Analysis of Ganciclovir-Resistant Cytomegalovirus Infection Caused by the UL97 Gene Mutation in Codons 460 and 520 in Pediatric Patients: A Case Series

Sophida Boonsathorn,1 Ekawat Pasomsub,2 Chonnamet Techasaensiri,1 and Nopporn Apiwattanakul1,6

1Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, and 2Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Background. Drug-resistant cytomegalovirus (CMV) infection has been increasingly recognized. However, there are limited data in pediatric patients. In this study, the prevalence and factors associated with CMV infection with UL97 mutations in pediatric patients treated with ganciclovir but not responding to treatment were evaluated.

Methods. This retrospective study was conducted from January 2013 to December 2017. All patients who were suspected of having ganciclovir-resistant CMV infection and had never had ganciclovir prophylaxis were included. Genotypic assay for UL97 mutations in codons 460 and 520 conferring ganciclovir resistance was performed. Factors associated with the presence of UL97 mutations were analyzed.

Results. Of 34 patients included, 10 patients (29.4%) had a genotypically confirmed UL97 mutation. The median age (inter-quartile range [IQR]) was 3 (0.85–8.68) years. Ganciclovir resistance was tested at a median time (IQR) of 22.5 (14.3–31) days after initiation of ganciclovir. All resistant isolates harbored a UL97 mutation in codon 460. Compared with patients infected with CMV without UL97 mutation, those infected with UL97 mutation strains were younger (median age [IQR], 3.02 [0.85–8.68] vs 10.45 [2.7–16.4] years) and had a higher maximum viral load (median [IQR], 5.06 [4.74–6.05] vs 4.42 [4.03–4.87] copies/mL). Six of 10 (60%) patients were successfully treated with high-dose ganciclovir (7.5 mg/kg twice daily).

Conclusions. UL97 mutation ganciclovir-resistant CMV infection was not uncommon in the pediatric population. Screening for this mutation should be considered in patients experiencing virological worsening while ganciclovir is given, even if patients have not previously received ganciclovir prophylaxis.

Keywords. cytomegalovirus; ganciclovir-resistant; pediatric.

Cytomegalovirus (CMV) is a ubiquitous double-stranded enveloped DNA virus of the Herpesviridae family. Primary infection in immunocompetent individuals is usually asymptomatic or a self-limited disease. CMV resides in various cells as latent infection, which can be reactivated during a period of immunosuppression. CMV seroprevalence varies among different populations, with a higher rate of seroprevalence in developing countries, including Thailand [1–3].

CMV infection remains a major cause of morbidity and mortality in solid organ and hematopoietic stem cell transplant recipients [4]. Ganciclovir is the antiviral drug of choice for the treatment and prevention of CMV infection. However, the emergence of ganciclovir-resistant CMV infection has been increasingly reported worldwide, particularly among recipients of solid organ transplants [5–8]. The occurrence of ganciclovir-resistant CMV infection is associated with longer hospitalization and increased morbidity and mortality [5, 9]. Previous studies have shown that the risk of developing ganciclovir-resistant CMV is associated with several factors, including but not limited to prolonged exposure to ganciclovir, CMV serostatus of donors and recipients, and type of transplantation [8–11].

The major mechanisms of ganciclovir resistance in CMV are mutations in the viral kinase and/or DNA polymerase genes. Mutations in the viral UL97 kinase gene confer resistance to ganciclovir, whereas mutations in the UL54 DNA polymerase gene are typically associated with high-level resistance to ganciclovir and cross-resistance to cidofovir and/or foscarin [12–14]. Common mechanisms of CMV resistance to ganciclovir have been predominantly described with the UL97 mutation [15–18]. The majority of UL97 mutations occur at codons 460–607, with mutations at codons 460 and 520 resulting in at least a 5-fold increase in IC50 [19, 20]. Despite the currently available information on risk factors and clinical
outcomes of patients who are infected with ganciclovir-resistant CMV, data in pediatric patients are still limited. As ganciclovir is the frontline treatment for CMV infection, this study aimed to describe the clinical characteristics of pediatric patients with ganciclovir-resistant CMV infection with UL97 mutations. Additionally, the risk factors and outcomes attributable to this ganciclovir-resistant CMV infection were examined.

METHODS

Study Population

This was a retrospective study in which patients’ data were collected from January 2013 to September 2017 at a tertiary hospital in Bangkok, Thailand. All pediatric patients who were virologically suspected of ganciclovir-resistant CMV infection were included. Inclusion criteria included patients with CMV reactivation/infection who had not been exposed to ganciclovir or valganciclovir before initiation of treatment and had increasing CMV load or suboptimal responses (increased or failed to decrease to half of the initial viral load) after at least 2 weeks of appropriately dosed intravenous ganciclovir (5-mg/kg/dose twice daily). Patients’ demographics, clinical manifestations, laboratory data, antiviral treatment, and outcomes were collected from electronic medical records. The following definitions were used according to the published guidelines [21, 22]. CMV infection was defined as evidence of CMV replication regardless of symptoms. CMV disease was defined as patients with evidence of CMV infection with attributable symptoms. CMV disease can be further classified as a viral syndrome (ie, fever, leukopenia, and/or thrombocytopenia), or as a tissue-invasive disease. The study was approved by the Institutional Review Board of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

CMV Prevention Strategies

For solid organ transplant recipients and stem cell transplant recipients, we used preemptive therapy as the main approach for prevention of CMV diseases in all cases in this study. Patients who underwent kidney transplant with serostatus of D+/R− received intravenous ganciclovir prophylaxis for 2 weeks and had preemptive therapy after that. Patients who underwent liver transplant received only preemptive therapy regardless of CMV serostatus. Weekly monitoring for plasma CMV load was implemented in all patients. During the study period, we used a viral load of 1000 copies/mL as a cutoff value to initiate preemptive therapy.

For patients with hematologic malignancies, plasma CMV load was measured if CMV infection was suspected, and ganciclovir was administered as a preemptive therapy. In addition, if patients required blood transfusion, only leukocyte-poor blood components were used to reduce the risk of transfusion reactions and transmission of CMV.

Laboratory Confirmation of CMV Infection and Ganciclovir-Resistant Diagnosis

Plasma CMV load was quantified using the quantitative real-time polymerase chain reaction (PCR) technique by COBAS AmpliPrep/COBAS Taqman (Taqman CMV Test; Roche Molecular Diagnostics, Branchburg, NJ, USA). Detection of UL97 gene mutations was achieved by using the LightCycler real-time PCR assay for the simultaneous detection of different UL97 mutations in codons 460 and 520 (Roche Diagnostic, Mannheim, Germany), as previously described [23].

Statistical Analysis

Descriptive statistics were used for demographic and clinical characteristics. The independent t test and Mann-Whitney U test were used for comparison of continuous variables between groups. A P value of <.05 was considered statistically significant.

RESULTS

Demographic Data

A total of 34 patients who had virologically suspected ganciclovir-resistant CMV infection and blood analyses for UL97 gene mutations were included. Approximately half of all patients were boys. The median age of the patients (interquartile range [IQR]) was 7.85 (1.69–14.03) years. The majority of patients were hematopoietic stem cell transplantation recipients. Eleven patients had CMV tissue invasive diseases, and 9 patients died.

Characteristics of Patients With Ganciclovir-Resistant CMV Infection

Of 34 patients who were suspected of having ganciclovir-resistant CMV infection, 10 patients (29.4%) had genotypically confirmed ganciclovir-resistant CMV infection. None of these patients had received ganciclovir for CMV prophylaxis before diagnosis. The median age (IQR) was 3.02 (0.85–8.68) years. Underlying diseases included 4 solid organ transplant recipients (3 livers, 1 kidney), 1 haploidentical stem cell transplant patient, and 5 hematopoietic stem cell transplant patients with leukemia and/or infection-associated hemophagocytic syndrome. Five patients had asymptomatic CMV reactivation, and the other patients had tissue-invasive diseases. Among these solid organ recipients, 2 (50%) patients were D+/R−, and a patient who underwent hematopoietic stem cell transplantation was D−/R−. The clinical characteristics of the patients with ganciclovir-resistant CMV infection are shown in Table 1.

Ganciclovir-Resistant CMV Infection and Associated Factors

Ganciclovir resistance was tested at a median time (IQR) of 22.5 (14.3–31) days after initiation of ganciclovir, with a median CMV load (IQR) of 41 100 (13 575–102 346) copies/mL or log 4.61 (4.13–5). All ganciclovir-resistant isolates harbored a UL97 mutation in codon 460. No gene mutation at codon 520 was
Pediatric Drug-Resistant CMV Infection

The median maximum CMV load (IQR) was 113,245 (54,900–1,184,634) copies/mL or log 5.06 (4.74–6.05). Several variables as potential associated factors for development of ganciclovir-resistant CMV infection were examined. Among all patients, those with ganciclovir-resistant CMV infection were significantly younger than those without the mutation (P = .049). The median CMV load at diagnosis and the mean CMV load at the time of gene mutation analysis were not significantly different between those with and those without mutations. In contrast, the maximum CMV viral load was significantly higher in those who harbored a UL97 gene mutation compared with those without this mutation (P = .004).

Although patients with ganciclovir-resistant CMV infection had a higher rate of end-organ involvement and worse outcomes, these were not significantly different from those with wild-type infection. The demographic data and outcomes of the patients with and without ganciclovir-resistant CMV infection are shown in Table 2.

### Treatment and Outcome of Ganciclovir-Resistant CMV Infection

Reduction of immunosuppression, along with high-dose ganciclovir up to 7.5 mg/kg per dose twice daily, was implemented in all patients. Six of 10 (60%) patients were successfully treated with high-dose ganciclovir, and the plasma CMV load became undetectable at a median time (IQR) of 23 (17–56) days.

No complications of nephrotoxicity were observed in all patients treated with high-dose ganciclovir. The values for baseline and maximal serum creatinine are shown in Table 1. Neutropenia with an absolute neutrophil count (ANC) of <1000/µL was observed in 6 patients. Granulocyte-colony stimulating factor (G-CSF) was administered in all patients with an ANC of <500/µL. However, it is worth mentioning that 5 of these 6 patients had underlying diseases of hematologic malignancies and had had neutropenia before initiation of high-dose ganciclovir.

Ganciclovir was switched to foscarnet or cidofovir in 4 patients. Artesunate was added in 1 patient as an adjunctive therapy. Four (40%) patients died, but only 1 patient died from a CMV-related condition (disseminated CMV infection with infection-associated hemophagocytosis syndrome). The remaining patients died from complications that were not directly related to CMV infection, including Pseudomonas aeruginosa septicemia, Klebsiella pneumoniae septicemia, and post-transplant lymphoproliferative disorder.

### DISCUSSION

This single-center study examined ganciclovir-resistant CMV infection in a pediatric patients who had never been exposed to ganciclovir and focused on clinical predictors associated with this infection. Almost one-third of the patients who failed to respond to standard ganciclovir treatment developed genotypically confirmed ganciclovir resistance. All ganciclovir-resistant CMV cases in this study harbored a codon 460 mutation in UL97, which was confirmed by a genotypic assay. Interestingly, none of the patients received ganciclovir or valganciclovir for CMV prophylaxis before development of resistance. Therefore, the current data support the assertion that children who are not exposed to anti-CMV drugs may also be at risk of early emergence of ganciclovir-resistant CMV infection.

### Table 1. Clinical Characteristics of Patients With Ganciclovir-Resistant CMV Infection

| Patient No. | Age, y | Sex | Underlying Diseases | CMV Serostatus | Days of Resistance Testing | Treatment | Baseline/Maximum Creatinine, mg/dL | Outcome |
|-------------|-------|-----|---------------------|----------------|--------------------------|-----------|-----------------------------------|---------|
| 1           | 15    | M   | KT                  | D+/R+          | 15           | HDIV ganciclovir                | 0.72/0.95 | Survived                          |
| 2           | 0.96  | F   | LT                  | D+/R−          | 23           | HDIV ganciclovir, IV cidofovir  | 0.13/0.2  | Survived                          |
| 3           | 7     | F   | ALL                 | −              | 204          | HDIV ganciclovir, IV foscarnet, IV cidofovir | 0.25/0.35 | Survived                          |
| 4           | 1.5   | M   | ANLL                | −              | 16           | HDIV ganciclovir                | 0.31/0.57 | Survived                          |
| 5           | 1.75  | M   | LT                  | D+/R+          | 22           | HDIV ganciclovir                | 0.36/0.46 | Survived                          |
| 6           | 13.7  | M   | ALL s/p haplo-HSCT  | D−R−           | 12           | HDIV ganciclovir                | 0.51/0.77 | Survived                          |
| 7           | 4.6   | F   | LT                  | D+R−           | 11           | HDIV ganciclovir                | 0.19/0.32 | Died from PTLD                     |
| 8           | 0.5   | F   | ANLL                | −              | 31           | HDIV ganciclovir, IV foscarnet, PO artesunate | 0.12/0.15 | Died from septicemia              |
| 9           | 0.17  | F   | IAHS                | −              | 31           | HDIV ganciclovir, IV foscarnet  | 0.15/0.16 | Died from disseminated CMV disease |
| 10          | 4.30  | M   | ALL                 | −              | 26           | HDIV ganciclovir                | 0.13/0.18 | Died from septicemia              |

Abbreviations: +, seropositivity; −, seronegativity; ALL, acute lymphocytic leukemia; ANLL, acute nonlymphocytic leukemia; CMV, cytomegalovirus; D, donor; f, female; HDIV, high-dose intravenous ganciclovir; IAHS, infection-associated hemophagocytic syndrome; IV, intravenous; KT, kidney transplantation; LT, liver transplantation; M, male; PO, per oral; PTLD, post-transplant lymphoproliferative disorder; R, recipient; s/p haplo-HSCT, status post-haploidentical hematopoietic stem cell transplantation.

*Days of resistance testing after initiation of appropriately dosed intravenous ganciclovir.
Although the ganciclovir-resistant phenotype caused by UL97 mutations has been widely described in adults, data on CMV infection with UL97 mutations in pediatric patients are scarce. Previous studies of pediatric patients have reported that the incidence of drug-resistant CMV infection was 2%–5% [18, 24–26]. However, most cases were hematopoietic stem cell and solid organ transplant recipients. To the best of the authors’ knowledge, this is the first study to show development of CMV resistance in the pediatric population without prior ganciclovir/valganciclovir exposure. This supports the fact that mutant strain viruses have already existed before initiation of ganciclovir therapy. It is possible that a high initial viral load comprises more resistant strain viruses than a low initial viral load. Thus, a higher number of resistant strain viruses could rapidly predominate after selective pressure from antiviral drugs. This mechanism operates in HIV patients exposed to zidovudine [27].

Generally, a 1-log decline in CMV viral load is expected after at least 2 weeks of appropriate antiviral treatment. Ganciclovir resistance should be considered if patients have persistent viremia, DNAemia, or the CMV viral load continues to increase beyond 2 weeks after initiation of corrected therapy [6, 28, 29]. Potential risk factors for emergence of CMV drug resistance include prior antiviral exposure, high initial viral load, persistent viral replication, intense immunosuppression, and recurrent episodes of CMV infection [6, 18, 26, 30, 31]. The current study shows that patients who were infected with a ganciclovir-resistant CMV strain had a significantly higher maximum viral load than those without the mutation strain. This finding supports previously published studies that have described a high peak CMV viral load as a potential contributor to development of drug-resistant CMV [15, 32]. Our results also show that patients infected with the ganciclovir-resistant CMV strain tended to harbor a higher viral load at diagnosis of CMV infection and a higher viral load at the time when ganciclovir resistance was suspected. However, the small sample size of the study precluded the power to demonstrate a statistically significant difference. Our study also demonstrated that patients with ganciclovir-resistant CMV infection were younger than those without resistance. This may reflect the fact that immunity to CMV infection was less active in younger patients. It is well known that patients with absence of preexisting immunity to CMV such as D+/R– patients in solid organ transplant have a higher risk of acquisition of ganciclovir-resistant CMV infection [5, 8]. Therefore, this conclusion requires further confirmation in a study with a greater sample size with multivariate analysis.

The duration of all forms of ganciclovir exposure is also a significant risk factor for development of drug-resistant CMV strains. Drug resistance typically occurs after months of antiviral prophylaxis or therapy. In an adult cohort study, the median accumulative duration of ganciclovir treatment was 151 days before development of virological resistance [16]. Fisher et al. showed that the median time of ganciclovir/valganciclovir exposure in solid organ transplant recipients was 153 days before development of a drug-resistant CMV strain [15]. Another study by Shmueli et al. showed the appearance of resistance after a cumulative treatment duration of 70 days [30]. In contrast, the present study showed that resistance could develop in the early phase after treatment initiation (a median time of ≥22.5 days).

In all cases of suspected or confirmed drug-resistant CMV infection, reduction of immunosuppression remains the first-line strategy to optimize the host immune response. However, this approach may be problematic in some complicated cases. The choices of anti-CMV drugs should be guided by the results of CMV genotypic assays [29]. Generally, CMV isolates that harbor UL97 gene mutations remain susceptible to foscarnet.

| Characteristic                                      | Patients With a UL97 Gene Mutation (n = 10) | Patients Without a UL97 Gene Mutation (n = 24) | P Value |
|-----------------------------------------------------|--------------------------------------------|------------------------------------------------|--------|
| Male sex, No. (%)                                   | 5 (50)                                     | 11 (45.8)                                      | .83    |
| Age, median (IQR), y                                | 3.02 (0.85–8.68)                          | 10.45 (2.7–16.4)                               | .049   |
| Underlying diseases, No. (%)                        |                                            |                                               |        |
| Hematological malignancy                            | 6 (60)                                     | 17 (70.8)                                      | .553   |
| Solid organ transplantation                          | 4 (40)                                     | 7 (29.2)                                       |        |
| CMV load at diagnosis of CMV infection, median (IQR), log copies/mL | 3.8 (2.74–4.48)                          | 2.92 (2.61–3.75)                               | .064   |
| CMV load at the time of gene mutation analysis, median (IQR), log copies/mL | 4.61 (4.13–5)                            | 3.96 (3.0–4.6)                                 | .061   |
| Maximum CMV load, median (IQR), log copies/mL       | 5.06 (4.74–6.05)                          | 4.42 (4.03–4.87)                               | .004   |
| Days of anti-CMV resistance testing after initiation of ganciclovir, median (IQR) | 22.5 (14.3–31)                           | 20.5 (16–28.5)                                 | .461   |
| End-organ involvement, No. (%)                      | 5 (50)                                     | 6 (25)                                         | .165   |
| Mortality, No. (%)                                  | 4 (40)                                     | 5 (20.8)                                       | .315   |

Abbreviations: CMV, cytomegalovirus; IQR, interquartile range.
and cidofovir. However, isolates with UL54 gene mutations are more likely to confer cross-resistance to cidofovir. Therefore, foscarnet is recommended as the first option for empirical treatment of suspected ganciclovir-resistant CMV diseases. However, a disadvantage of this option is its potential nephrotoxicity, which requires close monitoring of fluid and electrolytes. Previous studies have demonstrated that dose escalation of ganciclovir (up to 7.5–10 mg/kg twice daily) is also an appealing option to avoid adverse effects associated with foscarnet [29, 33, 34]. In the absence of UL54 gene mutations, cidofovir is another therapeutic option for drug-resistant CMV, but nephrotoxicity is also a concern. Because foscarnet and cidofovir were not consistently available during the study period, 6 (60%) patients achieved eradication of the virus with high-dose ganciclovir (7.5 mg/kg/dose twice daily) therapy without any evidence of nephrotoxicity. Therefore, high-dose ganciclovir might be a feasible treatment option for infection caused by CMV with UL97 mutation.

The present study has some limitations associated with bias of a retrospective study. First, not all patients, but only those with viologically suspected resistance, were tested for resistance. In addition, the magnitude of ganciclovir-resistant CMV infection among patients who were treated with this drug could not be accurately determined, as the total number of patients who were treated with ganciclovir during this time period was not completely available. Recent data from our center have shown that the overall prevalence of CMV reactivation (CMV viral load ≥200 copies/mL) among pediatric acute lymphoblastic leukemia patients was 50%. However, all treated patients in this study responded well to ganciclovir [35]. For pediatric patients who underwent hematopoietic stem cell transplant, only 1 of 47 patients (2%) treated with ganciclovir had documented ganciclovir-resistant CMV infection. For liver transplant patients, 2 of 40 patients (5%) who were treated with ganciclovir were proven to have ganciclovir-resistant CMV infection. Prospective data collection is needed to elaborate the overall magnitude of ganciclovir-resistant CMV infection in pediatric patients who are being treated with ganciclovir. Second, only mutations in codons 460 and 520 were analyzed in this study, as the primers for detection of other mutations were not available during the study period. Although most UL97 mutations conferring ganciclovir resistance are strongly clustered at codons 460, 520, or 590–607 [17, 36], the true incidence of drug-resistant CMV infection was underrated, as only 2 codons were evaluated. Therefore, whole-genome sequencing should be performed in the future. Finally, UL54 gene mutations, which confer cross-resistance to cidofovir, were not evaluated in the current study.

In conclusion, ganciclovir-resistant CMV infection is an emerging problem, not only in pediatric transplanted recipients, but also in hematopo-oncological patients. Therefore, screening for mutations should be considered in patients with persistent viremia, even if patients have not previously received ganciclovir prophylaxis. High-dose ganciclovir might be a feasible option for the treatment of ganciclovir-resistant CMV infection when other anti-CMV drugs are not available.

**Acknowledgments**

We thank the Division of Virology, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, for providing laboratory data. **Financial support.** This work was supported by Faculty of Medicine Ramathibodi Hospital, Mahidol University. **Potential conflicts of interest.** All authors: no reported conflicts of interest. 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.

**References**

1. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010; 20:202–13.
2. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the National Health and Nutrition Examination Surveys, 1988-2004. Clin Infect Dis 2010; 50:1439–47.
3. Urwijitaroon Y, Teawpatanatawoen S, Kitireonart A. Prevalence of cytomegalovirus antibody in Thai-northeastern blood donors. Southeast Asian J Trop Med Public Health 1993; 24(Suppl 1):180–2.
4. Razonable RR. Epidemiology of cytomegalovirus disease in solid organ and hematopoietic stem cell transplant recipients. Am J Health Syst Pharm 2005; 62:57–13.
5. Lamaye AP, Corey L, Kocell DM, et al. Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants. Lancet 2000; 356:645–9.
6. Young PG, Rubin J, Angarone M, et al. Ganciclovir-resistant cytomegalovirus infection in solid organ transplant recipients: a single-center retrospective cohort study. Transpl Infect Dis 2016; 18:390–5.
7. Lamaye AP. Ganciclovir-resistant cytomegalovirus in transplant recipients. Clin Infect Dis 2002; 35:866–72.
8. Lamaye AP, Raghu G, Kocell DM, et al. High incidence of ganciclovir-resistant cytomegalovirus infection among lung transplant recipients receiving preemptive therapy. J Infect Dis 2002; 185:20–7.
9. Li F, Kenyon KW, Kirby KA, et al. Incidence and clinical features of ganciclovir-resistant cytomegalovirus disease in heart transplant recipients. Clin Infect Dis 2007; 45:459–47.
10. Bhorade SM, Lurain NS, Jordan A, et al. Emergence of ganciclovir-resistant cytomegalovirus disease in lung transplant recipients. J Heart Lung Transplant 2002; 21:1274–82.
11. Myhre HA, Haug Dorenborg D, Kristiansen KI, et al. Incidence and outcomes of ganciclovir-resistant cytomegalovirus infections in 1244 kidney transplant recipients. Transplantation 2011; 92:217–23.
12. Chou S. Antiviral drug resistance in human cytomegalovirus. Transpl Infect Dis 1999; 1:105–14.
13. Chou S, Waldemer RH, Senters AE, et al. Cytomegalovirus UL97 phosphotransferase mutations that affect susceptibility to ganciclovir. J Infect Dis 2002; 185:162–9.
14. Baldanti F, Lurain N, Gerna G. Clinical and biologic aspects of human cytomegalovirus resistance to antiviral drugs. Hum Immunol 2004; 65:403–9.
15. Fisher CE, Knudsen JL, Less ED, et al. Risk factors and outcomes of ganciclovir-resistant cytomegalovirus infection in solid organ transplant recipients. Clin Infect Dis 2017; 65:57–63.
16. Hantz S, Garnier-Geoffroy F, Mazeron MC, et al; French CMV Resistance Survey Study Group. Drug-resistant cytomegalovirus in transplant recipients: a French cohort study. J Antimicrob Chemother 2010; 65:2628–40.
17. Laurin NS, Bhorade SM, Pursell KI, et al. Analysis and characterization of antiviral drug-resistant cytomegalovirus isolates from solid organ transplant recipients. J Infect Dis 2002; 186:760–8.
18. Kim YJ, Boeckh M, Cook L, et al. Cytomegalovirus infection and ganciclovir resistance caused by UL97 mutations in pediatric transplant recipients. Transpl Infect Dis 2012; 14:611–7.
19. Chou S, Van Wechel LC, Lichy HM, Marousek GI. Phenotyping of cytomegalovirus drug resistance mutations by using recombinant viruses incorporating a reporter gene. Antimicrob Agents Chemother 2005; 49:2710–5.
20. Martin M, Gilbert C, Covington E, Boivin G. Characterization of human cytomegalovirus (HCMV) UL97 mutations found in a valganciclovir/oral ganciclovir prophylactic trial by use of a bacterial artificial chromosome containing the HCMV genome. J Infect Dis 2006; 194:579–83.
21. Humar A. Reactivation of viruses in solid organ transplant patients receiving cytomegalovirus prophylaxis. Transplantation 2006; 82:59–514.
22. Ljungman P, Boeckh M, Hirsch HH, et al; Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis 2017; 64:87–91.
23. Göhring K, Mikeler E, Jahn G, Hamprecht K. Rapid simultaneous detection by real-time PCR of cytomegalovirus UL97 mutations in codons 460 and 520 conferring ganciclovir resistance. J Clin Microbiol 2006; 44:4541–4.
24. 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; 16:919–29.
25. Martin M, Goyette N, Ives J, Boivin G. Incidence and characterization of cytomegalovirus resistance mutations among pediatric solid organ transplant patients who received valganciclovir prophylaxis. J Clin Virol 2010; 47:321–4.
26. Eckle T, Preis I, Jahn G, et al. Drug-resistant human cytomegalovirus infection in children after allogeneic stem cell transplantation may have different clinical outcomes. Blood 2000; 96:3286–9.
27. Frost SD, mclean AR. Quasispecies dynamics and the emergence of drug resistance during zidovudine therapy of HIV infection. AIDS 1994; 8:323–32.
28. Chemaly RF, Chou S, Einsele H, et al; Resistant Definitions Working Group of the Cytomegalovirus Drug Development Forum. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. Clin Infect Dis 2019; 68:1420–6.
29. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients—guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant 2019; 33:e13512.
30. 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; 209:557–61.
31. Cunha-Bang Cd, Kirkby N, Sanderholm M, et al. The course of development and impact from viral resistance against ganciclovir in cytomegalovirus infection. Am J Transplant 2013; 13:458–66.
32. Couzi L, Helou S, Bachelet T, et al. High incidence of anticytomegalovirus drug resistance among D+R- kidney transplant recipients receiving preemptive therapy. Am J Transplant 2012; 12:202–9.
33. Gracia-Ahufinger I, Gutiérrez-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; 95:1015–20.
34. Kotton CN, Kumar D, Caliendo AM, et al; The Transplantation Society International CMV Consensus Group. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation 2018; 102:900–31.
35. Phasuk N, Keatkla J, Rattanasiri S, et al. Monitoring of cytomegalovirus infection in non-transplant pediatric acute lymphoblastic leukemia patients during chemotherapy. Medicine (Baltimore) 2019; 98:e14256.
36. 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.