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Short Communication

Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus

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ABSTRACT

Cytomegalovirus (CMV) strains resistant to ganciclovir, cidofovir and/or foscarnet were genotypically and phenotypically characterised in two haematopoietic stem cell transplant recipients and three solid-organ transplant recipients with CMV disease. The anti-malaria drug artesunate led to a favourable virological and clinical response in three cases with mild CMV diseases (fever and neutropaenia) but was ineffective in two fatal CMV diseases with lung involvement in spite of a decrease in the CMV DNA load in blood and bronchoalveolar fluid.

Multidrug resistance of cytomegalovirus (CMV) against the commercially available antiviral drugs (i.e. valaciclovir (VACV), ganciclovir (GCV)/valganciclovir (VGCV), cidofovir (CDV) and foscarnet (FOS)) characterised by amino-acid substitutions within the viral phosphotransferase (UL97) and/or the viral DNA polymerase (UL54) is a difficult challenge in haematopoietic stem cell or solid-organ transplantations (HSCT, SOT) (Boeckh and Ljungman, 2009; Kotton et al., 2010; Chou, 2008; Scott et al., 2007). The anti-malarial drug artesunate has demonstrated *in vitro* antiviral activity against multidrug-resistant CMV (Chou et al., 2011; Härter and Michel, 2012; He et al., 2012). Currently, clinical reports of treating drug-resistant CMV infections with artesunate are rare and controversial (Shapira et al., 2008; Lau et al., 2011; Wolf et al., 2011). Here we report five cases of virologically-confirmed drug-resistant CMV diseases in HSCT and SOT treated with artesunate with either favourable (three cases) or unfavourable (two cases) outcomes.

1. Reports of three cases with favourable outcomes

A 47-year-old female patient received a phenoidentical HSCT in 2007 for acute myeloid leukaemia (Table 1, patient 1). She was CMV-seropositive and the donor was CMV-seronegative (R+/D−). Valaciclovir (500 mg bid) was given from day 1 (D1) for herpes simplex prophylaxis. CMV DNA in whole blood (WB) was detected without symptoms of CMV disease on D27 by qPCR performed as described elsewhere (Gault et al., 2001). A preemptive treatment with VGCV (450 mg bid, 21 days) resulted in undetectable CMV DNA load (CMV DL) and two further episodes of asymptomatic CMV reactivation on D93 and D336 were successfully treated with VGCV (450 mg bid) (Fig. 1). On D420, within the treatment of chronic graft-versus-host disease, fever, myelosuppression and a significant increase of CMV DL were unsuccessfully treated with different anti-CMV regimens over the following 6 months (FOS 100 mg/kg/day, CDV 5 mg/kg/week, intravenous (IV) GCV 5 mg/kg bid). On D576, the direct PCR sequencing of the *UL97* and *UL54* genes performed by the French national reference center for CMV revealed drug-resistant CMV in blood with a C592G substitution in *UL97* responsible for low-level GCV resistance, associated with two substitutions in *UL54*: L545S conferring GCV and CDV cross-resistance and D588N known to confer resistance to

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Table 1
Patient characteristics.

	TX[a]	CMV[c] antibody status (D/R[d])	Onset of CMV infection from TX[a] (days)	Anti-viral treatment received	Peak VL[f] in whole blood (log copies/mL)	Symptoms	Time to characterisation of virological resistance (days from TX[a])	Resistance mutation to GCV: *UL97*	Resistance mutation to anti-virals: *UL97*	Phenotypic resistance (SI_{50})[h]	Clincal and virological outcome	Time to undetectable VL[f] under artesunate treatment
Patient 1	HSCT[b]	D−/R+	27	VACV, VGCV, GCV, CDV, FOS[e]	>4	Fever, neutropaenia	576	C592G	L545S, D588N	GCV (3.7), CDV (3.1), FOS (3.4)	CMV viraemia neg[i], favourable[j]	1 month
Patient 2	Lung	D+/R−	472	VGCV, GCV[e]	>4	Fever, neutropaenia	543	None	L545S	GCV (3.4), CDV (8.4)	CMV viraemia neg[i], favourable[j]	3 months
Patient 3	Lung	D+/R−	347	VGCV, GCV[e]	>4	Fever, neutropaenia	536	A594V	None	GCV (3.7)	CMV viraemia neg[i], favourable[j]	1 month
Patient 4	Kidney	D+/R−	55	VGCV, GCV, FOS[e]	>5 (BAL[g]>4)	Pneumonia	113	C592G	A987G, N408K, G841A	GCV (7.7), CDV (5.7), FOS (3.9)	Unfavourable, death	
Patient 5	HSCT[b]	D+/R+	124	VGCV, GCV, FOS[e]	>7 (BAL[g]>7)	Pneumonia	261	L595S, M460I, 601–603 deletion	None		Unfavourable, death	

[a] Tx, transplant.
[b] HSCT, haematopoietic stem cell transplant.
[c] CMV, cytomegalovirus.
[d] D/R, donor/recipient.
[e] VACV, valaciclovir; GCV, ganciclovir; VGCV, valganciclovir; CDV, cidofovir; FOS, foscarnet.
[f] VL, viral load.
[g] BAL, broncho-alveolar fluid.
[h] SI_{50} = sensitivity index = IC_{50} of the isolate/IC_{50} of AD169 reference strain.
[i] CMV viraemia neg, means that DNAemia became negative after artesunate treatment (alone or in association).
[j] Favourable clinical outcome, means no further clinical events attributable to CMV infection.

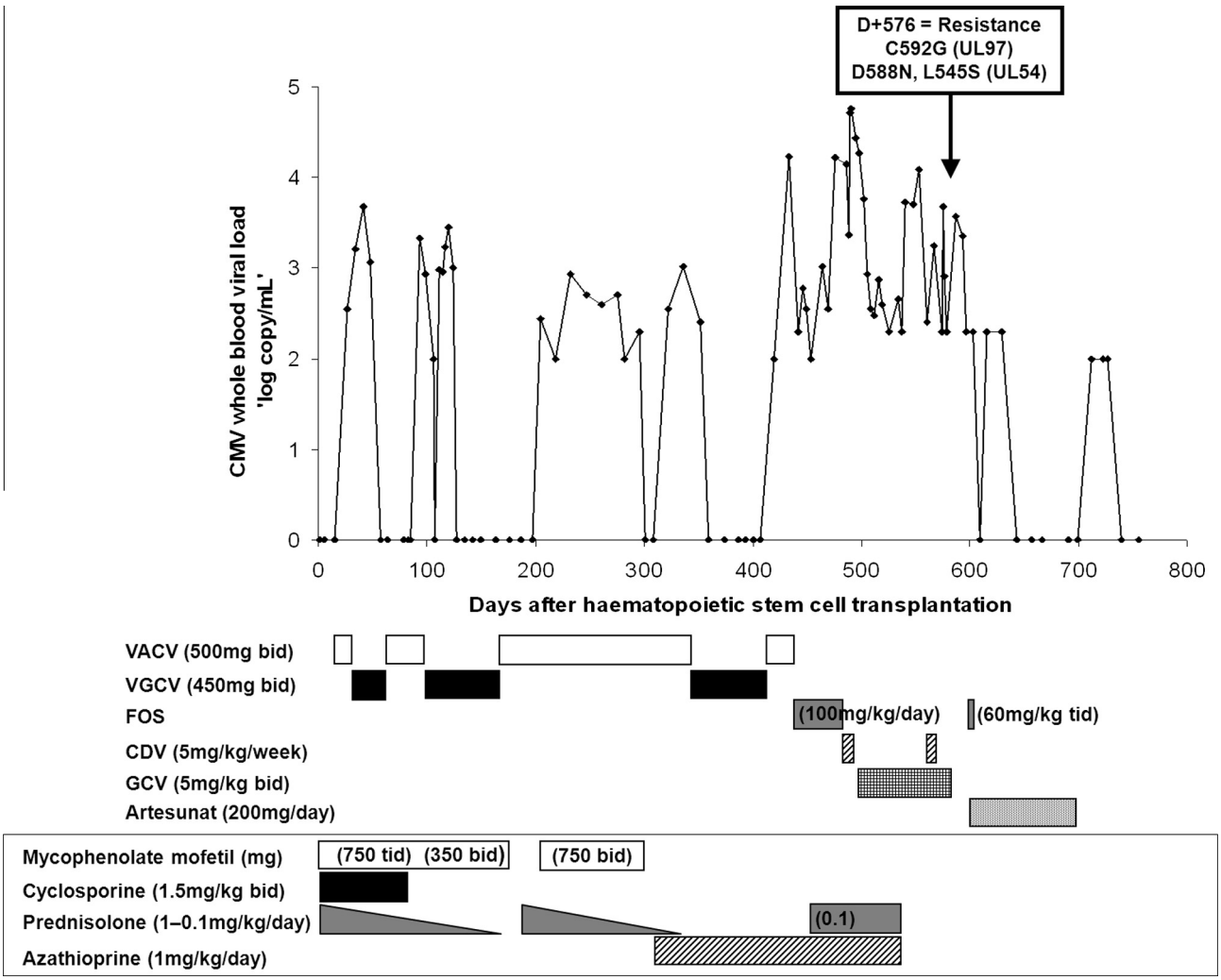

Fig. 1. Follow-up of CMV DNA load and associated antiviral treatment in a haematopoietic stem cell transplant recipient with multidrug-resistant CMV strain (patient 1). VACV, valaciclovir; VGCV, valganciclovir; FOS, foscarnet IV; CDV, cidofovir IV; GCV, ganciclovir, artesunate (temporary approval). D + 576, 576 days after transplantation. Resistance, presence of three resistance mutations, one in the *UL97* gene (C592G) responsible for ganciclovir resistance and two in the *UL54* gene (D588N, L545S) responsible for foscarnet and cidofovir resistance, respectively. Three-drug resistance was confirmed by a phenotypic assay.

FOS (Alain et al., 2004; Chou et al., 2003; Chou, 2008; Springer et al., 2005). The strain isolated from WB harboured the same mutations as previously described. Its phenotypic susceptibility was tested by the French national reference center for CMV, using a standardised 24-well-plate focus reduction assay (Cotin et al., 2012). Phenotypic resistance was defined using a sensitivity index (SI_{50}), which is the IC_{50} (drug concentration that inhibits 50% of virus growth) of the isolate divided by the IC_{50} of reference strain AD169, tested in parallel. Isolates were considered as resistant if their SI_{50} was >3. The SI_{50} was 3.7 for GCV, 3.1 for CDV and 3.4 for FOS, evidencing the phenotypic resistance against the three anti-virals. The strain was phenotypically sensitive to artesunate (SI_{50} = 1.18) (Schnepf et al., 2011). On D588, an oral treatment with artesunate (200 mg/day) was initiated in association with FOS (60 mg/kg tid stopped on D609), leading to an undetectable CMV DL within 1 month and normalisation of haematopoiesis. Artesunate was stopped on D691 because of digestive adverse effects (nausea and vomiting) without any rebound of CMV viraemia or CMV disease.

The two additional cases of drug-resistant CMV successfully treated with artesunate were observed in two lung-recipient patients (engraftment in 2007 and 2008) (Table 1, patients 2 and 3). Both were D+/R− and received VGCV prophylaxis (450 mg bid). They experienced late CMV disease (1 year after the transplantation) with fever and neutropaenia without organ involvement. CMV DL in WB ranged from 2.4 to 4.9 log copies/mL. Treatment with IV GCV (5 mg/kg bid) was unsuccessful, and genotypically- and phenotypically-proven drug-resistant CMV was detected in the WB of both patients. The first patient harboured a L545S substitution in *UL54* responsible for GCV and CDV resistance and the second patient harboured an A594V substitution in *UL97* involving GCV resistance. The culture isolate harboured the same mutations as previously described and its phenotypical analysis confirmed the genotyping data (SI_{50}; patient 2: GCV = 3.4, CDV = 8.38; patient 3: GCV = 3.7). Both patients 2 and 3 received oral artesunate (200 mg/day) for 4 and 7 months, respectively, with favourable clinical and virological responses obtained within 1–3 months after the introduction of artesunate.

2. Two case reports with unfavourable outcomes

A 60-year-old male patient with Wegener granulomatosis received a kidney transplant in 2008 (D+/R−) (Table 1, patient 4). In spite of anti-CMV prophylaxis with IV GCV (2.5–5 mg/kg/day adjusted to the renal function-ARF) followed by VGCV (225–450 mg/

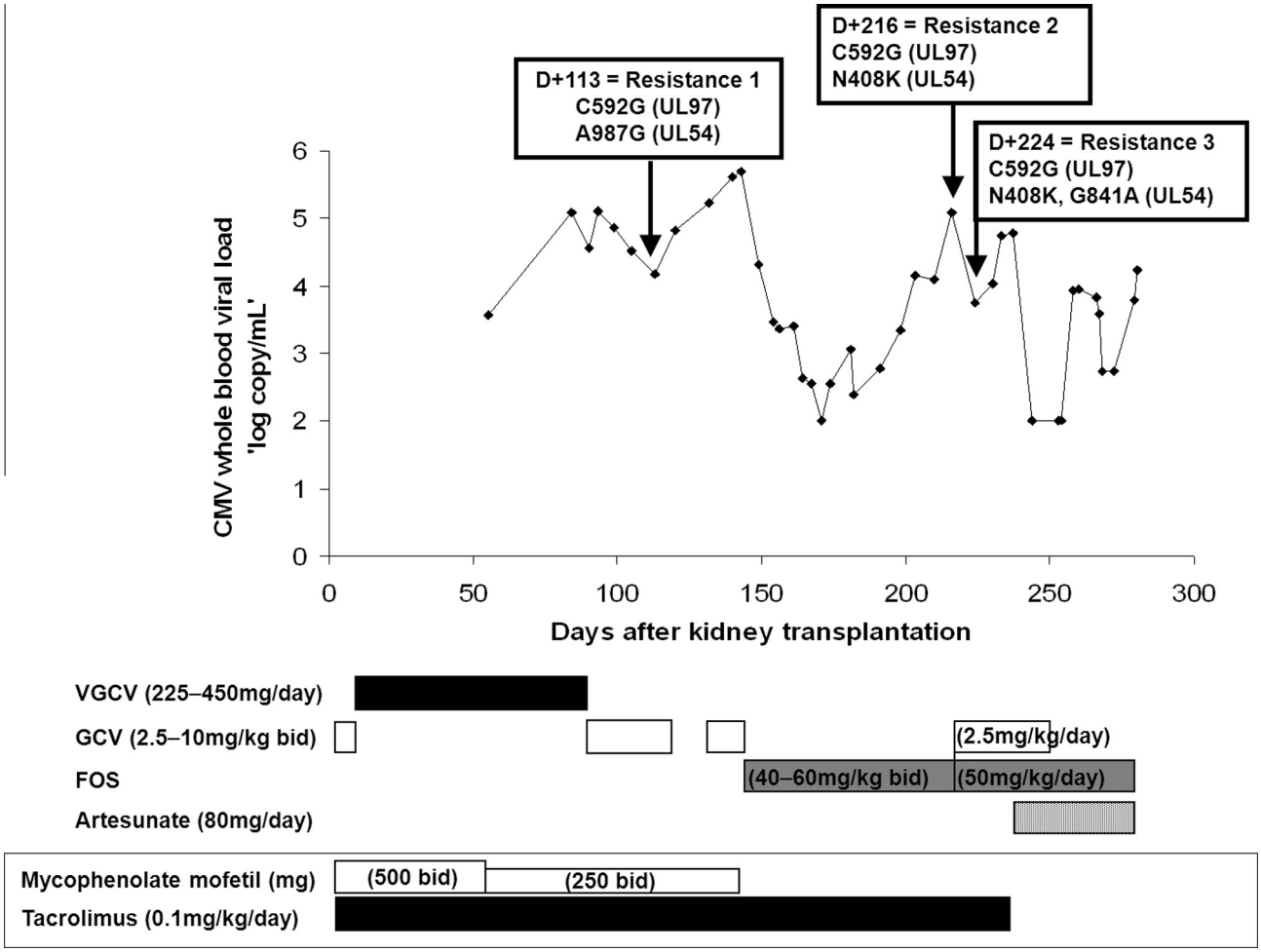

Fig. 2. Follow-up of CMV DNA load and associated antiviral treatment in a kidney transplant recipient with a multidrug-resistant CMV strain (Patient 4). All the drugs were adapted to the impaired renal function and the haematological parameters VGCV, valganciclovir; GCV, ganciclovir IV; FOS, foscarnet IV; artesunate (temporary approval). Resistance 1, presence of two resistance mutations, one in the *UL97* gene (C592G), responsible for ganciclovir resistance and one in the *UL54* gene (A987G), responsible for ganciclovir and cidofovir resistance. Ganciclovir resistance was also documented with phenotyping. Resistance 2 and 3, presence of two resistance mutations, one in the *UL97* gene (C592G), responsible for ganciclovir resistance and one in the *UL54* gene (N408K), responsible for ganciclovir and cidofovir resistance. Phenotypic test showed the resistance to ganciclovir, cidofovir and foscarnet (isolated strain of resistance 3 also harboured G841A mutations in the *UL54* gene).

day ARF), an asymptomatic CMV primary infection was diagnosed by qPCR in blood on D55. An increased dose of VGCV (450 mg/day) followed by IV GCV (2.5–5 mg/kg bid ARF) failed to decrease CMV DL (Fig. 2). On D113, CMV genotyping on a blood sample detected a C592G substitution in *UL97* and an A987G substitution in *UL54*, responsible for GCV and CDV resistance (Chou et al., 2003; Chou, 2008). The strain isolated from WB (carrying only the C592G substitution) was phenotypically resistant to GCV (SI_{50} = 5.1) but remained sensitive to CDV (SI_{50} = 2.1) and FOS (SI_{50} = 0.5). From D132 to D142, the patient developed fever, hepatic cytolysis pancytopaenia and encephalitis. The CMV DL was 5.7 log copies/mL in WB and 2.8 log copies/mL in cerebrospinal fluid. GCV was replaced with FOS (40–60 mg/kg bid ARF) with a transient decrease of CMV DL in WB. A subsequent significant progressive rebound of CMV DL (5 log copies/mL) occurred and an association of GCV (2.5 mg/kg/day) and FOS (50 mg/kg/day ARF) was not effective. Genotypic assays for CMV resistance were performed on WB specimens sampled on D216 and D224. Both specimens harboured a C592G substitution in *UL97* and a N408K substitution in *UL54*, conferring GCV and CDV resistance, respectively (Scott et al., 2007). The strain cultured from the blood on D224 exhibited an additional mutation in *UL54* associated with FOS resistance: G841A (Chou et al., 2007) correlated with the phenotypical resistance to GCV, CDV and FOS and sensitivity to artesunate (SI_{50}; D216: GCV = 3.2, CDV = 3.1, FOS = 3.3; D224: GCV = 7.7, CDV = 5.7, FOS = 3.9). On D233, the patient developed Guillain–Barré syndrome and oral artesunate (80 mg/day) was associated with the FOS and GCV treatments. This regimen led to a rapid decrease (>2 log copies/mL) of CMV DL in WB within 7 days of treatment without clinical improvement of the Guillain–Barré syndrome. GCV was stopped due to severe cytopaenia. On D252 the patient developed acute respiratory distress syndrome. The CMV DL was higher in the bronchoalveolar fluid (BAL) (4 log copies/mL) than in blood (2 log copies/mL). The CMV resistance mutation pattern detected in BAL was identical to the pattern observed in WB on D224. Despite a decrease of CMV DNA load in two further BAL samples, the patient died on D280 due to intractable lung disease after 43 days of artesunate treatment.

The second case concerned a HSCT patient (D+/R+, engraftment in 2012 for Hodgkin lymphoma) (Table 1, patient 5). The first episode of asymptomatic CMV infection occurred on D15, successfully treated with IV GCV (5 mg/kg bid) and oral VGCV (450–900 mg bid). The CMV infection recurred on D124 with CMV DL in WB ranging from 3.3 to 6.9 log copies/mL. IV GCV (2.5–5 mg/kg bid ARF) was unsuccessful and CMV pneumonia was clinically suspected with a CMV DL in BAL (7.1 log copies/mL). CMV genotyping

showed three modifications in the *UL97* gene expected to be associated with GCV resistance (Lurain and Chou, 2010): the WB harboured an L595S substitution and a 601–603 deletion; the BAL carried, in addition, an M460I substitution. The phenotypic analysis could not be performed. Artesunate (120 mg/day) was associated with FOS (85 mg/kg/day ARF) for 5 days. Because of intolerable adverse effects and severe renal impairment, the artesunate/FOS association was replaced with an artesunate/GCV association (2.5–5 mg/kg bid ARF). Despite a 1-log decrease in the viral load in the WB, the patient died of respiratory failure 24 days after beginning artesunate.

3. Discussion

Although it is mode of action against CMV is still not clearly elucidated, we report the clinical use of artesunate for the management of virologically-confirmed multidrug-resistant CMV diseases in HSCT or SOT (Chou et al., 2011; He et al., 2012, 2013; Shapira et al., 2008). We observed favourable outcomes mainly during mild CMV disease without organ involvement. Of note, the viral decay half-lives were much higher than those described by Wolf et al. (2011) (4–20 days versus 0.9–1.9 days for Wolf et al., 2011; result not shown). By contrast, artesunate treatment was not successful in the two cases of severe CMV diseases presenting with high CMV DL (5.7 and 6.9 log copies/mL) and lung involvement, despite a weak or moderate effect on CMV DL in WB or BAL. We cannot rule out that the two patients' underlying disease (Wegener granulomatosis, Hodgkin lymphoma) or Guillain–Barré syndrome were in part responsible for the fatal outcome. One could also speculate that a low level of artesunate diffusion in lung tissues, as reported in animal models (Zhao and Song, 1989), could hamper its antiviral activity. In the seminal clinical study reported by Shapira et al. (2008), artesunate was effectively active against a CMV-resistant disease with fever and myelosuppression but did not prevent the subsequent development of CMV retinitis in spite of good diffusion in the central nervous system (Shapira et al., 2008). Intravenous artesunate has also been reported as unsuccessful during associated CMV-resistant colitis in SOT (Lau et al., 2011). The level of anti-CMV T-cell immunity is also likely to be crucial for the efficacy of artesunate. Wolf et al. reported that artesunate used as pre-emptive therapy for a non-drug-resistant CMV strain in HSCT patients failed to decrease CMV DL in the blood of four out of six patients. These failures appeared to be the result of complex mechanisms depending on the reconstitution of the anti-CMV immune response and on the virus's fitness (Wolf et al., 2011). Gantt et al. (2013) recently reported that a very short course of artesunate used as antimalarial regimen had no effect on CMV viraemia in African children, highlighting the importance of the dose and duration of artesunate treatment to circumvent CMV replication.

In conclusion, artesunate may be useful in treating mild CMV disease due to multidrug-resistant CMV strains. The mechanisms and the risk factors associated with artesunate failure require additional data, but clinicians should be aware that artesunate may not be effective enough against severe CMV diseases with pulmonary involvement.

Potential conflicts of interest

All authors state no conflicts of interest.

The patients were included in a prospective survey for CMV resistance (and gave informed consent for the study (clinical trial.gov: NCT01008540) (Hantz et al., 2010).

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