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Dynamics of coexisting HCMV-UL97 and UL54 drug-resistance associated mutations in patients after haematopoietic cell transplantation

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ABSTRACT

Background: Resistance to antiviral drugs can be a severe problem in transplant recipients. Mutations in the HCMV phosphotransferase-gene (UL97) and the polymerase-gene (UL54) are responsible for resistance against ganciclovir (GCV), cidofovir (CDV) and foscarnet (PFA). Most frequently mutations in the UL97-gene are associated with resistance to GCV. Resistance against PFA and CDV is associated to mutations in the UL54-gene. There are only few reports about multidrug-resistance with mutations in both genes in patients after allogeneic haematopoietic cell transplantation (HCT).
Objectives: To asses retrospectively the role of UL97/UL54-mutations for clinical deterioration.
Study design: We present here three patients after HCT developing multidrug-resistance with coexisting UL97 and UL54-mutations. Genotypical resistance screening was done with restriction-fragment-length-polymorphism (RFLP), sequencing of UL97/UL54, and LightCycler real-time PCR. Phenotyipcal testing was performed by a cell-associated plaque-reduction-assay. Plasma viral-load (VL) was determined longitudinally using Roche Cobas-Amplicor-System (Roche Diagnostics). In one case VL was also correlated to different ratios of coexisting UL97-wildtype and mutant variants.
Results: All three patients developed multidrug resistant HCMV-infections with one or more UL97 and UL54-mutation detected by RFLP, sequencing and LightCycler-analysis. Two out of three patients showed biphasic VL kinetics with manifestation of UL97 drug-resistance prior/or at peak VL. UL54-mutations emerged also in all three patients either at increasing VL levels of $\geq 10^5$ copies/ml or at peak VL.
Conclusions: The development of coexisting HCMV UL97 and UL54-mutations conferring drug-resistance after HCT is not strictly associated with fatal outcome in one of our three patients. Manifestation of drug resistant combined UL97/UL54-mutations occurred prior to a second VL peak under (V)GCV/PFA co-treatment.

1. Background

The development of multidrug-resistant HCMV infection and disease after allogeneic haematopoietic cell transplantation (HCT) can be life-threatening. Therefore a rapid and close meshed monitoring of HCMV infection and viral load under therapy after HCT as well as after organ transplantation is mandatory.[1–3] Mutations in the HCMV phosphotransferase-gene (UL97) and the polymerase gene (UL54) are responsible for resistance to ganciclovir (GCV), cidofovir (CDV) and foscarnet (PFA). Over 90% of all GCV-resistant clinical isolates have mutations in the UL97 gene.[4]

Many of the GCV-resistant HCMV-isolates contain UL97 defined mutations, mostly the "canonical" point mutations M460 V/I, H520Q, C592G, and C603W.[5] A new web-based HCMV drug resistance data base may help to discriminate between UL97 and UL54 mutations associated either to drug-resistance or to polymorphism. In context of UL97, 43 of 58 mutations seamed to be linked to resistance, while only 66 of total 159 mutations in the pol gene were linked to drug resistance in 2009.[6]

Special mutations in conserved subdomaines of the UL54-gene can cause resistance to GCV, CDV and PFA as well as cross-resistance between all three antiviral drugs. There are only few reports on multiple drug resistant HCMV infections in HCT recipients with UL97 and UL54 mutations.[2,7,8] We present here a case series of three patients developing a multidrug-resistance with mutations in both

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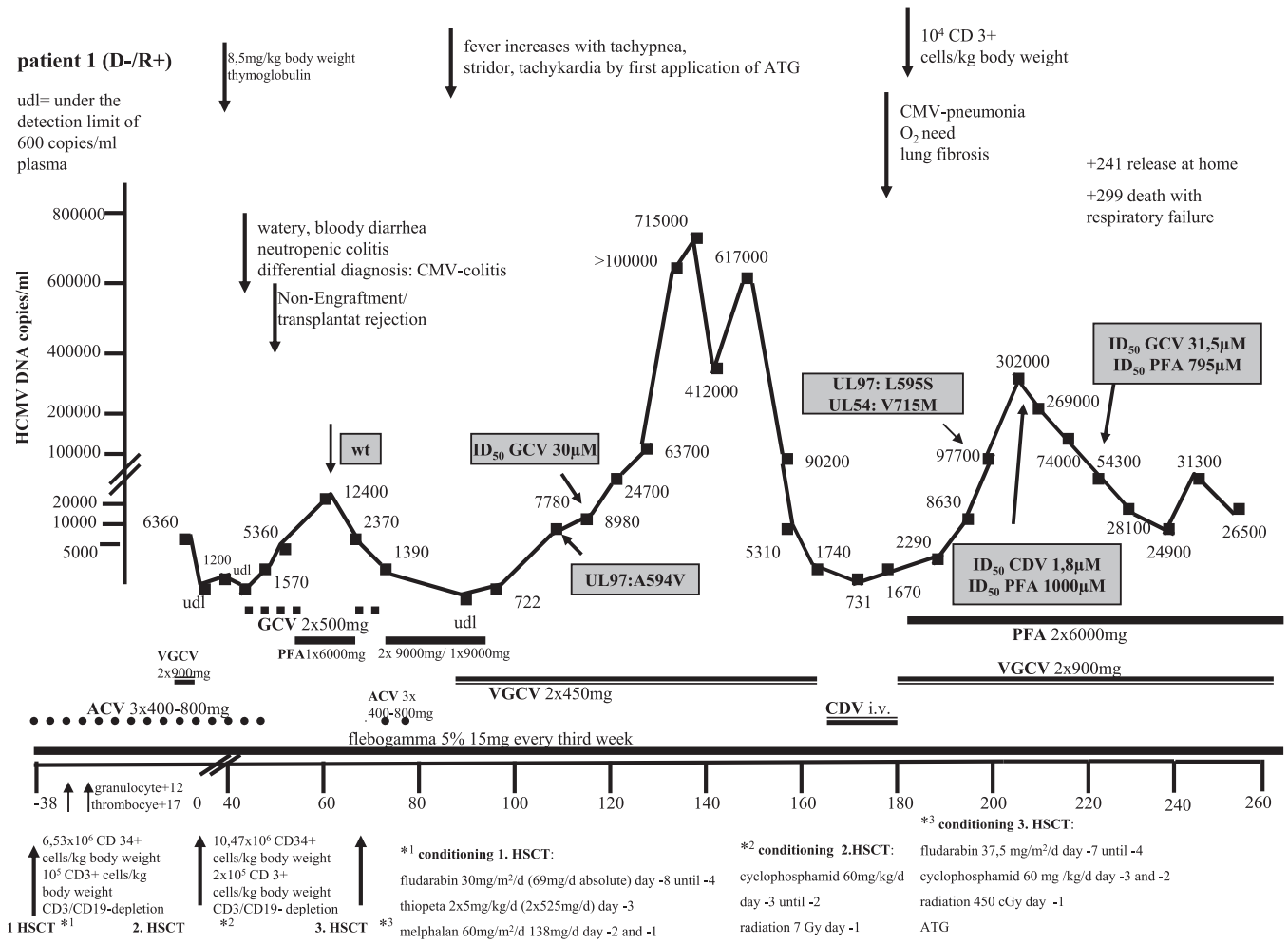

Fig. 1. Patient 1: clinical synopsis, antiviral treatment and biphasic VL kinetics.

genes. Therapy was genotypically monitored by RFLP analysis and sequencing of the UL97 region. Retrospectively, a new genotypic real-time-PCR approach for the detection of the UL97 mutations was evaluated for semiquantification of the corresponding mutant-wildtype sequence ratio.

2. Objectives

We aimed to evaluate retrospectively the concise role of UL97/UL54-mutations during clinical deterioration of severe disseminated HCMV disease with manifestation of HCMV pneumonia.

3. Study design

Virologic characterisation of multidrug resistant HCMV infection in three HCT recipients was performed prospectively and in part retrospectively.

3.1. Virus strains

HCMV laboratory strains AD169 and Towne and clinical isolates used as reference strains were propagated in human foreskin fibroblasts.

3.2. DNA extraction

When a pronounced cytopathic effect of at least 50% of the monolayer was visible, viral DNA was extracted by the Hirt-lysis method. DNA extraction from patient samples was performed with the proteinase K/phenol-chloroform procedure.

3.3. RFLP

The RFLP analysis for the detection of mutations A591 V, A594 V and L595S were performed as described elsewere.[9,10]

3.4. Sequencing

Sequencing analysis was performed including codons 439 to 696 of the UL97 gene, and codons 365–1084 of the UL54 gene.[1,2] PCR fragments were cleaned up with the Qiagen-PCR-purification-Kit (Qiagen, Hilden, Germany).

3.5. Real-time PCR

The assay for the simultaneous detection of mutations in codon 594 and 595 was performed with one pair of hybridisation probes labelled with LCRed-dye-640 and Fluorescein. The detection of mutations was achieved by melting-curve-analysis following PCR as decribed elsewhere.[11]

3.6. Viral-load

The viral load was determined using Cobas Amplicor (Roche Diagnostics). The samples were extracted as described by the