

Artesunate as a Potent Antiviral Agent in a Patient with Late Drug-Resistant Cytomegalovirus Infection after Hematopoietic Stem Cell Transplantation

Michael Y. Shapira,¹ Igor B. Resnick,¹ Sunwen Chou,³ Avidan U. Neumann,² Nell S. Lurain,⁴ Thomas Stamminger,⁵ Orit Caplan,¹ Niveen Saleh,¹ Thomas Efferth,⁶ Manfred Marschall,^{5,a} and Dana G. Wolf^{1,a}

¹Hadassah Hebrew University Medical Center, Jerusalem, and ²Bar-Ilan University, Ramat-Gan, Israel; ³VA Medical Center and Oregon Health and Science University, Portland, Oregon; ⁴Rush University Medical Center, Chicago, Illinois; and ⁵Virological Institute of the University Hospital Erlangen, University of Erlangen-Nuremberg, and ⁶German Cancer Research Center, Heidelberg, Germany

This is the first report of treatment of cytomegalovirus infection with artesunate, for a stem cell transplant recipient with a newly identified foscarnet-resistant and ganciclovir-resistant DNA polymerase L776M mutation. Artesunate treatment resulted in a 1.7–2.1-log reduction in viral load by treatment day 7, with a viral half-life of 0.9–1.9 days, indicating a highly effective block in viral replication.

Despite diagnostic and therapeutic advances, cytomegalovirus (CMV) infection has remained a significant complication after hematopoietic stem cell transplantation (HSCT). The widespread use of preemptive antiviral therapy has reduced the occurrence of early CMV infection; however, the development of late infection is increasingly recognized [1, 2].

Major risk factors for late infection include haploidentical HSCT, graft-versus-host disease, and high-dose steroid treatment. Delayed immune reconstitution could allow for continued virus replication, with emergence of drug-resistant strains under drug pressure [1, 2].

All currently available anti-CMV drugs—including ganciclovir,

foscarnet, and cidofovir—target the viral DNA polymerase. Their use is limited because of toxicity, low oral bioavailability, and drug resistance. Ganciclovir resistance results mainly from mutations in the CMV UL97 kinase [3, 4] and, less frequently, from mutations in the viral DNA polymerase (*pol*). Foscarnet-resistance and cidofovir-resistance mutations are found in the *pol* gene, and mutations that confer cross-resistance to multiple drugs have been reported [3–5].

The epidemiological shift of CMV infection, requiring repeated and prolonged treatment courses, creates an increasing need for new antiviral drugs. The benzimidazole L-riboside maribavir, which targets the UL97 kinase, holds promise as an alternative treatment for infection [6]. Recently, the antimalaria drug artesunate was shown to be an effective inhibitor of human CMV infection in vitro [7]. Its antiviral activity was further demonstrated in a rat model of CMV infection [8]. Although its mechanism of action is not yet understood, the mechanism differs from that of the currently available drugs and may be mediated by inhibition of cellular activation pathways that play an essential role in virus replication [7, 8]. Importantly, the extensive use of artesunate in patients with malaria has not been associated with significant adverse effects. These findings raise the possibility that artesunate represents a safe therapeutic option for treatment of CMV infection. Here, we describe, to our knowledge, the first clinical use of artesunate for the treatment of CMV infection in a patient who developed drug-resistant infection during preemptive antiviral therapy after HSCT.

Patient and methods. The patient is a 12-year-old boy with X-linked adrenoleukodystrophy who received haploidentical T cell-depleted hematopoietic stem cells from his father. The patient's conditioning regimen included fludarabine busulfex, antithymocytic globulin, and total body irradiation. Starting from day 15 after transplantation, CMV viremia was noted. The patient was treated with foscarnet (induction dose, 60 mg/kg 3 times per day) because of myelosuppression, which precluded ganciclovir therapy, with initial favorable virological response (see figure 1 for the patient's detailed viral load measurements and treatment course). From day 119, a recurrent episode of CMV infection was noted, with continuous increase in viral load during preemptive foscarnet therapy (maintenance dose, 120 mg/kg/day, followed by an induction dose), accompanied by fever and myelosuppression. Bone marrow failure persisted despite the patient's receipt of a second stem cell transplant. Treatment attempts with ganciclovir (induction dose, 5 mg/kg 2 times per day) and cidofovir (5 mg/kg/week) combined with

Received 12 November 2007; accepted 9 January 2008; electronically published 20 March 2008.

^a M.M. and D.G.W. contributed equally as senior authors.

Reprints or correspondence: Dr. Dana G. Wolf, Clinical Virology Unit, Dept. of Clinical Microbiology & Infectious Diseases, Hadassah Hebrew University Medical Center, Jerusalem, Israel 91120 (dana.wolf@ekmd.huji.ac.il).

Clinical Infectious Diseases 2008;46:1455–7

© 2008 by the Infectious Diseases Society of America. All rights reserved.

1058-4838/2008/4609-0023\$15.00

DOI: 10.1086/587106

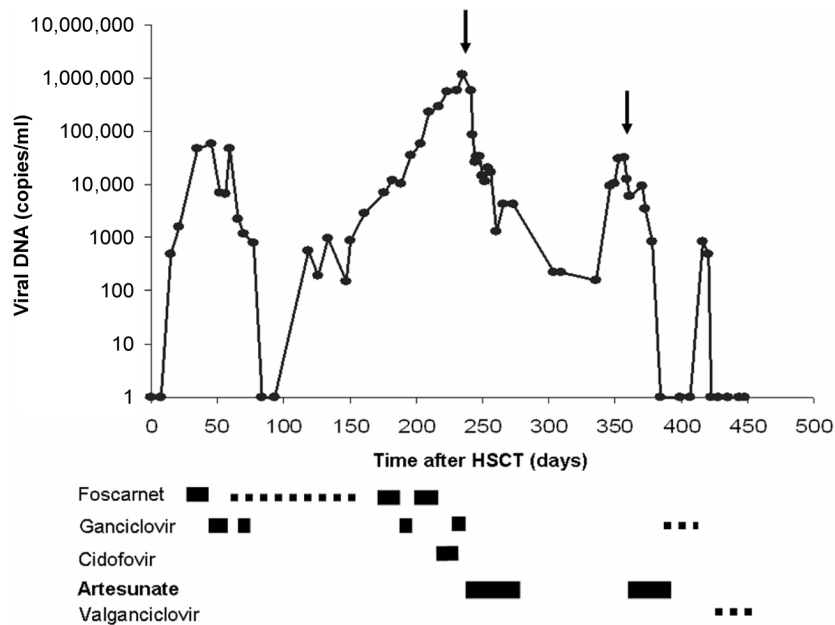


Figure 1. Cytomegalovirus DNA load kinetics and antiviral treatment in a patient with recurrent cytomegalovirus infection during preemptive treatment. *Thick line*, induction treatment; *dotted line*, maintenance treatment; *arrows*, time of initiation of artesunate treatment. HSCT, hematopoietic stem cell transplantation.

intravenous immunoglobulin were unsuccessful, and the viral DNA load increased to 1.15×10^6 copies/mL. At this point, treatment with all conventional anti-CMV drugs was discontinued, and oral treatment with artesunate (100 mg/day; approved by the institutional ethics committee) was initiated on a compassionate basis. A favorable response was noted, with rapid reduction in viral load (figure 1) and improved hematopoiesis within 10 days. No adverse effects developed during 30 days of treatment. There was no rebound of viremia for 76 days after completion of therapy. On day 346, 15 days after the patient received a third haploidentical transplant from his mother, he had a recurrent episode of viremia. A second treatment course with artesunate resulted in a rapid decrease in the peripheral blood viral load; however, CMV retinitis was subsequently diagnosed (day 378) during treatment. Combined treatment with systemic artesunate and intravitreal ganciclovir resulted in resolution of the retinitis, with sustained virological response (observed until day 665).

Quantification of CMV DNA. Viral DNA load in whole blood samples (genome copies per milliliter) was determined by real-time PCR assay, as described elsewhere [9], with the use of primers and a probe derived from the CMV gB gene. The assay demonstrated a linear quantitation over a 6-log range, with a sensitivity of 50 copies/mL. The decay half-life ($T_{1/2}$) of CMV DNA load was calculated on the basis of the best-fit curve by use of the equation $(\ln 2)/a$, where a is the growth constant [10].

Amplification and sequencing of the CMV UL97 and pol

genes. Direct PCR sequencing was performed, as described elsewhere [2], by using overlapping primer pairs encompassing nucleotides 1207–1979 (*UL97*) and 900–3000 (*pol*). These regions include all of the known resistance mutations.

Marker transfer experiments. To confirm the resistance phenotype of the observed *pol* mutation, it was transferred into the reference drug-sensitive strain T2211, which contains a secreted alkaline phosphatase reporter gene, as described elsewhere [5]. Antiviral drug susceptibility of the recombinant plaque-purified virus was assayed by determining the drug concentration required to reduce the supernatant secreted alkaline phosphatase activity by 50% (IC₅₀), with use of 13–25 replicate experiments per drug.

Results and discussion. With the improved survival after HSCT, change in immunosuppressive regimens, and longer antiviral drug exposure, drug resistance has become a growing problem in this context [1, 2, 11]. The consistent increase in viral load during foscarnet treatment in a high-risk patient after haploidentical T cell-depleted HSCT suggested the presence of resistant virus. Direct genotypic analysis revealed no mutations in the *UL97* gene. Mixed wild-type and mutant *pol* gene viral populations containing the *pol* L776M amino acid substitution were found after cumulative foscarnet therapy for 116 days. L776M is located in the conserved region VI of *pol*, in proximity to mutations at codons 756, 781, and 787, which are known to be associated with foscarnet resistance [3, 4]. Indeed, recombinant phenotyping revealed a 3.5-fold and a 2.5-fold increase of the L776M mutant's IC₅₀ for foscarnet and ganci-

clovir, respectively, and no increase for cidofovir, confirming the foscarnet-resistant and borderline ganciclovir-resistant phenotype of this new mutation. In cell culture, growth of the L776M mutant was slightly retarded, as was also observed with some other *pol* mutations [5]. However, the L776M variant persisted for >100 days after cessation of foscarnet therapy, suggesting its in vivo stability.

Importantly, the institution of artesunate therapy resulted in a rapid decrease in viral load. Although there was no live clinical isolate available for in vitro susceptibility testing with artesunate, a 1.7–2.1-log reduction of the patient's viral load by day 7 was demonstrated in the repeated treatment courses. This extent of response was comparable to that observed during the patient's initial ganciclovir treatment (1-log reduction by day 7; figure 1) and to the previously reported response to ganciclovir and foscarnet [10]. Furthermore, analysis of CMV load kinetics during artesunate treatment courses revealed a short viral decay ($T_{1/2}^1$, 0.9–1.9 days), a range similar to that shown during ganciclovir therapy [10]. Although the mechanism of artesunate antiviral activity is not yet understood, these consistent findings argue for a highly effective block of virus production and are in line with the reported in vitro results. Interestingly, frequent sampling during the first artesunate treatment episode identified for the first time, a biphasic CMV decline curve, where a first phase of rapid decline ($T_{1/2}^1$, 0.9 days) was followed by a slower decline phase ($T_{1/2}^2$, 8 days). On the basis of detailed viral dynamics studies of hepatitis C and B viruses [12], one could speculate that artesunate blocks 95%–99% of CMV virion production or release from infected cells; thus, the initial rapid decline corresponds to the clearance rate of free viral particles, whereas the second phase could represent the loss rate of infected cells.

In the patient described here, artesunate was well tolerated over prolonged treatment courses and induced a stable suppression of viral replication. In this regard, drug resistance is not expected to develop during artesunate therapy, because the proposed antiviral mechanism involves cellular rather than viral targets.

The development of retinitis during artesunate treatment could reflect limited penetration into the eye. Although artesunate has been shown to be effective in the treatment of cerebral malaria, implicating penetration into the CNS, higher

oral doses may be required to achieve a local concentration above the IC50.

In conclusion, in this case, artesunate proved to be a highly effective inhibitor of CMV replication. Additional studies are needed to examine the role of artesunate in the treatment of CMV infection in transplant recipients. A clinical trial of preemptive artesunate treatment in HSCT recipients is currently under way at our center.

Acknowledgments

Financial support. Israeli Ministry of Science, Israel Science Foundation, and National Institutes of Health (AI39938).

Potential conflict of interest. All authors: no conflicts

References

1. Boeckh M, Fries B, Nichols WG. Recent advances in the prevention of CMV infection and disease after hematopoietic stem cell transplantation. *Pediatr Transplant* **2004**; 8(Suppl 5):19–27.
2. Wolf DG, Lurain NS, Zuckerman T, et al. Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients. *Blood* **2003**; 101:463–5.
3. Gilbert C, Boivin G. Human cytomegalovirus resistance to antiviral drugs. *Antimicrob Agents Chemother* **2005**; 49:873–83.
4. Baldanti F, Lurain N, Gerna G. Clinical and biologic aspects of human cytomegalovirus resistance to antiviral drugs. *Hum Immunol* **2004**; 65: 403–9.
5. Scott GM, Weinberg A, Rawlinson WD, Chou S. Multidrug resistance conferred by novel DNA polymerase mutations in human cytomegalovirus isolates. *Antimicrob Agents Chemother* **2007**; 51:89–94.
6. Wang LH, Peck RW, Yin Y, Allanson J, Wiggs R, Wire MB. Phase I safety and pharmacokinetic trials of 1263W94, a novel oral anti-human cytomegalovirus agent, in healthy and human immunodeficiency virus-infected subjects. *Antimicrob Agents Chemother* **2003**; 47:1334–42.
7. Efferth T, Marschall M, Wang X, et al. Antiviral activity of artesunate towards wild-type, recombinant, and ganciclovir-resistant human cytomegaloviruses. *J Mol Med* **2002**; 80:233–42.
8. Kaptein SJ, Efferth T, Leis M, et al. The anti-malaria drug artesunate inhibits replication of cytomegalovirus in vitro and in vivo. *Antiviral Res* **2006**; 69:60–9.
9. Boeckh M, Huang M, Ferrenberg J, et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. *J Clin Microbiol* **2004**; 42:1142–8.
10. Emery VC, Cope AV, Bowen EF, Gor D, Griffith PD. The dynamics of human cytomegalovirus replication in vivo. *J Exp Med* 1999; 190: 177–82.
11. Marfori JE, Exner MM, Marousek GI, Chou S, Drew WL. Development of new cytomegalovirus UL97 and DNA polymerase mutations conferring drug resistance after valganciclovir therapy in allogeneic stem cell recipients. *J Clin Virol* **2007**; 38:120–5.
12. Neumann AU, Lam N, Dahari H, Gretch DR, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon- α therapy. *Science* **1998**; 282:103–7.