Selection and Recombinant Phenotyping of a Novel CMX001/Cidofovir HCMV Resistance Mutation

Scott H. James, MD, Carol B. Hartline, BS, Randall Lanier, PhD, and Mark N. Prichard, PhD

1University of Alabama at Birmingham, Birmingham, AL, USA, 2Chimerix, Inc., Durham NC, USA

Abstract

Background: CMX001 is a novel, orally available lipid ester of the acyclic nucleotide analogue cidofovir (CDV) that exhibits enhanced in vitro antiviral activity against a wide range of DNA viruses, including human cytomegalovirus (HCMV), BK virus, and adenovirus. Mutations in the DNA polymerase gene of HCMV that impart resistance to CDV also render the virus resistant to CMX001. Here, we report a novel resistance mutation that arose under the selective pressure of CMX001. Methods: The HCMV strain AD169 was propagated in low passage human foreskin fibroblasts at increasing concentrations of CMX001 over the course of 10 months, with a final CMX001 concentration of 0.5µM. Reduced susceptibility of the resulting strain (CMX001®) to CDV and CMX001 was demonstrated by standard plaque reduction assays. Genotypic analysis of CMX001® was performed with Sanger sequencing of the DNA polymerase gene (UL54) and the UL97 kinase gene (UL97). Recombinant phenotyping of a novel UL54 mutation was performed via bacterial artificial chromosome mediated marker transfer experiments using a kanamycin selectable marker. Results: A resistant isolate (CMX001®) was generated under the selective pressure of CMX001. Compared to the parent virus, the isolate was resistant to both CMX001 and CDV, with 50% effective concentration (EC50) values that were elevated by approximately 10-fold for each drug. Genotypic resistance analysis of CMX001® demonstrated a novel UL54 mutation (D542E) that had not been reported in CDV resistant isolates. There were no other mutations in UL54 or UL97. A recombinant virus with the D542E mutation (RC556) was generated and subjected to further phenotypic analysis that confirmed it conferred approximately 10-fold resistance to both CDV and CMX001. CMX001® and RC556 isolates both demonstrated a small plaque phenotype. Conclusion: This represents the first resistance mutation generated under the selective pressure of CMX001. This mutation arose after an extended period of selective pressure, and affected isolates demonstrate a plaque phenotype consistent with a replicative defect. D542E was not a previously identified mutation associated with HCMV resistance, which indicates that CMX001 may have a unique resistance profile.

Materials and Methods

Resistant Strain Selection

HCMV strain AD169 was used to infect human foreskin fibroblasts (HFF) cells at a low multiplicity of infection (0.1 PFU/cell) in the presence of 0.01 µM CMX001. The culture was passaged 10 times with increasing concentrations of CMX001 up to a final concentration of 0.5 µM. Total passage time was 10 months. After subsequent plaque purification, the resultant virus (CMX001®) underwent plaque reduction assays to determine the EC50 values of CMX001 and the control compounds CDV, ganciclovir (GCV), and foscarnet (PFA).

Genotypic Analysis

The UL97 and UL54 genes were amplified from CMX001® using double nested polymerase chain reaction methods and the resulting DNA amplicons underwent Sanger sequencing at the UAB Department of Genetics Core Sequencing Facility to identify any amino acid changes. Polymorphisms were identified by comparing consensus DNA sequences to AD169 (GenBank accession number X17403).

Recombinant Phenotyping

To further investigate a novel UL54 mutation identified during genotypic resistance analysis, the mutation was reconstructed in the AD169 HCMV baculovirus artificial chromosome (BAC) using methods similar to those previously reported. A kanamycin resistance marker was inserted adjacent to the UL54 gene segment in the plasmid used as a template for mutagenesis (UL54kan pEXPs5 NT), allowing for selection of BAC colonies in which recombination correctly occurred. Recombinant virus was reconstructed via transfection into HFF cell culture and underwent plaque reduction assay to determine EC50 values against CMX001 and the panel of control compounds. A second recombinant virus (RC556) was engineered with the kanamycin marker in the same locus, but with no UL54 mutations, was used as a control for phenotypic studies.

Results

- The resistant isolate obtained at the end of 10 months of passage in the presence of CMX001 was designated CMX001®.
- CMX001® exhibited a small plaque phenotype indicative of a replicative defect.
- The CMX001® isolate demonstrated an EC50 for CMX001 that was 17-fold higher than the EC50 of its AD169 parent virus, CMX001® was also resistant to CDV (7.6-fold increase over parent virus), but retained susceptibility to GCV and PFA (Table 1).

<table>
<thead>
<tr>
<th>EC50 (µM)</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMX001®</td>
<td>0.017 ± 0.003</td>
</tr>
<tr>
<td>AD169</td>
<td>0.001 ± 0.0005</td>
</tr>
<tr>
<td>CDV</td>
<td>8.4 ± 5.5</td>
</tr>
<tr>
<td>GCV</td>
<td>4.3 ± 2.0</td>
</tr>
<tr>
<td>PFA</td>
<td>59 ± 12</td>
</tr>
</tbody>
</table>

Conclusions

A CMX001-resistant HCMV strain was generated under prolonged selective pressure in cell culture. The resistant strain exhibited a small plaque phenotype. Genotypic resistance analysis of this CMX001-resistant strain identified a novel UL54 mutation, D542E.

Based upon recombinant phenotyping studies, we conclude that the HCMV UL54 D542E mutation is sufficient to confer resistance to both CDV and CMX001.

D542E has not previously been identified in CDV-resistant HCMV strains. Though it occurs within the conserved 6C/ExoIII region of UL54, D542E does not confer GCV or PFA cross resistance, as do a majority of other known resistance mutations in this region, indicating that CMX001 may drive the selection of unique resistance mutations.

References


Acknowledgements

This research was supported by a research grant from Chimerix, Inc.