Antiviral drug resistance of Human Cytomegalovirus:genotypic characterization of UL97 polymorphisms by viral DNA sequencing

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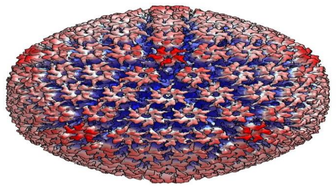
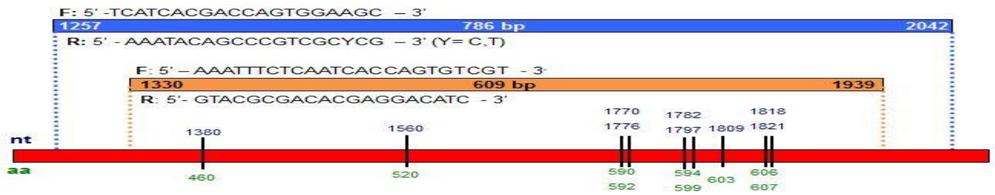


UL97 primers



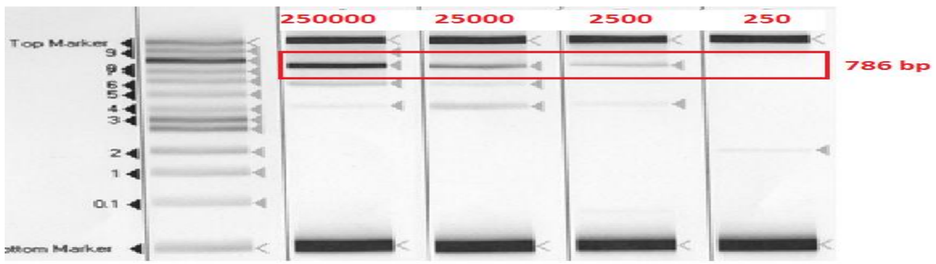
Background

Human cytomegalovirus (CMV) is an important pathogen that causes significant morbidity and mortality in transplant recipients. The availability of anti-CMV drugs has improved treatment but prolonged antiviral therapy with ganciclovir (GCV) may result in the emergence of drug-resistant CMV strains, mainly due to the presence of mutations within the protein kinase UL97 gene. Timely identification of viral mutations conferring resistance is essential for effective patient management. Here, we describe an improved, rapid, sequencing-based assay for the identification of mutations in the UL97 gene in order to recognize clinically-relevant resistant CMV subpopulations.

Materials/ methods

The test was based on viral DNA Sanger sequencing, characterized by two principal PCR steps: locus specific amplifications of UL97 gene, followed by sequence reactions with di-deoxy nucleotides. Primers were designed by using the *Primer-BLAST* (NCBI) software. Results obtained with capillary analysis of fragments originated with di-deoxyterminators have been analysed with Bioedit software, for manually alignment, and Mutation Resistance Analyzer online software, for automated alignment. Sensibility of method has been tested on serial dilutions of HCMV strain Merlin, while specificity has been performed analyzing QCMD panels (Quality Control for Molecular Diagnostics, CMVDR14, CMVDR15). Finally, three clinical samples from transplanted patients (SOT) have been preliminarily analysed.

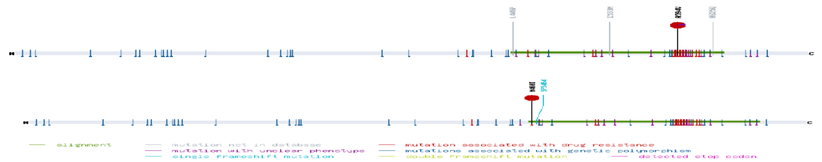
Sensibility of UL97 assay



Results

The test has shown a good sensibility (10,000 HCMV DNA copies/ml whole blood) and a good specificity, detecting all known mutations in 8/9 QCMD. Moreover, ganciclovir resistance-associated mutations (A594V and M460I) have been detected in 2/3 patients.

MRA software alignment and detection of con riscontro A594V e M460I mutations



Conclusion

UL97 assay will allow a rapid, sensible and specific identification of drug resistant viral populations, positively influencing therapeutic choice and prognosis for severe HCMV infections. Moreover, the analysis on greater number of patients will allow a comparison between our patients and patients of other Italian and international realities described in literature.