Poster 812

Virologic analysis of genotype-1-infected patients treated with once-daily TMC435 during the Optimal Protease inhibitor Enhancement of Response to TherApy (OPERA)-1 study

O. Lenz,[1] L. Vijgen,[1] T. Lin,[1] M. Peeters,[1] G. De Smedt,[1] G. Picchio[2]

[1]Tibotec, Beerse, Belgium; [2]Tibotec Inc., Yardley, PA, USA

Disclosure: all authors are employees of Tibotec.

Corresponding author:
Oliver Lenz
Tibotec
Turnhoutseweg 30
2340 Beerse
Belgium
+3214641624
e-mail olenz@its.jnj.com

1. Premise

- TMC435 is a potent, once-daily hepatitis C virus (HCV) NS3/4A protease inhibitor with an *in vitro* 50% effective concentration (EC_{50}) value of 8 nM in a genotype-1b replicon cell line.[1]
- *In vitro* resistance studies identified changes at amino acid positions 43, 80, 155, 156, and 168 within the NS3 protease region that confer variable degrees of reduced susceptibility to TMC435.[2]
- In a Phase IIa proof-of-concept study (OPERA-1; TMC435-C201; NCT00561353), HCV genotype-1-infected treatment-naïve and -experienced patients were treated for four weeks with TMC435 at doses between 25 mg and 200 mg once daily (QD) in combination with pegylated (Peg) interferon (IFN)α-2a and ribavirin (RBV), followed by PegIFNα-2a/RBV up to Week 48. At the four-week interim analysis:[3-5]
  - All doses of TMC435 were generally safe and well tolerated.
  - TMC435 demonstrated potent, dose-dependent antiviral activity in both treatment-naïve and -experienced patients.
  - All treatment-naïve patients receiving TMC435 at doses of 75 mg or 200 mg QD in combination with PegIFNα-2a/RBV; and 4/9, 7/9, and 7/10 treatment-experienced patients receiving TMC435 at doses of 75 mg, 150 mg, or 200 mg QD in combination with PegIFNα-2a/RBV, respectively, achieved HCV ribonucleic acid (RNA) levels of <25 IU/mL at Week 4.
  - Mean trough TMC435 concentrations at Day 28 following TMC435 doses of 25 mg, 75 mg, and 200 mg QD were approximately 10-fold, 50-fold, and 800-fold higher than the EC_{50} value of 8 nM (6 ng/mL).
- TMC435 is currently in Phase IIb development.
- For patients included in the OPERA-1 study, we investigated the relationship between specific NS3 variants at baseline and:
  - *In vitro* susceptibility to TMC435
  - Response during four weeks of treatment with TMC435.
- Emerging viral variants in patients with viral breakthrough were characterized using population sequencing and genotype-1b transient chimeric replicon assays.

2. Methods

2.1 Study design

- The OPERA-1 study design is summarized in Figure 1.
  - Patients from Cohorts 1, 2, and 4 were included in this analysis.
- OPERA-1 was a double-blind, placebo-controlled Phase IIa study to assess the antiviral activity, safety, and pharmacokinetics (PK) of TMC435 in HCV genotype-1-infected patients. Treatment-naïve patients (Cohorts 1 and 2) were randomized to receive either 7 days of monotherapy with TMC435 at doses of 25 mg, 75 mg, or 200 mg QD, or placebo, followed by 21 days of triple therapy with TMC435 or placebo in combination with PegIFNα-2a/RBV (Panel A), or 28 days of triple therapy with TMC435 (at the same doses as in Panel A) or placebo (Panel B). In Cohort 4, patients who had failed previous (Peg)IFN/RBV therapy received 28 days of triple therapy with TMC435 at doses of 75 mg, 150 mg, or 200 mg QD, or placebo.

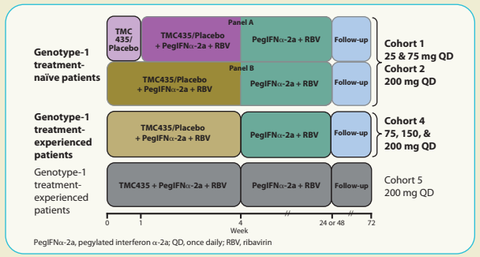

Figure 1. Study design of OPERA-1.

2.2 Virologic analysis

- HCV RNA was extracted from plasma samples and the NS3/4A region was sequenced using standard population sequencing.
- For phenotypic analyses, NS3 protease regions were introduced into a genotype-1b replicon backbone (clone ET). Antiviral activity of TMC435 against the mutant or chimeric replicons compared with the parental (wild type) replicon was assessed in a standard transient replicon assay.

3. Results

3.1 Effect of baseline mutations on response to TMC435 therapy

- The overall prevalence of Q80K and R155K mutations at baseline in OPERA-1 was 18 (15.9%) and 1 (0.9%) of 133 patients, respectively, with a higher prevalence in genotype-1a than -1b.
- The presence of mutation Q80K in patient-derived NS3 protease domains resulted in fold changes (FC) in EC_{50} values ranging from 0.8–15.5 in a transient replicon assay.
- The sample with an R155K mutation (T40A and L153I mutations were also present) resulted in an FC of 95 for TMC435.
- No clear correlation between FC for TMC435 at baseline and HCV RNA levels at Day 28 was observed. Baseline samples from all patients with viral breakthrough were fully susceptible to TMC435.

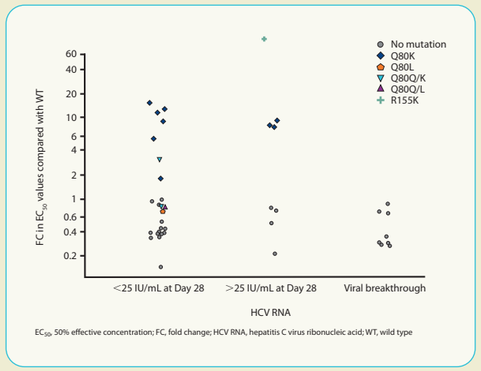

Figure 2. TMC435 fold changes in EC_{50} values compared with WT assessed in a transient replicon assay for chimeric genotype-1b replicons engineered with the NS3 protease domain of baseline samples from patients included in the OPERA-1 study versus HCV RNA levels (<25 and >25 IU/mL) at Day 28 or viral breakthrough. The selection of samples shown is biased towards those with Q80K and R155K variants and does not represent the natural distribution. Mutations at NS3 positions 43, 80, 155, 156, or 168 (i.e. changes from the reference sequence con1 for genotype-1b and H77 for genotype-1a) are indicated.

- Six out of eight patients harboring a Q80K variant at baseline and treated with TMC435 at doses of ≥75 mg QD achieved HCV RNA levels <25 IU/mL detectable or undetectable at Day 28.
- HCV RNA levels in the remaining two patients (patients 0346 and 0281; both previous non-responders to IFN-based therapy) declined >2 log_{10} IU/mL from baseline at Day 3 and reached 30 and 411 IU/mL at Day 28, respectively. Population-based sequencing of viral RNA from the latter subject revealed emerging F43S, R155K, and D168E mutations in addition to the already-present Q80K mutation, which could have contributed to the plateau effect on observed HCV RNA levels.
- None of the four patients with a Q80K mutation at baseline treated with TMC435 25 mg QD had a >2 log_{10} IU/mL decline in HCV RNA at Day 3, and two of four achieved HCV RNA levels of <25 IU/mL at Day 28.
- A limited decline in HCV RNA levels was observed in the one patient with a pre-existing R155K variant in combination with T40A and L153I mutations (patient 0535; prior non-responder to PegIFN/RBV therapy) who received triple therapy with TMC435 75 mg in the OPERA-1 study. The FC of the baseline sample (95) was similar to that reported in a genotype-1a replicon harboring the R155K, T40A, and L153I mutations.

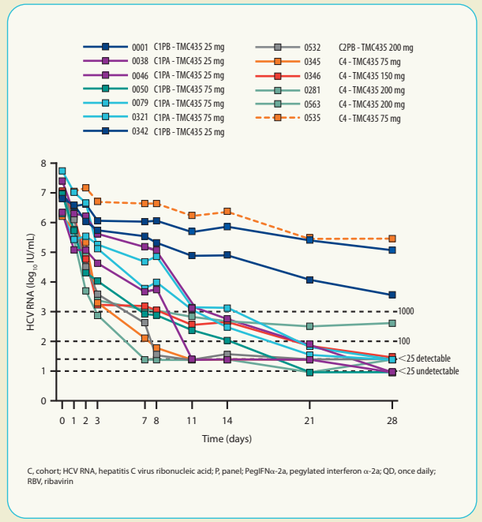

Figure 3. Individual changes in plasma HCV RNA over time in patients with pre-existing mutations during four weeks of treatment with TMC435 25–200 mg QD plus PegIFNα-2a/RBV in the OPERA-1 study. Patients with a baseline Q80K variant are represented by a solid line; patients with a baseline R155K variant are represented by a dashed line. HCV RNA levels of 1000 IU/mL, 100 IU/mL, and <25 IU/mL detectable or undetectable are indicated.

3.2 Viral breakthrough and associated mutations during the first four weeks of treatment in the OPERA-1 study

- No viral breakthroughs (defined as a >1 log_{10} IU/mL increase in HCV RNA from the lowest level reached) were observed during the 28-day TMC435 treatment period in treatment-naïve patients receiving 28 days of triple therapy (Panel B) or in treatment-experienced patients receiving triple therapy with TMC435 200 mg QD.
- Overall, 8/82 (10%) patients who received TMC435 had viral breakthrough, and in all of them, one or more of the following mutations emerged in the virus population during viral breakthrough: Q80R or K; R155K; or D168A, E, N, or V (Figure 4).
- None of the patients with viral breakthrough harbored virus with a pre-existing mutation known to confer reduced susceptibility to TMC435 *in vitro* (based on population sequencing).
- Emerging mutations in those same patients with viral breakthrough during treatment with TMC435 as monotherapy or as part of triple therapy conferred intermediate to high FC in EC_{50} values in a transient replicon assay (Figure 5).

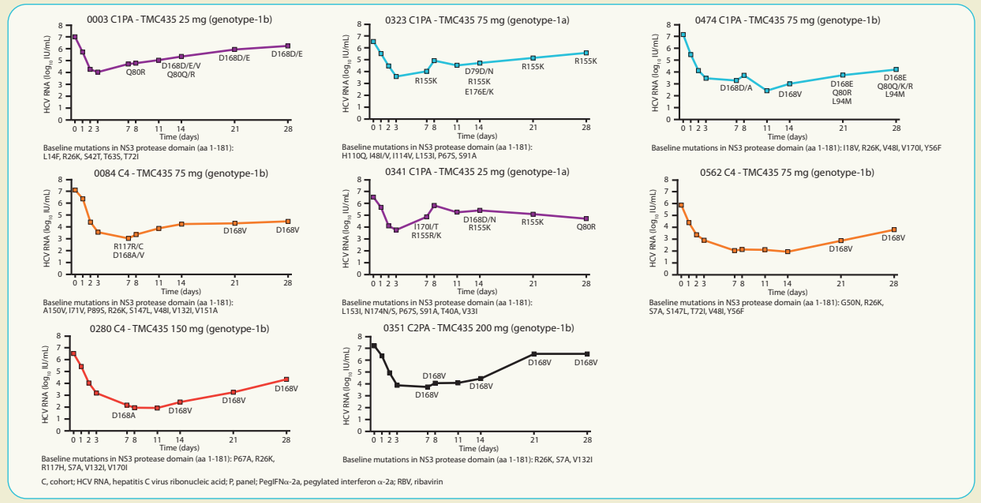

Figure 4. Individual changes in plasma HCV RNA over time for all patients who had viral breakthrough during four weeks of treatment with TMC435 plus PegIFNα-2a/RBV in the OPERA-1 study. Baseline mutations, defined as changes from reference sequence (con1 for genotype-1b and H77 for genotype-1a), are depicted below the graph. Emerging mutations in NS3 protease domain (aa 1-181) are indicated in each graph at the time points with sequence information available.

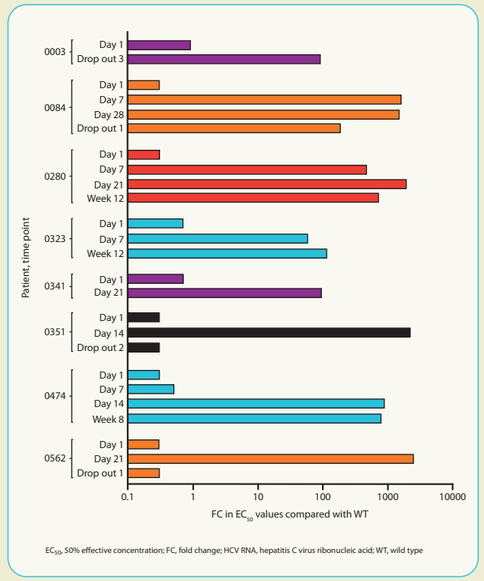

Figure 5. Fold change in EC_{50} values for TMC435 compared with WT assessed in a transient replicon assay using chimeric genotype-1b replicons engineered with the NS3 protease domain.

4. Conclusions

- **The presence of Q80K variants at baseline did not appear to affect the antiviral activity observed at Day 28 with TMC435 at doses of ≥75 mg QD.**
- **No viral breakthroughs were observed during the 28-day TMC435 treatment period in treatment-naïve patients receiving 28 days of triple therapy (Panel B) or in treatment-experienced patients receiving triple therapy with TMC435 200 mg QD.**
- **Viral breakthrough was infrequent, was observed in patients receiving initial monotherapy or in those who had failed prior Peg(IFN) therapy, and was characterized by the emergence of variants with reduced susceptibility to TMC435 *in vitro*.**
- **These findings provide the first insights into the relevance of viral variants with reduced susceptibility to TMC435 and treatment response.**

5. Acknowledgments

Special thanks to our colleagues from Tibotec and Virco who contributed to this work. Medical writing support was provided by Angela Corstorphine on behalf of Complete Medical Communications and funded by Tibotec.

6. References

1. Lin TI et al. Antimicrob Agents Chemother 2009; 53: 1377-1385.
2. Lenz O et al. Antimicrob Agents Chemother 2010; 54: 1878-1887.
3. Marcellin P et al. Poster presented at the 44th Annual Meeting of European Association for the Study of the Liver (EASL), Copenhagen, Denmark, April 22-26, 2009.
4. Manns M et al. Presented at the 44th Annual Meeting of European Association for the Study of the Liver (EASL), Copenhagen, Denmark, April 22-26, 2009.
5. Sekar V et al. Poster presented at the 45th Annual Meeting of European Association for the Study of the Liver (EASL), Vienna, Austria, April 14-18, 2010.

Poster presented at the 61st AASLD Meeting, Boston, MA, USA, October 29-November 2, 2010