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Case Report

Dynamics of cytomegalovirus populations harbouring mutations in genes UL54 and UL97 in a haematopoietic stem cell transplant recipient

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ABSTRACT

We characterised by pyrosequencing, the dynamics of cytomegalovirus populations harbouring mutations A594V in gene *UL97* and A834P and Q578H in gene *UL54* in a haematopoietic stem cell transplant recipient. Unexpected re-emergence of A594V and decrease of A834P under CMX001 were shown to depend on both the selection pressure exerted by the antiviral treatments and the immune response.
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Resistance of cytomegalovirus (CMV) to antiviral drugs is rarely encountered in adult recipients of haematopoietic stem cell transplant (HSCT) [1–3]. However, in some patients with persistent viraemia, successive courses of antiviral treatment could lead to emergence of resistance to the available antiviral drugs ganciclovir (GCV) or valganciclovir (vGCV), foscarnet (FOS) and cidofovir (CDV) [4]. Resistance to GCV results mainly from mutations within the protein kinase *UL97* gene and less frequently from mutations in the DNA polymerase *UL54* gene. FOS and CDV resistance-associated mutations are located in gene *UL54* and mutations that confer cross-resistance to two or more drugs have been described [5]. Alternatives to the currently licensed drugs include CMX001, an orally administered lipid conjugate of CDV that exhibits in vitro activity against GCV-, FOS- and some CDV-resistant mutants and artesunate, an antimalarial drug able to inhibit replication of GCV-susceptible or resistant strains [6–9]. How mutated populations evolve under selection pressure and after cessation of treatment is of great concern for treatment strategy. As pyrosequencing methods allow a deep discrimination of resistance-associated mutations, we studied the emergence and persistence kinetics of gene mutations in serial samples from a HSCT recipient according to the given anti-CMV drugs including CMX001 and artesunate.

1. Case report

Patient Rec2 was diagnosed with Fanconi disease at 5.5 years of age. Ten months before transplantation she experienced a primary CMV infection which resulted in a prolonged neutropenia. At 20-years she received a bone marrow transplant from a HLA-matched CMV-seronegative unrelated donor. She was given a non-myeloablative conditioning regimen with 2 Gy body irradiation. She was monitored once weekly with CMV Real Time

Abbreviations: CDV, cidofovir; CMV, cytomegalovirus; CTSQ, direct dideoxy chain termination sequencing; FOS, foscarnet; GCV, ganciclovir; HSCT, haematopoietic stem cell transplant; PSQ, pyrosequencing; vGCV, valganciclovir.

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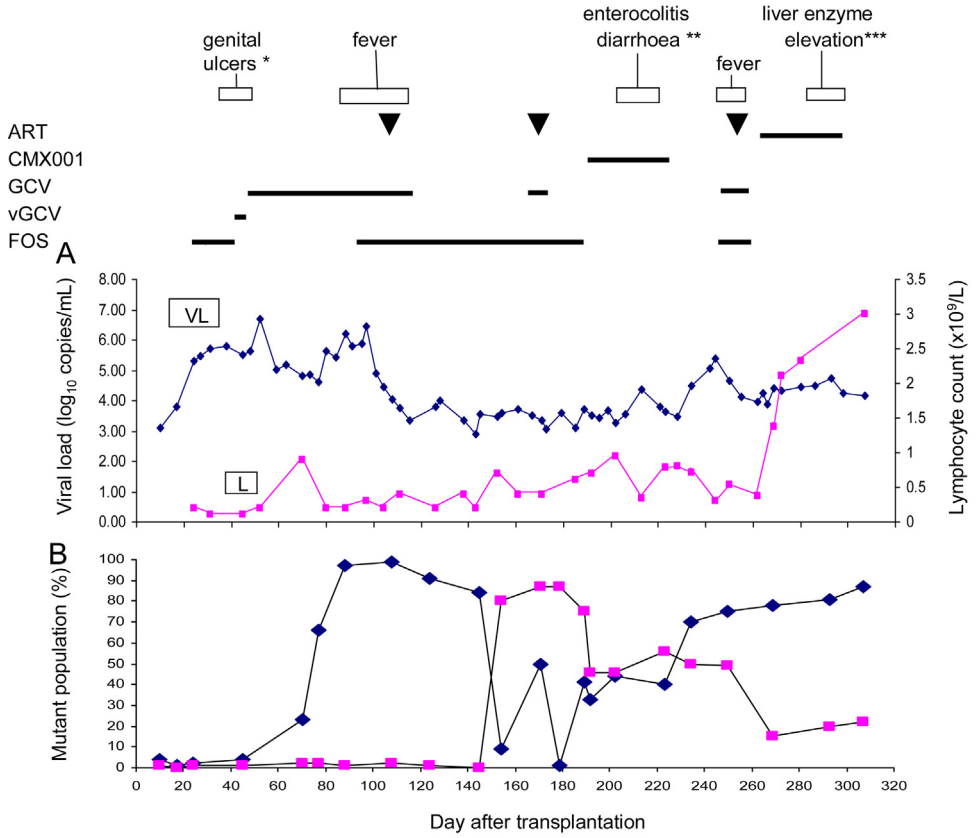

Fig. 1. Time course of antiviral treatments and events. (A) Evolution of cytomegalovirus DNA load levels and lymphocyte counts according to antiviral drugs given during the follow-up. (B) Dynamics of emergence of resistance-associated mutations. The percentages of mutant genomes with regard to wild type genomes are indicated (square: A834P, diamond: A594V). The clinical events associated with cytomegalovirus infection or drug side effects are shown. VL: viral load, L: lymphocyte count, ART: artesunate, GCV: intravenous ganciclovir, vGCV: valganciclovir and FOS: foscarnet. Side effects attributed to *foscarnet, **CMX001 and ***artesunte. The arrows indicate profound pancytopenia episodes.

quantitative PCR in whole blood (Abbott CMV PCR Kit produced by Qiagen GmbH, Hilde Germany for Abbott Diagnostics, limit of detection: 100 copies/mL) (Fig. 1A). At day 10 post transplantation, the viral load was 3.11 $\log_{10}$ copies/mL and FOS was initiated at day 19. The viral load reached 5.8 log copies/mL 19 days after initiation of treatment. FOS was switched to vGCV and then to intra venous GCV (10 mg/kg/day). Sequencing of the viral genes after 19 days of FOS and 11 days of vGCV and GCV did not reveal drug-resistance associated mutations. The CMV load decreased and maintenance GCV treatment (5 mg/kg/d) was started. After 12 days, as CMV load had risen to 6.21 $\log_{10}$ copies/mL, FOS was added to the regimen. The CMV load decreased under combination therapy and GCV was subsequently stopped. The viral load persisted at levels between 3.07 and 3.73 $\log_{10}$ copies/mL despite reintroduction of GCV over a ten day duration. FOS was stopped at day 185 and replaced by CMX001 (2 mg/kg twice weekly). However CMV DNA levels remained unchanged. CMX001 was sopped after 36 days because the patient experienced profuse diarrhoea episodes. The viral load reached 5.39 $\log_{10}$ copies/mL. Genotypic resistance testing performed on post transplant day 222 showed UL97 mutation A594V responsible for resistance to GCV and UL54 mutations A834P and Q578H that conferred moderate resistance to GCV, FOS and CDV [10–12]. With a combination of GCV and FOS treatment (both at maintenance doses) for 12 days (day 244–day 255) followed by two days of FOS a 1 $\log_{10}$ copies/mL decrease was observed. Artesunate was then started (100 mg/day) and maintained for 38 days. CMV DNA levels remained unchanged. Her lymphocyte counts had fluctuated between 0.1×10^9 cells/L and 0.94×10^9 cells/L during the first 8 months after transplantation (Fig. 1A). Analysis of the lymphocyte populations at day 112 post transplantation showed a profound lymphopenia with 8×10^6 CD4+ cells/L and 81×10^6 CD8+ cells/L. The patient was not given antiviral treatment after artesunate stopping and she recovered from CMV infection after immune reconstitution.

In order to determine the dynamics of resistance-associated mutations A594V in UL97, and A834P and Q578H in UL54, a retrospective analysis was performed from blood samples kept stored at −80 °C obtained between day 10 and day 307 post transplantation. Direct dideoxy chain termination sequencing (CTSQ) and pyrosequencing (PSQ) were applied to 13 and 22 samples respectively. Genes *UL97* and *UL54* were sequenced in entirety as already described [13]. For PSQ, three primer sets (two PCR primers and one pyrosequencing primer) to analyse the three codons of interest were designed using the Pyrosequencing Assay Design software (Qiagen, Courtaboeuf, France) according to the sequences established by CTSQ. The PSQ reaction was performed using a specific nucleotide dispensation strategy on the PyroMark ID machine.

CTSQ showed no change in polymorphisms (N108S in *UL97*, S685N and S897L in *UL54*) and no double populations outside the resistance-associated sites during the follow up suggesting that the patient was infected by a single strain (Table 1). To evaluate the specificity of the PSQ assay wild-type CMV DNA (reference strains AD169 and TB40/E, and clinical strains from non-treated newborns) was tested in parallel with the patient samples in three PSQ assays (data not shown). Mutant signals of 1–2% (mean 1.2 ± 0.5) for position 594 in UL97 and 0–1% (mean 0.5 ± 0.5) for position 834 in UL54, 7–9% (mean 8.25 ± 0.9) for position 578 in UL54 were taken as false positive. Mutant detection limits (above mean + 3 standard deviations) of 5% for positions 594 and 834 and 12% for the position 578 were defined according to Armbruster and Pry (Table 2) [14].

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