

# LOW SYSTEMIC GANCICLOVIR EXPOSURE AND PREEMPTIVE TREATMENT FAILURE OF CYTOMEGALOVIRUS REACTIVATION IN A TRANSPLANTED CHILD

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## ABSTRACT

Prevention of cytomegalovirus (CMV) disease with ganciclovir has led to decrease morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. In the present report, we describe a case of ganciclovir treatment failure in a HSCT child who presented a refractory CMV infection despite harbouring a susceptible strain. The failure was partly attributed to sub-therapeutic plasma ganciclovir levels. Our experience emphasizes the importance of drug monitoring in immunocompromised patients.

**Key Words:** *Cytomegalovirus; pediatric; pharmacokinetic; ganciclovir; resistance*

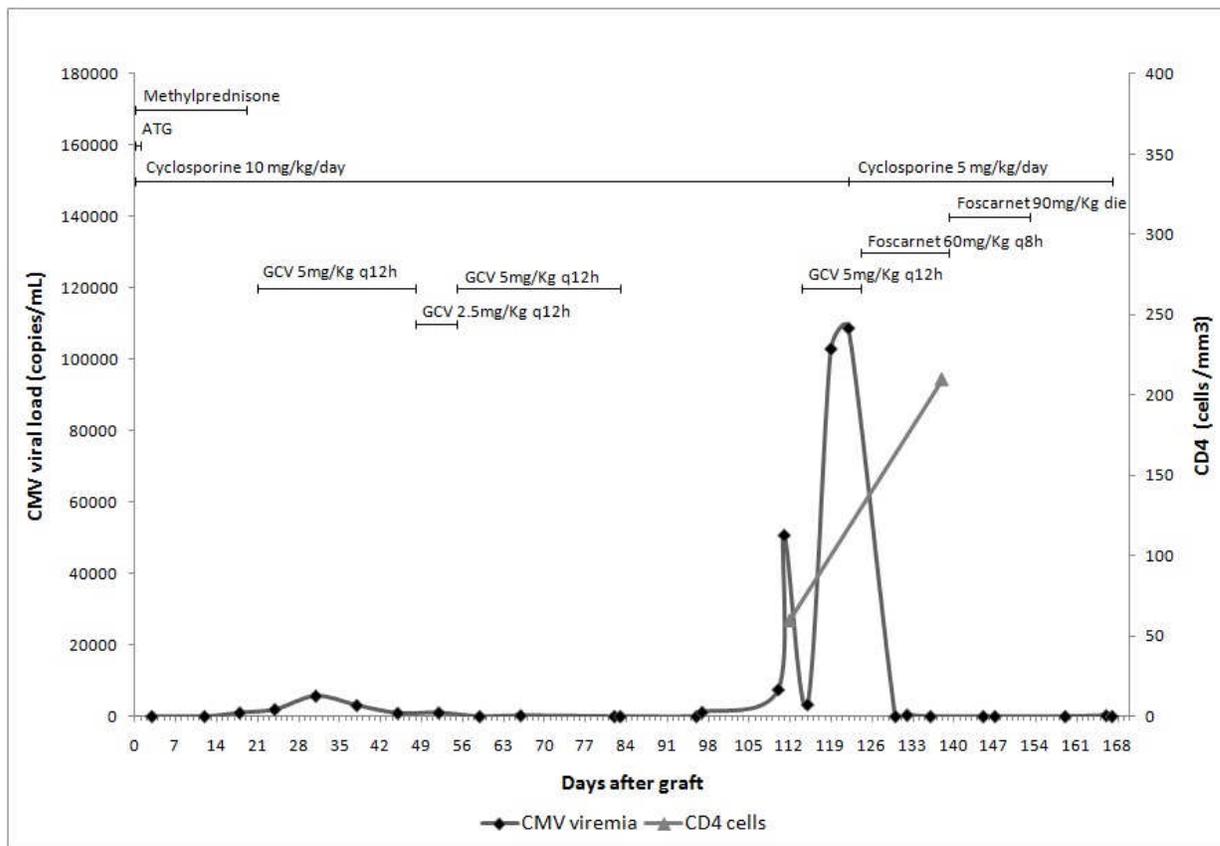
## CASE REPORT

Cytomegalovirus (CMV) infection remains a major cause of transplant-associated morbidity and mortality, particularly in allogeneic hematopoietic stem cell transplant (HSCT) recipients.<sup>1-2</sup> It has been reported in 23% of unrelated umbilical cord blood (UCB) recipients<sup>3</sup>, the most important risk factors being the recipient's CMV-seropositive status, immunosuppression state and the occurrence of graft-versus-host disease.<sup>1</sup> CMV disease can be prevented by either antiviral prophylaxis or preemptive therapies.<sup>4-5</sup> Ganciclovir (GCV), a synthetic nucleoside analogue with in vitro activity against most human herpes virus, including CMV, is the drug of choice for treatment in HSCT recipients<sup>6</sup> but selection of viral mutants may lead to treatment failure.<sup>7</sup> However, drug resistance is not always the cause of treatment failure.

We report the case of a 4-year-old boy, treated at Sainte-Justine Hospital for a condition similar to Wiskott-Aldrich disease with severe eczema and congenital thrombocytopenia. For this reason, he was given two umbilical cord blood

transplantations (UCBT). As the first resulted in graft rejection, he had a second UCBT two years later for which he was given a conditioning regimen including busulfan and cyclophosphamide, as well as anti-thymocyte globulin, methylprednisone and cyclosporine as graft-versus-host disease prophylaxis (Figure 1). Being seropositive for CMV, he was monitored with weekly CMV Real Time quantitative PCR (RT-PCR) in whole blood (limit of detection: 300 copies/mL). On day 18 after the second UCBT, the patient was asymptomatic and the viral load was estimated at 1025 copies/mL. Intravenous (IV) GCV 5mg/kg twice daily was started as preemptive therapy. The viremia returned to undetectable levels after 30 days of treatment and GCV was discontinued on day 83 post-UCBT after two undetectable CMV viral load tests two weeks apart. On day 97, the patient presented a second asymptomatic CMV reactivation (viral load: 1166 copies/mL) for which he was initially treated with IV GCV 5mg/kg twice daily (Figure 1).

**FIG. 1** Temporal Course



**Legend:** ATG, anti-thymocyte globulin; CMV, cytomegalovirus; GCV, ganciclovir

The boy was still immunosuppressed as he was receiving cyclosporine (10 mg/kg/day), with a CD4 count of  $60 \times 10^6$  cells/L. However, unlike the first episode, CMV viral load continued to rise despite therapy and reached a peak viremia of 109000 copies/mL on day 122, after 11 days of treatment. A GCV resistant strain was then suspected. Therefore, GCV was replaced by IV foscarnet 60 mg/kg every 8 hours and cyclosporine dosage was reduced to 5 mg/kg/day. A week later, on day 130, CMV viral clearance was obtained with undetectable viral load. Simultaneously, we observed a rise in the CD4 cell count to  $260 \times 10^6$  cells/L. A genotypic assay on the CMV strains from day 119 and day 122 was performed based on PCR-amplification and DNA sequencing of two viral genes: UL54 and UL97. By comparing their DNA sequence with a list of common mutations known to confer GCV resistance<sup>8</sup>, we found that these mutations were absent suggesting this CMV strain should be

susceptible to GCV. Using high performance liquid chromatography (HPLC) technique with diode array detection, GCV levels were also measured in blood samples collected while the patient was at steady state with the recommended dosage of 5 mg/kg every 12 hours. GCV area under the curve over 12h ( $AUC_{0-12}$ ), peak and trough levels were 16.50 h.µg/mL, 5.54 µg/mL and 0.15 µg/mL, respectively. Measurements of trough levels were also done after reinitiating GCV treatment on day 97 and showed trough values between 0.13 and 0.20 µg/mL.

GCV is a potent antiviral drug that is primarily active against CMV, with a 50% inhibitory concentration ( $IC_{50}$ ) ranging from 0.4 to 1.5 µg/ml. Oral bioavailability of GCV is poor (<10%) thus only intravenous formulations are used for preemptive treatment.<sup>9</sup> GCV is eliminated by renal excretion and dose reduction is required in patients with altered renal function. GCV has led to reduction of CMV disease in

HSCT recipients.<sup>4</sup> The most common methods consist of universal prophylaxis or preemptive strategies, which have been shown to be equally effective.<sup>5</sup> According to the preemptive strategy used at our institution, a weekly CMV RT-PCR is performed and IV GCV (5 mg/kg every 12h) is started as soon as CMV viremia is detected (300 copies/mL). This regimen is pursued until two consecutive negative CMV RT-PCR tests.<sup>6</sup>

Although rare, CMV antiviral resistance has resulted in treatment failure in children with HSCT.<sup>7</sup> GCV-resistant CMV isolates may emerge rapidly in bone marrow transplant recipients especially after receiving antiviral prophylaxis.<sup>10</sup> Increase in viral load under GCV treatment is often suggested as a marker of resistance and a modification in therapy should be considered.<sup>11</sup> In this case, suspicion of a resistant CMV strain led us to change treatment from GCV to foscarnet.<sup>12</sup> In order to assess CMV drug resistance, we used genotypic assays that detect mutations in viral UL97 or UL54 genes, known to be associated with drug resistance.<sup>13</sup> In our case, these mutations were absent suggesting this CMV strain should have been susceptible to GCV. However, we could not confirm it by phenotypic assay due to failure to isolate the virus. Therefore, phenotypic resistance due to novel mutations cannot be excluded.

Another hypothesis for such GCV treatment failure was that drug plasma concentrations failed to achieve therapeutic levels despite recommended dosages. Intravenous administration of a 5 mg/kg dose of GCV in adults produces peak plasma levels of 8 to 11 µg/mL.<sup>14-15</sup> Similar values were observed in paediatric kidney transplant recipients after IV GCV administration (5 mg/kg every 12h).<sup>9</sup> Mean plasma peak and trough concentrations were found to be 11,7 µg/mL and 0,84 µg/mL, respectively, with a mean AUC<sub>0-12</sub> of 42.3 ± 17.5 µg.h/mL and were above those required to inhibit 50% of viral replication *in vitro* (IC<sub>50</sub>). These concentrations were also much higher than those measured in our patient. Whereas IC<sub>50</sub> has been determined, there are no firm data correlating *in vitro* susceptibility and *in vivo* pharmacokinetic parameters to predict efficacy. Since no therapeutic concentrations have been formally established, systematic therapeutic drug monitoring cannot be recommended at the

moment. However, some authors suggest that GCV treatment could be monitored with trough levels.<sup>15</sup> The recommended target range for trough levels is 0.5-2.0 µg/ml, higher than trough concentration measured in our patient. Indeed, Piketty et al. have reported increased risk of CMV progression in AIDS patients with GCV trough plasma levels below 0.6 µg/mL.<sup>16</sup> Moreover, in solid organ transplant recipients, systemic GCV exposure determined by AUC<sub>0-24</sub> has been correlated with CMV viral load showing viremia to be suppressed when AUC<sub>0-24</sub> reached 40 to 50h.µg/mL<sup>17</sup>, values which are much higher than those observed in our patient.

The real cause for the low systemic GCV exposure observed in our patient remains unclear. Because GCV is eliminated almost exclusively unchanged in the urine, renal function may contribute to inter- and inpatient variability of GCV levels in children.<sup>18</sup> In a study of nine transplant recipients with a mean age of 5.6 years and receiving IV GCV 5 mg/kg every 12h, Vethamutu et al observed trough levels ranging from undetectable to 0.84 µg/ml (median of 0.20 µg/ml). For some renal transplant children who received a kidney from a parent, they suggested that the large renal reserve of the parental kidney may account for the higher glomerular filtration rate and, thus higher GCV renal clearance. Population pharmacokinetics of GCV following oral administration of valganciclovir in paediatric renal transplant patients identified both creatinine clearance and bodyweight as the most important factors influencing GCV clearance. Since GCV administration in our patient was solely based on a bodyweight-normalized dosage, this may have contributed to its reduced exposure to GCV. While some indicate that dosing per body surface area may have advantages over dosing per kg body weight, others claim that may lead to underexposure to GCV.<sup>18</sup> Factors that could explain low GCV plasma levels include drug-drug interaction. However, at present, there are no such interactions known to exist that could alter GCV levels and explain the low systemic exposure in our patient.

This case illustrates a GCV treatment failure despite an apparent drug-susceptible CMV strain. We hypothesise that the low systemic GCV exposure along with the profound state of immunosuppression could have promoted CMV

progression and treatment failure. Therefore, given the inter- and intra-patient pharmacokinetic variability described in the literature, GCV plasma levels monitoring may have a role. Pharmacokinetic/ pharmacodynamic parameters could be a useful clinical tool when faced with CMV treatment failure in highly immunocompromised paediatric patients.

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