Direct-acting antiviral therapy for chronic hepatitis C

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Efficacy of re-treatment with TMC435, in combination therapy, for patients infected with hepatitis C virus genotype 1

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ABSTRACT

Background and Methods: In the TMC435-C101 study, 6 patients infected with HCV genotype 1 were given the protease inhibitor TMC435 (200 mg, once daily) as monotherapy for 5 days. Approximately 1.5 years later, 5 of these patients were re-treated with TMC435 (200 mg, once daily) plus pegylated interferon-a-2a and ribavirin (peg-IFNa-2a and RBV) for 4 weeks, followed by peg-IFNa-2a and RBV until week 48 (in the optimal protease inhibitor enhancement of response to therapy [OPERA-1] study).

Results and Conclusion: TMC435-resistant variants, which emerged in all 5 patients during the TMC435-C101 study, were no longer detected at the beginning of the OPERA-1 study, based on virus population sequencing. During the OPERA-1 study, 3 patients had a sustained viral response; deep sequencing indicated low-level persistence of resistant variants in the remaining 2 patients, which might have affected their response to re-treatment.

INTRODUCTION

New treatments for patients infected with HCV genotype 1 are now available including HCV NS3/4A protease inhibitors (PIs). Drug-resistant viral variants can emerge in patients treated with direct-acting antivirals (DAAs) who do not achieve sustained virologic response (SVR). Although it has been described that variants become undetectable after treatment failure in most patients, it is unclear whether low level variants persist and affect re-treatment options. TMC435 is an investigational, oral, once-daily (QD) HCV PI. In Study TMC435-C101, six patients infected with HCV genotype 1 received TMC435 (200 mg QD) as monotherapy for five days. Approximately 1.5 years later, five of the six patients participated in the Optimal Protease inhibitor Enhancement of Response to TherApy (OPERA-1; TMC435-C201) study and were treated with TMC435 (200 mg QD) plus peginterferon alpha-2a/ribavirin (peg-IFNa-2a/RBV) for 4 weeks, followed by peg-IFNa-2a/RBV up to Week 48. Patients were Caucasian males who were non-responders or relapsers to previous interferon-based therapy (Table 1).

Table 1. Baseline patient characteristics and virologic response in the OPERA-1 study (Cohort S).

<table>
<thead>
<tr>
<th>Patient / disease characteristics</th>
<th>Patient 140</th>
<th>Patient 141</th>
<th>Patient 142</th>
<th>Patient 143</th>
<th>Patient 144</th>
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<tbody>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>24.6</td>
<td>26.3</td>
<td>23.8</td>
<td>20.5</td>
<td>27.5</td>
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<td>Metavir score</td>
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<td>F4</td>
<td>F1</td>
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<tr>
<td>IL28B genotype</td>
<td>CC</td>
<td>TT</td>
<td>TT</td>
<td>CT</td>
<td>CT</td>
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<tr>
<td>HCV genotype subtype</td>
<td>1a</td>
<td>1b</td>
<td>1a</td>
<td>1b</td>
<td>1a</td>
</tr>
<tr>
<td>Previous IFN-based therapy</td>
<td>IFN/RBV</td>
<td>peg-IFN/RBV</td>
<td>peg-IFN/RBV</td>
<td>peg-IFN/RBV</td>
<td>peg-IFN/RBV/ amantadine</td>
</tr>
<tr>
<td>Response to previous IFN-based therapy</td>
<td>Relapser</td>
<td>Non Responder</td>
<td>Non Responder</td>
<td>Relapser</td>
<td>Non Responder</td>
</tr>
<tr>
<td>HCV RNA plasma concentration</td>
<td>Baseline</td>
<td>&lt;25 IU/mL (detectable)</td>
<td>24 IU/mL (detectable)</td>
<td>&lt;25 IU/mL (detectable)</td>
<td>&lt;25 IU/mL (detectable)</td>
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<tr>
<td>Day 14</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (detectable)</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (detectable)</td>
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<td>Day 28</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>NA</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (undetectable)</td>
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<td>Week 12</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>NA</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (undetectable)</td>
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<tr>
<td>Week 72 (SVR)</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>NA</td>
<td>NA</td>
<td>&lt;25 IU/mL (undetectable)</td>
<td>&lt;25 IU/mL (undetectable)</td>
</tr>
</tbody>
</table>

*Sub-haemophilia A
*Categorized by investigator

n129979860; NA, not applicable; peg-IFN, peginterferon; RBV, ribavirin; SVR, sustained virologic response; Patient 141 stopped treatment at day 14 due to elevated bilirubin levels; Patient 142 experienced viral breakthrough at Week 28 during peg-IFN/RBV treatment.
In TMC435-C101, all patients had a rapid and pronounced decline in HCV RNA during TMC435 monotherapy. Although no viral breakthrough was observed, mutations at NS3 amino acid positions 80, 155, 156, and/or 168 emerged in all patients. Deep sequencing at baseline of TMC435-C101 showed that there had been no additional pre-existing NS3 mutations at positions known to affect TMC435 activity in vitro (80, 155, 156, and 168). At baseline of OPERA-1, these variants which emerged during C101 were no longer detectable using population sequencing. By deep sequencing, additional Q80L and R155G were observed at baseline of OPERA-1 at low frequency (1-2%) in two out of three patients (Patients 140 and 144, respectively) who reached undetectable HCV RNA after four weeks of triple therapy and ultimately achieved SVR (Table 1). Q80L had been previously detected in Patient 140 as an emerging minor variant in TMC435-C101 (frequency <5%), while in patient 144, R155G had not been previously observed.

In the remaining two patients who did not achieve SVR and who were previous nonresponders to peg-IFN/RBV and of IL28B TT genotype (polymorphism rs2979860), HCV RNA decreases from baseline were slower in OPERA-1 where TMC435 was given in combination with peg-IFN/RBV compared with TMC435-C101 where TMC435 was given alone. In Patient 141, decreases at Day 7 were -2.8 IU/mL (OPERA-1) versus -3.5 log_{10} IU/mL (TMC435-C101). In Patient 142, decreases at Day 3 were -2.9 IU/mL (OPERA-1) versus -3.7 log_{10} IU/mL (TMC435-C101). Patient 142 achieved HCV RNA <25 IU/mL at the end of triple therapy (OPERA-1) and experienced viral breakthrough at Week 28 during peg-IFN/RBV treatment. Population sequencing identified emerging D168A/V and R155K mutations during TMC435-C101, and R155K at Day 7, Day 14 and time of viral breakthrough in OPERA-1. Based on deep sequencing (Figure 1), R155K became detectable (frequency 9%) at Day 3 of TMC435-C101 before becoming the major variant, and was detected 4 weeks after the end of TMC435 dosing (frequency 64%). R155K was still detectable at baseline of OPERA-1 (frequency 1%) and became the major variant from Day 7 until time of viral breakthrough, 24 weeks after end of TMC435 dosing. The ability of R155K to persist is consistent with reports showing the relatively high fitness of variants harboring this mutation. In addition to R155K, multiple mutations emerged at position 80 and 168 (Q80L, K and D168V, A, E, H, N) in parallel in TMC435-C101 and/or OPERA-1 as minor variants.

Patient 141 had a TMC435 dose reduction at Day 10 from 200 to 100 mg QD and stopped all treatment at Day 14 due to elevated bilirubin levels. HCV RNA levels did not decline further from Day 7 until Day 14. Q80R+D168E and Q80K+D168E mutations were detected at Day 7 and 4 weeks after the end of the 14-day treatment period in OPERA-1, respectively. In TMC435-C101, population-based sequencing demonstrated the emergence of D168V. Based on deep sequencing (Figure 1), D168V became detectable (frequency 2%) at Day 4 of TMC435-C101 before becoming the major variant, and D168E emerged (frequency 6%) at the last follow-up visit, 4 weeks after the end of dosing with TMC435. At baseline of OPERA-1, only the wild-type residue aspartate was observed at position 168, while during OPERA-1 D168E became the major variant (frequency 83% at Day 7) and D168V was only transiently detectable (frequency 12% at Day 7). In parallel to the emergence of D168E, Q80R and subsequently Q80K became detectable. This suggests that previous exposure to TMC435 facilitated the emergence of a

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**Figure 1.** Evolution of viral variants over time (NS3 amino acid 80, 122, or 156, 155, and 168 are shown) in patient 141 (A) and 142 (B) assessed using deep sequencing.

- **A**: Frequencies of individual amino acids (indicated in the figure) were calculated as number of sequence reads per amino acid divided by total number of reads at that position. Dotted lines connect points where the respective amino acid was not detected at intermittent time points. Amino acid frequency to a level of 0.1% is shown but frequency below 0.5% should be considered below the sensitivity of the assay (data not shown).

- **B**: Frequencies of individual amino acids (indicated in the figure) were calculated as number of sequence reads per amino acid divided by total number of reads at that position. Dotted lines connect points where the respective amino acid was not detected at intermittent time points. Amino acid frequency to a level of 0.1% is shown but frequency below 0.5% should be considered below the sensitivity of the assay (data not shown).
Q80R/K+D168E double mutant variant, which confers a similar level of resistance to TMC435 as the single D168V mutation but displays higher fitness. In summary, although only five patients were analyzed and their first exposure to TMC435 was short, all patients had TMC435-resistant variants at the end of their first treatment which were no longer detectable (by population sequencing) at baseline of a second study 1.5 years later. A similar pattern may occur in patients who fail a PI-based regimen and are later evaluated for retreatment. The data presented here suggest that in some patients the frequency of emerging resistant viral variants may decrease over time, in the absence of selective pressure imposed by a DAA, to levels that do not negatively impact outcome upon subsequent re-treatment with a regimen containing the same DAA. However, in other patients, resistant variants might persist at low levels, potentially below the detection limit of deep sequencing assays, which may impact the efficacy of a second course of treatment. It is important to note that the impact of pre-existing resistant variants on treatment outcome is intimately linked to the intrinsic peg-IFN/RBV responsiveness of the patient, as shown in this and other studies, since their sole presence does not automatically translate into treatment failure.12,13 The significance of pre-existing variants on SVR may be different when considering interferon-free regimens.

REFERENCES