Treatment outcome and resistance analysis in HCV genotype 1 patients previously exposed to TMC435 monotherapy and re-treated with TMC435 in combination with PegIFNα-2a/ribavirin

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INTRODUCTION

- TMC435 is a once daily (QD) oral NS3/4A protease inhibitor currently in Phase III clinical development for the treatment of hepatitis C virus (HCV) infection.
- TMC435 is an investigational potent and selective inhibitor of the NS3/4A protease, with an *in vitro* 50% effective concentration (EC_{50}) of 8 nM (6 ng/mL) in a genotype 1b replicon cell line.[1]
- Findings from Phase I and II studies have shown that TMC435 is well tolerated, with promising efficacy reported in combination with pegylated interferon α-2a/ribavirin (PegIFNα-2a/RBV).[2-6]
- *In vitro* resistance studies identified changes at amino acid positions 43, 80, 155, 156 and 168 within the NS3 protease domain that confer variable degrees of reduced susceptibility to TMC435.[7]
- Median/mean trough TMC435 concentrations observed following doses of 75 mg and 200 mg QD were approximately 25-50 and 350-950 fold higher, respectively, than the EC_{50} values obtained in the genotype 1b-replicon cell line (6 ng/mL).[2,8,9]
- In a Phase I study (TMC435-C101; NCT00938899), six HCV genotype 1-infected patients who failed previous IFN-based therapy received TMC435 200 mg QD for 5 days.[2] Approximately 1.5 years later 5/6 patients (1 patient died 1 year after study TMC435-C101; unrelated to HCV therapy) were enrolled into Cohort 5 of the Phase IIa **O**ptimal **P**rotease inhibitor **E**nhancement of **R**esponse to Ther**A**py (OPERA)-1 study (TMC435-C201; NCT00561353) and were re-treated with TMC435 (200 mg QD) in combination with PegIFNα-2a/RBV for 28 days followed by PegIFNα-2a/RBV up to Week 48.[3]
- Here we report the final clinical outcomes for the 5 patients included in the TMC435-C101 study who were re-treated in TMC435-C201, and present a detailed genotypic and phenotypic analysis of HCV genotype 1 isolates obtained from these patients.

METHODS

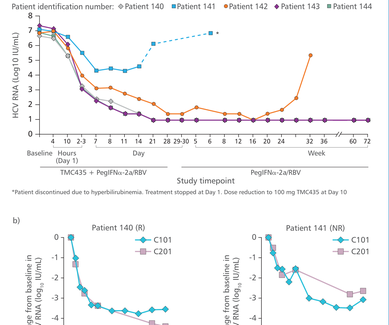
- Plasma samples were collected during the TMC435-C101 and TMC435-C201 studies and HCV ribonucleic acid (RNA) levels were assessed with a Roche COBAS® TaqMan® HCV/HPS v2.0 assay. This assay has a lower limit of quantification (LLOQ) of <25 IU/mL. HCV RNA results below the LLOQ are reported as either <25 IU/mL detectable or <25 IU/mL undetectable.
- HCV RNA was extracted from plasma samples and the NS3/4A protease region was sequenced using standard population sequencing, if HCV RNA was >100 IU/mL. In addition, selected samples were analysed using clonal single genome sequencing (20-80 clones per sample) or were subjected to massive parallel pyrosequencing (ultra-deep sequencing) using the 454 life science platform (GS-FLX, Roche Applied Science).
- For phenotypic analysis, selected mutations or patient-derived NS3 protease regions were introduced into a genotype 1b replicon backbone. The antiviral activity of TMC435 was tested and compared with the parental replicon in a standard transient replicon assay.

RESULTS

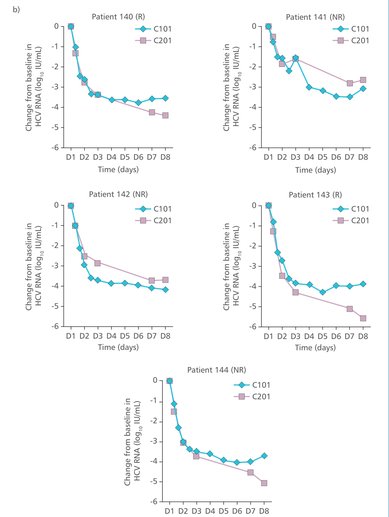
Antiviral Activity and Efficacy

- At Day 6 in study TMC435-C101, a consistent and rapid decline in plasma HCV RNA was observed in all six patients, with a maximal median reduction from baseline of 3.9 log_{10} IU/mL.[2]
- Of the five patients who were re-treated in study TMC435-C201,[3] one (patient 141, previous non-responder) stopped all treatment at Day 14 due to an adverse event (AE) (increase in bilirubin level). Three of the remaining four patients (one non-responder and two patients with viral relapse) who completed the 28-day TMC435 in combination with PegIFNα-2a/RBV treatment had undetectable HCV RNA levels at Day 28 and ultimately achieved a sustained virologic response (SVR). One patient (patient 142) achieved HCV RNA <25 IU/mL detectable at Day 28, followed by a viral breakthrough during PegIFNα-2a/RBV therapy at Week 28 (Figure 1a).
- Overall changes in HCV RNA during the first week of therapy were similar between study TMC435-C101 and TMC435-C201. In two patients, the HCV RNA reduction from baseline was slower in TMC435-C201 versus study TMC435-C101: in patient 141 at Day 3 (-2.9 versus -3.7 log_{10} IU/mL) and patient 142 at Day 7 (-2.8 versus -3.5 log_{10} IU/mL) (Figure 1b). This difference could be attributable to persistent virus and/or suboptimal response to PegIFNα-2a/RBV in study C201.

FIGURE 1: a) Changes in plasma HCV RNA in genotype 1-infected IFN-experienced patients who participated in study TMC435-C101 and were re-treated with TMC435 200 mg QD in combination with PegIFNα-2a/RBV in study TMC435-C201 (Day 0 to Week 72). b) Individual changes from baseline in HCV RNA during study TMC435-C101 and TMC435-C201 (Days 1 to 8).



*Patient discontinued due to hyperbilirubinemia. Treatment stopped at Day 1. Dose reduction to 100 mg TMC435 at Day 10



D, day; HCV, hepatitis C virus; NR, non-responder to previous IFN-based therapy; PegIFNα-2a, pegylated interferon α-2a; QD, once daily; R, relapser to previous IFN-based therapy; RBV, ribavirin; RNA, ribonucleic acid

Genotypic/Phenotypic Changes in the HCV NS3 Protease Region During Study TMC435-C101 and TMC435-C201

- Mutations at one or more of the amino acid positions 80, 155, 156 and 168, which were absent at baseline, were detected in all subjects as early as on Day 3 of study TMC435-C101 using population sequencing (Figure 2a).
- Population sequencing and phenotypic analysis of the HCV isolates obtained at baseline of TMC435-C201 suggested that the dominant viral variants returned to the characteristics observed prior to study TMC435-C101 in all 5 patients.
- Mutations S122R plus R155K (patient 142), and Q80R plus D168E plus F169L (patient 141), were detected in TMC435-C201 during treatment.
- The *in vitro* activity of TMC435 against the treatment emerging NS3 variants identified revealed fold changes in EC_{50} values (FC) >100 in all patients in study TMC435-C101 and in patient 141 and 142 in TMC435-C201 (Figure 2b).

FIGURE 2: a) Mutations detected in the NS3 protease domain defined as changes from reference sequence (Con1 for genotype 1b and H77 for genotype 1a). b) Fold changes in EC_{50} values compared with wild-type assessed in a transient replicon assay using chimeric replicons harbouring the NS3 protease region derived from patient samples.

a)

	Genotype 1a	I018	T054	V055	P067	Q080	S122	S133	R155	D168	I170
Patient 140 (R)	C101 Day 1	V	S	I/V	S		N/S	F/S		D/E	
	C101 Day 3	V	S	I	S	Q/R	N/S			E	I/T
	C101 Day 6	V	S	I	P/S	Q/R			K/R	E	
	C101 Follow up 2	V	S	I	P/S	Q/R				E	
	C201 Day 1	V	S	I/V	S		N/S			D/E	

	Genotype 1b	S007	R026	V048	Y056	Q80	P089	A150	D168	F169
Patient 141 (NR)*	C101 Day 1	A	K	I	F		S	V		
	C101 Day 3	A	K	I	F		S	V		
	C101 Day 6	A	K	I	F		S	V	V	
	C101 Follow up 2	A	K	I	F		S	V	V	
	C201 Day 1	A	K	I	F		S	V		
	C201 Day 7	A	K	I	F	R	S	V	E	
	C201 Day 14	A	K	I	F	R	S	V	E	L
	C201 Discontinued Visit 1	A	K	I	F	R/K/Q	S	V	E	F/L
	C201 Discontinued Visit 2	A	K	I	F	K	S		E	

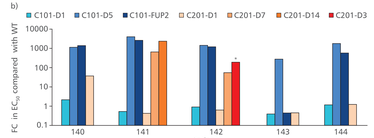
	Genotype 1a	G15	V029	S122	I132	R155	D168
Patient 142 (NR)	C101 Day 1		A				
	C101 Day 3		A				D/V
	C101 Day 5		A			K/R	A/D
	C101 Day 6		A				V/A
	C101 Follow up 2		A			K/R	
	C201 Day 1		A				
	C201 Day 7		A			K	
	C201 Day 14	G/W	A		L	K	
	C201 Discontinued Visit 3		A	R		K	

	Genotype 1b	R026	D168
Patient 143 (R)	C101 Day 1	K	
	C101 Day 3	K/R	D/V
	C101 Day 6	K	V
	C101 Follow up 2	K	
	C201 Day 1	K	

	Genotype 1a	P067	K068	S122	R155	D168
Patient 144 (NR)	C101 Day 1	S	K/N	N/S		
	C101 Day 3	S	K/N	N/S		
	C101 Day 6	S	N	N		D/V
	C101 Follow up 1	S		N/S	K/R	A/V
	C101 Follow up 2	S		N/S	K	D/E
	C201 Day 1	S				

*Patient discontinued due to hyperbilirubinemia. Mutations T408, S917I/A and L153I were found in all HCV genotype 1a patients at all time points analysed; the mutation R26K was found in all HCV genotype 1b patients at all time points analysed; A156A/V was present in patient 143 at Day 4.
NR, non-responder to previous IFN-based therapy; R, relapser to previous IFN-based therapy. Day 1 = Baseline; C101 follow-up 1 = 2 weeks after end of dosing; C101 follow-up 2 = 4 weeks after end of dosing. Missing values were below the lower limit of the assay

b)

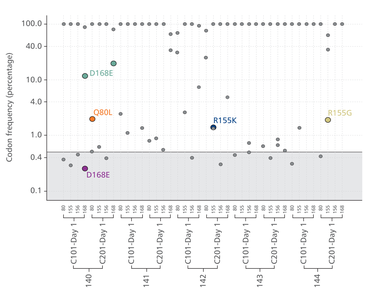


*Fold change determined using site directed Mutants S122R and R155K. Patient discontinued at Week 32
C201=OPERA-1
D, day; EC_{50}, 50% effective concentration; FC, fold change; FUP, follow-up; HCV, hepatitis C virus; NR, non-responder; OPERA, Optimal Protease inhibitor Enhancement of Response to TherApy; R, relapser; WT, wild type

Presence of Viral Variants at Baseline in Studies TMC435-C101 and TMC435-C201 based on Deep Sequencing

- No additional NS3 mutations at key binding pocket residues (80, 155, 156 and 168) were detected at baseline of the TMC435-C101 study using ultra-deep sequencing (D168D/E was also detected by population sequencing).
- At baseline of the TMC435-C201 study, mutations at key binding positions were observed at low frequency (<2%) in 3 patients: Q80L, R155G and R155K, in patients 140, 144 and 142, respectively (Figure 3).
  - Patient 142 showed a slower initial viral decline in TMC435-C201 compared with TMC435-C101, while patients 140 and 144 showed a comparable viral decline in both studies.

FIGURE 3: The sequence of the HCV NS3 protease domain was determined for baseline samples of studies TMC435-C101 and TMC435-C201 using ultra-deep sequencing. Percentage of codon frequency at NS3 positions 80, 155, 156 and 168 are shown. Grey dots represent codons encoding wild-type amino acids. Coloured dots represent codons encoding mutations (defined as changes from reference sequence: Con1 for genotype 1b and H77 for genotype 1a).



HCV, hepatitis C virus

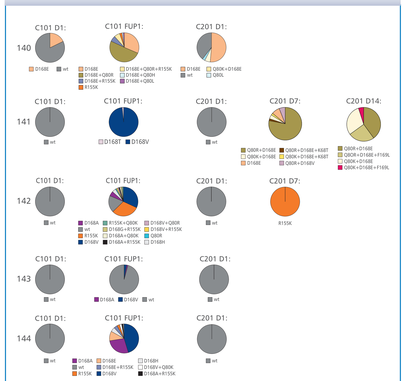
Single Genome (Clonal) Sequencing of NS3 Protease Domain

- In addition to single mutations, variants encoding 2 or 3 amino acid changes on the same genome were observed (Figure 4).
- In general, when introduced into the replicon, most of the single mutations detected resulted in a lower fold change in EC_{50} value compared with replicons with 2 or 3 mutations (data not shown).

Evolution of Amino Acid Changes over Time Based on Deep Sequencing

- In patient 141, ultra-deep sequencing detected a Q80R and a D168E mutation four weeks after the end of dosing for study TMC435-C101, which were no longer detectable at baseline of the TMC435-C201 study. These two mutations emerged as major variants upon re-exposure to TMC435 in the TMC435-C201 study (Figure 5a). This patient also showed a slower initial viral decline in TMC435-C201 compared with in TMC435-C101.
- In patient 142, ultra-deep sequencing detected variant R155K persisting at low frequency (<2%) at baseline of the TMC435-C201 study. This mutation became detectable by population sequencing at Day 7 and 14 of the study while HCV RNA was still declining. An S122R and an R155K mutation became detectable by population and 454 sequencing at the time of viral breakthrough (Week 28) (Figure 5b).

FIGURE 4: NS3 protease domain was amplified, cloned into standard cloning vectors and sequenced to determine linkage of mutations. Mutations at position 80, 155, 156, 168 and 169 were considered for this analysis. Frequency of clones is represented.



D, day; FUP, follow-up

CONCLUSIONS

- **Treatment with TMC435 200 mg QD resulted in potent antiviral activity in study TMC435-C101, during five days of monotherapy, and subsequently in the same patients when combined with PegIFNα-2a/RBV in the TMC435-C201 study.**
- **Viral variants that emerged during first exposure to TMC435 in study TMC435-C101 were no longer detectable in most cases over time, while in some they persisted at low frequencies based on deep-sequencing analysis.**
- **Persistence of viral variants in two patients at low frequency was associated with slightly reduced antiviral activity of TMC435 during the first seven days of the TMC435-C201 study.**
- **In TMC435-C201, successful re-treatment (SVR) with TMC435 in combination with PegIFNα-2a/RBV for 4 weeks followed by PegIFNα-2a/RBV up to Week 48 after prior exposure to TMC435 with emergence of resistance variants could be achieved in 3 out of 4 patients who completed therapy.**
- **The clinical implications of these findings warrant further investigation.**

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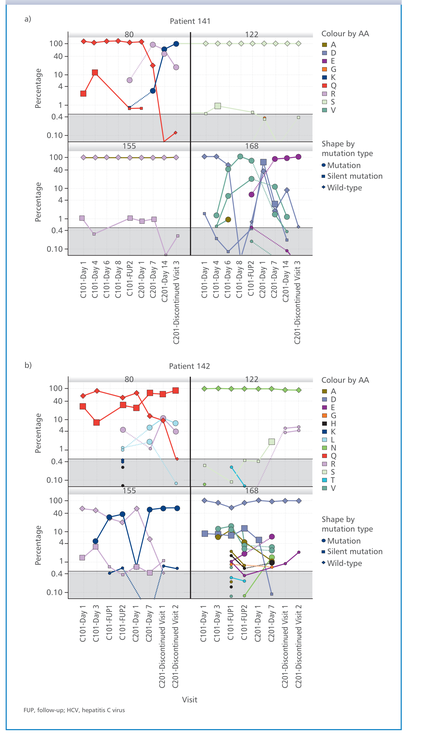
Some of these data have been presented previously at the following meetings:

- Lenz O et al. Poster presented at the International HIV & Hepatitis Virus Drug Resistance Workshop, Dubrovnik, Croatia, 8-12 June, 2010.
- Reesink H et al. Poster presented at the 60th American Association for the Study of Liver Diseases (AASLD) meeting, Boston, MA, USA, 30 October–3 November, 2009.

FIGURE 5: The sequence of the HCV NS3 protease domain was analysed from multiple samples using ultra-deep sequencing. Changes of codon frequency over time for NS3 at positions 80, 122, 155 and 168 are shown for two patients a) 141 b) 142.



FUP, follow-up; HCV, hepatitis C virus

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9. Data on file. Tibotec Inc. 2010.

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