HHS Public Access

Author manuscript

Curr Opin Infect Dis. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Curr Opin Infect Dis. 2015 August ; 28(4): 293–299. doi:10.1097/QCO.0000000000000170.

Approach to Drug-Resistant Cytomegalovirus in Transplant Recipients

Sunwen Chou

Division of Infectious Diseases, Oregon Health and Science University and, Department of Veterans Affairs Medical Center, Portland, Oregon, USA

Abstract

Purpose of review—updated information on diagnosis of CMV drug resistance, treatments for drug-resistant infection and potential uses of experimental antiviral compounds.

Recent findings—For established CMV antivirals, uncommon viral UL97 kinase and UL54 DNA polymerase drug resistance mutations are sporadically described that expand an extensive existing database. Some novel mutations reported from treated patients have no drug resistant phenotype and may be genotyping artifacts. Next generation sequencing technology may enable earlier detection of emerging resistance mutations in treated patients. Management options for drug-resistant infection include optimization of host defenses, antiviral dose escalation, substitutions or combinations of standard or experimental antivirals. Maribavir and letermovir have antiviral targets distinct from the classic DNA polymerase. UL97 mutations elicited by ganciclovir and maribavir are different, although a single p-loop mutation can confer significant cross-resistance. High-grade resistance mutations in the UL56 terminase gene are readily selected in vitro under letermovir and await clinical correlation.

Summary—Technical advancements can enhance the accurate and timely genotypic detection of drug resistance. Antivirals undergoing clinical trial offer the prospect of new viral targets and drug combinations, but unresolved issues exist with regard to their therapeutic potential for drug-resistant CMV and their genetic barriers to resistance.

Keywords

Cytomegalovirus; antiviral drug resistance; maribavir; letermovir; brincidofovir

Introduction

CMV antiviral drug resistance may be suspected in transplant recipients who have received several weeks of full dose standard antiviral treatment yet show a persistent or increasing CMV load or disease progression. Because this scenario can also result from fluctuation in host conditions and antiviral drug delivery, clinical management involves assessment of patient risk factors, antiviral treatment history and sequential viral loads, followed as

Corresponding Author: Sunwen Chou, Division of Infectious Diseases L457, 3181 SW Sam Jackson Park Road, Portland, Oregon, USA, 97239, Tel +1 503 273 5115, chous@ohsu.edu.

Conflicts of Interest: None

appropriate by laboratory confirmation and empiric or genotype-guided changes in antiviral regimen. This update is intended to integrate original data published in years 2013 and 2014 into the framework of existing reviews and guidelines [1–5].

Risk factors for CMV drug resistance

Continued CMV replication in the presence of antiviral therapy poses a risk for development of drug resistance, rising in proportion to the level of viral replication and duration of drug exposure. As new drugs with different antiviral targets are being introduced, one should also consider their relative barriers to the development of resistance, reflecting the extent to which viral mutations that confer drug resistance also impair viral growth fitness. While extensive experience with existing CMV DNA polymerase inhibitors indicates that drug resistance typically occurs after months of antiviral drug exposure [1,5], the timing and incidence of resistance may vary with newly introduced treatments.

The most prominent risk group for CMV drug resistance consists of CMV-seronegative recipients of seropositive donor organs (D+R− subset), where the absence of pre-existing immunity and post-transplant immunosuppression combine to increase the intensity and duration of viral replication [3]. Currently available antiviral therapy is often incompletely effective in these circumstances and resistance may develop in 5–12% [2]. Recent literature has drawn attention to other groups at risk of drug resistance, including haploidentical or allogeneic stem cell transplant recipients [6,7] and congenital CMV infection [8].

Because of the difficulty in treating established end-organ CMV disease (especially pneumonia), longstanding emphasis has been given to suppressing post-transplant CMV activity at or before the anticipated time of onset [5]. Antiviral prophylaxis can be given for months to all patients within defined risk groups, or serial viral load monitoring can be used to initiate treatment at defined viral activity thresholds, hopefully before tissue invasive disease occurs. A prophylaxis approach exposes a larger number of patients to longer total durations of treatment, but because most patients will have no active viral loads, the incidence of resistance remains low (up to 3%) [3]. The higher frequency of drug resistance after pre-emptive treatment offsets the benefit of more selective antiviral usage [2,5,6].

Adequate delivery of active drug to intracellular sites of viral replication is important. Selection of resistant mutants may follow subtherapeutic drug levels [9] resulting from insufficient dosing, inadequate absorption or conversion of oral prodrugs, and variation in drug clearance. Greater use of therapeutic drug level monitoring has been suggested [10] but is not routine and has not been validated by prospective studies [3]. Intracellular levels and half lives of active drug, e.g. ganciclovir triphosphate or cidofovir diphosphate may vary significantly from plasma levels of precursor molecules.

Genetic mechanisms of CMV drug resistance

Seven viral UL97 kinase mutations (amino acid substitutions M460V/I, H520Q, C592G, A594V, L595S and C603W) are established as the genetic basis of ganciclovir resistance in most cases [1]. A recent tally from a large diagnostic laboratory also listed other mutations at codon 595 (L595F/W) as relatively common [11]. These mutations decrease the

phosphorylation of ganciclovir required for antiviral activity, while preserving biologically important UL97 kinase function [1]. They confer a 5- to 15-fold increase in the ganciclovir concentration required to reduce viral growth by 50% (EC50), except 3-fold for C592G [1]. Other UL97 mutations in the codon range 590–607, including in-frame deletions of one or more codons, occasionally emerge to confer varying levels of ganciclovir resistance. Rare mutations are described elsewhere in UL97, as in recent reports listed in Table 1 [12–16]. These are notable for unusual features such as being detected only after in vitro selection experiments, as minor sequence subpopulations, association with significant growth impairment or borderline resistance phenotypes.

As an ATP-competitive UL97 kinase inhibitor, the experimental drug maribavir elicits a different set of UL97 mutations clustered around the ATP binding site, usually amino acid substitutions V353A, T409M and H411Y/N/L which were initially observed in vitro and confer 9- to 80-fold increased maribavir EC50 without ganciclovir cross-resistance [1,2]. The second reported clinical case of maribavir resistance evolved the same associated UL97 mutations as the first, T409M and H411Y/N [17]. Mutations that knock out UL97 kinase activity, e.g. by altering a critical residue such as K355, are inherently highly resistant to maribavir and cross-resistant to ganciclovir, but have no demonstrated clinical relevance because of the resulting severe viral growth impairment [12,15]. This includes some of the mutants in Table 1 that show high-level maribavir resistance (>200-fold increased EC50). However, the p-loop mutation F342S confers moderate dual ganciclovir and maribavir resistance while preserving biochemical kinase activity and relatively normal growth in cell culture [12]. It has not so far been observed in vivo, although the usual clinical diagnostic assays [11] do not detect it.

Viral UL54 DNA polymerase gene mutations can confer resistance to any or all of the traditional CMV polymerase inhibitors ganciclovir, foscarnet and cidofovir. Unlike UL97, there is no compact list of canonical resistance mutations; instead diverse mutations cluster in certain functional domains with characteristic associated resistance phenotypes [1,2,4,5]. New UL54 mutations affecting drug susceptibility continue to be reported regularly (Table 2) [13,18–21]. In most cases they are close to known mutations of similar phenotype but are sometimes in unexpected locations, e.g. at codon 726 in Table 2. In general, mutations conferring ganciclovir and cidofovir cross-resistance map to the exonuclease and thumb domains. Although such mutations were speculated to favor the excision of misincorporated nucleotide analogs from replicating DNA, a recent biochemical study [22] suggests that these mutations actually overcome the stalling of the polymerase complex to enable continued chain extension beyond the misincorporated base. Since the experimental drug brincidofovir (formerly CMX001) is a lipid conjugate of cidofovir and intracellularly converted to the active form of the parent drug, resistance is expected to involve the same UL54 mutations, although in vitro propagation under brincidofovir may select for novel cidofovir resistance mutations such as D542E [21]. Foscarnet resistance mutations encountered in clinical practice tend to cluster in the palm and finger polymerase structure domains and may confer a low-grade ganciclovir ± cidofovir cross-resistance [5]. These mutations differ from those preferentially selected under ganciclovir, are often growth-attenuated, and high-grade foscarnet resistance is rare, with the typical mutations conferring only 3- to 5-fold increased EC50s.

The experimental drug letermovir (formerly AIC246) is a CMV terminase complex inhibitor [23]. This complex includes the products of viral genes UL56, UL89 and UL51, acting in concert with others involved in viral DNA packaging. Against wild type CMV, letermovir is potently inhibitory, with nanomolar EC50 values. However, in vitro propagation under the drug readily selects for UL56 mutations in the codon range 231–369 that can confer impressive levels of resistance [24]. For example, mutation C325Y effectively confers absolute resistance to non-cytotoxic letermovir concentrations (EC50 >5000 fold over baseline), with no perceptible in vitro growth impairment [24]. This suggests that critical letermovir binding residues are not critical to the biological function of the terminase complex, implying a low genetic barrier to resistance. Our understanding remains quite incomplete of the diversity, relative frequency and therapeutic implications of letermovir resistance mutations.

Laboratory diagnosis of CMV drug resistance

Confirmation of drug resistance is desirable because suboptimal responses to treatment may have other causes, and empiric changes in therapy may have adverse effects. Laboratory testing is based on genotypic analysis of clinical specimens such as blood or plasma for the presence of viral mutations known to confer drug resistant phenotypes. A recent study of serial specimens received for genotypic testing indicated that the first submitted specimen tested negative for resistance mutations in more than half of the patients, although about two-thirds eventually turned positive with repeated testing [11]. A consensus guideline [5] suggests a criterion of at least 6 weeks of antiviral drug exposure including 2 or more weeks of full dose therapy before testing for suspected drug resistance, although the schedule may be accelerated by poor host factors and extreme viral loads (e.g. $>10^6$ IU/mL), and may vary with newer drugs of different antiviral mechanism.

For ganciclovir, genotypic testing is usually straightforward in that a canonical UL97 kinase mutation is expected, corresponding to a known level of drug resistance. UL54 DNA polymerase mutations are typically selected after prolonged ganciclovir exposure to increase the level of drug resistance and add cross-resistance to cidofovir or sometimes to foscarnet [1]. Interpretation becomes more complicated when unrecognized mutations are detected in UL97 and especially in UL54. New genotype-phenotype correlations are still being reported after more than 20 years of experience with existing antivirals (Tables 1 and 2). A recent analysis of atypical mutations encountered (especially as partial sequence populations) in a large valganciclovir trial concluded that many of them were not reproducible on repeated testing of the same sample and were rarely authenticated as new resistance mutations [18]. Many were probably artifacts of PCR amplification. A report of an unrecognized mutation or one known to confer a severe growth defect should be followed by independent confirmatory testing, particularly if mixed with wild type sequence and the tested specimen had a low viral load.

Conversely, the current diagnostic Sanger sequencing technology lacks sensitivity to detect mutant sequence subpopulations of less than 20–30%. Evolving next-generation deep sequencing technologies [25] can detect much smaller subpopulations of 1%–3%, with suitable precautions for errors introduced during amplification and sequencing. Recent pilot

studies demonstrate increased sensitivity of detection of resistance mutations in samples tested in parallel using old and new technologies [26], including detection of emerging resistant mutant subpopulations weeks earlier in serial samples, along with a greater variety of mutations than traditional testing [19]. However, the new technologies are in flux and have not yet been adequately standardized or quality controlled to enter routine clinical diagnostic practice.

Phenotypic assays of clinical CMV culture isolates directly against antiviral compounds are impractical [1]. Instead, the drug susceptibility phenotypes of newly encountered mutations are determined by mutagenesis of cloned baseline CMV laboratory strains followed by quantitative growth assays, often using reporter genes under increasing drug concentrations, a process known as recombinant phenotyping [1,13,27]. Newly introduced phenotyping assays need to be calibrated using mutations conferring known levels of drug susceptibility.

Treatment strategies for drug-resistant CMV

Consensus management guidelines were recently updated [5] but remain undocumented by controlled studies. Variation in host factors greatly influences the outcome of CMV infection, drug-resistant or not, and along with individual differences in antiviral drug exposure and viral loads will make it very difficult to perform and interpret any controlled comparisons of treatment strategies. Proposed treatment algorithms [3,5] must also take these factors into account.

The first consideration is to optimize host defenses by reducing immunosuppression as feasible. Adoptive T-cell transfers have been described as beneficial [28] but may be logistically complicated. For UL97 mutations that confer lower levels of ganciclovir resistance, dose escalation may be an attractive interim option as used in some recently published cases [29]. The idea is that a limited but beneficial amount of ganciclovir phosphorylation may still occur in the presence of UL97 mutants and a higher ganciclovir level. This option is less appealing if there are additional UL54 mutations increasing the overall level of resistance. Hematologic toxicity must be closely monitored when using nonstandard high doses.

Despite doubt about its ultimate efficacy as salvage therapy, e.g. in lung transplant recipients [30], foscarnet is currently the principal alternative to ganciclovir [5], as the extent of cross-resistance is limited and usually low-grade. Foscarnet therapy is complicated by metabolic and renal adverse effects, and intravenous administration of high fluid volumes. Cidofovir is the least documented treatment option for drug-resistant CMV. It tends to appear in reports of multiply relapsing cases as a salvage treatment of transient efficacy, with resistance seemingly selected fairly quickly [19], probably accelerated by prior exposure to ganciclovir, since significant ganciclovir-cidofovir cross-resistance is the rule among UL54 mutations [1,5]. Nephrotoxicity becomes dose-limiting after a few weeks.

Given the limited options for standard CMV antivirals, there are ongoing suggestions to use drugs that alter cellular metabolic pathways to reduce the rate of CMV replication, such as mTor inhibitors, artesunate [31] and leflunomide [32]. These adjunctive measures have had mixed success in overcoming negative host factors that contributed to the development of

drug resistance, but adjusting post-transplant immunosuppression in favor of an mTor inhibitor may be practical in the absence of adverse drug interactions or other concerns [5].

Experimental CMV antiviral compounds

Three experimental oral CMV antivirals have entered late stage clinical trial: the cidofovir lipid conjugate brincidofovir, the UL97 kinase inhibitor maribavir, and the terminase inhibitor letermovir.

Despite overlapping drug resistance mechanisms, brincidofovir may have a role in treating drug-resistant CMV because of its oral bioavailability, in vitro potency, intracellular drug concentrations, and antiviral activity shown in a phase II trial [33], where no development of resistance mutations was noted. Higher than expected doses were needed for full effect and were limited by gastrointestinal toxicity. Although brincidofovir resistance is not rapidly selected in vitro [21], continued viral replication during treatment may be a risk, as prior ganciclovir therapy may have selected for undetected subpopulations of cross-resistant mutants.

Maribavir has been at various stages of clinical trial over an extended period. After failed phase III prophylaxis trials [5], the drug has completed a phase II trial for the salvage treatment of resistant CMV infection, with no results announced as of early 2015. Uncontrolled reports suggest mixed success as salvage therapy [34]. Therapeutic utility is more probable in cases with lower viral loads and less impaired host immune function, given the fast emergence of maribavir resistance in two cases with high viral loads [17].

Letermovir recently entered phase III trials as CMV prophylaxis after stem cell transplantation following a successful phase II trial [23]. As with brincidofovir, higher doses were needed than projected from the nanomolar in vitro EC50s. The distinct viral target is appealing but the apparently low genetic barrier to letermovir resistance may compromise utility as a single agent in treating high-grade active infection. The phase II report [23] provided no information on letermovir resistance mutations in the observed cases of CMV breakthrough during prophylaxis.

Combination antiviral therapy involving multiple antiviral targets is a well-established concept for treatment of HIV and hepatitis C infections that should in principle apply to CMV. Historical attempts to combine polymerase inhibitors such as ganciclovir and foscarnet were limited by tolerability and cross-resistance [5], but the advent of newer options involving different antiviral targets may provide new opportunities for rational combinations that lower the risk of treatment failure and drug resistance.

Conclusion

Risk factors and approaches to diagnosis of CMV drug resistance are now quite well established and updated with periodic published details of specific patient populations and relevant mutations. Genotypic diagnosis may benefit from quality control and improved detection sensitivity of newer technology. Because of limited treatment options using currently licensed drugs, all targeting the viral DNA polymerase, there is great interest in

developing alternative antiviral targets. The genetic barriers and pathways to resistance for experimental antivirals need better definition, and information is awaited on the efficacy of these compounds singly or in combination for the treatment of drug-resistant CMV disease.

Acknowledgments

Financial support and sponsorship: Work in the author's laboratory was supported by grants from the National Institutes of Health (AI39938) and the Department of Veterans Affairs (BX00925).

References

1. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev. 2010; 23:689–712. [PubMed: 20930070]
2. Hakki M, Chou S. The biology of cytomegalovirus drug resistance. Curr Opin Infect Dis. 2011; 24:605–611. [PubMed: 22001948]
3. Le Page AK, Jager MM, Iwasenko JM, et al. Clinical aspects of cytomegalovirus antiviral resistance in solid organ transplant recipients. Clin Infect Dis. 2013; 56:1018–1029. [PubMed: 23243176]
4. Komatsu TE, Pikis A, Naeger LK, Harrington PR. Resistance of human cytomegalovirus to ganciclovir/valganciclovir: a comprehensive review of putative resistance pathways. Antiviral Res. 2014; 101:12–25. [PubMed: 24184129]
5. Kotton CN, Kumar D, Caliendo AM, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2013; 96:333–360. [PubMed: 23896556]
6*. Shmueli E, Or R, Shapira MY, et al. High rate of cytomegalovirus drug resistance among patients receiving preemptive antiviral treatment after haploidentical stem cell transplantation. J Infect Dis. 2014; 209:557–561. The incidence of ganciclovir resistance in this patient population at 14.5% is comparable to that in D+/R− solid organ recipients considered to be high risk. [PubMed: 23983215]
7. Choi SH, Hwang JY, Park KS, et al. The impact of drug-resistant cytomegalovirus in pediatric allogeneic hematopoietic cell transplant recipients: a prospective monitoring of UL97 and UL54 gene mutations. Transpl Infect Dis. 2014
8. Choi KY, Sharon B, Balfour HH Jr, et al. Emergence of antiviral resistance during oral valganciclovir treatment of an infant with congenital cytomegalovirus (CMV) infection. J Clin Virol. 2013; 57:356–360. [PubMed: 23688863]
9. Gagermeier JP, Rusinak JD, Lurain NS, et al. Subtherapeutic ganciclovir (GCV) levels and GCV-resistant cytomegalovirus in lung transplant recipients. Transpl Infect Dis. 2014; 16:941–950. [PubMed: 25491023]
10. Bedino G, Esposito P, Bosio F, et al. The role of therapeutic drug monitoring in the treatment of cytomegalovirus disease in kidney transplantation. Int Urol Nephrol. 2013; 45:1809–1813. [PubMed: 23054314]
11*. Kleiboeker S, Nutt J, Schindel B, et al. Cytomegalovirus antiviral resistance: characterization of results from clinical specimens. Transpl Infect Dis. 2014; 16:561–567. Informative statistics from a large diagnostic laboratory on the relative frequency of UL97 and UL54 resistance mutations in specimens sent out for testing. [PubMed: 24903568]
12. Chou S, Ercolani RJ, Marousek G, Bowlin TL. Cytomegalovirus UL97 kinase catalytic domain mutations that confer multidrug resistance. Antimicrob Agents Chemother. 2013; 57:3375–3379. [PubMed: 23650173]
13. Drouot E, Piret J, Lebel MH, Boivin G. Characterization of multiple cytomegalovirus drug resistance mutations detected in a hematopoietic stem cell transplant recipient by recombinant phenotyping. J Clin Microbiol. 2014; 52:4043–4046. [PubMed: 25143583]
14. Fischer L, Laib Sampaio K, Jahn G, et al. Generation and characterization of a GCV resistant HCMV UL97-mutation and a drug sensitive UL54-mutation. Antiviral Res. 2013; 100:575–577. [PubMed: 24120366]

15. Komazin-Meredith G, Chou S, Prichard MN, et al. Human cytomegalovirus UL97 kinase is involved in the mechanism of action of methylenecyclopropane analogs with 6-ether and -thioether substitutions. Antimicrob Agents Chemother. 2014; 58:274–278. [PubMed: 24145545]

16. Zhang Y, Zhao Z, Sun J, et al. A new mutation in the human cytomegalovirus UL97 gene may confer ganciclovir resistance in Chinese kidney transplant recipients. Arch Virol. 2013; 158:247–250. [PubMed: 23011309]

17. Schubert A, Ehlert K, Schuler-Luettmann S, et al. Fast selection of maribavir resistant cytomegalovirus in a bone marrow transplant recipient. BMC Infect Dis. 2013; 13:330. [PubMed: 23870704]

18. Chou S, Boivin G, Ives J, Elston R. Phenotypic evaluation of previously uncharacterized cytomegalovirus DNA polymerase sequence variants detected in a valganciclovir treatment trial. J Infect Dis. 2014; 209:1219–1226. [PubMed: 24273181]

19*. Chou S, Ercolani RJ, Sahoo MK, et al. Improved detection of emerging drug-resistant mutant cytomegalovirus subpopulations by deep sequencing. Antimicrob Agents Chemother. 2014; 58:4697–4702. An early case report on the use of next generation sequencing for the earlier detection of a greater variety of emerging resistance mutations in serial specimens. [PubMed: 24890586]

20. Hantz S, Cotin S, Borst E, et al. Novel DNA polymerase mutations conferring cytomegalovirus resistance: input of BAC-recombinant phenotyping and 3D model. Antiviral Res. 2013; 98:130–134. [PubMed: 23415883]

21. James SH, Price NB, Hartline CB, et al. Selection and recombinant phenotyping of a novel CMX001 and cidofovir resistance mutation in human cytomegalovirus. Antimicrob Agents Chemother. 2013; 57:3321–3325. [PubMed: 23650158]

22*. Chen H, Beardsley GP, Coen DM. Mechanism of ganciclovir-induced chain termination revealed by resistant viral polymerase mutants with reduced exonuclease activity. Proc Natl Acad Sci U S A. 2014; 111:17462–17467. A new model for the biochemical effects of exonuclease mutations conferring dual ganciclovir and cidofovir resistance. [PubMed: 25422422]

23*. Chemaly RF, Ullmann AJ, Stoelben S, et al. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. N Engl J Med. 2014; 370:1781–1789. Phase II clinical trial supporting continued testing of this experimental terminase inhibitor. Longstanding interest in this alternative antiviral drug target has finally advanced into later stage clinical trials. [PubMed: 24806159]

24**. Goldner T, Hempel C, Ruebsamen-Schaeff H, et al. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. Antimicrob Agents Chemother. 2014; 58:610–613. First impression of the range of UL56 mutations selected in vitro under letermovir and their associated resistance phenotypes. These are the interim basis for genotypic diagnosis of letermovir resistance, but additional resistance mutations are expected to be discovered. Clinical correlation is pending; none available so far. [PubMed: 24189264]

25. Sijmons S, Van Ranst M, Maes P. Genomic and functional characteristics of human cytomegalovirus revealed by next-generation sequencing. Viruses. 2014; 6:1049–1072. [PubMed: 24603756]

26. Sahoo MK, Lefterova MI, Yamamoto F, et al. Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. J Clin Microbiol. 2013; 51:3700–3710. [PubMed: 23985916]

27. Drouot E, Piret J, Boivin G. Novel method based on “en passant” mutagenesis coupled with a gaussia luciferase reporter assay for studying the combined effects of human cytomegalovirus mutations. J Clin Microbiol. 2013; 51:3216–3224. [PubMed: 23863570]

28. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014; 6:242ra283.

29. Gracia-Ahufinger I, Gutierrez-Aroca J, Cordero E, et al. Use of high-dose ganciclovir for the treatment of cytomegalovirus replication in solid organ transplant patients with ganciclovir resistance-inducing mutations. Transplantation. 2013; 95:1015–1020. [PubMed: 23407543]

30. Minces LR, Nguyen MH, Mitsani D, et al. Ganciclovir-resistant cytomegalovirus infections among lung transplant recipients are associated with poor outcomes despite treatment with foscarnet-containing regimens. Antimicrob Agents Chemother. 2014; 58:128–135. [PubMed: 24145525]

31. Germi R, Mariette C, Alain S, et al. Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus. Antiviral Res. 2014; 101:57–61. [PubMed: 24184983]

32. Verkaik NJ, Hoek RA, van Bergeijk H, et al. Leflunomide as part of the treatment for multidrug-resistant cytomegalovirus disease after lung transplantation: case report and review of the literature. Transpl Infect Dis. 2013; 15:E243–249. [PubMed: 24298985]

33. Marty FM, Winston DJ, Rowley SD, et al. CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation. N Engl J Med. 2013; 369:1227–1236. [PubMed: 24066743]

34. Alain S, Revest M, Veyer D, et al. Maribavir use in practice for cytomegalovirus infection in French transplantation centers. Transplant Proc. 2013; 45:1603–1607. [PubMed: 23726629]

Key Points

- Incomplete suppression of CMV replication during therapy risks the eventual development of drug resistance
- Resistance to current DNA polymerase inhibitors typically requires months of antiviral drug exposure, but the interval may be shortened by adverse host or drug factors permitting higher ongoing levels of viral replication
- Genotypic testing for relevant viral mutations can be improved with attention to quality control and use of newer technologies offering higher sensitivity of detection of mutant subpopulations
- Experimental drugs with different antiviral mechanisms, used singly or in combination, may offer better options for treating drug-resistant CMV infection
- Further definition is needed of the mutations and genetic barriers to resistance applicable to drugs targeting different antiviral gene products.

Table 1

Recently published CMV UL97 kinase mutations that decrease drug susceptibility

Amino acid change	Fold increase EC50[1] for		Reference	Circumstances of detection[2]
	Ganciclovir	Maribavir		
F342S	7.8	18	[12]	in vitro, cyclopropavir
V356G	5.5	108	[12]	in vitro, cyclopropavir minor subpopulation
D456N	12	278	[15]	in vitro, methylenecyclopropane nucleoside
C480R	9	243	[15]	in vitro, methylenecyclopropane nucleoside
C518Y	12		[16]	clinical isolate
P521L	17	428	[12]	clinical specimen, genotypic test result
A613V	2.3		[14]	clinical specimen, genotypic test result
Y617del[3]	10	372	[15]	in vitro, methylenecyclopropane nucleoside
E655K	1.7		[13]	clinical specimen, genotypic test result

[1] Increase in drug concentration required to reduce viral growth 50% (EC50) compared with wild type

[2] Partial or complete mutant populations in clinical specimens or after in vitro passage under drug

[3] In frame deletion of codon 617

Table 2

Recently published CMV UL54 polymerase mutations that decrease drug susceptibility

Amino acid change	Fold increase EC50[1] for			Reference	Circumstances of detection[2]
	Ganciclovir	Foscarnet	Cidofovir		
N408S	3.1	1	7.5	[20]	clinical specimen, genotypic test result
D413N	3.8	1	10	[19]	clinical specimen, sequence subpopulation
A505V	1.8	1.1	2	[18]	clinical specimen, genotypic test result
K513R	3.7	1.1	10	[19]	clinical specimen, sequence subpopulation
C524del[3]	3.5	1.1	9.7	[20]	clinical specimen, genotypic test result
V526L	5.5	1.8	2.5	[13]	clinical specimen, genotypic test result
C539G	3.1	1	4.4	[19]	clinical specimen, sequence subpopulation
D542E	1.5	1.7	12	[21]	in vitro, brincidofovir
Q578L	1.9	3	0.8	[18]	clinical specimen, genotypic test result[4]
I726T	2	1.1	1.7	[18]	clinical specimen, genotypic test result
I726V	1.9	1.2	1.9	[18]	clinical specimen, genotypic test result
L773V	3	4.4	2.5	[19]	clinical specimen, genotypic test result
G841S	2.2	2.1	1.1	[18]	clinical specimen, genotypic test result[4]

[1] In constructed mutant virus, increase in drug concentration required to reduce viral growth 50% (EC50) compared with wild type

[2] Detection of partial or complete mutant populations in clinical specimens or after in vitro passage under indicated compound

[3] In frame deletion of codon 524

[4] Presence of mutation not confirmed on retesting of same specimen