Combination of TMC435 with two novel NSSB inhibitors increases anti-HCV activity and results in a higher genetic barrier in vitro


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Poster presented at the 45th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria, 14–18 April, 2010

Introduction

TMC435 is a macrocyclic NS3/4A protease inhibitor currently in Phase IIb clinical development for the treatment of hepatitis C virus (HCV) infection. It is a potent and selective inhibitor of NS3/4A in vitro, with a 50% effective concentration (EC50) of 0.4 nM in a geno-1b replicon cell line.1

Recent studies from Phase I and IIa studies have demonstrated that TMC435 is well tolerated, has a pharmacokinetic profile that supports once-daily (QD) dosing regimens, and demonstrates potent antiviral activity in both treatment-naïve and experienced geno-1b patients.2,3

Since combinations of specifically targeted antiviral therapies for HCV (‘cocktails’) with different mechanisms of action may provide more efficacious HCV treatment regimens, we performed in vitro replication studies to assess the potential of combining TMC435 with one or two novel HCV NSSB polymerase inhibitors (a non-nucleoside inhibitor (Tib-NI) and a nucleoside inhibitor (Tib-NNI)) here we report these findings.

Methods

The effect of combining TMC435 with Tib-NI and/or Tib-NNI on anti-HCV activity, genetic barrier to resistance and replicon clearance was assessed using three different assays.

Anti-HCV activity

This was a 3-day antiviral assay conducted using HCV genotype-1b replicon-containing cells with luciferase (Luc) readout, followed by combination analysis.

Half-Luc replicon-containing cells were seeded at a density of 2,500 cells/well in a 96-well plate in Dulbecco Modified Eagles Medium (DMEM) plus 10% fetal calf serum (FCS) and incubated in the absence of G418 with a range of serially diluted combinations of TMC435, Tib-NNI and Tib-NI, according to the checkerboard method.

After 72 hours of incubation, the luc signal was measured with a Victor2 multiskip (PerkinElmer), and the effect of each combination was assessed using the Bliss independence model based on the algorithm developed by Prichard and Shipman6 using the MacSynergy™ II software for representative experiments are shown in Figure 1 and Table 1.

Colony formation

Colonies were incubated with TMC435 in combination with Tib-NNI and/or Tib-NI in the absence of G418 with a range of serially diluted combinations of TMC435, Tib-NNI and Tib-NI, according to the checkerboard method.

After 14 days, colonies were stained with neutral red and counted.

Replicon clearance-rebound assay

To assess the potential of TMC435 in combination with Tib-NNI or Tib-NI in the suppression of resistant replication, Huh7-Luc replicon cells (300,000) were seeded in a 10 cm dish medium (DMEM) plus 10% fetal calf serum (FCS) and incubated in the absence of G418 with a range of serially diluted combinations of TMC435, Tib-NNI and Tib-NI, according to the checkerboard method.

The number of surviving cell colonies is indicated on the right lower corner of each graph. The number of surviving colonies is indicated on the right lower corner of each graph.

Results

Effect of inhibitor combinations in an antiviral assay

The effect of combining TMC435, Tib-NI and Tib-NNI on anti-HCV activity is shown in Figure 1 and Table 1.

Table 1. Antiviral activity of different combinations of TMC435, Tib-NNI and Tib-NI.

<table>
<thead>
<tr>
<th>Combination</th>
<th>RNA levels (log10)</th>
<th>Synergistic (%)</th>
<th>CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMC435 + Tib-NNI</td>
<td>0.5</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>TMC435 + Tib-NI</td>
<td>2.5</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>TMC435 + Tib-NNI + Tib-NI</td>
<td>1.5</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

Combination Effect

- Treatment of the cells with TMC435 in combination with Tib-NI or Tib-NNI resulted in additive or synergistic anti-HCV activity, respectively.
- Treatment with Tib-NI in combination with Tib-NI resulted in additive anti-HCV activity.
- The number of surviving colonies is indicated on the right lower corner of each graph. The number of surviving colonies is indicated on the right lower corner of each graph.

Conclusions

- In vitro replication studies reported here show that combined treatment with an HCV NS3/4A protease inhibitor (TMC435) and an HCV NSSP polymerase NNi or NNi:
  - suppresses HCV replication,
  - is additive or synergistic, with no antagonism observed,
  - increases anti-HCV activity and raises the genetic barrier to resistance,
  - results in improved clearance of replicon HCV RNA.

- In vitro treatment with a combination of all three inhibitors at low concentration further increases replicon HCV RNA clearance.

- These in vitro replication studies support further evaluation of TMC435 in combination with HCV NSSP inhibitors.

References