Poster 1390

In vitro activity and preclinical pharmacokinetics of the HCV protease inhibitor TMC435350

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Abstract

Background: As a class, HCV NS3/4A protease inhibitors have shown promise in clinical trials for the treatment of chronic hepatitis C virus infection. TMC435350 is a novel and potent macrocyclic NS3/4A protease inhibitor. To further assess the potential of TMC435350, we characterized the *in vitro* activity of TMC435350 alone or in combination with different classes of HCV inhibitors. The preclinical plasma pharmacokinetics and tissue distribution were also studied *in vivo*.

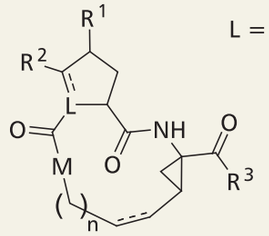
Methods: The effect on HCV RNA level and the emergence of drug-resistant colonies was analyzed in the replicon model with TMC435350 alone, or in combination with interferon alpha, ribavirin, or an HCV polymerase thumb domain inhibitor. Pharmacokinetic profiles were evaluated following single or repeated dosing in rats. The tissue distribution of TMC435350 was studied in male rats at time points from 0.5 up to 31 hours after a single oral dose of 40 mg/kg.

Results: In biochemical HCV NS3/4A protease assays, TMC435350 exhibited K_i values of 0.5 and 0.4 nM for subtypes 1a (H77) and 1b (con1) enzymes respectively. TMC435350 was found in the subgenomic genotype1b replicon model to have an EC_{50} of 8 nM and a selectivity index (SI) of > 2000. The combination of TMC435350 with different classes of HCV inhibitors further increased its activity in reducing HCV RNA in an additive to synergistic manner, and further reduced the emergence of resistant replicon colonies. After single oral administration of a PEG400-based solution of TMC435350 at 40 mg/kg in rats, the mean peak plasma concentration (C_{max}) was 1430 ng/ml and was observed at two hours post-dose (t_{max}). The absolute bioavailability of TMC435350 was calculated at 44% after single oral administration of a 40 mg/kg dose. TMC435350 was found to be extensively distributed to the liver, small- and large intestines (tissue/plasma ratios >35). Concentrations in other organs were similar to plasma. Notably, TMC435350 was still quantifiable in the liver tissue up to 31 hours post-dosing.

Conclusions: TMC435350 is a novel potent and specific HCV protease inhibitor, with good oral bioavailabilty and a favorable liver distribution. In addition, *in vitro* studies support the potential use of TMC435350 in combination with other HCV inhibitors.

Introduction

Fig. 1: TMC435350 is a novel HCV NS3/4A serine protease inhibitor targeting HCV replication.



TMC435350 was identified as a potent inhibitor from a series of macrocyclic analogs in enzymatic assays using HCV genotype 1 NS3 proteases and cellular replicon models. Selectivity and cytoxicity were further characterized, and studies with IFN-α, ribavirin and an NS5B polymerase thumb domain inhibitor[1] in the replicon demonstrated the potential of these combinations.

Results

HCV enzymatic activity

Table 1: Inhibitory activity of TMC435350 in genotype 1a and 1b HCV NS3/4A protease assays (A), and against a panel of human proteases (% inhibition at 10 μM (B) or as IC_{50} (C)). All data was generated using standard FRET based assays.

A)

HCV NS3	genotype 1a*	K_i = 0.5 nM
	genotype 1b*	K_i = 0.4 nM

* Protease catalytic domains aa 1-181 cloned with a C-terminal His_6 tag, was expressed in *E. coli* and purified. NS4A peptide was added for the biochemical assay.

B)

Name	Family	% inhibition at 10 μM
Tryptase	serine	0.0
Chymotrypsin	serine	6.8
Tissue plasminogen activator	serine	36.6
Kallikrein	serine	18.4
Chymase	serine	-18.1
Proteinase 3	serine	-11.3
Factor VIIa	serine	8.5
Urokinase	serine	-17.0
Streptokinase	serine	4.6
Factor Xa	serine	10.4

C)

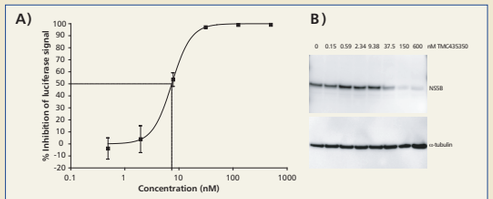
Name	Family	IC_{50} (μM)
Leukocyte-Elastase	serine	1.5
Plasmin	serine	5.8
Thrombin	serine	5.6
Trypsin	serine	5.7
Cathepsin G	serine	3.8
Cathepsin S*	cysteine	0.8
Cathepsin L	cysteine	>20
Cathepsin B	cysteine	>20
Cathepsin E	aspartic	>20
Cathepsin D	aspartic	>20

* 1% Triton-X100: IC_{50} > 10μM

- Potent inhibitor of both genotype 1a and 1b HCV NS3/4A proteases.
- Inhibition of HCV NS3/4A protease was > 1,000 times stronger than inhibition of tested host proteases.

HCV cellular activity and selectivity

Fig. 2: Inhibition of HCV replication in genotype 1 replicon cells treated for 72 h with TMC435350.



C)

Replicon	Detection	EC_{50} (nM)	EC_{90} (nM)
genotype 1b Huh7-Luc-ET	Luciferase	8	24
genotype 1b Huh7-Luc-ET	RNA	14	29
genotype 1b Huh7-Con1b	RNA	32	116
genotype 1a Huh7-SG-1a	RNA	39	147

Dose-dependent inhibition of HCV replication was monitored with genotype 1b Huh7-Luc-ET (kindly provided by R. Bartenschlager as HCV con1b bicistronic replicon clone ET), Huh7-con1b and Huh7-SG1a replicon cells (kindly provided by Apath L.L.C. as con1b and H77 replicons) using luciferase reporter read-out, immunoblotting and quantitative RNA detection by real-time PCR.

Table 2: Antiviral selectivity (A) and cytotoxicity (B) testing

A)

Virus	Family	Genome	TMC435350 EC_{50} (μM)
West Nile virus	Flaviviridae	ssRNA	>100
Bovine viral diarrhea virus	Flaviviridae	ssRNA	>100
Yellow fever virus	Flaviviridae	ssRNA	>100
Sindbis virus	Togaviridae	ssRNA	>100
HIV	Retroviridae	ssRNA	>10
Influenza	Orthomyxoviridae	ssRNA	>100
Vesicular stomatitis virus	Rhabdoviridae	ssRNA	>100
Respiratory syncytial virus	Paramyxoviridae	ssRNA	>32
Herpes simplex virus	Herpesviridae	dsDNA	>100
Hepatitis B	Hepadnaviridae	ds/ssDNA	>10

Antiviral selectivity was determined using a panel of RNA and DNA viruses.

B)

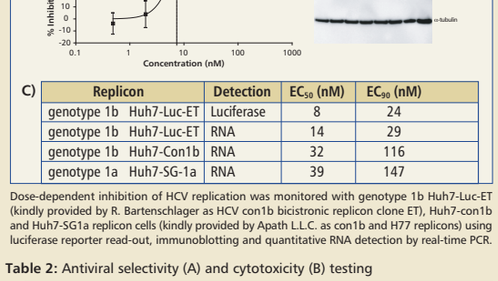
Cell line	Origin	CC_{50} (μM)	SI
Huh-7	liver	53	6806
HepG2	liver	>32	>4102
HEK-293	kidney	>32	>4102
MT-4	lymphocyte	24	3077
MRC-5	lung	17	2179
SAOS-2	bone	21	2692
HT-1080	epithelial like	16	2051

Cytotoxicity, measured by resazurin staining was assessed in several cell lines, and selectivity indexes (SI) were calculated using the CC_{50}/EC_{50} ratios.

- Potent and highly specific HCV inhibitor (EC_{50} = 8 nM).
- Demonstrated minimal cytotoxicity in tested human cell lines (SI>2,000).

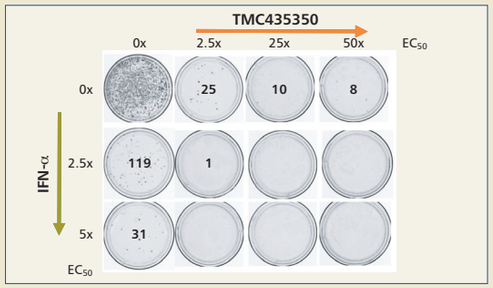
Combination studies with IFN-α

Fig. 3: Nine day incubation of HCV replicon cells with TMC435350 alone, IFN-α alone, and TMC435350 in combination with IFN-α.



Huh7-Luc-ET cells were treated for 9 days with IFN-α, TMC435350, and TMC435350 in combination with IFN-α (Biosource recombinant human IFN-α). Medium with compounds was refreshed every 3 days and cells were harvested on days 3, 6 and 9. HCV RNA and cellular RPL13A levels were measured by quantitative RNA detection.[2]

Fig. 4: Impact of combining IFN-α with TMC435350 on emergence of drug resistant replicon colonies.

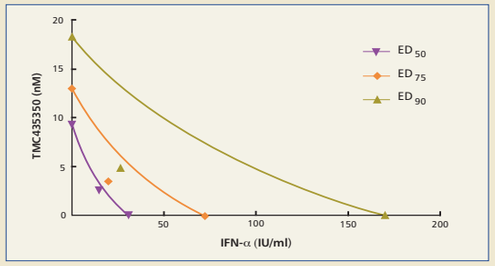


Huh7-Luc-ET cells were treated for 4-6 weeks with G418 and TMC435350 +/- IFN-α. Medium with compounds was refreshed every 3-4 days. Resistant Colonies were stained with neutral red and counted.[3]

In the replicon model, combination of TMC435350 with IFN-α resulted in:
- > 4 log_{10} reduction of HCV RNA in 9 days.
- increased suppression of drug resistant replicon colonies.

In vitro synergy studies

Fig. 5: Mutual influence between TMC435350 and IFN-α as analyzed by the Loewe additivity model.



	TMC435350 Combination Index			
Test Compound	ED_{50}	ED_{75}	ED_{90}	
IFN-α	0.77	0.57	0.43	Synergistic
ribavirin	0.98	0.94	0.92	Additive
NS5B thumb domain inhibitor	0.88	0.72	0.61	Synergistic

Huh7-Luc-ET cells were treated for 3 days with TMC435350 in combination with IFN-α, ribavirin, or an NS5B thumb domain inhibitor. The relationship between inhibitory activities of TMC435350 and test compound was explored using CalcuSyn (Biosoft, Ferguson, MO). CI values of <1, =1, and >1 indicate synergy, an additive effect, or antagonism, respectively.

- In replicon cells, TMC435350 showed additivity when combined with ribavirin, and synergy in combination with IFN-α or an NS5B polymerase thumb domain inhibitor (thiophene class)[1].

Preclinical ADME properties

Table 3: IV pharmacokinetics in rat (n=3)*

Dose (mg/kg)	C_0 (µg/ml)	$AUC_{0\text{-}inf}$ (µg.h/ml)	$t_{1/2}$ (h)	Cl (l/h/kg)
4	3.6	1.8	4.0	2.3

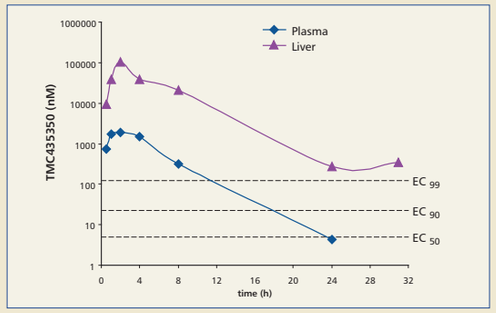
* vehicle: 20% (w/v) hydroxypropyl-β-cyclodextrin (HP-β-CD)

Table 4: oral pharmacokinetics in rat (n=3)**

Dose (mg/kg)	C_{max} (µg/ml)	t_{max} (h)	$AUC_{0\text{-}inf}$ (µg.h/ml)	$t_{1/2}$ (h)	F_{abs} (%)
40	1.4	2.0	7.8	2.6	44

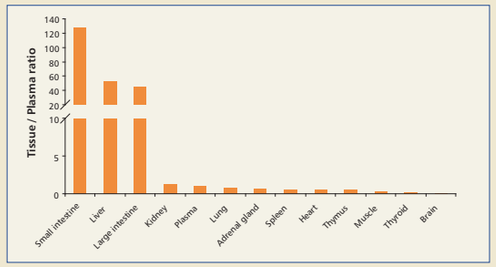
** vehicle: polyethylene glycol 400/D-α-tocopheryl polyethylene glycol 1000 succinate (PEG400/TPGS)

Fig. 6: Plasma kinetics and liver concentration of TMC435350 after a single oral administration of 40 mg/kg in Sprague Dawley rats (n=3, mean ± S.D.).



- Good systemic oral bioavailability.
- Plasma exposure: $C_{8h\text{-}plasma}$ > EC_{99}; liver exposure $C_{31h\text{-}liver}$ > EC_{99}.

Fig. 7: Tissue distribution of TMC435350 after a single oral administration of 40 mg/kg in Sprague Dawley rats.



$AUC_{0\text{-}31h}$ (except muscle $AUC_{0\text{-}8h}$) tissue to plasma ratios of TMC435350 in tissues of male Sprague-Dawley rats collected after single oral administration of a VitE-TPGS/PEG-400 solution of TMC435350 at 40 mg/kg. N = 3, analysis on pooled tissue.

- Extensive distribution to liver and GI tract.
- Concentrations in other organs similar to plasma.

Preclinical safety

- Negative in a battery of genotoxicity assays.
- Favorable profile in *in vitro* and *in vivo* safety pharmacology assays (CNS, cardiovascular, pulmonary functions).

Conclusions

TMC435350 is a novel small molecule HCV NS3/4A protease inhibitor with:

- **potent** anti-HCV activity in enzymatic and cellular assays.
- **good selectivity** to other viruses and human proteases.
- **synergy** in combination with IFN-α or an NS5B polymerase thumb domain inhibitor, and additivity with ribavirin.
- **drug-like** properties.
- **favorable liver exposure** as shown in pharmacokinetic and preclinical safety studies.

TMC435350 is currently being evaluated in phase I clinical trials.

References
(1) Chan, L. et al., 2004. Med. Chem. Lett. 14: 797
(2) Lin, K. et al., 2004. AAC 48: 4784
(3) Mo, H. et al., 2005. AAC 49: 4305

This poster will be available on-line at www.tibotec.com

58th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, MA, 2-6 November 2007

Poster 1318

Results of a Phase I Placebo-Controlled Trial in Healthy Volunteers to Examine the Safety, Tolerability and PK of the HCV Protease Inhibitor TMC435350 after Single and Repeated Dosing

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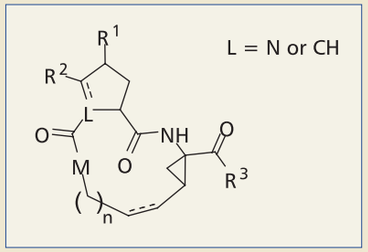
Introduction

This trial studied the safety, tolerability and pharmacokinetics (PK) of TMC435350, a novel HCV NS3/4A protease inhibitor, after single oral dosing and, in a second step, after 5 days of oral dosing in HCV-negative healthy volunteers.

Fig. 1: TMC435350 was selected from a novel series of HCV NS3/4A serine protease inhibitors targeting HCV replication.



TMC435350 (Simmen et al., 2007):

- Has potent and selective enzymatic HCV NS3/4A inhibiting activity *in vitro*:
  - genotype 1a: K_i = 0.5 nM
  - genotype 1b: K_i = 0.4 nM
  - inhibition of HCV NS3/4A protease was > 1,000 times stronger than inhibition of tested host proteases.
- Is potent and highly selective in genotype 1 cellular replicon assays:
  - EC_{50} = 8 nM
  - EC_{90} = 24 nM
  - minimal cytotoxicity in tested human cell lines (SI > 2,000).
- Highly selective for HCV inhibition (EC_{50} > 10 μM against a panel of DNA and RNA viruses)

Methods

Single and multiple ascending doses of TMC435350 were studied in HCV-negative healthy volunteers.

Single ascending dose (SAD)

- 50-600 mg TMC435350 oral solution in PEG400; 2 panels of 9 males or females, 6 of whom received TMC435350 and 3 received placebo (Figure 2A).
- Fed conditions for all doses tested followed by an investigation of effects of fasting on PK profile and exposure of a single dose of 200 mg of TMC435350 (same individuals in panel 1).
- Full PK profile was studied on the day of dosing with additional samples taken up to 72 h post-dose.

Multiple ascending dose (MAD)

- 100-400 mg TMC435350 oral solution in PEG400; 4 panels of 9 volunteers: 6 subjects receiving TMC435350 solution and 3 subjects receiving placebo; except for panel 3 that had 4 subjects receiving TMC435350 and 2 placebo, and panel 4 that had 5 subjects on TMC435350 and 2 on placebo (Figure 2B).
- Safety monitoring.
- Full PK profiles were studied on days 1 and 5, with predose samples on days 2, 3 and 4.

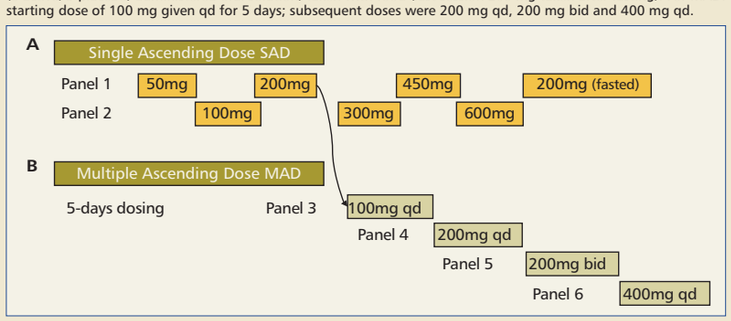
Assessments

- Safety was closely monitored throughout and up to one month after dosing with ambulatory visits. Safety parameters included physical examination, vital signs, laboratory parameters (routine hematology, biochemistry, urinalysis), and adverse events (AEs) monitoring.
- Cardiovascular safety was intensively monitored using triplicate 12-lead ECGs and, in the MAD part only, assessment of proANP, proBNP and BNP levels, and troponin cardiac enzymes. In addition, transcutaneous echocardiography was performed pre- and post-dose.
- Data review meetings were organized using interim safety reports, and the decision to escalate the dose was taken in full consensus and approved at each step by the local ethical committee.

Bioanalysis

Plasma concentrations of TMC435350 were determined by a validated liquid chromatography tandem mass spectrometry method (LC-MS/MS) which had a lower limit of quantification (LLOQ) of 2 ng/ml.

Fig. 2: Study design for the SAD (A) and the MAD (B) part in healthy volunteers. Each session had 9 volunteers (6 active, 3 placebo) administered oral solution, taken with food (last session 200 mg taken after fasting). For MAD: starting dose of 100 mg given qd for 5 days; subsequent doses were 200 mg qd, 200 mg bid and 400 mg qd.



Single ascending dose (SAD)

Table 1: Incidence of AEs following single oral doses of either TMC435350 or placebo.

Single Dose	Possibly Related AEs TMC435350	Possibly Related AEs Placebo
50 mg	None	Flatulence (1/3); Diarrhea (1/3)
100 mg	Frequent bowel movement (1/6)	None
200 mg	Flatulence (1/6)	None
200 mg, Fasted	None	None
300 mg	Dizziness (1/6); Somnolence (1/6)	None
450 mg	None	None
600 mg	Diarrhea (1/6); Fatigue (1/6)	Flatulence (1/3)

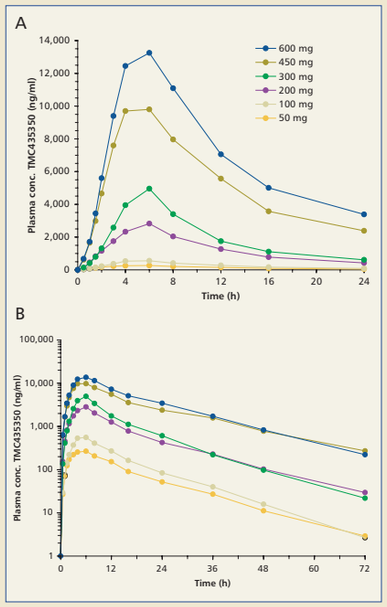
- Oral doses up to 600 mg were well tolerated.
- Only mild (grade 1) AEs noted.
- No grade 3 or 4 AEs noted.
- Minor effects observed were gastrointestinal tract related.
- No dose-limiting toxicity or determination of a maximum tolerated dose.
- No dose-dependent increase in AE incidence was observed.

Table 2: Mean (± standard deviation, n=6) PK parameters after single doses of TMC435350.

	50 mg	100 mg	200 mg	300 mg	450 mg	600 mg
t_{max}, hour*	5.0 (3.0 - 6.0)	5.0 (4.0 - 6.0)	6.0 (4.0 - 6.0)	6.0 (4.0 - 6.0)	6.0 (4.0 - 6.0)	6.0 (4.0 - 6.0)
C_{max}, μg/ml	0.29 ± 0.10	0.58 ± 0.09	2.96 ± 1.02	5.09 ± 0.79	10.46 ± 2.46	13.55 ± 1.79
AUC_{24h}, μg.h/ml	3.35 ± 1.36	6.28 ± 1.09	30.05 ± 10.02	46.38 ± 11.62	125.00 ± 31.82	166.70 ± 23.65
AUC_{last}, μg.h/ml	4.21 ± 2.14	7.55 ± 1.63	37.55 ± 14.82	54.72 ± 15.89	175.50 ± 69.21	225.00 ± 42.67
$t_{1/2}$, hour	9.9 ± 2.7	9.5 ± 1.4	10.9 ± 2.8	9.9 ± 1.6	13.4 ± 4.2	11.7 ± 2.0

* Median value + range

Fig. 3: Plasma concentrations of TMC435350 after single dosing. (A) Linear plot; (B) Semi-logarithmic plot.



Mean PK parameters and mean plasma concentration-time plots after single oral doses of TMC435350 are presented in Table 2 and Figure 3, respectively:

- Readily absorbed
- Delayed time to peak (t_{max}) indicates slow or prolonged absorption.
- Plasma exposure increased in more than dose proportional fashion. Half-life was independent of dose, indicating that apparent dose-disproportionality is not associated with dose-dependent clearance.
- Good plasma exposure with a t_{max} of 4-6 hours and a plasma elimination half-life of ~12 hours supported once daily dosing (qd) in the MAD phase.

Multiple ascending dose (MAD)

Table 3: Incidence of AEs following multiple oral doses of either TMC435350 or placebo, for 5 days.

Multiple doses	Possibly Related AEs TMC435350	Possibly Related AEs Placebo
100 mg qd	Headache (1/4)	None
200 mg qd	None	Frequent bowel movement (1/2)
200 mg bid	Abdominal distension (1/6)* Abdominal pain upper (2/6) Bowel sounds abnormal (1/6) Diarrhea (2/6) Paraesthesia (1/6) Paraesthesia oral (1/6) Photosensivity reaction (3/6) Skin burning sensation (1/6)	None
400 mg qd	Myalgia (1/6)	None

* Probably related AE

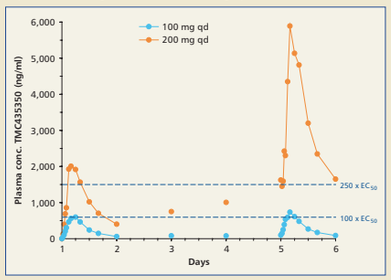
- Multiple oral doses of TMC435350 given for 5 days up to 400 mg or placebo were well tolerated.
- No discontinuations.
- Only mild (grade 1) AEs noted.
- No grade 3 or 4 AEs noted.
- Minor effects observed were mainly gastrointestinal tract related. Mild, short-lasting erythema was noted after sun exposure in 3 subjects receiving the 200 mg bid dose.
- No dose-limiting toxicity or determination of a maximum tolerated dose.
- No clinically relevant changes from baseline on laboratory parameters, vital signs, ECG recordings and echocardiographic evaluations.
- Generally well tolerated, without a dose relationship for AE incidence.

Table 4: Mean (± standard deviation) PK parameters after 5 days of once-daily dosing of TMC435350.

	100 mg qd (n = 4)	200 mg qd (n = 5)
Day 1		
C_{max}, μg/ml	0.68 ± 0.17	2.30 ± 0.92
t_{max}, hour*	5.0 (3.0-6.0)	4.0 (3.0-6.0)
AUC_{24h}, μg.h/ml	6.35 ± 1.61	24.6 ± 7.33
Day 5		
C_{min}, μg/ml (PreDose)	0.09 ± 0.03	1.40 ± 0.68
C_{max}, μg/ml	0.76 ± 0.21	6.17 ± 2.86
t_{max}, hour*	4.0 (4.0-6.0)	4.0 (4.0-8.0)
AUC_{24h}, μg.h/ml	7.62 ± 1.91	70.9 ± 36.5
C_{24h} μg/ml	0.09 ± 0.03	1.65 ± 1.10

* Median value + range

Fig. 4: Mean plasma concentrations of TMC435350 after repeated dosing at 100 and 200 mg qd.

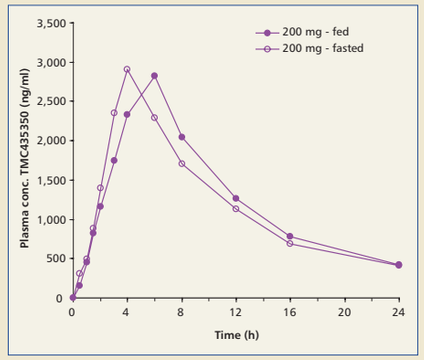


Once-daily oral TMC435350 dosing yielded excellent plasma exposure:

- Day 1 PK was similar to the single-dose PK in the SAD part of the study.
- Exposure increased in a substantially more than dose-proportional fashion from 100 to 200 mg qd.
- Steady state was attained within the 5 days-dosing period for the 100 mg qd regimen, but not for 200 mg qd. Pre-dose plasma concentrations for 200 mg qd tended to stabilize between day 4 and day 5, suggesting steady state to be achieved within approximately 7 days.
- Mean plasma levels detected 24 hours after day 5 dosing were substantially in excess of the HCV replicon EC_{50} value for both doses.
- Analysis of MAD panels 5 and 6 (200 mg bid and 400 mg qd) is ongoing.

Absence of food effect

Fig. 5: Mean plasma concentration-time plots after oral intake of 200 mg TMC435350, with and without food.



Food, consisting of a moderate fat breakfast, slightly delayed the absorption and did not influence the oral bioavailability of TMC435350.

Conclusions

- In this phase I study, TMC435350 was safe and well-tolerated when given to HCV-negative healthy volunteers at single oral doses up to 600 mg, and at 5 days of oral doses up to 400 mg once-daily.
- The pharmacokinetic profile of TMC435350 supports once-daily dosing.
- There is no food effect.
- The plasma levels of TMC435350 24 hours after day 5 dosing are substantially in excess of the replicon EC_{50} value for both 100 and 200 mg qd doses.
- Minor (grade 1 only) adverse events:
  - Mainly gastrointestinal tract related.
  - Transient photosensitive reaction in some subjects (mild, short-lasting erythema).
- TMC435350 will be further investigated following once-daily administration in HCV patients.

References
(1) Simmen, K. et al., The Liver Meeting 2007, November 2-6, Abstract 1390.

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58th Annual Meeting of the American Association for the Study of Liver Diseases, Boston, MA, 2-6 November 2007