Use of Illumina MiSeq Technology to Detect Drug Resistance Mutations in Human Cytomegalovirus

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Abstract

Background: Cytomegalovirus (CMV) is extremely common with a prevalence of 40-80% in the U.S. In clinical infections, the virus establishes a lifelong, latent infection with minor sequelae or subclinical manifestations. However, infection in immunocompromised hosts (i.e., transplant recipients) may be associated with severe morbidity and mortality, causing disease in multiple organ systems. CMV has a large genome and a complex life cycle. Additionally, mechanisms of drug resistance have been identified. However, prophylactic treatment is believed to have minimal increase in antiviral resistance. Currently, Sanger sequencing of the UL54 and UL97 genes of CMV is considered the gold standard for detection of resistance mutations. This method has inherent limits, and next generation sequencing (NGS) has been explored.

Methods: Long range PCR primers were designed to target the intragenic sequences of UL54 and UL97 and amplicons were subjected to targeted DNA sequencing techniques (DSI, Corvus, MiPhy). GenoBase (GNB) BLAST was used to assess analytic specificity. Clinical plasma samples (n=6) were tested. Isolated viral DNA was extracted using the GeneJet viral DNA and RNA purification kit (Thermo Scientific, Waltham, MA) and PCR performed using the Applied Biosystems OligoAnalyzer and NCBI Blast to assess sequence information. Sequences were then aligned to the reference CMV Merlin genome (NC_006273.2) with clingen, and the results compared to traditional Sanger sequencing.

Results: Three samples determined to harbor CMV with UL54 mutations containing parvovirus resistance by Sanger were tested by the MiSeq method, and resistance mutations were detected in all 3 samples by NGS. Furthermore, 2 clinical samples determined to harbor wild type CMV by Sanger were confirmed to be NGS positive.Interestingly, identical mutations (m520q, l595s) were detected among the clinical samples by the NGS method.

Conclusions: The novel NGS method detected mutations conferring parvovirus resistance in clinical samples accurately, characteristics of parvovirus resistance. However, due to the small sample size, further studies are warranted to confirm these findings.

Objectives

1. Develop and evaluate a sensitive and specific test using MiSeq platform to identify mutations in UL54 and/or UL97 that are responsible for antiviral resistance.

2. Compare the performance of the Illumina® MiSeq platform to Sanger sequencing for detecting CMV antiviral resistance.

Methods

- Long range nested PCR primers for UL54 and UL97 were designed using published sequences and ITD primer design software.
- Specificity checked with NCBI BLAST and by performing PCR with H1V 12, H1V 2, and ES6.
- Samples analyzed by routine Sanger sequencing for resistance by using the MiSeq platform.
- DNA isolated using the GeneJet viral DNA and RNA purification kit (Thermo Scientific, Waltham, MA). Sequences were then aligned to the reference CMV Merlin genome (NC_006273.2) with clingen, and the results compared to traditional Sanger sequencing.

Results: Three samples determined to harbor CMV with UL54 mutations containing parvovirus resistance by Sanger were tested by the MiSeq method, and resistance mutations were detected in all 3 samples by NGS. Furthermore, 2 clinical samples determined to harbor wild type CMV by Sanger were confirmed to be NGS positive. Interestingly, identical mutations (m520q, l595s) were detected among the clinical samples by the NGS method.

Conclusions: The novel NGS method detected mutations conferring parvovirus resistance in clinical samples accurately, characteristic of parvovirus resistance. However, due to the small sample size, further studies are warranted to confirm these findings.

Discussion/Future Directions

- The manuscript refers to the use of Illumina MiSeq technology for detecting drug resistance mutations in CMV, which is a sensitive and specific test that can be used to detect additional drug resistance mutations in clinical samples. Further studies are needed to assess the capability of the MiSeq platform.

Table 1: Mutants Detected by Each Method

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sanger</th>
<th>MiSeq</th>
</tr>
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<tbody>
<tr>
<td>M460I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L595S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Example of CMV Drug Resistance Genotype Patient Report

<table>
<thead>
<tr>
<th>Patient</th>
<th>UL54 Mutations</th>
<th>UL97 Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M460I</td>
<td>L595S</td>
</tr>
<tr>
<td>B</td>
<td>M460I</td>
<td>L595S</td>
</tr>
</tbody>
</table>

Fig 2: Jaxm files were submitted to Advanced Biological Laboratories (ABL) for analysis by DeepChek software. The report included an initial page that noted the mutations that were tested, where the information regarding the phenotype associated with genotypes is found and briefly what percentage of the results were susceptible or resistant to a given drug. Subsequent pages consisted of in-depth rotations of the position of the mutation codon, what mutation occurred and the prevalence of the mutation.

References

