Clinical Aspects of Cytomegalovirus Antiviral Resistance in Solid Organ Transplant Recipients

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Despite advances in the prophylaxis and acute treatment of cytomegalovirus (CMV), it remains an important pathogen affecting the short- and long-term clinical outcome of solid organ transplant. The emergence of CMV resistance in a patient reduces the clinical efficacy of antiviral therapy, complicates therapeutic and clinical management decisions, and in some cases results in loss of the allograft and/or death of the patient. There is increasing use of antiviral prophylaxis after transplant with little expansion in the range of antiviral agents effective in treatment of CMV. Further understanding is needed of the risk factors for development of CMV antiviral resistance and of therapeutic strategies for treating patients infected with resistant viruses. We review the current status of CMV resistance in solid organ transplant recipients, and provide diagnostic and therapeutic suggestions for the clinician in managing antiviral resistance.

Keywords. CMV; cytomegalovirus; resistance; transplant.

Human cytomegalovirus (CMV) is one of the most clinically significant viral pathogens causing infections after solid organ transplant (SOT), owing to the frequency of infection, the acute effects on patient and graft survival, and effects on long-term graft survival [1]. Developments in antiviral agents and preventive strategies over the past decade have significantly improved outcomes from CMV disease in SOT recipients. Kidney transplant recipients of high serologic risk, in which the donor is CMV seropositive (D+) and the recipient seronegative (R−), on prolonged prophylaxis have a cumulative disease incidence 2 years after transplant of approximately 22% [2]. More frequent and prolonged prophylaxis with ganciclovir and valganciclovir (valganciclovir [valGCV]) [2] has raised concern about antiviral resistance (AVR), with the potential for cross-resistance to currently available therapies [3, 4]. We here provide a brief update on CMV antiviral agents, with an in-depth focus on the clinical significance and risks of CMV AVR in SOT, and strategies to monitor, prevent, and treat AVR.

ANTIVIRAL AGENTS

The development of ganciclovir (GCV) in the mid-1980s was a significant breakthrough in CMV therapy. It is a nucleoside analogue requiring phosphorylation by the viral protein kinase (pUL97) prior to inhibition of the viral DNA polymerase (UL54) (reviewed in [5]). The poor oral bioavailability of GCV led to development of oral valGCV, a prodrug with enhanced bioavailability and comparable efficacy to intravenous GCV for treatment of CMV disease in SOT recipients [6]. Both agents have been used in CMV-preventive regimens in prophylactic, preemptive, or combination algorithms largely based on donor/recipient serologic
Table 1. Alternative Antivirals in Development or Under Consideration for Use in Cytomegalovirus Resistant to Ganciclovir, Foscarnet, or Cidofovir

<table>
<thead>
<tr>
<th>Drug Name (Class)</th>
<th>Dose Schedule (Phase of Development)</th>
<th>Target</th>
<th>Recent CMV Clinical Studies</th>
<th>Antiviral Resistance * Cross-Reactivity With GCV/FOS/CDV</th>
<th>Reference(s)</th>
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<tr>
<td>AIC246 – letermovir (3,4-dihydroquinazoline derivative)</td>
<td>80–240 mg/d dependent on clinical reason for use</td>
<td>UL56/UL89 terminase complex</td>
<td>Current (2012) phase II trial for prophylaxis in HSCT Recent single-case report of successful use in multidrug-resistant CMV in SOT</td>
<td>No with GCV, FOS, CDV Mutations in UL56 confer resistance</td>
<td>[59] [60]</td>
</tr>
<tr>
<td>Artesunate (antimalarial)</td>
<td>200 mg bd 1 d then 100 mg/d for 28 d</td>
<td>Acts on cellular pathways altering host cell kinase signaling and NF-kappa B and Sp1 activation paths with secondary inhibition of synthesis of viral immediate early proteins</td>
<td>Recent case series: used in preemptive CMV treatment in HSCT demonstrating reduced viral loads or plateau in viral load increase. Single case report of unsuccessful use in resistance</td>
<td>Unknown</td>
<td>[13] [14] [15]</td>
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<tr>
<td>CMX-001 (Cidofovir prodrug, orally bioavailable lipid conjugate. CDV is nucleotide analogue of cytidine)</td>
<td>Unknown</td>
<td>UL54</td>
<td>Current phase II–III in treatment of CMV disease</td>
<td>CDV – probably same UL54 exonuclease, A987G mutations as for CDV</td>
<td>[9]</td>
</tr>
<tr>
<td>Cyclopropavir – CPV (Methylenecyclopropane nucleoside analogue)</td>
<td>Unknown</td>
<td>Similar to GCV as phosphorylated by pUL97</td>
<td>Current phase I trial of oral use in healthy volunteers</td>
<td>Cross-resistance with GCV mutations UL97 M460I/T, H520Q,C603R with 8–20 increase in IC50 No cross-resistance with GCV UL97 mutation L595S Probable cross-resistance with FOS</td>
<td>[53] [61]</td>
</tr>
<tr>
<td>Fomivirsen (21-nucleotide [nt] antisense oligonucleotide with sequence complementary to CMV IE2 mRNA)</td>
<td>Current approval only for intraocular application for retinitis</td>
<td>IE2 mRNA</td>
<td>Nil known in nonretinitis disease</td>
<td>Unknown</td>
<td>[62]</td>
</tr>
<tr>
<td>Leflunomide (Inhibitor of pyrimidine synthesis and protein kinase activity)</td>
<td>Off-label use for CMV treatment in SOT using a loading dose of 100 mg/d orally for 5 d, then 40 mg/d orally, with dose adjustment according to levels of the teriflunomide metabolite</td>
<td>? Viral capsid assembly</td>
<td>Low-term suppressive response (~50%) in 2010 studies of use in CMV treatment in SOT</td>
<td>In vitro activity against GCV-resistant CMV</td>
<td>[16] [63]</td>
</tr>
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risk. Toxicity associated with prolonged use of GCV and valGCV, especially reversible bone marrow suppression, remains a limitation.

Foscarnet (FOS) and cidofovir (CDV) are intravenous CMV antivirals that also target the viral polymerase [7]. They are more commonly used as second-line treatment or for AVR, often limited by frequent nephrotoxicity. GCV and CDV, once phosphorylated, (CDV via cellular enzymes) compete with deoxyguanosine triphosphate binding of UL54 with consequent inhibition, with FOS blocking the UL54 pyrophosphate binding site (reviewed in [8]).

Alternative antivirals are few, although trials are being conducted for less toxic agents with different mechanisms of action (Table 1). They include an oral CDV prodrug (CMX-001) with lower nephrotoxicity [9], new antivirals with alternative targets (maribavir [MBV], letermovir) and drugs currently used in other settings (leflunomide, artesunate). MBV is a UL97 inhibitor with good oral bioavailability and low toxicity and has theoretical benefits for inhibiting cross-resistant viruses (reviewed in [7, 10]). However, in phase III clinical trials, as prophylaxis in hematopoietic stem cell transplant (HSCT) recipients, and more recently in D+/R− liver recipients, MBV prophylaxis has been demonstrated as inadequate [11, 12]. Orally administered artesunate has low toxicity and in vitro inhibits CMV, including GCV-resistant viruses [13], although clinical efficacy is currently unclear [14, 15]. Oral leflunomide has anti-CMV activity, although low (approximately 50%) response rates suggest it may best be used in combination [16].

CMV intravenous immunoglobulin (IVIG) may have some role in CMV therapy and prevention, especially where recipients have low gammaglobulin levels or in heart/lung transplant [17].

## CMV ANTIVIRAL RESISTANCE

### Definitions, Prevalence, and Outcome

A practical definition of CMV AVR is a clinical profile of increasing or high plateaued viral load in the presence of adequate antiviral therapy administered for >2 weeks, with confirmed resistance on genotypic and/or phenotypic testing. Most reports of AVR occur in patients with CMV disease on long-term (>6 weeks) GCV, although resistance may occur in asymptomatic viremia [18, 19]. Clinical resistance (clinical profile) and virologic resistance (laboratory evidence) are not always linked, with virologic resistance reported in up to 50% of cases of clinical resistance [3, 19].

Accurate estimates of SOT AVR risk and prevalence are confounded by differing AVR definitions, testing methods, the diversity of SOT subjects, and selection bias. Table 2 reviews resistance prevalence in publications reflecting the recent era.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Method of Assessment</th>
<th>Population Studied: SOT Type, (No. of Patients), Inclusion/Exclusion Criteria</th>
<th>Therapy Type, Duration, and Dose Where Stated</th>
<th>Resistance Definition/Testing Methods</th>
<th>Onset of Resistance (Posttherapy, Infection or Transplant as Indicated)</th>
<th>Resistance Risk Factors Where Reported</th>
<th>Resistance Rate, Overall and/or According to Specific Treatment Regimen or Organ Type</th>
<th>Outcomes for Resistant States</th>
<th>Mutations</th>
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<tbody>
<tr>
<td>Limaye 2002 [20]</td>
<td>Review including cohort and case studies of resistance, 1996–2000</td>
<td>Variable treatment in different centers, with IV or oral GCV</td>
<td>Genotypic and phenotypic methods for UL97 only. Antiviral load measurements not in common practice prior to 2002</td>
<td>51 to 510 d after therapy</td>
<td>SNR: D^R¬</td>
<td>Heart 0.28%, kidney 0.54%–1%, kidney-pancreas 13%, lung 3.3–9%, liver 0%</td>
<td>Symptomatic disease in 27/30 patients</td>
<td>Crude mortality rate 19%</td>
<td>UL97 only: predominantly L595S, A594V, M460V (12/23)</td>
</tr>
<tr>
<td>Boivin 2009 [21]</td>
<td>Prospective cohort study of resistance as part of RCT: the VICTOR study [6]</td>
<td>SOT: mixed. Numbers with CMV infection: heart (20), kidney (216), liver (23), lung (17) Adult recipients Life-threatening disease or GFR, 10 mL/min excluded</td>
<td>Rx for CMV disease randomized to valGCV (900 mg/bd) or IV GCV (5 mg/kg/bd), both GFR adjusted, for 21 d, followed by secondary prophylactic (maintenance) valGCV for 28 d Primary prophylaxis received by 44%–46% of patients</td>
<td>Genotypic testing of viremia of UL97* UL54 detected at days 0, 21, 49 of Rx and during CMV clinical recurrence Confirmed resistance defined as a previous report with marker transfer phenotypic resistance Probable genotypic resistance defined as new mutation at codon of previous identified resistance</td>
<td>Confirmed or probable: 5/13 demonstrated from day 0 of therapy Median time 21 d</td>
<td>Persistent VL day 21*: D^+R+ Lung recipients Baseline viral load at start of therapy* NS: antiviral (valGCV vs IV GCV), time from last transplant</td>
<td>2.3% (valGCV) of infected, 3.6% (GCV) of infected, 17.6% in lung cohort, 3.7% in kidney cohort</td>
<td>Failure to eradicate (VL &gt;600 copies/mL at days 21 and 49)* One-third recurrent CMV disease, 1 death attributed to recurrent CMV</td>
<td>10/13 UL97, 2/13 UL54, 1/13 UL97/UL54, MDR-nil</td>
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<td>Hantz 2010 [19]</td>
<td>Prospective cohort study Prospective monitoring after day 21 of therapy for infection or disease according to variable center practice</td>
<td>Rx according to local center practice: valGCV, IV GCV, or FOS, doses not defined Primary prophylaxis given at the discretion of the local center</td>
<td>Clinical (day 21 replication) with genotypic **/phenotypic resistance testing</td>
<td>Clinical resistance suspected (and subsequently confirmed with virologic testing) at median of 48 d after infection</td>
<td>D^+R¬ [virologic resistance] NS: induction antilymphocyte antibody therapy</td>
<td>Overall = 5.2% of those infected Lung 14.3%, kidney 6.2%</td>
<td>56.25%: persistent viremia (undefined) or severe disease</td>
<td>Of whole cohort including HSCT group: 17/18 patients UL97, 5/18 UL54, 3/18 UL97/UL54, 4 patients MDR</td>
<td>UL97 only; predominantly L595S, A594V, M460V (12/23)</td>
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<td>(Myhre 2011 [24]) Retrospective cohort study Retrospective review of cases already identified or subsequently identified as resistant</td>
<td>SOT: kidney (1244) Rx for disease: valGCV 900 mg/bd or IV GCV 6 mg/kg/bd (GFR adj) until CMV DNAemia below detection limit for at least 2 wk Generally 4/52 secondary prophylactic valGCV thereafter All patients had prevention: either preemptive: weekly screening and Rx with valGCV 900 mg daily (GFR adj) if viremia identified or prophylaxis (valGCV dose/duration not defined)</td>
<td>Genotypic testing of clinically suspected cases. Confirmed and probable resistance definitions as per Boivin 2009 used as reporting of resistance</td>
<td>Range of 59–219 d after infection (av 115 d) Range of 41–205 d after treatment (av 108 d)</td>
<td>D+/R− Preemptive therapy*</td>
<td>14.6% of infected patients with previous preemptive Rx, 6.3% of infected patients previously on prophylaxis</td>
<td>No resistance attributed deaths, no persistent viremia following diagnosis of resistance and subsequent Rx</td>
<td>All (27) UL97 mutations</td>
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<td>(Martin 2010 [18]) Prospective cohort study Prospective monitoring at day 100 of prophylaxis (end of prophylaxis), during suspicion of CMV disease, or at time of organ biopsy up to 6 mo after transplant</td>
<td>SOT: mixed Total patients studied: heart (12), kidney (33), liver (17), multiorgan (1) Pediatric recipients only 38% D+/R− ValGCV prophylaxis daily for 100 d according to BSA equation [57] Genotypic testing of UL97 and UL54, and recombinant phenotyping for new mutations</td>
<td>Day 100 of prophylaxis in 1/1 infected patients During viremia after 100 d of prophylaxis = 140–182 d</td>
<td>2.2% of all patients at 100 d of prophylaxis (1/1 of viremic patients) 3/3 patients with viremia after prophylaxis, during first 6 mo after transplant</td>
<td>All asymptomatic at detection of resistance and remained asymptomatic thereafter</td>
<td>All UL97 mutations</td>
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<tr>
<td>(Van der Beek 2010 [58]) Retrospective cohort study Retrospective review with resistance tested during occurrence of any viremia up to 1 y after transplant</td>
<td>Total patients studied: kidney-pancreas (11) Only D+/R− Rx: valGCV 900 mg bd for 14 d or IV GCV 5 mg/kg/d for 14 d (if symptomatic) All patients had a prevention strategy: either preemptive: valGCV 900 mg/bd for 14 d or prophylaxis 900 mg/d for 90 d followed by preemptive regimen All adj for GFR</td>
<td>Genotypic testing (UL97 **/ UL54) according to antivirals used Not able to be accurately inferred</td>
<td>19% (4/21) of patients with treatment failure (defined as CMV load of ≥ 1000 copies/mL after 2 wk of Rx) 0% of patients at initial detection of viremia</td>
<td>Viremia and symptoms resolved on valGCV or GCV Rx</td>
<td>All UL97 mutations</td>
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Table 2 continued.

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</tr>
</thead>
<tbody>
<tr>
<td>(Couzi 2012 [30]) Retrospective cohort study Retrospective review with resistance tested if increasing viremia during therapy up to 1 y after transplant</td>
<td>Kidney (112) Only D^+/R^−</td>
<td>ValGCV prophylaxis: 900 mg/d for 3 mo ValGCV preemptive (initiated at 2000 copies/mL or therapeutic; dose 900 mg bd) GCV preemptive or therapeutic: 5 mg/kg bd, duration determined by viremia. GFR adj</td>
<td>Genotypic testing of both UL97 and UL54, requested with increasing viremia during therapy</td>
<td>Mean of 134 ± 61 d</td>
<td>Peak viral load^a (multiv) (5.25 log_{10} copies/mL) Preemptive therapy^b (univ) Viral eradication &gt;8 wk^c (univ) NS: induction with ATG</td>
<td>13% of infected patients, 3% for those with prophylaxis, 16% for PET (but where considered as % of only those infected and Rx, prophylaxis = 13% and PET = 27% NS)</td>
<td>Lower eGFR at 1 y NR</td>
</tr>
<tr>
<td>(Eid 2008 [33]) Retrospective cohort study Retrospective review of identified cases of CMV resistance-associated disease</td>
<td>SOT: mixed Total patients studied: kidney (138), kidney-pancreas (4), liver (58), and heart (25) Only D^+/R^− Variable/Individualized Rx of disease. All received valGCV prophylaxis (900 mg/d GFR adj. 90–100 d)</td>
<td>Genotypic testing of UL97 and UL54</td>
<td>Range of 132–300 d duration of (val)GCV exposure at suspicion of resistance</td>
<td>N/A</td>
<td>6.2% (4) of patients with CMV disease Drug-associated nephrotoxicity with allograft loss in 2/4 patients with resistance</td>
<td>3/4 UL97, 3/4 UL54</td>
<td>3/4 UL97, 3/4 UL54</td>
</tr>
<tr>
<td>(Boivin 2012 [27]) Prospective cohort study of resistance as part of RCT, the IMPACT study [2] Monitoring from the IMPACT study during and postprophylaxis</td>
<td>Kidney (318) Only D^+/R^−</td>
<td>ValGCV prophylaxis (randomized in IMPACT to 100–200 d: 900 mg/d GFR adj. Therapy for disease not defined by study)</td>
<td>Genotypic testing of both UL97 and UL54 (** recombiant phenotyping if (1) identified viremia (&gt;600 copies/mL) at 100 d or end of prophylaxis (+ post hoc including any viremia during prophylaxis) or 2) with CMV disease up to month 12 after transplant</td>
<td>5/6 occurred during 100 or 200 d of prophylaxis, 1/6 after prophylaxis (within 12 mo after transplant)</td>
<td>5/6 with resistance had persistent viral replication prior to detection of resistance Overall: 2.2% (of all patients): 1.8% (3) with 100 d of prophylaxis, 2.6% (4) with 200 d prophylaxis</td>
<td>3/6 were asymptomatic and cleared virus without Rx, 3/6 CMVS with resolution (including viremia) with Rx</td>
<td>5/6 UL97, 1/6 UL54</td>
</tr>
<tr>
<td>(Iwasenko 2011 [3]) Case series Review of AVR in specimens sent to an Australian testing facility (included HSCT recipients)</td>
<td>SOT: mixed Total patients studied: kidney (110), heart (8), lung (18), liver (2) Variable: GCV, valGCV ** FOS, CDV</td>
<td>Genotypic testing of both UL97 and UL54</td>
<td>Not able to be accurately inferred</td>
<td>N/A</td>
<td>50% of tested patients Not able to be accurately inferred</td>
<td>9/32 UL97, 7/32 L97 + UL54</td>
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</table>

Studies include valganciclovir therapy and/or prevention and examine antiviral resistance incidence, risk factors, and outcomes.

Abbreviations: adj, adjusted; ATG, antithymocyte globulin; av, average; AVR, antiviral resistance; bd, twice daily; BSA, body surface area; CMV, cytomegalovirus; CMVS, CMV syndrome; eGFR, estimated glomerular filtration rate; FOS, foscarnet; GCV, ganciclovir; GFR, glomerular filtration rate; HSCT, hematopoietic stem cell transplant; IV, intravenous; MDR, multidrug resistance; multiv, multivariate; N/A, not applicable; NR, not reported; NS, not significant; PET, preemptive therapy; RCT, randomized controlled trial; Rx, therapy; SNR, statistical significance not reported; SOT, solid organ transplant; TID, tissue invasive disease; univ, univariate; VL, viral load; valGCV, valganciclovir.

^a Statistical significance at $P \leq .05$.

^b Approaching significance $P = .05–.07$. 

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of valGCV prophylaxis. A pre-valGCV review summarizes retrospective highly selected populations [20]. Of prospective SOT studies examining resistance across organ types using both clinical and virologic definitions, resistance is noted in up to 17.6% of viremic lung transplant recipients [21] and up to 6.2% of viremic kidney recipients [19]. Clinical outcome in resistance-associated disease is generally poor, although confounded by selection bias. Higher rates of progressive viremia, CMV-associated mortality, and recurrent CMV are reported. This is most marked for lung recipients [21]. Crude mortality rates of up to 19%–22% have been reported in SOT recipients with CMV AVR [20].

Risk Factors
A cohort study of oral GCV prophylaxis demonstrated the interdependence of host-, virus- and antiviral therapy–associated risk factors for CMV disease, therapeutic failure, and AVR [22]. Among the SOT recipients, resistance was more common in D+R− kidney-pancreas recipients who had received potent immunosuppression (OKT3). The pathway to resistance commenced with D+R− transplant, leading to higher viral loads, with the additional factors of relative high immunosuppression, low drug concentration, and prolonged subclinical viremia, proposed to have led to selective pressure growth advantage for resistant strains [23].

Host-associated risk factors for AVR include type of transplant, with highest risk consistently demonstrated for lung and kidney-pancreas recipients [21, 22]. This may be associated with specific immunosuppression protocol risk, local organ CMV load, or anatomical factors. Degree of immunosuppression has been shown to influence AVR [22], although in studies over the past 2 decades (Table 2), use of specific antilymphocyte agent induction has not been demonstrated as a significant risk. Absence of preexisting CMV specific immunity (D+/R−) is the most consistently identified AVR risk factor [19, 21, 22, 24].

Antiviral therapy factors associated with AVR risk include delay in commencement of prophylaxis [25] and increased duration of antiviral exposure, which is shown using sequential GCV susceptibility assays [26]. Although resistance has been described both during prophylaxis and at commencement of therapy [18, 21], prolonged duration of valGCV prophylaxis has not been shown to increase AVR [27]. Recent studies have demonstrated benefits of such prolonged prophylaxis with reduced CMV disease [2] and infection [28], especially for high-risk recipients, and this has been incorporated into guidelines [29]. Preemptive antiviral prevention has been advocated to reduce AVR, although surprisingly, AVR rates have been observed to be higher in preemptive therapy [30].

Most AVR has been reported in association with GCV or valGCV long-term use (usually >6 weeks), although exposure to other antivirals may contribute to resistance. Prior use of UL97-activated antivirals (aciclovir, valaciclovir) with higher IC50 (see “Laboratory Monitoring” below) for CMV and hence lower CMV inhibition may be a risk for AVR, although this has been uncommonly reported [31, 32]. Suboptimal drug concentration, especially with use of oral GCV, has been associated with AVR through in vitro modeling studies, with these results also reflected in case series [22, 23]. With use of intravenous GCV and valGCV, adequate GCV levels have been demonstrated in most patients with AVR [19]. There are, however, circumstances in which suboptimal exposure is more common with consequent greater potential AVR risk, such as in scenarios of malabsorption, poor compliance, intolerance, or in recovery of glomerular filtration rate (GFR) after transplant [33]. ValGCV dose alteration with GFR change or the occurrence of neutropenia has been identified more frequently in AVR [19].

Significant post-SOT AVR risk factors in the era of valGCV and preventive therapy are primary infection with D+/R− serostatus, day 21 persistent viremia, high peak viral load (>10⁵ copies/mL in peripheral blood), and lung transplant (Table 2).

Laboratory Monitoring
The phenotypic assay is the reference standard for assessing antiviral susceptibility, although it is rarely used in clinical practice [34]. This method defines IC₅₀ (the drug concentration that inhibits 50% of viral plaque number). The proposed cutoffs for antiviral susceptibilities are 6 μM for GCV, 2 μM for CDV, and 400 μM for FOS, although standardization of assays is difficult. Interlaboratory correlation may be improved by using a sensitivity index (SI₅₀), which is the IC₅₀ of the isolate divided by the IC₅₀ of the reference strain (usually strain Ad169). A >3-fold increase in SI₅₀ is defined as resistance [19]. The process of phenotypic testing, however, is lengthy (approximately 4 weeks), labor intensive, and subjective. Currently it is mostly performed to assess newly described genotypes, with recombinant virus phenotyping (see below) increasingly used owing to the difficulties of culturing viral isolates.

In the era of molecular diagnostics, CMV AVR testing is usually performed clinically using polymerase chain reaction amplification and sequencing of resistance loci direct from clinical specimens (genotypic testing), with a potential turn-around time of 2–3 days. Loci are compared to known resistant or wild-type strains. The recent development of Web-based mutation databases has facilitated this, by enabling laboratories to easily access and contribute to reports of polymorphisms or mutations and their significance [35]. Where an unknown mutation is found at a resistance codon “hot spot” in the setting of clinical resistance, the virus is considered as a probable resistant strain. Validation of the clinical effect of a given mutation or deletion is now usually performed through marker transfer.
to a wild-type strain [36]. This involves transfer of the mutation sequence to a reference CMV strain or a bacterial artificial chromosome virus clone (methods reviewed in [7]), and subsequent (recombinant) phenotyping. Using this technique, new resistance mutations and natural polymorphisms may be identified. It also allows categorization of mutation sensitivity to antivirals. Where the IC₅₀ ratio is >5, resistance (IC₅₀) is classed as major, with a change in antiviral therapy necessary [7].

Genotypic testing allows detection of mixed wild-type and mutant populations, although minorities of <10%–20% may not be identified using conventional DNA sequencing or restriction-length fragment polymorphism analyses [37]. These minority populations may be clinically relevant, with ultra-deep pyrosequencing able to detect resistant minorities in clinical resistance [37].

Because risk of resistance may be influenced by suboptimal antiviral exposure, therapeutic drug monitoring (TDM) of plasma GCV may be a useful tool to monitor such risk. There is also potential to examine toxicity risk, with implications for reducing unnecessary antiviral withdrawal or dose reductions that may contribute to AVR. GCV TDM it is not currently in common use, although specific populations may benefit from its application [38]. This includes pediatric kidney transplant populations where low plasma drug levels can be associated with the relatively high creatinine clearance of the transplant-ed adult kidney [38, 39]. Trough level parameters associated with clinical efficacy, however, have not been clearly defined. Original efficacy data derived from adult HIV-infected populations refer to optimal trough levels of >0.6 mg/L, although outcome correlation is not consistent at this level [40, 41]. Of the few studies that examined GCV levels and resistance, sub-optimal levels were infrequently found [19, 39, 40].

**Mutations, Mechanisms, and Multiple Strains**

The genetic changes associated with resistance in UL97 and UL54 genes of CMV have been reviewed in detail [42]. The most common (>95%) AVR strains contain mutations in the UL97 gene, conferring resistance to GCV and valGCV. This gene encodes the UL97 protein kinase responsible for GCV phosphorylation and activation. In the virus it may be involved with encapsidation, nuclear egress, and replication [7]. UL97 mutations involved in resistance, however, have not clearly been shown to have significant negative effects on replication, and clinical isolates may demonstrate full pathogenicity [7, 23, 43], despite amino acid substitutions or short deletions [7, 24]. The most frequently identified UL97 mutations occur at codons 460, 520, and 590–607 [43]. It is proposed that segments 591–607 confer resistance by modifying substrate recognition, and 460 and 520 by modifying ATP binding [43]. Multiple codon deletions are generally associated with higher levels of resistance [7]. Identified point mutations such as N597D and C592G demonstrate lower levels of resistance that can be overcome by optimized GCV dosing [23, 44].

The CMV UL54 gene encodes the DNA polymerase (pol) responsible for viral replication. UL54 mutations are proposed to cause resistance by 3 mechanisms—reduced antiviral affinity (the only proposed mechanism of FOS resistance), reduced DNA chain incorporation, or increased antiviral excision out of the DNA chain—and this may be predicted by UL54 mutation location [45]. Typically UL54 mutations evolve following UL97 mutations, in the presence of continued GCV exposure, with isolated UL54 gene mutations uncommon [19, 46]. This may reflect reduced fitness of pol mutants, possibly linked to the key replicative function of UL54. Mutations in UL54 can demonstrate cross-resistance between GCV and CDV (multi-drug resistance [MDR]), and less commonly FOS. CDV resistance thus may occur even in the absence of specific exposure, although FOS resistance will usually only be seen with exposure [47]. High degrees of GCV resistance (>30-fold) are common with dual UL97/UL54 mutations [36]. Overall, UL54 mutations may not be infrequent, as demonstrated by recent cohort studies where up to 28% of resistance cases had UL54 mutations, mostly in combination with UL97 (Table 2) [19, 21, 27].

Sequencing of clinical isolates has demonstrated the mutated codons that may be used to predict specific resistance patterns [42]. Mutations at codons in genes more critical to replication, such as UL54, tend to be less variable. For the UL97 gene, codons 591–607 are most commonly found mutated in SOT recipients, and are associated with a large number of heterogeneous mutations [43]. UL54 mutations resulting in resistance are more widespread in the gene with more extensive sequencing required [42].

The evolution of MDR may also occur as a result of the accumulation of different mutated strains under antiviral selective pressure. Multiple strains with different AVR profiles may be present, complicating diagnosis and prediction of therapeutic response [44]. Multiple strains have also been demonstrated to compartmentalize, with AVR variability across organs [48].

**CLINICAL STRATEGIES FOR AVR**

**Testing**

CMV AVR testing should be considered based on both clinical scenario and viral load monitoring during antiviral therapy. At present there is no clear benefit in testing for AVR during prophylaxis or early therapy [21]. During therapy, most guidelines recommend weekly quantitative viral load monitoring [29]. Because up to two-thirds of patients may exhibit increased quantitative CMV DNA within the first 3 weeks of
antiviral therapy, it is after this point, with persistent or increasing viral loads, that resistance must be considered [19]. Where there has been adequate compliance and antiviral exposure, potentially assessed through GCV TDM, genotypic resistance testing should be undertaken (Figure 1). A rapid result and appropriate change in antiviral therapy may improve outcome and reduce the chance of MDR. Continued monitoring of quantitative viral loads weekly during management of resistant infections is necessary.

Information on antiviral use at each resistance test helps laboratories target and expedite genotype testing. Where GCV has been used, the UL97 gene should first be analyzed, with codons 400–670 initially sequenced, as these are the sites of canonical UL97 mutations. The finding of UL97 mutations should always direct sequencing of UL54 to assess for additional GCV resistance and cross-resistance. In the absence of UL97 mutations with a background of sole GCV use, GCV therapy should be optimized. If clinical suspicion of resistance is high, UL54 testing should be performed, as lone UL54 mutations have been reported in this circumstance [46]. In the setting of FOS or CDV use, the UL54 gene should always be sequenced, with codons 300–1000 targeted. Even where GCV has not been used, UL97 should be sequenced, as this will provide most information in guiding alternative antivirals.

Sequencing results can be compared with Web-based databases that report SI50 AVR levels for mutations vs the wild-type strain. While CMV AVR definitions vary, most authors use a SI50 (or IC50 ratio) of >3 as indicating resistance, and an SI50 of >5 as indicating high-level resistance [7, 42]. The finding of unreported sequences in clinical resistance should be considered to indicate resistance-associated mutations and direct further assessment through phenotypic testing.

Figure 1. Therapeutic strategies for treatment of the immunosuppressed solid organ transplant recipient with suspected antiviral resistance. Abbreviations: ART, artesunate; AVR, antiviral resistance; CDV, cidofovir; FOS, foscarnet; GCV, ganciclovir; IC, inhibitory concentration; IVIG, intravenous immunoglobulin; SI50, IC50 of the isolate divided by the IC50 of the reference strain; TID, tissue invasive disease; VL, viral load.
Clinical specimens used for testing depend on the presence of viremia or tissue invasive disease. In lung transplant patients, CMV viral load is often more predictive when determined in lung specimens [3]. In gastrointestinal disease, blood results may be negative and colonoscopy specimens more accurate [3]. Persistent tissue invasive disease, despite lack of resistance in blood isolates, may necessitate local sampling due to strain compartmentalization, although the risk-benefit ratio of invasive sampling needs consideration.

Therapy
Several strategies have been proposed for the treatment of CMV AVR in the SOT recipient, although evidence is limited to case reports and case series. In Figure 1 we present a management algorithm that extends that of current consensus guidelines [29]. All strategies must include careful consideration of immunosuppressant reduction where possible. This may include considering immunosuppressive conversion to sirolimus (mTOR inhibitor), which has some limited clinical evidence of efficacy [49]. In the presence of significant, life-or sight-threatening CMV disease, an empiric change or addition to therapy should be made while awaiting testing. Where high-dose GCV has been already used [50], the addition or replacement with FOS is recommended. Multidrug therapies may be successful, with early studies of combination GCV and FOS showing promise [51].

Where UL97 mutations are found and can be categorized phenotypically at an SI50 or IC50 ratio of <5, GCV dose optimization is recommended. High-dose intravenous GCV (up to 10 mg/kg twice daily for normal renal function) may be used, although toxicity may be limiting or necessitate addition of granulocyte colony-stimulating factor [29, 50]. The presence of additional UL54 mutations conferring increased GCV resistance would direct a change in therapy to FOS. In cases of lone high-level UL97 mutations, therapy should also be changed to FOS. GCV-CDV cross-resistance from UL54 mutations is common and thus this agent would not be considered if UL54 mutations are present. If FOS resistance occurs during therapy, cross-resistance to GCV is uncommon and this agent could be used, provided there are no additional UL97 mutations.

Where multiresistance is confirmed and remaining options are limited, novel agents (Table 1) such as leflunomide or artemunate could be considered, although current evidence is case based. There is concern that leflunomide may not be adequate as a single agent and response may be slow; thus, early initiation is recommended [16]. The addition of CMV immunoglobulin is another measure that may be considered [52].

Future Strategies
Newer antivirals for use in resistance include a range of drugs with differing or undefined activity against CMV (Table 1). Many of these (such as cyclopropavir) are phosphorylated (as is GCV) by the UL97 kinase and act on the DNA pol [53], with cross-resistance thus possible.

Therapeutic immune manipulation through CMV specific adoptive T-cell therapy has reached phase II clinical trials in HSCT recipients [54], although the role is less defined in SOT [55]. Although currently experimental, these therapies may have future application in preventing and treating resistance, providing long-term immunity, and reducing drug toxicity.

CMV disease and resistance risk of may also be prevented by active immunization. Of several vaccines, the glycoprotein subunit vaccines have progressed farthest through clinical trials, with demonstrated reduction in viremia duration in D+/R- transplant recipients [56].

In conclusion, CMV AVR can be a challenging clinical problem in SOT. At present, there is little clinical evidence to guide decision making in managing these patients. We present an easy-to-follow algorithm for testing and managing resistance (Figure 1). Publication of cohort studies where such strategies are used is a necessary validation step.

Notes
Acknowledgment. We thank Dr Sunwen Chou for his review of this article.
Potential conflicts of interest. All authors: No reported conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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