this calculation, because the timing of onset of resistance cannot be ascertained, and resistance did not develop during treatment for CMV retinitis. By use of this threshold, the incidence of foscarnet resistance among patients receiving foscarnet therapy was 13% at 6 months, 24% at 9 months, and 37% at 1 year of foscarnet therapy.

**Clinical outcomes.** There were 7 episodes (3-month blocks of time) during follow-up among 4 patients who were receiving foscarnet when the foscarnet resistance genotype was detected. The resistance phenotype (IC_{50} ≥600 μM by the DHA assay) was detected in 6 of these 7 episodes, and, in the seventh episode, the IC_{50} was 545 μM. Retinitis progression occurred during each of the 7 episodes in which foscarnet resistant virus was isolated, compared to its occurrence during 52% of the 114 blocks of time in patients receiving foscarnet who had no evidence of resistance. Because progression occurred in all time blocks when resistance was detected, the odds ratio (OR) for the association of resistance with progression is infinite. Although this does not account for the correlation of repeated measures, the lower confidence limit is 1.27, and Fisher’s exact P value is .016. If the OR is estimated by the typical method of adding 0.5 to all the counts, the result is an OR of 14.0.

*Figure 1.* Proportion of patients without a foscarnet-resistant cytomegalovirus (CMV) isolate.
DISCUSSION

Foscarnet was the second drug approved by the US Food and Drug Administration and the second most commonly used agent for the treatment of CMV retinitis [40, 41]. Our data suggest that, similar to ganciclovir, foscarnet treatment failure is associated with foscarnet resistance mutations detected in virus in the blood or urine, as evidenced by the increased relative OR for retinitis progression. Because of the small number of specimens in this study, the size of the relative OR cannot be estimated with precision. Also, a generalized estimating equations analysis, which corrects for correlation among repeated measures, could not be performed. Hence, the statistical significance of the occurrence of progression in the face of foscarnet resistance may have been overestimated. Nevertheless, the fact that progression occurred whenever foscarnet resistance was detected suggests that resistance is associated with adverse ocular outcomes. This finding is particularly important for immunocompromised patients, who require prolonged antiviral therapy. Although there has been a 75% reduction in the incidence of CMV retinitis as a consequence of HAART [42–45], new cases of CMV continue to occur, and these patients typically are similar both clinically and immunologically to those with CMV retinitis in the era before HAART [46, 47]. Although there are case reports of patients developing CMV retinitis who have higher CD4+ T cell counts, most new cases of CMV retinitis still occur in patients with CD4+ T cell counts <50 cells/μL [46]. Therefore, it is likely that these patients with CMV retinitis will require long-term suppressive anti-CMV therapy and will benefit from monitoring for antiviral resistance.

In recent years, antiviral susceptibility monitors increasingly have used molecular detection of resistance mutations. However, interpretation of these assays relies on phenotypic characterization of mutations and clinical correlations. In this study, marker transfer experiments coupled with PRA were used to define the significance of E756Q and V787L mutations, which have not been characterized previously. Both mutations were shown to confer in vitro resistance to foscarnet, which is consistent with selection during foscarnet therapy.

We used genotypic analysis to validate the PRA and DHA criteria of CMV resistance to foscarnet. For PRA, the traditional 400 μM threshold for foscarnet resistance appears to be appropriate. However, for DHA, a 600 μM threshold agreed better with pol mutations than did the 400 μM one (κ = 0.68 vs. κ = 0.49). Nevertheless, 4 of 30 isolates tested by DHA and genotyping yielded discrepant results. In addition, 1 of 18 isolates tested by PRA and genotyping disagreed with respect to foscarnet resistance. These discrepancies might be caused by the presence of mixed viral populations in the clinical isolates [48–50]. In vitro reconstitution experiments showed that genotypic assays detect up to 10% mutant strains on a CMV AD169 background (A.W., unpublished observations). However, this population might not be sufficient to increase the IC_{50} of the viral mixture. Furthermore, the process of in vitro expansion of CMV clinical isolates required for DHA or PRA might select against those containing foscarnet resistance mutations, because it has been reported that foscarnet resistance may impair CMV growth in tissue culture [50]. Finally, phenotypic assays exhibit high interassay variability, which occasionally may generate inconsistent results. We observed an excellent correlation between DHA-established foscarnet resistance using the 600 μM threshold (κ = 0.68) and PRA results using the 400 μM threshold (κ = 0.72), superior to the correlation between DHA with a 400 μM threshold and PRA (κ = 0.56). These data collectively argue in favor of adopting the 600 μM threshold as a new standard for defining foscarnet resistance by DHA.

Because CMV continues to cause significant morbidity and mortality in immunocompromised patients, thereby requiring prolonged antiviral therapy, it is important to understand the kinetics of development of drug resistance. Using the new definition of foscarnet resistance by DHA, we reanalyzed the incidence of foscarnet resistance in the CRVR Study Group population. The proportion of patients harboring foscarnet resistance mutations after 6, 9, and 12 months of foscarnet therapy was 13%, 24%, and 37%, respectively. The estimated incidence of foscarnet resistance at 6 and 9 months was lower than the one previously reported by the CRVR Study Group for this same population (26% and 37%, respectively), owing to the use of an optimized phenotypic definition of resistance. However, the estimated incidence of foscarnet resistance was similar after 1 year of therapy. The newly estimated incidence of foscarnet resistance is similar to the incidence of ganciclovir resistance previously reported by the CRVR Study Group [15, 29].

Although foscarnet and ganciclovir have been successfully used in combination as therapy for patients with AIDS and relapse of retinitis, their combined use is limited by the fact that the patient is exposed to the potential toxicity from both agents [41]. A recent study of patients who had undergone transplantation attempted to address this issue by decreasing the doses of both antivirals to decrease the toxicity of each but failed to detect a beneficial effect with respect to toxicity or protection against CMV disease [51]. Other approaches need to be studied. Other areas that need further investigation include the ability of CMV to revert to a susceptible genotype and/or phenotype after discontinuation of the drug exercising the selective pressure, as well as the effect of reintroducing the same drug. Recent advances in molecular diagnostic techniques have made possible amplification and sequencing of CMV genes obtained from clinical samples. Comparison of genotypes obtained directly from clinical samples with those obtained from clinical isolates and with phenotypic results of culture susceptibility testing will be important to establish the accuracy and
utility of the direct approach, which can be performed more rapidly than conventional culture techniques. Caution must be taken in interpreting these data. The number of isolates available for sequencing was not large, particularly the number for comparison of genotype with phenotype by PRA. The small numbers precluded corrections for possibly linked data. Patients who had negative cultures were assumed to harbor a sensitive virus, which is a reasonable assumption, but one that gives minimum estimates for the incidence of resistance. Nevertheless, these data represent a major increase in information available on the phenotype-genotype correlation for foscanet resistance and on the clinical-virological correlation for foscanet resistance.

In conclusion, these data suggest that there is good phenotype-genotype correlation for the determination of foscanet resistance which a phenotypic definition of resistance as an $IC_{50} > 600 \mu M$ with DHA and as an $IC_{50} > 400 \mu M$ with PRA is used, that foscanet resistance is associated with poorer control of retinitis, and that the estimated incidence of foscanet resistance among patients being treated with foscanet for CMV retinitis is 13% by 6 months and 24% by 9 months of therapy.

**CYTOMEGALOVIRUS RETINITIS AND VIRAL RESISTANCE STUDY GROUP**

**Clinical centers.** Johns Hopkins University School of Medicine, Baltimore (D.A.J. [principal investigator]), John G. Bartlett, Stephen G. Bolton, Diane M. Brown, Lisa M. Brune, J. P. Dunn, John H. Kempen, Laura G. Neisser, George B. Peters, Richard D. Semba, and Jennifer E. Thorne; former members: Paul A. Latkany, Susan M. LaSalvia, Tracey Miller, Quon Dong Nguyen, and Eva M. Rorer; Northwestern University Medical School, Chicago (David V. Weinberg, Alice T. Lyon, and Annie Muñana); and University of Miami, Florida (Janet L. Davis and Patricia Vera; former members, Elizabeth Cruz and Tina A. Rhee).

**Data center.** Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Baltimore (B.K.M., Michelle O. Ricks, and Lynn M. Hutt; former members, Cheryl Enger, Shirley Quaskey, and Judy Southall).

**Flow cytometry laboratory.** Johns Hopkins University Bloomberg School of Public Health, Baltimore (Joseph B. Margolick and Fred Menendez).

**Fundus photograph reading center.** University of Wisconsin, Madison (Matthew D. Davis, Larry Hubbard, Jane Armstrong, Dolores Hurlburt, Sheri Glaeser, Jeff Joyce, Linda Kastorff, Nancy Robinson, and Marilyn Vanderhoof-Young; former member, Judy Brickbauer).

**Virology laboratory.** Johns Hopkins Medical Institutions, Baltimore (J. Brooks Jackson, Michael Forman, Linda Gluck, and Avareena Schools-Cropper; former members, Tamica Hamlin, Huiling Hu, and Alicja Rylka).

**ADULT AIDS CLINICAL TRIALS GROUP CYTOMEGALOVIRUS LABORATORIES**

University of Colorado Health Sciences Center, Denver (A.W., Julia Clark, and Shaoqin Li); Veterans Administration Medical Center, Oregon Health Sciences University, Portland (S.C., Rachel H. Wademer, and Anne E. Senters); Department of Immunology/Microbiology, Rush Medical College, Chicago (N.S.L.); and Beth Israel–Deaconess Medical Center, Boston (C.S.C., Eve Burns, and Jze Lin Zhang).

**References**


