Rapid HCV-RNA Decline With Once Daily TMC435: A Phase I Study in Healthy Volunteers and Hepatitis C Patients

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BACKGROUND & AIMS: The search for targeted anti-hepatitis C virus (HCV) drugs is driven by the adverse effect profile and limited efficacy of the current standard of care (pegylated interferon-α/ribavirin). In a first-in-human trial, we tested the safety, tolerability, and pharmacokinetics of the macrocyclic HCV NS3/4A protease inhibitor TMC435 in healthy volunteers, followed by HCV genotype 1-infected patients to assess antiviral activity. **METHODS:** The TMC435350-C101 study was a phase I, randomized, double-blind, placebo-controlled trial in 49 healthy volunteers, followed by an open-label, nonplacebo-controlled panel in 6 genotype 1 hepatitis C patients. Healthy volunteers received oral, single, ascending doses (up to 600 mg) or 5-day multiple ascending doses (200 mg twice daily or 100, 200, or 400 mg once daily). Patients received 200 mg once daily for 5 days. Pharmacokinetics and safety were evaluated for all panels, and plasma HCV-RNA levels were determined in patients. **RESULTS:** There were no serious adverse events, no grade 3 reactions, and no treatment-related discontinuations; pharmacokinetics supported a once daily dosing regimen. Plasma HCV-RNA levels dropped rapidly in all patients, with a median maximal reduction of 3.9-$\log_{10}$ IU/mL and a median of 6 days to maximal reduction. The initial steep reduction of HCV-RNA (median 3.5-$\log_{10}$ IU/mL at day 3) was followed by a more gradual decline that was maintained over the dosing period. No viral breakthroughs (>1-$\log_{10}$ IU/mL HCV-RNA increase from nadir) were observed during treatment nor in the 3 days posttreatment; HCV-RNA returned to pretreatment levels by week 4. **CONCLUSIONS: Once daily TMC435 given orally was generally safe and well tolerated and demonstrated potent antiviral activity.**

Keywords: TMC435; HCV; Protease Inhibitor; Phase-1 Study.

The number of individuals chronically infected with hepatitis C virus (HCV) globally has been estimated at 170 million, with 3–4 million new infections occurring each year.[1] Approximately 20%–45% of patients clear the virus in the acute phase,[2] a process that depends on an early, strong, and broad CD4 and CD8 cell immune response to viral antigens.[3] For chronically infected patients, treatment success is primarily determined by viral genotype. Standard of care for genotype 1 patients, the most refractory to therapy, is a 48-week course of pegylated interferon α (Peg-IFN) and ribavirin (RBV).[4] HCV clearance is achieved in approximately 45% of patients infected with genotype 1, in 50%–60% of genotype 4 patients, and in up to 80% of patients infected with genotype 2 or 3.[5,6] The low response rate for genotype 1 has prompted the development of specific targeted antiviral drugs that, in combination with Peg-IFN and RBV, can lead to increased cure rates and shorter treatment durations.[7]

Small molecule drugs targeting the HCV NS3/4A serine protease have been optimized from the natural substrate and show potent antiviral activity in HCV patients.[8] There are 2 protease inhibitors currently in phase III development: telaprevir and boceprevir. Telaprevir monotherapy (750 mg, once every 8 hours) resulted in a rapid decline in HCV-RNA with a median maximal reduction from baseline of 4.4-$\log_{10}$ IU/mL over a 14-day dosing period.[9] Subsequent studies combined protease inhibitors with Peg-IFN and RBV, which resulted in higher HCV-RNA reduction and improving cure rates.[10,11]

The macrocyclic protease inhibitor TMC435 has been identified as a potent and specific inhibitor of HCV replication.[12] In biochemical assays using NS3/4A proteases of HCV genotypes 1–6, half maximal inhibitory concentration values below 13 nmol/L were observed, with the exception of genotype 3a protease (37 nmol/L).[13] Different genotype 1a and 1b replicons were inhibited by TMC435, with half maximal effective concentration (EC_{50}) values ranging between 8 and 28 nmol/L and half maximal cytotoxicity concentration values above 16 μmol/L in a range of human cell lines (selectivity indexes

Abbreviations used in this paper: aa, amino acid; AE, adverse event; AUC_{24h}, area under the plasma concentration-time curve; EC_{50}, half maximal effective concentration; HCV, hepatitis C virus; Peg-IFN, pegylated interferon α; QD, once daily; RBV, ribavirin.

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above 2000). Addition of human serum or serum components in the replicon assay increased EC_{50} values less than 2-fold, suggesting a limited effect of functional protein binding on TMC435 activity. In replicon cells, TMC435 was synergistic with IFN and an HCV NS5B polymerase inhibitor and additive with RBV. In rats, the compound was extensively distributed to the liver and intestinal tract, and the absolute bioavailability was 44% after a single oral administration. Compound concentrations detected in both plasma and liver at 8 hours postdosing in rats were above the EC_{99} value measured in the replicon assay and around the EC_{50} value at 24 hours, suggesting feasibility of once daily (QD) dosing.[14]

The TMC435350-C101 study described here was designed to investigate the safety, tolerability, and pharmacokinetic profile of TMC435 in single and multiple ascending doses in healthy volunteers. These results supported the inclusion of a separate panel enrolling 6 chronic hepatitis C patients with genotype 1 who had failed previous IFN-based treatment regimens. A potent antiviral activity was observed in all patients with a QD schedule for 5 days.

Materials and Methods

TMC435350-C101 Study Design

This trial was a randomized, double-blind, placebo-controlled trial to determine the safety, tolerability, and pharmacokinetics of TMC435 after single and multiple oral intakes in 49 healthy non-HCV-infected subjects, followed by an open-label, repeated dosing session in 6 chronic hepatitis C patients with genotype 1 (nonplacebo controlled) (Figure 1).

The trial was conducted from January 23, 2007, until September 4, 2007, at PRA International EDS (Zuidlaren, The Netherlands) and Academic Medical Center, Department of Gastroenterology and Hepatology (Amsterdam, The Netherlands). Study details were made available beforehand on the ClinTrials.gov Web site. The study was performed in accordance with the principles of Good Clinical Practice as outlined in 21 CFR Parts 50, 56, and 312; the Declaration of Helsinki and its subsequent revisions; and the European Union (EU) Clinical Trials Directive. Prior to study initiation, the final protocol and amendments were reviewed and approved by independent ethics committees and the Regulatory Authority in The Netherlands. All subjects provided written informed consent before participating in any study-related activity. Candidates were informed about the nature and purpose of the trial, participation and termination conditions, and risks and benefits. The study was conducted as an inpatient study, with all subjects admitted to the clinical facility 2 days prior to the first dose and discharged 72 hours after the last dose of test drug.

The single dose escalation part of the trial was designed with 6 dose groups and included 2 panels of 9 healthy non-HCV-infected subjects. In each dose group, 6 subjects received active treatment and 3 received placebo after a standardized breakfast. The doses of the test compound were consecutively escalated, following interim data reviews for safety, tolerability, and pharmacokinetics. A washout period of at least 10 days was respected between consecutive TMC435 or placebo doses within each panel. The effect of food or fasting was studied on exposure of a 200-mg QD dose. Single doses up to 600 mg were found to be generally safe and well tolerated. However, data will not be presented in detail here.

Subsequently, safety, tolerability, and pharmacokinetic profiles were studied in healthy non-HCV-infected subjects during 5-day oral multiple ascending doses (200 mg twice daily or 100, 200, or 400 mg QD) in different cohorts of 6 TMC435 + 3 placebo subjects. These re-

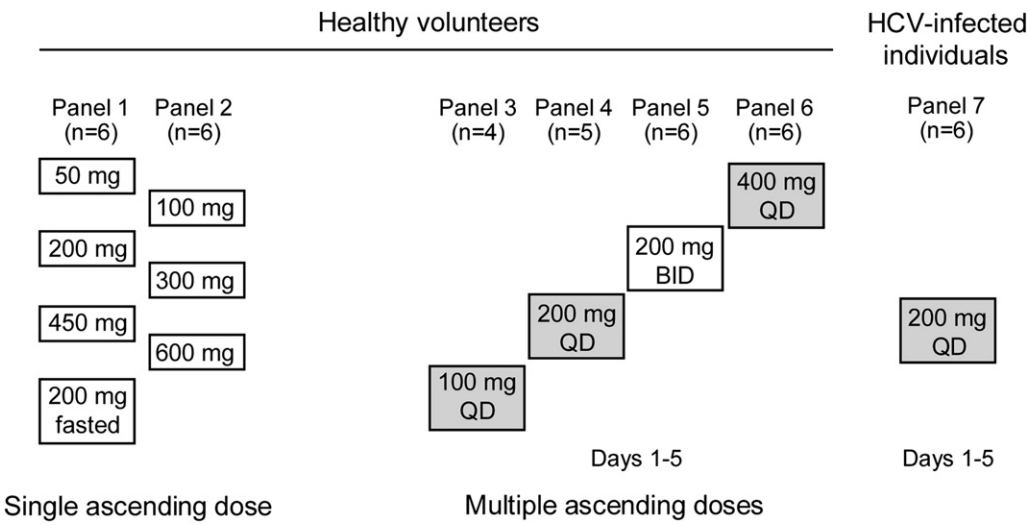

Figure 1. Design of the TMC435350-C101 trial.

peated doses were found to be generally safe and well tolerated.

Thereafter, an open-label panel including chronic hepatitis C patients with genotype 1 was added. A 200-mg, QD regimen was selected, which was shown to be safe in healthy subjects when administered for 5 days. In addition to drug safety and pharmacokinetic parameters, HCV-RNA levels were determined in plasma and followed up to 30–35 days after the last drug intake.

Inclusion Criteria of Healthy Volunteers and Hepatitis C Patients

Forty-nine healthy non-HCV-infected subjects were selected and divided into 6 panels of 6 to 9 subjects each. Six hepatitis C patients infected with genotype 1 virus were assigned to panel 7 (Table 1).

Volunteers were determined healthy on the basis of a medical evaluation that included a physical examination, medical history, electrocardiogram, transcutaneous echocardiogram (multiple dose panels only), blood biochemistry, blood coagulation, hematology tests, urinalysis, and an HCV/human immunodeficiency virus serology test carried out at screening.

Chronic hepatitis C patients should be nonresponders or relapsers to previous IFN-based therapy and needed plasma HCV-RNA levels of at least 50,000 IU/mL at screening. Patients with compensated cirrhosis were also allowed to participate.

TMC435 Administration

TMC435 and placebo were manufactured and provided under the responsibility of Tibotec Pharmaceuticals Ltd (Eastgate Village, Ireland). TMC435 was formulated as an oral solution containing (100 mg/mL) in polyethylene glycol 400 (PEG400), sodium hydroxide, and hydrochloric acid. The placebo treatment consisted of the oral vehicle solution PEG400. TMC435 or placebo was dosed after a standardized meal and completed by drinking a glass of water.

Safety Assessments

Safety was closely monitored throughout and up to 1 month after dosing. Safety parameters included physical examination, vital signs, laboratory parameters (routine hematology, biochemistry, urinalysis), cardiovascular safety, and adverse events (AEs) monitoring. Safety and tolerability were evaluated continuously and documented. Interim pharmacokinetic data were evaluated 24 hours after the last dose of each multiple dosing regimen. Dose escalation was continued only if the previous dose was found to be safe and tolerated by study participants and approved by the medical ethics committee.

Pharmacokinetic Assessments of TMC435

To evaluate the plasma pharmacokinetics of TMC435, multiple blood samples were taken on days 1 and 5, with a follow-up of 72 hours after the last dose. Additional sampling was performed prior to dosing on days 2, 3, and 4. Following a selective plasma sample cleanup, plasma concentrations of TMC435 were determined using a validated liquid chromatography mass spectrometry/mass spectrometry method, with a lower limit of quantification of 2.00 ng/mL.

Antiviral Assessments

Plasma HCV-RNA levels were determined using the Roche Cobas Taqman HCV/HPS assay v2.0 (Roche Molecular Diagnostics, Basel, Switzerland) (performed at Covance, Switzerland) with a dynamic range of 25–391,000,000 IU/mL with a lower limit of quantification of 25 IU/mL and a lower limit of detection of approximately 10 IU/mL. Samples were taken at screening; day −1; day 1 (every 8 hours, including predose); day 2 (every 12 hours, including predose); days 3 to 5 (predose); and at 24 hours, 48 hours, and 72 hours after the last dose to coincide with sampling for pharmacokinetic

Table 1. Baseline Characteristics of the Study Subjects: Healthy Volunteers

	Part I			Part II				
Parameter	Panel 1, n = 9	Panel 2, n = 9	Total, n = 18	Panel 3, n = 6	Panel 4, n = 7	Panel 5, n = 9	Panel 6, n = 9	Total, n = 31
Age, *y*, median (range)	37.0 (21–55)	38.0 (23–55)	37.5 (21–55)	40.5 (22–53)	25.0 (23–51)	38.0 (25–54)	24.0 (20–44)	27.0 (20–54)
BMI (kg/m^2), median (range)	24.7 (19–30)	24.4 (21–28)	24.5 (19–30)	22.5 (19–27)	26.0 (21–30)	24.3 (22–29)	22.1 (19–27)	23.6 (19–30)
Sex, *n*								
Male	8	9	17	3	7	8	8	26
Female	1	0	1	3	0	1	1	5
Ethnicity, *n*								
White	8	7	15	6	7	9	8	30
Oriental/Asian	0	1	1	0	0	0	1	1
Black	0	1	1	0	0	0	0	0
Other	1	0	1	0	0	0	0	0

Table 2. Baseline Characteristics of the Study Subjects: Hepatitis C Patients

	Panel 7: HCV patients, n = 6
Age, *y*, median (range)	56.5 (32–67)
BMI (kg/m^2), median (range)	23.3 (21–27)
Sex, *n*	
Male	6
Female	0
Ethnicity, *n*	
White	6
Hemophilia	2
ALT (ULN, 69 U/L)	107.3
Range	34–262
AST (ULN, 52 U/L)	81.7
Range	31–189
Viral subtype	
1a	4
1b	2
Baseline viral load, median (range)	6.75 (6.47–7.03)

BMI, body mass index; ULN, upper limit of normal.

measurements. HCV-RNA levels were also determined at 10–14 days and 30–35 days after last drug intake.

NS3/4A Sequence and HCV Genotype Determination

The sequence of the NS3/4A region or the NS3 protease domain (amino acids [aa] 1–181) of the HCV genome obtained from patient sera was determined on a population level using polymerase chain reaction and standard Sanger sequencing-based methods. HCV genotype subtyping was conducted by standard Sanger sequencing followed by automated BLAST analysis of a 329-base pair region within the HCV NS5B gene.[15]

Results

Baseline Demographic of Hepatitis C Patients and Healthy Volunteers

Forty-nine healthy volunteers and 6 hepatitis C patients were included in the study according to the scheme outlined in Figure 1 and described in the Material and Methods section. Baseline demographic data are summarized for healthy volunteers (Table 1) and for the hepatitis C patients (Table 2). The median age of the hepatitis C patients was higher than the healthy volunteers and ranged from 32 to 67 years; other nonviral related parameters were similar. All hepatitis C patients were male and of white ethnicity, and the median plasma HCV-RNA level was 6.75-$\log_{10}$ IU/mL (range, 6.47- to 7.03-$\log_{10}$ IU/mL) at baseline. Four hepatitis C patients were infected with genotype 1a and 2 patients with genotype 1b as assessed by NS5B-based sequence analysis. All 6 hepatitis C patients had failed at least 1 previous IFN-based therapy, with 4 of them never reaching undetectable HCV-RNA during treatment (nonresponders) and 2 having relapsed after therapy (relapsers). Two patients had hemophilia A.

Plasma Pharmacokinetics of TMC435 in Healthy Volunteers

The pharmacokinetics of TMC435 in the single and multiple ascending dose groups (panels 1–6) in healthy volunteers and of the hepatitis C patients (panel 7) were assessed (Table 3). The variability in pharmacokinetic parameters was moderate in healthy volunteers but more substantial in HCV-infected patients. The TMC435 exposure increased more than proportionally to the dose, both in comparing the 100-mg and 200-mg QD

Table 3. Pharmacokinetic Parameters of TMC435 After 5 Days of 200 mg QD Dosing in Healthy Volunteers and in Hepatitis C Patients

Pharmacokinetics of multiple dose TMC435, mean ± SD; t_{max}: median (range)	200 mg TMC435 QD for 5 days: healthy subjects	200 mg TMC435 QD for 5 days: hepatitis C patients
n	5	6
Day 1		
t_{max}, h	4.0 (3.0–6.0)	6.0 (4.0–8.0)
C_{max}, ng/mL	2304 ± 918	4067 ± 1479
AUC_{24h}, ng/h/mL	24,630 ± 7331	56,430 ± 22,470
Day 3		
C_{0h}, ng/mL	749.0 ± 373.7	3015 ± 1971
Day 4		
C_{0h}, ng/mL	1005 ± 560	4610 ± 3205
Day 5		
t_{max}, h	4.0 (3.9–8.0)	4.0 (4.0–8.0)
C_{0h}, ng/mL	1482 ± 791	6057 ± 4213
C_{min}, ng/mL	1445 ± 767	5743 ± 4089
C_{max}, ng/mL	6172 ± 2859	11,470 ± 5337
AUC_{24h}, ng/h/mL	79,710 ± 37,230	206,000 ± 113,600
$t_{1/2term}$, h	16.04 ± 5.11	41.32 ± 32.99
Ratio $AUC_{24h,\ day\ 5/day\ 1}$ (%)	316.0 ± 101.2	344.8 ± 67.2

AUC_{24h}, area under the curve at 24 hours; C_{max}, mean peak concentrations; C_{min}, mean trough concentrations; C_{0h}, concentration prior to dose; $t_{1/2term}$, terminal half-life; t_{max}, time to mean peak concentration.

regimen and the 200-mg and 400-mg QD dosing. After the last dose on day 5, the area under the plasma concentration-time curve (AUC_{24h}) for the 200-mg QD dose group was approximately 10 times higher than that for the 100-mg QD dose, whereas the increase in mean AUC_{24h} value was 4.7-fold for the dose increase from 200 mg to 400 mg QD. Steady state was attained on day 3 for the 100-mg QD regimen but was not reached during the 5-day evaluation period for both higher doses. The pharmacokinetic profile of TMC435 observed in this study suggests a slow or prolonged absorption, with a late T_{max}, of 4 to 6 hours. Terminal half-lives could not be accurately assessed for the higher dose levels within the evaluation period of 72 hours. The mean trough concentrations of TMC435 achieved in the 200-mg QD regimen on day 5 of 1445 ng/mL represented a considerable exposure, more than 275 times the EC_{50} value determined in an in vitro genotype 1b replicon assay (8 nmol/L or 6 ng/mL).[14]

Safety in Healthy Volunteers

No serious adverse events or adverse events (AEs) leading to discontinuation were observed. A complete listing of AEs recorded for the 5-day dosing schedules is shown in Table 4. In the multiple dose part of the trial, 3 (out of 4), 4 (out of 5), and 4 (out of 6) subjects developed at least 1 AE during TMC435 100-mg, 200-mg, and 400-mg QD administration, respectively. No volunteer developed a serious adverse event or an AE that was considered very likely related to TMC435 by the investigator. In panel 5 (dose group, 200 mg twice daily), mild and transient photosensitivity was observed in 3 (out of 6) healthy volunteers. Photosensitivity was not observed in any of the other panels.

Dose Selection for Hepatitis C Patients

The safety profile and exposure levels of TMC435 achieved in healthy volunteers provided an adequate range to select a dose for investigating safety, pharmacokinetic, and proof-of-concept antiviral response in patients infected with genotype 1 hepatitis C. The pharmacokinetic properties of TMC435 supported once daily dosing (Figure 2). The minimal exposure levels for the 200-mg QD regimen in the healthy volunteers were well in excess (>275-fold) of the in vitro replicon EC_{50} value and therefore considered adequate for proof-of-concept evaluation. Given the safety profile of TMC435, both for the 200-mg and 400-mg QD regimens in healthy volunteers and anticipating a higher exposure in patients as compared with healthy volunteers, a dose of 200 mg QD was selected for hepatitis C patients.

Pharmacokinetics of 5-Day Dosing of 200 Milligrams QD TMC435 in Hepatitis C Patients

Hepatitis C patients received a QD dose of 200 mg of TMC435 for 5 days (Table 3). They presented a similarly shaped pharmacokinetics profile as compared with healthy volunteers. However, the overall TMC435 expo-

Table 4. Summary of AEs by Body System and Preferred Term (regardless of severity and drug relatedness) Observed During the 5-Day Multiple Ascending Dose of TMC435 in Healthy Volunteers

Safety part II (multiple dose escalation part)	100 mg QD (panel 3)		200 mg QD (panel 4)		200 mg BID (panel 5)		400 mg QD (panel 6)	
Healthy subjects (n = number of subjects with data)	TMC435 (n = 4)	Placebo (n = 2)	TMC435 (n = 5)	Placebo (n = 2)	TMC435 (n = 6)	Placebo (n = 3)	TMC435 (n = 6)	Placebo (n = 3)
Adverse events								
Most frequently reported AEs (reported in >1 subject), *n*								
Headache	1	0	3	1	0	0	0	0
Photosensitivity reaction	0	0	0	0	3	0	0	0
Diarrhea	0	0	0	0	2	0	0	0
Abdominal pain, upper	0	0	0	0	2	0	0	0
Abdominal distension	1	0	0	0	1	0	0	0
No. with 1 or more AEs	3	1	4	1	6	1	4	1
No. with 1 or more grade 3 or 4 AEs	0	0	0	0	0	0	0	0
No. of deaths	0	0	0	0	0	0	0	0
No. with 1 or more other serious AEs	0	0	0	0	0	0	0	0
No. of treatment stopped because of AEs	0	0	0	0	0	0	0	0
No. with 1 or more AEs considered at least possibly related to TMC435	1	0	0	0	5	1	1	0
Most frequently reported AEs possibly related to TMC435 (reported in >1 subject), *n*								
Photosensitivity reaction	0	0	0	0	3	0	0	0
Diarrhea	0	0	0	0	2	0	0	0
Abdominal pain, upper	0	0	0	0	2	0	0	0

NOTE. Summary of AEs by body system and preferred term regardless of severity and drug relatedness.

sure with a 200-mg QD dose of TMC435 was higher in patients, which was in between the exposure observed at 200 mg and 400 mg QD in healthy volunteers (Figure 2). As with healthy volunteers, a relatively long absorption phase was apparent from the T_{max} of 4 to 6 hours. Mean plasma concentrations after day 5 declined faster for healthy volunteers than for patients. As in healthy volunteers, steady state was not attained by day 5 with a dose of 200 mg QD of TMC435.

Safety in Hepatitis C Patients

No serious adverse event or AEs leading to treatment discontinuation were reported in hepatitis C patients, and no grade 3 or 4 AEs were reported. In the patient panel, the most common AEs were headache and fatigue (respectively, 3 and 2 out of 6 patients), and no events were considered very likely related to TMC435 (Table 5). No consistent or clinically relevant changes over time in laboratory parameters were observed. Median changes in vital signs, electrocardiogram, and chest echocardiography parameters were minor and not considered clinically relevant. Finally, no abnormal findings in physical examinations were observed during TMC435 administration.

HCV-RNA Levels

HCV-RNA levels declined rapidly in all 6 patients with more than 3-$\log_{10}$ IU/mL reductions in plasma HCV-RNA compared with baseline for all patients during treatment (Figure 3). The median HCV-RNA reduction at day 3 was 3.46-$\log_{10}$ IU/mL (range, 1.6-$\log_{10}$ to 3.8-$\log_{10}$ IU/mL) and reached a median maximum reduction of 3.9-$\log_{10}$ IU/mL (range, 2.9-$\log_{10}$ to 4.1-$\log_{10}$ IU/mL). The time to maximal reduction of viral load varied among patients, with a median value of 6 days, 24 hours after the last dose of TMC435. HCV-RNA levels in the 3 days after the last dose remained relatively constant, changing less than 0.5-$\log_{10}$ IU/mL in all patients, suggesting continued suppression of viral replication. No viral breakthroughs (increase of >1-$\log_{10}$ IU/mL from nadir) were observed during the 5-day dosing or over the 3-day follow-up period. Viral decline appeared similar in patients with genotype 1a and 1b viruses, previous nonresponders or relapsers, or between patients presenting with or without hemophilia. HCV-RNA levels returned to pretreatment levels in all patients 4 weeks after the final dose. Using the HCV-RNA measurements from the 6 hepatitis C-infected patients as input in a viral kinetics model,[16]

Table 5. Summary of AEs Observed in the 200 mg QD 5-Day Patient Panel of the Trial

Safety part III (multiple dose part) Hepatitis C patients (n = number of patients with data)	200 mg QD TMC435 (panel 7) (n = 6)
Adverse events	
Most frequently reported AEs (reported in >1 patient), *n*	
Headache	3
Fatigue	2
No. with 1 or more AEs	5
No. with 1 or more grade 3 or 4 AEs	0
No. of deaths	0
No. with 1 or more other serious AEs	0
No. of treatment stopped because of AEs	0
No. with 1 or more AEs considered at least possibly related to TMC435	1

NOTE. Summary of AEs by body system and preferred term regardless of severity and drug relatedness. In the multiple dose part in hepatitis C patients (panel 7) of the trial, 5 patients developed at least 1 AE. No events were considered probably or very likely related to study medication. One patient reported an AE considered possibly related to TMC435 (ie, rash). All AEs reported were grade 1.

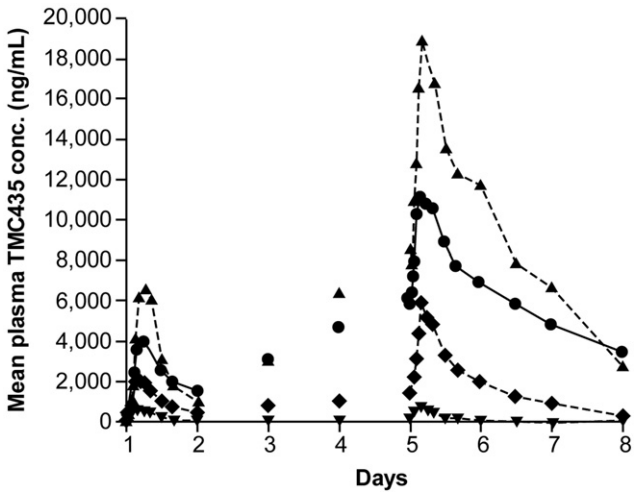

Figure 2. Mean plasma concentration-time profile during 5-day TMC435 in hepatitis C patients (●, 200 mg QD) and in healthy volunteers (▼, 100 mg QD; ♦, 200 mg QD; ▲, 400 mg QD).

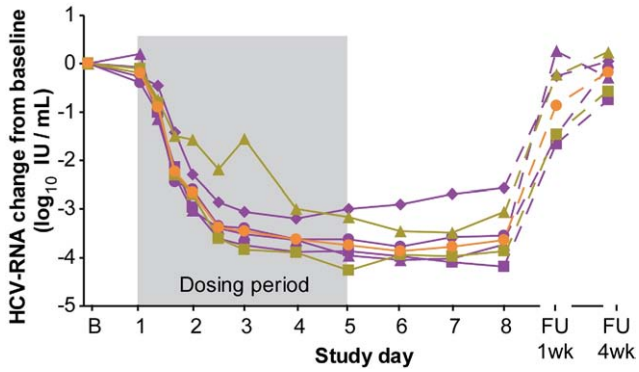

Figure 3. Change in plasma HCV-RNA ($\log_{10}$ IU/mL) from baseline observed in 6 hepatitis C patients receiving 5 days, dosing of TMC435 200 mg QD. Four patients indicated in *purple* had genotype 1a HCV infection (■, patient 173; ▲, patient 175; ●, patient 176; ♦, patient 177), whereas 2 patients indicated in *olive* were infected with HCV genotype 1b (■, patient 174; ▲, patient 178). Median values (●) are shown in *orange*. Follow-up times were day 18 and day 34 (patient 173), day 18 and day 34 (patient 174), day 14 and day 24 (patient 175), day 14 and day 39 (patient 176), day 14 and day 35 (patient 177), day 14 and day 35 (patient 178).

the mean half-life of free virus was estimated at 2.66 hours, clearance of infected cells at 2.8 days, and the in vivo potency (ε) value for TMC435 at 0.9993.

Sequence Analyses

Viral population sequencing was performed on plasma samples for all patients at all time points collected. Because previous in vitro work identified changes at aa positions 80, 155, 156, and 168 within NS3 as being involved in decreased susceptibility to TMC435, we focused on changes at these positions for this analysis.[17] In line with previous in vitro work, differences at the amino acid level were observed among baseline, on-treatment, and follow-up samples mainly on NS3 aa positions 80, 155, and 168 (Table 6) (Oliver Lenz, personal communication, October 2008). At baseline, 4 patients had no detectable mutations at aa positions 80, 155, 156, or 168, whereas, in 2 patients, a mutation at aa position 80 and 168, respectively, was observed. Both these patients were infected with genotype 1a virus. These sequence variations present at baseline had no apparent negative effect on the response to TMC435 treatment during the 5-day dosing period or in the 3 days follow-up. Mutations at one or more of the aa positions 80, 155, or 168 were newly detected in each patient as early as day 3 after treatment initiation. There was no temporal relationship between the detection of mutations and an increase in HCV-RNA levels. At the week 4 follow-up time point, the NS3 sequence of 1 patient was identical to the pretreatment sample by population sequencing. For 5 patients, the NS3/4A sequence was determined approximately 1 year after the end of TMC435 dosing; population sequence analysis for all 5 patients showed a similar sequence pattern as before the first TMC435 dose was given, suggesting a return of wild-type variants within this time frame. The exact timing of reversion to wild type is not known because interim sampling was not foreseen as part of the original trial.

Table 6. Mutations at NS3 Amino Acid Positions 80, 155, and 168 Observed During the C101 Trial at Baseline; Day 3, Day 5, Day 8; and During Follow-Up Sampling at 2 Weeks and Approximately 1 Year After End of TMC435 Dosing

Patient	Q080	R155	D168
Prototype 1a			
173			
Day 1	WT	WT	WT
G1a			
Day 3	WT	WT	D/V
Day 5	WT	K/R	A/D
Day 8	WT	WT	V
Follow-up week 2	WT	K/R	WT
Follow-up year 1	WT	WT	WT
175			
Day 1	WT	WT	WT
G1a			
Day 3	WT	WT	WT
Day 8	WT	WT	V
Follow-up week 2	WT	K	D/E
Follow-up year 1	WT	WT	WT
176			
Day 1	WT	WT	D/E
G1a			
Day 3	Q/R	WT	E
Day 5	Q/R	K/R	E
Day 8	Q/R	WT	E
Follow-up week 2	Q/R	WT	E
Follow-up year 1	WT	WT	D/E
177			
Day 1	K	WT	WT
G1a			
Day 3	K	K/R	WT
Day 5	K	K	WT
Day 8	K	K	WT
Follow-up week 2	K	K	WT
Prototype 1b			
174			
Day 1	WT	WT	WT
G1b			
Day 3	WT	WT	D/V
Day 5	WT	WT	V
Day 8	WT	WT	V
Follow-up week 2	WT	WT	WT
Follow-up year 1	WT	WT	WT
178			
Day 1	WT	WT	WT
G1b			
Day 3	WT	WT	WT
Day 5	WT	WT	WT
Day 8	WT	WT	V
Follow-up week 2	WT	WT	V
Follow-up year 1	WT	WT	WT

NOTE. Mutations at NS3 amino acid positions 80, 155, and 168 (defined as changes from reference sequence H77 [AF009606] for genotype 1a and Con1 [AJ238799] for genotype 1b).
WT, indicates wild-type residues: 80Q, 155R, 168D.
A, alanine; D, aspartic acid; E, glutamic acid; K, lystine; Q, glutamine; R, arginine.

Discussion

TMC435 is a potent and selective inhibitor of HCV replication in replicon cells. Pharmacokinetic studies in rats and dogs showed high levels of TMC435 in plasma and, in particular, in liver 24 hours after dosing. These data suggested a favorable systemic exposure and a substantial liver-to-plasma ratio. The plasma and liver concentrations achieved in these animal species 24 hours after last dosing were well in excess of the anticipated therapeutic levels and were thus supportive of QD dosing.[14] The TMC435350-C101 trial investigated the safety and pharmacokinetics of TMC435 in healthy volunteers and aimed to define a suitable dose for a first evaluation of the early antiviral response in a separate treatment panel encompassing 6 chronic hepatitis C patients infected with genotype 1 virus. Overall, this study demonstrated that QD TMC435 given orally for 5 days was well tolerated and resulted in a rapid HCV-RNA decline in all patients. Interestingly, although mutations in NS3 could be newly detected in patients from day 3 onwards using

population sequencing, no viral breakthroughs were observed during dosing and 3 days after the last dose of TMC435.

In healthy volunteers, QD dosing of 100 mg, 200 mg, and 400 mg TMC435 for 5 days was generally safe and well tolerated. Trough plasma concentrations well above the replicon EC_{50} value were observed for all 3 dose levels. Although steady state was attained for 100 mg QD within 3 days, it was not reached within the 5 days of dosing for subjects in the 200-mg and 400-mg QD panel. Recently presented data demonstrated that steady state for a 200-mg QD dose in healthy volunteers was attained within 7 days.[18] A time to steady state of 7 days predicts an elimination half-life of approximately 30 hours, which is consistent with observed values. Differences in accumulation for the 3 doses are associated with the more than dose-proportional increase in TMC435 exposure. The dose-disproportional pharmacokinetics can in part be explained by an apparent prolonged absorption (extending over 32 hours from dosing), which becomes more evident at higher dose.[19] This also impacted the terminal half-life, which was assessed over only a 72-hour period, but a dose effect on the elimination of TMC435 may also have contributed to the prolongation.

Liver functions such as drug metabolism and biliary excretion may be impaired in hepatitis C patients. Because these functions are involved in the elimination of TMC435, higher exposures were expected in patients. Thus, a dose level of 200 mg QD was selected for the patient panel, and safety data were available for 400 mg QD in healthy volunteers. The first dose of 200 mg in patients gave a similar exposure as a single dose of 300 mg in healthy volunteers,[18] and the mean TMC435 plasma exposure (AUC_{24h}) at day 5 for a 200-mg QD regimen in patients was close to 3-fold the value in healthy volunteers. It should be noted that there was variability in TMC435 pharmacokinetics in hepatitis C patients; whereas the individual pharmacokinetics of 3 patients was similar to healthy volunteers, 2 patients with hemophilia had a higher exposure. It is stipulated that liver functions including drug elimination mechanisms could explain these differences.

TMC435 induced a rapid reduction in viral RNA over the first 3 days of dosing in all 6 patients ($\varepsilon = 0.9993$) studied. The 6 patients included 4 nonresponders and 2 relapsers to previous IFN-based treatment, 4 genotype 1a and 2 genotype 1b virus-infected patients, and 2 patients with hemophilia. However, HCV-RNA kinetics appeared similar among the patients. A median maximal reduction in HCV-RNA levels of 3.9-$\log_{10}$ IU/mL was achieved. The time to maximal reduction of viral load varied among patients, with a median value of 6 days.

TMC435 has shown a preferential distribution to the liver, with levels 30-fold higher than plasma in animal studies. This favorable distribution to the liver may provide an even greater antiviral suppression at the main site of infection.[14] The mean plasma concentration at day 8 (72 hours after the last dose) was still 3360 ng/mL and thus 280 times above the replicon EC_{50} value (plasma protein binding corrected), which could explain the sustained suppression of HCV-RNA levels after end of therapy. More extensive studies will be needed to assess fully the importance of this observation. However, it is possible that high plasma trough levels of TMC435 could explain the ensuing suppression of viral variants. In addition, the high trough levels infer that patients of a higher body mass index, as would be the case in US patients, would have sufficient drug available to maintain viral suppression; moreover, our phase II trial confirmed antiviral activity of lower drug doses.[20]

Viral variants with aa changes at one or more of the aa positions 80, 155, and/or 168 of NS3 were detected in each of the treated patients as early as day 3. Mutations at these positions were previously characterized in vitro to confer reduced susceptibility to TMC435 in a transient replicon system, with changes in EC_{50} values ranging from low (below 10-fold, for example, for Q80K and Q80R) to high (above 100-fold, for example, for D168V and D168A) depending on the residue.[17] Additional geno- and phenotypic analysis of samples is ongoing to further characterize viral variants and to assess fully their impact on TMC435 activity. Of note, after a 1-year follow-up, viral sequences in all of the 5 patients analyzed had reverted back to the pretreatment sequence patterns, suggesting that the persistence of these mutant viruses may be limited in time. In addition, in vitro work has confirmed that replicons with variations at aa positions 80, 155, and 168 of NS3 remain susceptible to Peg-IFN. Longer duration of TMC435 treatment in combination with Peg-IFN and RBV will be conducted in future clinical studies, which should increase the antiviral potency of the treatment regimen and add a further level of protection against potential viral breakthrough and emergence of viral variants.

Different classes of anti-HCV drugs are currently in development, and 3- and 4-drug combinations can be envisaged to cure patients of HCV infection.[21] Proof-of-concept data for combining HCV NS3/4A protease inhibitors with Peg-IFN and RBV are very encouraging, with sustained virologic response of up to 74% realized in phase II studies.[7,22] The challenges for protease inhibitors including TMC435 are to optimize sustained virologic response rates in combination with standard of care while at the same time shortening treatment duration.[7,21] The potency of TMC435 and in vitro work showing synergy with Peg-IFN and additivity with RBV[14] are consistent with its use in combination with the current standard of care. Additionally, its simple dosing schedule and low pill burden may become important attributes in future combination studies with other specifically targeted therapies for hepatitis C. The protease inhibitor TMC435 is a promising addition to the growing arsenal

of specifically targeted antivirals against HCV undergoing development.

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Conflicts of interest

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