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Short Communication

Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation

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ABSTRACT

The anti-malaria drug artesunate has been shown to be an effective inhibitor of cytomegalovirus (CMV) *in vitro*, in an experimental animal model, and in a recent single-case clinical use. In this first case-series of 6 stem cell transplant recipients who received preemptive artesunate treatment for CMV infection, we have examined the viral kinetics following institution of artesunate, and employed first-phase viral kinetics studies to calculate its antiviral effectiveness. Two patients demonstrated a rapid 0.8–2.1 log viral load decline by 7 days, with a viral decay half-live of 0.9–1.9 days. Four patients demonstrated a continued yet stalled viral growth slope during treatment. No adverse events were noted in treatment courses of up to 28 days. Overall, a divergent antiviral efficacy was revealed, ranging from 43% to 90%, which appeared to be primarily dependent on the virus baseline growth dynamics. Further dose escalation studies are needed to examine the role of artesunate in the treatment of CMV infection in the transplantation setting.

Despite the availability of effective antiviral therapy and reliable diagnostic assays, cytomegalovirus (CMV) has remained a significant complication after hematopoietic stem cell transplantation (HSCT) (Boeckh and Ljungman, 2009).

All currently available anti-CMV drugs, including ganciclovir, foscarnet, and cidofovir, target the viral DNA polymerase. Their use is limited by toxicity, low oral bioavailability (with the exception of the oral prodrug valganciclovir), and drug resistance (Boeckh and Ljungman, 2009; Lurain and Chou, 2010). These limitations, along with the epidemiological shift of CMV infection, requiring repeated and prolonged treatment courses, create an increasing need for new, effective, and better-tolerated antiviral drugs.

The benzimidazole L-riboside maribavir which targets the UL97 kinase has held promise as an alternative treatment for CMV infection (Winston et al., 2008). However, recent results from a phase III study have not revealed a significant impact on the rate of CMV disease following HSCT.

Recently, the anti-malaria drug artesunate has been shown to be an effective inhibitor of human CMV *in vitro* and in an experimental animal model (Efferth et al., 2002, 2008; Kaptein et al., 2006; Schnepf et al., 2010). Importantly, the extensive use of artesunate in malaria patients has not been associated with significant adverse effects (Efferth et al., 2008). These characteristics raise the possibility that artesunate could represent a safe therapeutic option for CMV infection in immunocompromised patients.

We have recently described the successful clinical use of artesunate for the treatment of CMV in a single patient who developed drug-resistant infection during preemptive antiviral therapy after HSCT (Shapira et al., 2008).

Here we report the first case-series of 6 HSCT recipients who received preemptive artesunate treatment for CMV infection; utilizing frequent viral load monitoring, we have examined the viral kinetics following institution of artesunate, and further employed first-phase viral kinetics studies to determine its antiviral effectiveness.

Of the 6 patients, one (Table 1, Patient #1) received preemptive artesunate treatment on a compassionate basis due to increasing viral load with emergence of multi-drug-resistant L776M DNA

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Table 1
Demographic, clinical, and virological characteristics of HSCT recipients receiving preemptive artesunate treatment

Pt #	Gender/ age, y	Underlying disease	Type of HSCT	Time of viremia post HSCT, days	Baseline Viral load, (copies/ml)/ DT (days)/S_0	viral load kinetics at 7 days of artesunate treatment	Calculated antiviral effectiveness (ε) (%)[a]	Artesunate treatment outcome	Clinical & virological outcome following treatment[b]
1[c]	M/12	X-linked adrenoleuk-odystrophy	Haploidentical T-cell-depleted	147; 357	1.15×10^6; 32,500/7.6	1.7–2.1 log decline; *T½* 0.9–1.9 days; D 0.98	90	Completed 56 days (2 treatment courses)	Asymptomatic; no rebound viremia for 76d
2	F/66	Diffuse large B cell lymphoma	Autologous	28	2,500/1.8	0.8 log decline; *T½* 1.8 days; D 0.75	82	Completed 28 days	Asymptomatic; no rebound viremia for 1 y
3	M/42	Acute lymphocytic leukemia	Mismatched	90	50,000/1.4/0.56	Stabilization; S_A 0.25	57	Discontinued at 7 days	CMV disease; Continued deterioration with foscarnet
4	M/65	Acute myelocytic leukemia	Matched unrelated	81	24,000/1.8/0.39	Stabilization; S_A 0.06	84	Discontinued at 7 days	Asymptomatic; Rapid response to ganciclovir
5	M/46	Acute myelocytic leukemia	haploidentical non-T-cell-depleted	35	12,400/0.98/0.71	0.86 log icrease; S_A 0.28	60	discontinued at 7 days	Asymptomatic; Rapid response to ganciclovir
6	F/52	Acute myelocytic leukemia	Matched unrelated	34	12,000/1.3/0.52	0.65 log icrease; S_A 0.30	43	Discontinued at 7 days	Asymptomatic; Rapid response to ganciclovir

DT indicates doubling time; S_0 and S_A represent the exponential vial growth slopes before and immediately after the initiation of artesunate treatment (relevant for patients #3–6).; *T½* indicates viral decay half life; D represents the magnitude of the first-phase viral decline in log10 base (relevant for patients #1,2)

[a] Calculated as indicated in "Patients and Methods" section: for patients #1,2, $\varepsilon = 1 - 10^{-D}$, for patients #3–6, $\varepsilon = 1 - S_A/S_0$

[b] Following completion or discontinuation of artesunate treatment.

[c] Patient 1 had received 2 courses of preemptive artesunate on a compassionate basis as previously reported (7).

polymerase (*pol*) mutant (Shapira et al., 2008). Five patients (Table 1, patients #2–6) were enrolled in a pilot study aimed to evaluate the safety and efficacy of artesunate in preemptive treatment of CMV infection in HSCT recipients >18 years, who had detectable CMV DNA with ⩾2000 DNA copies/ml. Eligible patients in this study received preemptive treatment with oral artesunate (Dafra Pharma, Belgium; 200 mg × 2/day for one day, followed by 100 mg × 1/day for 28 days). CMV DNA load was determined on days 0, 3, 7, 14, 21, 28 of treatment by real-time PCR assay as described (Boeckh et al., 2004). Artesunate was discontinued upon lack of clear virological response (defined as viral load increase or decrease by <0.5 log DNA copies/ml) on days 7, 14 and 21. These strict criteria were employed to prevent deterioration during treatment. (For more details of the study design, see ClinicalTrials.gov NCT00284687; The study was approved by the Institutional and National Ethics Committees and performed according to the Declaration of Helsinki, Good Clinical Practice guidelines, and the Human-Experimentation Guidelines of the Israeli Ministry of Health. All participants gave written informed consent).

The viral doubling time and decay half life (*T½*) were calculated on the basis of the best-fit curve by use of the equation (ln2)/*a*, where "*a*" is the logarithmic slope (Emery et al., 1999). The antiviral effectiveness of artesunate (ε) was calculated in two ways: For patients with an early decline in viremia, we used the magnitude of the first-phase viral decline (D in log10 base) in the equation $\varepsilon = 1 - 10^{-D}$ (Neumann et al., 1998). For patients with delayed virological response we used the equation $\varepsilon = 1 - S_A/S_0$ where "S_A" and "S_0" represent the exponential vial growth slopes immediately after and before the initiation of treatment, respectively, according to the model developed by Neumann et al. for viral kinetics (Neumann et al., 1998).

Two patients (patients #1 and #2; Table 1 and Fig. 1) successfully completed 28 days of artesunate treatment. These patients exhibited a rapid decline in viral load, with 0.8–2.1 log decline by 7 days of treatment and a viral *T½* of 0.9–1.9 days (see Table 1). These viral decay kinetics are consistent with those previously reported for ganciclovir and foscarnet (Emery et al., 1999). Based on the first-phase viral decline, a high antiviral effectiveness (82–90%) was calculated.

In the four remaining patients (patients #3–6), artesunate was discontinued at 7 days of treatment, in accordance with the study criteria, due to the development of CMV disease (patient #3) or lack of clear virological response (patients #4–6). While no viral load decline was observed in these patients by 7 days of artesunate treatment, all four demonstrated a stalled viral growth slope during treatment (S_A) when compared to baseline growth rate (S_0) (see Table 1, Fig. 1). These viral dynamics revealed some, albeit variable and limited antiviral effectiveness, ranging from 43% to 84% in patients #3–6 (Table 1). Notably, three of these patients (patients #4–6) rapidly responded to ganciclovir (Fig. 1), with a 1.4–1.8 log decline at day 7 of ganciclovir treatment – suggesting a lower antiviral efficacy of artesunate, in its current dosing regimen, when compared to ganciclovir. However, it is important to interpret these findings with caution, as an initial lag phase of virologic response has been also reported in high-risk patients receiving ganciclovir (Buyck et al., 2010; Nichols et al., 2001). Thus, the small number of patients, and the early discontinuation of artesunate in 4 of the 6 patients preclude conclusions regarding its relative antiviral efficacy in heavily immunosuppressed patients.

We sought to elucidate the basis for the enhanced response to artesunate in patients #1 and #2 when compared to patients #3–6; The rapid viral load decrease in patient #2 could be attributed to an earlier reconstitution of the host immune response following autologous HSCT, along with a low baseline viral load (Table 1), a well-known predictor of viral eradication. Yet, these factors could not account for the effective block of viral replication by artesunate in patient #1, who had received a T-cell depleted haploidentical HSCT, and exhibited a high baseline viral load of $>10^6$ copies/ml. Limited analysis of artesunate and dihydroartemisinin concentrations in available plasma samples by the liquid chromatography–mass spectrometry method (Van Quekelberghe et al., 2008), did not show significant differences between the patients (data not shown), and thus could not explain the different response rates. Importantly, patient #1 harbored a mutant virus containing a

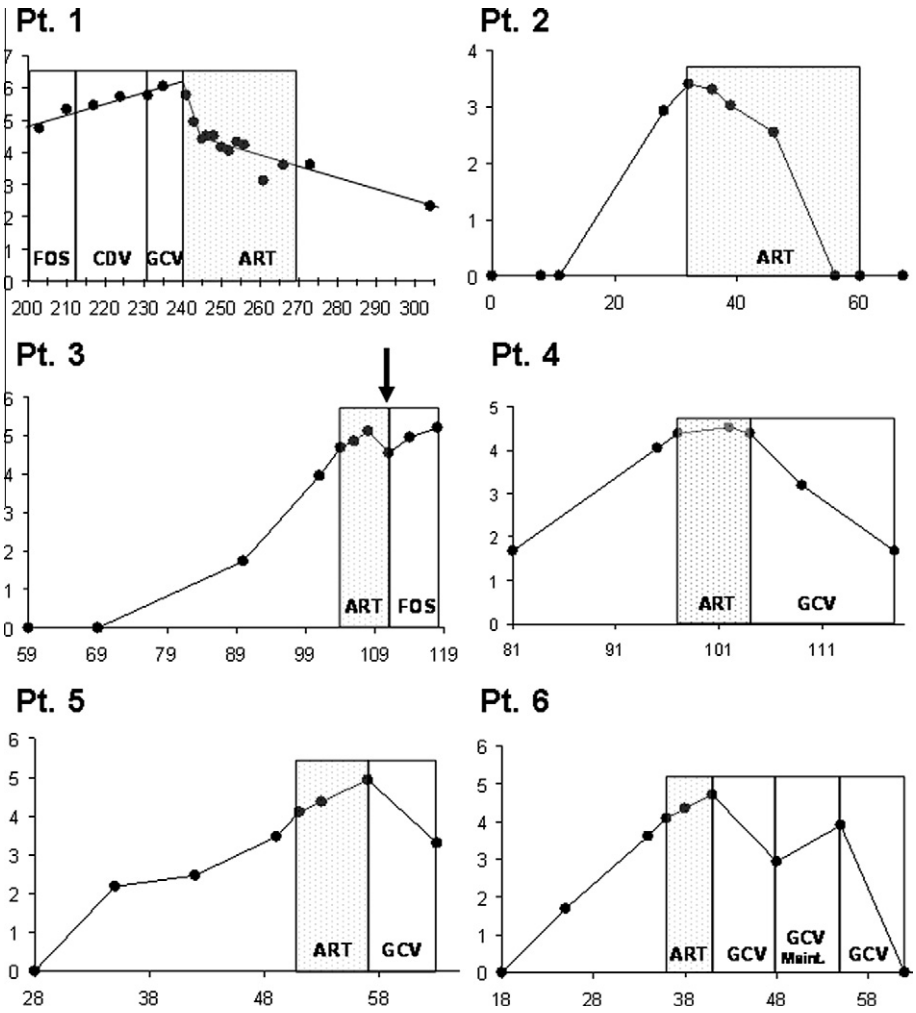

Fig. 1. CMV DNA load kinetics and antiviral treatment in HSCT recipients receiving preemptive artesunate treatment. Y axis values, log DNA copies/ml; X axis values, days after HSCT; arrow, time of disease development; ART, artesunate; GCV, ganciclovir; CDV, cidofovir; FOS, foscarnet; Maint., maintenance treatment.

pol L776M substitution, previously shown to confer a slight replication defect in cell culture (Shapira et al., 2008). Furthermore, analysis of the mutant-virus baseline growth kinetics revealed a slow *in vivo* growth rate, with a prolonged doubling time of 7.6 days (Table 1). Thus, the attenuated growth of the mutant could have accounted for the enhanced response to artesunate in this case.

To further examine if the L776M *pol* mutation confirmed increased artesunate susceptibility, we compared the artesunate IC50 of a recombinant mutant strain, containing the L776M mutation and a wild type control virus, using a secreted alkaline phosphatase (SEAP) activity assay as described (Scott et al., 2007; Shapira et al., 2008). The artesunate IC50 of the mutant (1.68 ± 0.30 μM) was slightly lower than that of the wild type (2.53 ± 0.96 μM), as revealed in 14 replicate assays spread over 4 setup dates. Further testing with artemisinin demonstrated similar trend for increased susceptibility of the mutant (IC50 14.6 ± 4.1 μM versus 35.3 ± 11.1 μM, in 4–7 replicates over 2 setup dates). These findings may be relevant towards the future use of artesunate in patients with drug-resistant CMV mutants, especially those demonstrating reduced fitness. Experiments are currently underway to examine its activity against various drug-sensitive and drug-resistant clinical isolates.

In view of the limited and diverse response to artesunate as demonstrated herein, a prophylactic rather than preemptive treatment study design, and a dose escalation study with close pharmacokinetic monitoring should be considered in future trials. The safety of this approach is supported by the low rate of toxicity reported for artesunate (Efferth et al., 2008), and the lack of adverse events in the 6 HSCT recipients treated over 7–56 days. We believe that the favorable safety profile of artesunate, along with its unique mechanism of antiviral activity, involving inhibition of early replication steps (Efferth et al., 2002, 2008; Kaptein et al., 2006), will make it an attractive candidate for combination antiviral drug therapy. In this regard, artesunate, has been shown to exert an additive *in vitro* antiviral effect when combined with any of the currently available anti-CMV drugs (Efferth et al., 2008; Kaptein et al., 2006). Combined ganciclovir–artesunate treatment could potentially allow for reduced dosage of ganciclovir and thus limit its bone marrow toxicity.

In conclusion, these first-phase viral kinetics studies in 6 HSCT recipients who received preemptive artesunate treatment revealed a divergent antiviral efficacy of artesunate, ranging from 43% to 90%, which appeared to be primarily dependent on the virus baseline growth dynamics. Further dose escalation studies are needed to examine the role of artesunate in the treatment of CMV infection in the transplantation setting.

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