Research Article

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# Virologic response and characterisation of HCV genotype 2–6 in patients receiving TMC435 monotherapy (study TMC435-C202)

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**Background & Aims**: TMC435 is a potent, once-daily, investigational hepatitis C virus (HCV) NS3/4A protease inhibitor in phase III clinical development. In the phase II trial TMC435-C202 (NCT00812331), TMC435 displayed potent activity in genotype 4, 5 and 6 patients and in 3/6 genotype 2 patients, whereas no activity was observed with genotype 3.

**Methods**: Thirty-seven patients received TMC435 monotherapy (200 mg once daily) for 7 days. HCV RNA, NS3 protease sequences and the corresponding phenotypes were evaluated.

**Results**: Genotype and isolate-specific baseline polymorphisms at NS3 positions known to affect HCV protease inhibitor activity were present in all genotypes. Consistent with the antiviral activity observed in genotypes 4 and 6, TMC435 was active *in vitro* against all genotype 4 isolates, and against most genotype 6 polymorphisms when tested as single or double mutants. In contrast, in genotype 3 where no HCV RNA decline was observed, isolates displayed >700-fold increases in $EC_{50}$ attributed to the D168Q polymorphism. In genotypes 2 and 5, HCV RNA changes from baseline to Day 3 ranged between −0.3 to −3.6 and −1.5 to −4.0 $\log_{10}$ IU/ml, respectively, and isolates or site-directed mutants displayed intermediate *in vitro* susceptibility to TMC435 with fold changes in $EC_{50}$ between 15 and 78. Viral breakthrough in genotypes 4–6 was associated with emerging mutations including Q80R, R155K and/or D168E/V.

**Conclusions**: Sequence and phenotypic analyses of baseline isolates identified polymorphisms which could explain the differences in antiviral activity between genotypes. Pathways of TMC435 resistance in genotypes 2–6 were similar to those identified in genotype 1.

Keywords: Hepatitis C; Virologic response; Non-genotype 1; TMC435 monotherapy; HCV NS3/4A protease inhibitor; Polymorphism.

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*Abbreviations:* HCV, hepatitis C virus; PegIFN, pegylated interferon; RBV, ribavirin; SVR, sustained virologic response; DAAs, direct-acting antiviral agents; PIs, protease inhibitors; BID, twice daily; QD, once daily; LLOQ, lower limit of quantification; RT-PCR, reverse transcriptase-polymerase chain reaction; BLAST, basic local alignment search tool; $EC_{50}$, half maximal effective concentration; FC, fold changes; $C_{min}$, minimum plasma concentration (TMC435 plasma exposure).



## Introduction

An estimated 3% (170 million people) of the global population is infected with hepatitis C virus (HCV), a leading cause of chronic liver disease [1]. At least six major genotypes of HCV have been identified with distinct geographical distributions [2,3].

Until recently, the standard of care for the treatment of chronic HCV infection consisted of 24–48 weeks of pegylated interferon (PegIFN) and ribavirin (RBV) [4]. Sustained virologic response (SVR, undetectable plasma HCV RNA 24 weeks after end of treatment) with this regimen ranged between 40% and 80% depending upon multiple factors such as HCV genotype, patient *IL28B* genotype and race.

Novel direct-acting antiviral agents (DAAs) are being developed in combination with PegIFN/RBV or in IFN-free DAA combinations to improve efficacy and shorten treatment duration. Two HCV NS3/4A protease inhibitors (PIs), boceprevir [5,6] and telaprevir [7,8] have demonstrated significantly improved SVR rates when given in combination with PegIFN/RBV in genotype 1 patients. Their approval for the treatment of HCV genotype 1 infection has led to expectations of a paradigm shift in the way this disease is managed [9]. However, these new agents require thrice-daily dosing, are associated with more frequent and severe anaemia and rash, and have only been investigated *in vivo* in a limited number of patients infected with HCV genotypes 2-4 [10–12].

Improved *in vitro* replication and infection models have allowed assessment of antiviral activity in non-genotype 1 HCV [11,13,14]. Nucleotide inhibitors generally have *in vitro* activity across genotypes and show promising results in non-genotype 1 patients [15]. Pan-genotypic activity is more difficult to achieve with NS3/4A, NS5A and non-nucleoside NS5B inhibitors due to the sequence variation observed between genotypes and subtypes, occurring in sequences encoding the binding pocket of the respective inhibitors [16]. The first PI to advance to clinical evaluation, BILN2061, demonstrated good antiviral activity against genotype 1 [17] but had little or no activity against genotypes 2 and 3 [18]. Variation at the inhibitor binding pocket

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residues was prevalent in these genotypes and may explain the reduced activity [19,20]. Recent clinical studies with telaprevir monotherapy suggested activity in genotype 2, limited activity in genotype 4 and no activity against genotype 3 [10,12]. In a short monotherapy study with boceprevir, some antiviral activity was demonstrated in patients infected with genotypes 2 and 3 [21].

TMC435 is a potent, investigational HCV NS3/4A PI in phase III clinical development for the treatment of HCV genotypes 1 and 4 infection with a once-daily (QD) 150 mg dose in combination with PegIFN/RBV. Phase I and II trials in patients infected with HCV genotype 1 have demonstrated that TMC435 is a highly potent and efficacious antiviral which is generally well tolerated [22–24].

A phase IIa, open-label, proof-of-concept study (TMC435-C202; clinicaltrials.gov ID: NCT00812331), assessed antiviral activity, safety, tolerability and pharmacokinetics of TMC435 (200 mg QD for 7 days) in treatment-naïve patients infected with HCV genotypes 2–6 [25]. Here we describe the virologic analysis of this study, including the role of baseline genotype and subtype specific polymorphisms and their effect on virologic response to TMC435, and characterisation of viral variants in patients with viral breakthrough.

## Patients and methods

### *Study design and patient population*

Thirty-seven treatment-naïve patients, enrolled across Germany, Belgium, and Thailand (genotypes 2 [n = 6], 3 [n = 8], 4 [n = 8], 5 [n = 7], and 6 [n = 8]), received oral TMC435 200 mg QD monotherapy for 7 days. After the TMC435 treatment period, all patients received PegIFN/RBV. The follow-up period was up to Day 42 (35 days after the last TMC435 administration) including two assessments: follow-up 1 (Day 21) and follow-up 2 (Days 37–42). Multiple subtypes were included in cohorts for genotype 2 (2, 2b, 2c, 2i, 2k), genotype 4 (4, 4c, 4d), and genotype 6 (6a, 6c–l, 6j, 6n).

### *Antiviral activity*

Plasma samples were obtained at baseline, Days 1–11, and follow-ups 1 and 2. HCV RNA levels were quantified using the COBAS Taqman HCV v2 assay, (lower limit of quantification [LLOQ], 25 IU/ml).

The primary end point was change from baseline in HCV RNA at Day 8. Secondary efficacy end points included change from baseline in HCV RNA at other time points and proportion of patients with viral breakthrough (>1 $\log_{10}$ IU/ml increase in HCV RNA from nadir, or >100 IU/ml in those with prior HCV RNA <25 IU/ml undetectable).

### *HCV NS3/4A sequence analysis and subtype determination*

HCV RNA was extracted from plasma samples obtained during the study and the HCV NS3/4A region was sequenced using reverse transcription-polymerase chain reaction (RT-PCR) and standard Sanger population sequencing. HCV geno/subtypes were determined by Trugene, Versant LiPA v2 assay and/or sequencing followed by basic local alignment search tool (BLAST) analysis of a 329 bp region within the HCV *NS5B* gene.

### *Phenotypic characterisation using a transient replicon assay*

Mutations were engineered into a genotype 1b replicon, or for the chimeric replicon assay, sequences of the NS3 protease domain (aa7–192) derived from patient isolates were introduced into a genotype 1b replicon backbone for genotype 3 and 4 isolates or genotype 2a backbone for genotype 2 isolates. Antiviral activity of TMC435 against the mutants or chimeric replicons, as indicated by the half maximal effective concentration ($EC_{50}$) values, was compared with that of the parental wild type replicon using luciferase read-out, as described previously [26]. Fold changes (FC) in $EC_{50}$ were calculated.

To assess the effect of baseline polymorphisms on antiviral activity, the FC in $EC_{50}$ values were plotted against change in HCV RNA from baseline to Day 3. For each patient isolate, a ratio of TMC435 plasma exposure ($C_{min}$) value at Day 7 over $EC_{50}$ determined in the replicon assay was calculated and plotted against HCV RNA change from baseline.

## Results

### *Antiviral activity*

Antiviral activity and safety results from this study have been reported previously. TMC435 demonstrated activity against multiple HCV genotypes, except for genotype 3 (Fig. 1) [25]. The greatest reduction in HCV RNA was observed for genotypes 4 and 6, followed by genotype 5. A rapid and substantial decline in HCV RNA was evident for 3/6 genotype 2 patients.

### *NS3 protease polymorphisms present at baseline*

Genotype- and isolate-specific baseline polymorphisms include changes associated with reduced *in vitro* susceptibility to PIs (Fig. 2) [27]. These polymorphisms were located at NS3 positions 36, 54, 80, 122, 168, 169 and 170 (defined as difference from genotype 1a reference sequence H77). V36L, Q80G, and S122R were present in all genotype 2 patients for which sequence information was available. All genotype 3 patients carried V36L and D168Q polymorphisms, and V36L, S122T, and I170V were found in all genotype 4 patients. Of note, a valine at position 170 is present in the genotype 1b reference sequence. In addition, T54S was observed in one patient (subtype 4a) and F169L in another patient (subtype 4d). The combination of V36L and Q80K was present in all genotype 5a-infected patients and, in addition, S122A (n = 4) or S122G (n = 1) in some of these patients. All genotype 6 patients carried S122T or S122N while V36L, Q80K, and I170V were observed in 3, 1, and 6 patients, respectively.

### *Effect of baseline polymorphisms on TMC435 activity in vitro*

The effect of baseline polymorphisms on TMC435 *in vitro* activity was assessed in a transient replicon assay with a genotype 1b or 2a replicon backbone, in which the NS3 protease domain derived from patient isolates (chimeric replicon assay) or selected mutations were introduced. TMC435 activity against genotype 2 baseline isolates was reduced 22- to 78-fold compared with the reference wild type replicon. The S122R polymorphism, present in all genotype 2 patients in this study as a single mutation, resulted in an FC of 20 (Fig. 3A). In contrast, four other amino acid changes at position 122 (S122A, G, N, and T) observed in genotypes 5 and 6 did not affect TMC435 activity (FC of $\leqslant$1.1) (Fig. 3D and E). TMC435 activity was reduced compared to genotype 1 reference for the two genotype 3 isolates (778- and 1250-fold) tested. This can be largely attributed to the D168Q mutation, which as a single mutation confers an FC of 385 (Fig. 3B). In contrast, all genotype 4 isolates remained fully susceptible to TMC435 (FC of <1) (Fig. 3C). Consistently, no effect on TMC435 activity was observed (FC of $\leqslant$1.3) when the baseline genotype 4 polymorphisms were tested individually. The V36L and Q80K polymorphisms present in all genotype 5 patients