Efficacy of Re-treatment With TMC435 as Combination Therapy in Hepatitis C Virus–Infected Patients Following TMC435 Monotherapy

OLIVER LENZ,* JOEP DE BRUIJNE,‡ LEEN VIJGEN,* THIERRY VERBINNEN,* CHRISTINE WEEGINK,‡ HERWIG VAN MARCK,* INA VANDENBROUCKE,* MONIKA PEETERS,* KENNETH SIMMEN,* GREG FANNING,* RENE VERLOES,* GASTON PICCHIO,§ and HENDRIK REESINK‡

*Janssen Infectious Diseases, Beerse, Belgium; ‡Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, The Netherlands; and §Janssen Research and Development, Titusville, New Jersey

In the TMC435-C101 study, 6 patients infected with hepatitis C virus genotype 1 were treated with the protease inhibitor TMC435 (200 mg once daily) as monotherapy for 5 days. Approximately 1.5 years later, 5 of these patients were re-treated with TMC435 (200 mg once daily) plus pegylated interferon alfa-2a and ribavirin (PegIFNα-2a and RBV) for 4 weeks, followed by PegIFNα-2a and RBV until week 48 (in the Optimal Protease inhibitor Enhancement of Response to therApy [OPERA-1] study). TMC435-resistant variants, which emerged in all 5 patients during the TMC435-C101 study, were no longer detected at the beginning of the OPERA-1 study based on virus population sequencing. During the OPERA-1 study, 3 patients had a sustained virologic response; deep sequencing indicated low-level persistence of resistant variants in the remaining 2 patients, which might have affected their response to re-treatment. Clinical trials.gov Number, NCT00561353.

Keywords: TMC435-C101; Clinical Trial; Sustained Virologic Response; HCV NS3/4A; Direct-Acting Antiviral.

New treatments for patients infected with hepatitis C virus (HCV) genotype 1 are now available, including HCV NS3/4A protease inhibitors (PIs).[1,2] Drug-resistant viral variants can emerge in patients treated with direct-acting antivirals who do not achieve a sustained virologic response.[3,4] Although it has been described that variants become undetectable after treatment failure in most patients, it is unclear whether low-level variants persist and affect re-treatment options.[5,6]

TMC435 is an investigational HCV PI administered orally once daily.[7–9] In study TMC435-C101, 6 patients infected with HCV genotype 1 received TMC435 (200 mg once daily) as monotherapy for 5 days.[10] Approximately 1.5 years later, 5 of the 6 patients participated in the Optimal Protease inhibitor Enhancement of Response to TherApy (OPERA-1; TMC435-C201) study and were treated with TMC435 (200 mg once daily) plus peginterferon alfa-2a/ribavirin (PegIFNα-2a/RBV) for 4 weeks, followed by PegIFNα-2a/RBV up to week 48 (Supplementary Materials and Methods and Supplementary Figure 1). Patients were white men previously treated with interferon-based therapy who did not respond to treatment or experienced a relapse (Table 1).

In TMC435-C101, all patients had a rapid and pronounced decline in HCV RNA during TMC435 monotherapy.[10] Although no viral breakthrough was observed, mutations at NS3 amino acid positions 80, 155, 156, and/or 168 emerged in all patients. Deep sequencing at baseline of TMC435-C101 showed that there had been no additional preexisting NS3 mutations at positions known to affect TMC435 activity in vitro (80, 155, 156, and 168).[11] At baseline of OPERA-1, these variants that emerged during C101 were no longer detectable using population sequencing (Supplementary Table 1). With deep sequencing, Q80L and R155G were observed at baseline of OPERA-1 at low frequency (1%–2%) in 2 of 3 patients (patients 140 and 144, respectively) who achieved undetectable HCV RNA after 4 weeks of triple therapy and ultimately achieved a sustained virologic response (Table 1 and Supplementary Figures 2*A* and 3). Q80L had been previously detected in patient 140 as an emerging minority variant in TMC435-C101 (frequency <5%), while in patient 144, R155G had not been previously observed.

In the remaining 2 patients who did not achieve a sustained virologic response and who were previous non-responders to PegIFN/RBV and of *IL28B* TT genotype (polymorphism rs12979860), decreases in HCV RNA levels from baseline were slower in OPERA-1, in which TMC435 was given in combination with PegIFN/RBV, compared with TMC435-C101, in which TMC435 was given alone. In patient 141, decreases at day 7 were −2.8 IU/mL (OPERA-1) vs −3.5 $\log_{10}$ IU/mL (TMC435-C101) (Supplementary Figure 1*B*). In patient 142, decreases at day 3 were −2.9 IU/mL (OPERA-1) vs −3.7 $\log_{10}$ IU/mL (TMC435-C101) (Supplementary Figure 2*B*).

Patient 142 achieved an HCV RNA level <25 IU/mL at the end of triple therapy (OPERA-1) and experienced viral breakthrough at week 28 during PegIFN/RBV treatment. Population sequencing identified emerging D168A/V and

Abbreviations used in this paper: HCV, hepatitis C virus; OPERA, Optimal Protease inhibitor Enhancement of Response to TherApy, Study TMC435-C201; PegIFNα-2a, pegylated interferon alfa-2a; PI, protease inhibitor; RBV, ribavirin.

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2012.07.117

Table 1. Baseline Patient Characteristics and Virologic Response in the OPERA-1 Study (Cohort 5)

	Patient 140	Patient 141[a,b]	Patient 142[c]	Patient 143	Patient 144
Patient/disease characteristics					
Body mass index (kg/m^2)	24.6	26.3	23.8	20.5	27.5
METAVIR score	F2	F4	F1	F1	F1
IL28B genotype	CC	TT	TT	CT	CT
HCV genotype subtype	1a	1b	1a	1b	1a
Previous IFN-based therapy[d]	IFN α-2a/RBV	PegIFN/RBV	PegIFN/RBV	PegIFN/RBV	PegIFN/RBV/ amantadine
Response to previous IFN-based therapy[e]	Relapser	Nonresponder	Nonresponder	Relapser	Nonresponder
HCV RNA plasma concentration					
Baseline	6.64 $\log_{10}$IU/mL	7.10 $\log_{10}$IU/mL	6.85 $\log_{10}$IU/mL	7.34 $\log_{10}$IU/mL	6.87 $\log_{10}$IU/mL
Day 14	<25 IU/mL (detectable)	4.59 $\log_{10}$IU/mL	244 IU/mL	<25 IU/mL (detectable)	<25 IU/mL (detectable)
Day 28	<25 IU/mL (undetectable)	NA	<25 IU/mL (detectable)	<25 IU/mL (undetectable)	<25 IU/mL (undetectable)
Week 12	<25 IU/mL (undetectable)	NA	<25 IU/mL (detectable)	<25 IU/mL (undetectable)	<25 IU/mL (undetectable)
Week 72 (sustained virologic response)	<25 IU/mL (undetectable)	NA	NA	<25 IU/mL (undetectable)	<25 IU/mL (undetectable)

IL28B, polymorphism rs12979860; IFN, interferon; NA, not applicable.
[a]Subhemophilia.
[b]Patient 141 stopped treatment at day 14 due to elevated bilirubin levels.
[c]Patient 142 experienced viral breakthrough at week 28 during PegIFN/RBV treatment.
[d]Different doses of PegIFN and RBV were used in previous therapy.
[e]Categorized by investigator.

R155K mutations during TMC435-C101 and R155K at day 7, day 14, and time of viral breakthrough in OPERA-1. Based on deep sequencing (Figure 1), R155K became detectable (frequency of 9%) at day 3 of TMC435-C101 before becoming the major variant and was detected 4 weeks after the end of TMC435 dosing (frequency of 64%). R155K was still detectable at baseline of OPERA-1 (frequency of 1%) and became the major variant from day 7 to time of viral breakthrough, 24 weeks after the end of TMC435 dosing. The ability of R155K to persist is consistent with reports showing the relatively high fitness of variants harboring this mutation. In addition to R155K, multiple mutations emerged at position 80 and 168 (Q80L, K, R and D168V, A, E, H, N, G) as well as in TMC435-C101 and/or OPERA-1 as minor variants.

Patient 141 had a TMC435 dose reduction at day 10 from 200 to 100 mg once daily and stopped all treatment at day 14 due to elevated bilirubin levels. HCV RNA levels did not decline further from day 7 until day 14. Q80R + D168E and Q80K + D168E mutations were detected at day 7 and 4 weeks after the end of the 14-day treatment period in OPERA-1, respectively. In TMC435-C101, population-based sequencing showed the emergence of D168V (Supplementary Table 1). Based on deep sequencing (Figure 1), D168V became detectable (frequency of 2%) at day 4 of TMC435-C101 before becoming the major variant, and D168E emerged (frequency of 6%) at the last follow-up visit, 4 weeks after the end of dosing with TMC435. At baseline of OPERA-1, only the wild-type residue aspartate was observed at position 168, while during OPERA-1 D168E became the major variant (frequency of 83% at day 7) and D168V was only transiently detectable (frequency of 12% at day 7). In parallel to the emergence of D168E, Q80R and subsequently Q80K became detectable. This suggests that previous exposure to TMC435 facilitated the emergence of a Q80R/K + D168E double mutant variant, which confers a similar level of resistance to TMC435 as the single D168V mutation but displays higher fitness.

In summary, although only 5 patients were analyzed and their first exposure to TMC435 was short, all patients had TMC435-resistant variants at the end of their first treatment that were no longer detectable (by population sequencing) at baseline of a second study 1.5 years later. A similar pattern may occur in patients who fail to respond to a PI-based regimen and are later evaluated for re-treatment. The data presented here suggest that in some patients the frequency of emerging resistant viral variants may decrease over time, in the absence of selective pressure imposed by a direct-acting antiviral, to levels that do not negatively affect outcome on subsequent re-treatment with a regimen containing the same direct-acting antiviral. However, in other patients, resistant variants might persist at low levels, potentially below the detection limit of deep sequencing assays, which may affect the efficacy of a second course of treatment. The impact of preexisting resistant variants on treatment outcome is intimately linked to the intrinsic PegIFN/RBV responsiveness of the patient, as shown in this and other studies, because their sole

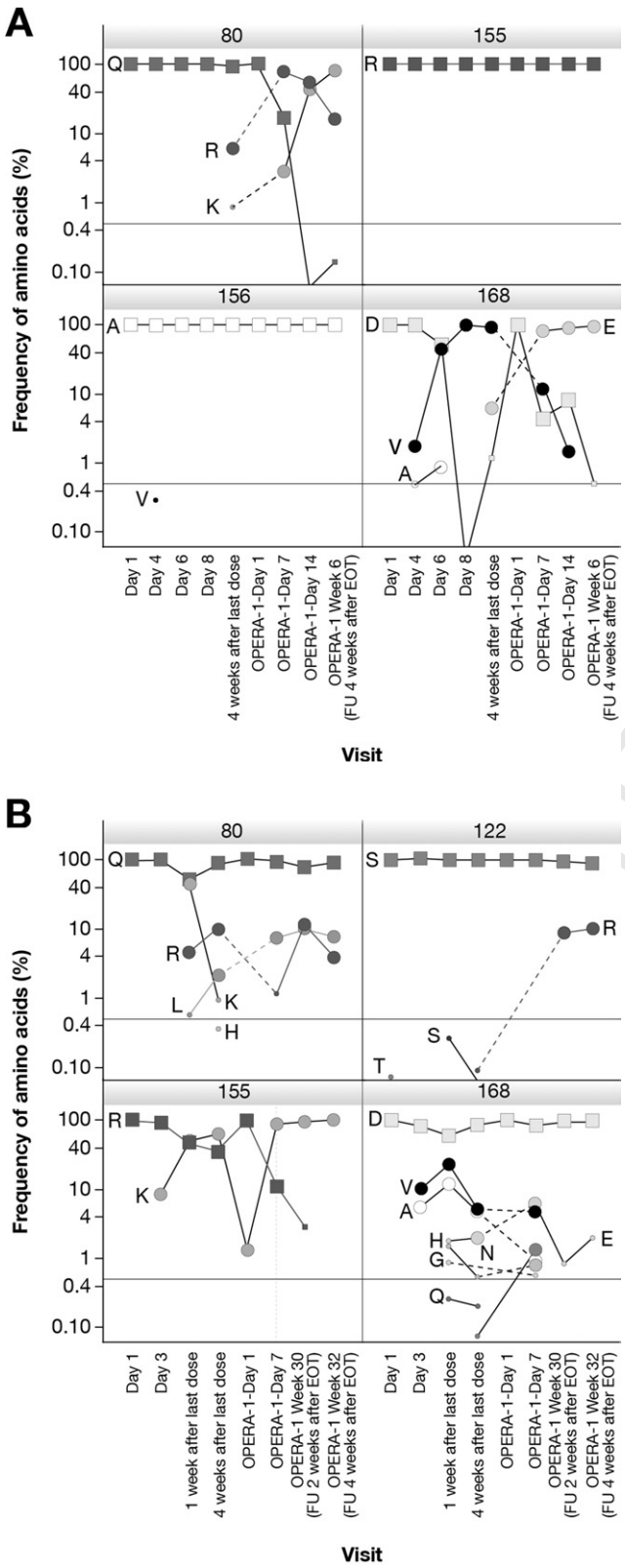


Figure 1. Evolution of viral variants over time (NS3 amino acid 80, 122, or 156, 155, and 168 are shown) in (*A*) patient 141 and (*B*) patient 142 assessed using deep sequencing.

presence does not automatically translate into treatment failure.[12,13] The significance of preexisting variants on sustained virologic response may be different when considering interferon-free regimens.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.07.117.

References

1. Jacobson IM, et al. N Engl J Med 2011;364:2405–2416.
2. Poordad F, et al. N Engl J Med 2011;364:1195–1206.
3. Pawlotsky JM. Hepatology 2011;53:1742–1751.
4. Sarrazin C, et al. Gastroenterology 2010;138:447–462.
5. Zeuzem S, et al. Hepatology 2010;52:436A, Abstract 227.
6. Barnard RJ, et al. Hepatology 2011;54:Abstract 164.
7. Fried MW, et al. American Association for the Study of Liver Diseases, 2011.
8. Lenz O, et al. Antimicrob Agents Chemother 2010;54:1878–1887.
9. Manns M, et al. Antivir Ther 2011;16:1021–1033.
10. Reesink HW, et al. Gastroenterology 2010;138:913–921.
11. Verbinnen T, et al. J Virol 2010;84:11124–11133.
12. Lenz O, et al. American Association for the Study of Liver Diseases, 2011.
13. Marcellin P, et al. Gastroenterology 2011;140:459–468.

Received December 14, 2011. Accepted July 27, 2012.

Reprint requests

Address requests for reprints to: Oliver Lenz, PhD, Janssen Infectious Diseases, Turnhoutseweg 30, Beerse, Belgium. e-mail: olenz@its.jnj.com; fax: (32) 0 14 602 841.

Acknowledgments

O.L. and J.D. contributed equally to this work.

Editorial support was provided by Phil Matthews, PhD, of Complete Medical Communications, funded by Janssen Research and Development. The authors thank the patients and the study staff, as well as all the colleagues from Janssen Infectious Diseases who contributed to this work.

The authors thank C. J. Schinkel and R. Molenkanp (Department of Clinical Virology, Academic Medical Center, Amsterdam, The Netherlands) for performing *IL28B* testing.

Conflicts of interest

The authors disclose the following: Oliver Lenz, Leen Vijgen, Thierry Verbinnen, Herwig Van Marck, Ina Vandenbroucke, Monika Peeters, Kenneth Simmen, Greg Fanning, Rene Verloes, and Gaston Picchio are or were employees of Janssen Infectious Diseases or Janssen Research and Development. Hendrik Reesink has received research funding and/or consultancy fees from Anadys, Astex, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Idenix, Janssen-Cilag, Merck, PRA International, Roche, Santaris, SGS, and Vertex. The remaining authors disclose no conflicts.

Funding

Supported by Janssen Research and Development.

Supplementary Materials and Methods

Study Design and Objectives

Results for cohorts 1–4 of OPERA-1 (TMC435-C201, NCT00561353) have been reported previously.[1] In cohort 5 of OPERA-1, subjects who were nonresponders to and experienced a relapse after previous interferon-based therapy who had subsequently received 5 days of treatment with TMC435 (200 mg once daily) in study C101[2] were enrolled and received TMC435 (200 mg once daily) in combination with PegIFN/RBV for 4 weeks, followed by up to 44 weeks of PegIFN/RBV only. The cohort 5 study was conducted in The Netherlands (Department of Gastroenterology and Hepatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands).

PegIFNα-2a was given subcutaneously at a dosage of 180 μg once weekly, and RBV was orally administered at a dosage of 1000–1200 mg twice daily (body weight dependent).

The objective of cohort 5 was to assess the antiviral activity, safety, and tolerability of a TMC435-containing treatment regimen in patients who had previously received TMC435 monotherapy.

Assessments

HCV RNA level. Plasma samples were obtained throughout the study and HCV RNA levels were assessed using TaqMan HCV/HPS assay v2.0 (Roche Molecular Diagnostics, Basel, Switzerland), with a lower limit of quantification of 25 IU/mL and a lower limit of detection of approximately 10–15 IU/mL. Samples in which no target HCV RNA was detected below the lower limit of quantification were reported as <25 IU/mL undetectable. Viral breakthrough was defined as an increase of >1 $\log_{10}$ IU/mL from nadir (lowest HCV RNA level obtained before breakthrough) or >100 IU/mL in patients whose HCV RNA had previously been <25 IU/mL undetectable.

HCV NS3/4A population sequence analysis and subtype determination. HCV RNA was extracted from plasma samples, and the HCV NS3/4A region was sequenced at the population level using reverse-transcription polymerase chain reaction (PCR) and standard Sanger sequencing. HCV genotypes/subtypes were determined by sequencing and basic local alignment search tool (BLAST) analysis of a 329–base pair region within the HCV NS5B gene.

Clonal and 454 deep sequencing. In addition, selected samples were analyzed using clonal single genome sequencing or were subjected to massively parallel pyrosequencing (deep sequencing) using the 454 GS-FLX platform (454 Life Sciences, Roche Applied Science) essentially as previously described.[3] For clonal sequencing, NS3/4A amplicons were prepared and bacterially cloned, and 20–80 clones were sequenced.

For 454 sequencing, complementary DNA was generated using random primers, followed by PCR generating 2 amplicons covering NS3 amino acid positions 1–99 and 85–192, respectively.

Sequence adaptors and additional multiplex identifiers (ie, short barcode sequences) were attached to the primers and PCR using Phusion Hot Start High-Fidelity DNA polymerase (Finnzymes, Vantaa, Finnland) was performed. To maximize the amount of input templates and to minimize variation due to PCR drift, 7 replicate reverse-transcription PCRs were performed for each sample and subsequently pooled for sequence analysis using the 454 GS-FLX amplicon approach followed by analyses with Amplicon Variant Analysis software (Roche).

The median (Q1–Q3) number of sequence reads per position (coverage) for NS3 positions of interest (36, 43, 54, 80, 138, 155, 156, 168, 169, and 170) for the subjects and visits included in this report was 5849 (3611–9176). The sensitivity limit was set as a frequency of 0.5% (data not shown).

Determination of IL28B genotype. Consent for *IL28B* testing[4] was obtained retrospectively. *IL28B* polymorphism (rs12979860 and rs17809917) was determined from patient serum by high-resolution melting curve analysis using the LightCycler 480 (Roche Applied Science, Penzberg, Germany) and High Resolution Melting Master (Roche Applied Science).

Safety and tolerability. Adverse events were monitored during all study visits. Vital signs, electrocardiogram recordings, and laboratory parameters were assessed at screening and on days 1, 7, 14, 21, and 28.

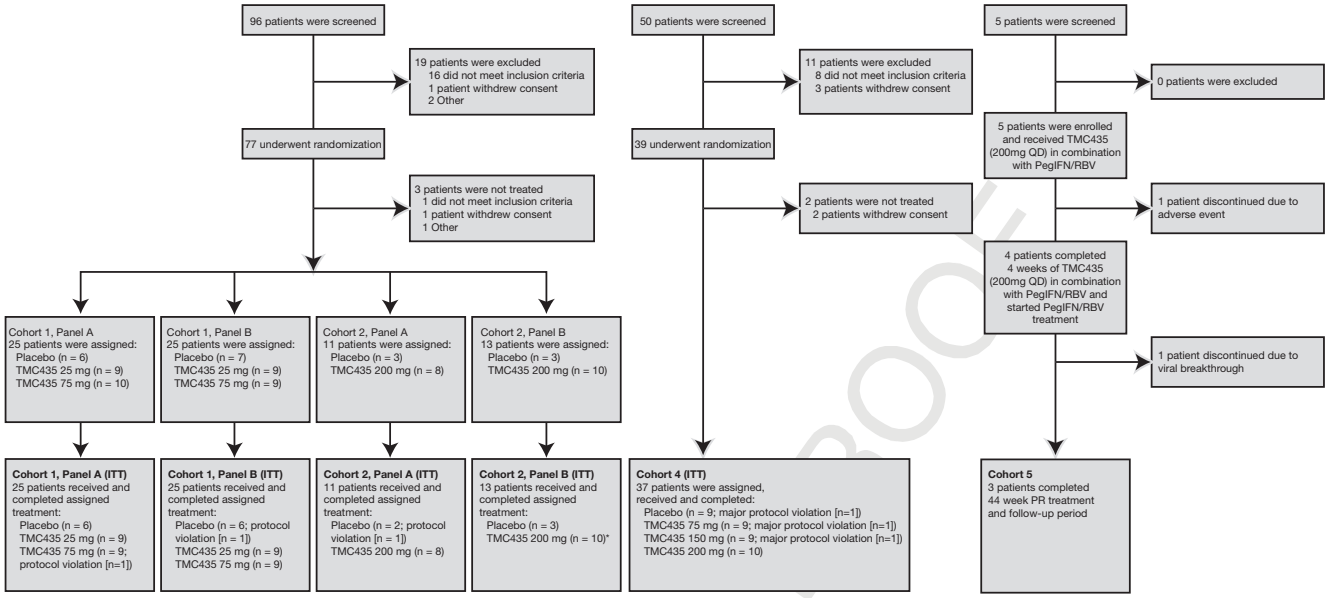
Statistical analysis. Final analysis was performed when all patients had completed week 72 of treatment or discontinued earlier. Descriptive analyses were performed.

Supplementary Results

During TMC435 dosing, there were no serious adverse events. Influenza-like illness was the most frequently reported adverse event, reported in 4 of 5 patients. All but one of the observed adverse events was mild to moderate in severity and not, or doubtfully, related to TMC435. One patient discontinued treatment after 14 days (following a reduction of the dose of TMC435 at day 10 to 100 mg once daily) due to an increase in blood bilirubin level (grade 4). This patient had an elevated bilirubin level (grade 2) at baseline, although the value at screening was within normal limits. Bilirubin level normalized after discontinuation of treatment. There were no other grade 3 or 4 adverse events during treatment with TMC435. No clinically relevant changes were observed in any other laboratory parameters, electrocardiogram parameters, or vital signs. During the PegIFNα-2a/RBV treatment from day 28 onward, aspartate aminotransferase and alanine aminotransferase levels decreased in all 5 patients during therapy.

Supplementary References

1. Manns M, et al. Antivir Ther 2011;16:1021–1033.
2. Reesink HW, et al. Gastroenterology 2010;138:913–921.
3. Verbinnen T, et al. J Virol 2010;84:11124–11133.
4. Ge D, et al. Nature 2009;461:399–401.



Cohort 3 TMC435 400 mg investigation was cancelled prior to the initiation of treatment based on the antiviral activity observed in Cohorts 1 and 2
*Intent-to-treat population for Cohorts 1 and 2 combined includes 1 patient allocated to Panel B, 200 mg, when they should have been randomized to Panel A, 200 mg

Supplementary Figure 1. CONSORT diagram.

Supplementary Table 1. Amino Acid Substitution in NS3 Protease Domain (Defined as Changes From Reference Sequence: H77 for Genotype 1a and con1 for Genotype 1b) Detected in Studies C101 and OPERA-1 Using Population-Based Sequencing

	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 4 weeks after last dose	V	S	I	P/S	Q/R				E	
	OPERA-1 Day 1	V	S	I/V	S		N/S			D/E	

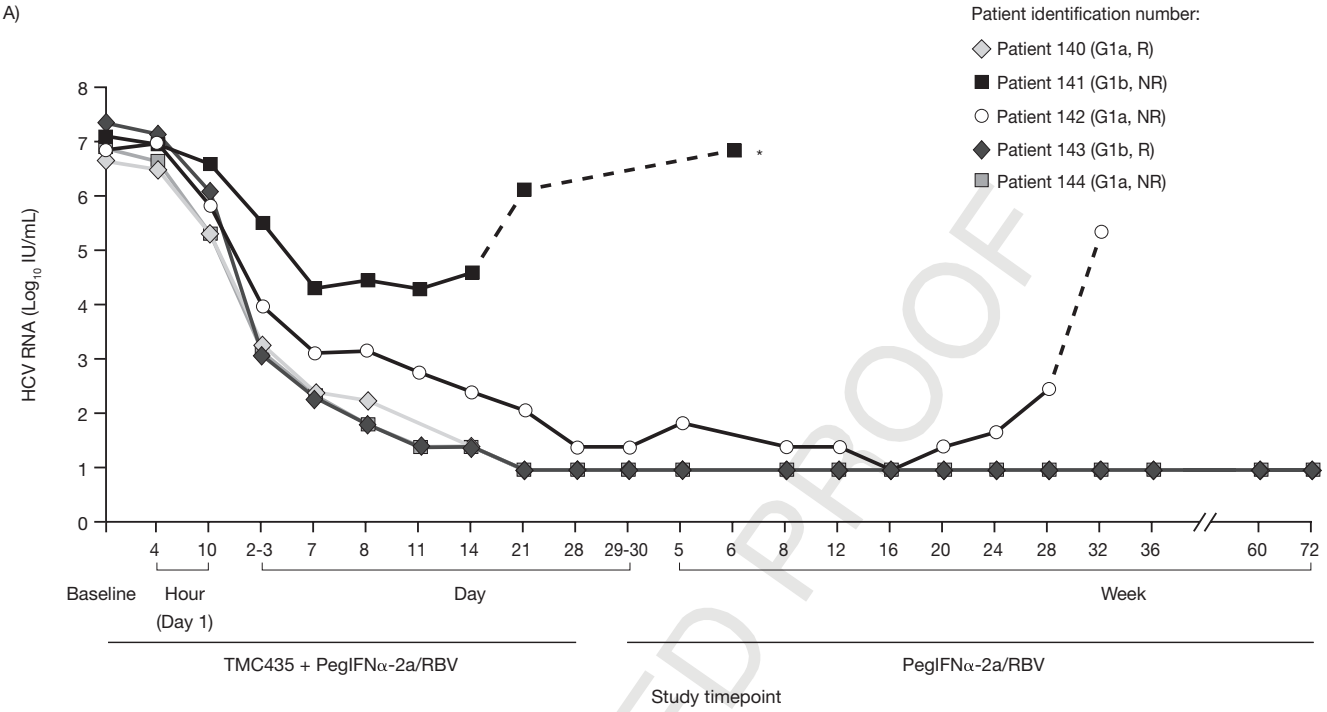
	Genotype 1b	S007	R026	V048	Y056	Q80	P089	A150	D168	F169
Patient 141 (NR)	C101 Day 1	A	K	I	F		S	V		
	C101 Day 3	A	K	I	F		S	V		
	C101 Day 6	A	K	I	F		S	V	V	
	C101 4 weeks after last dose	A	K	I	F		S	V	V	
	OPERA-1 Day 1	A	K	I	F		S	V		
	OPERA-1 Day 7	A	K	I	F	R	S	V	E	
	OPERA-1 Day 14	A	K	I	F	R	S	V	E	L
	OPERA-1 Day 21 (1-week follow-up)	A	K	I	F	R/K/Q	S	V	E	F/L
	OPERA-1 Week 6 (4-week follow-up)	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 4 weeks after last dose		A			K/R	
	OPERA-1 Day 1		A				
	OPERA-1 Day 7		A			K	
	OPERA-1 Day 14	G/W	A		L	K	
	OPERA-1 Week 32 (4-week follow-up)		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 4 weeks after last dose	K	
	OPERA-1 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 1 week after last dose	S		N/S	K/R	A/V
	C101 4 weeks after last dose	S		N/S	K	D/E
	OPERA-1 Day 1	S				

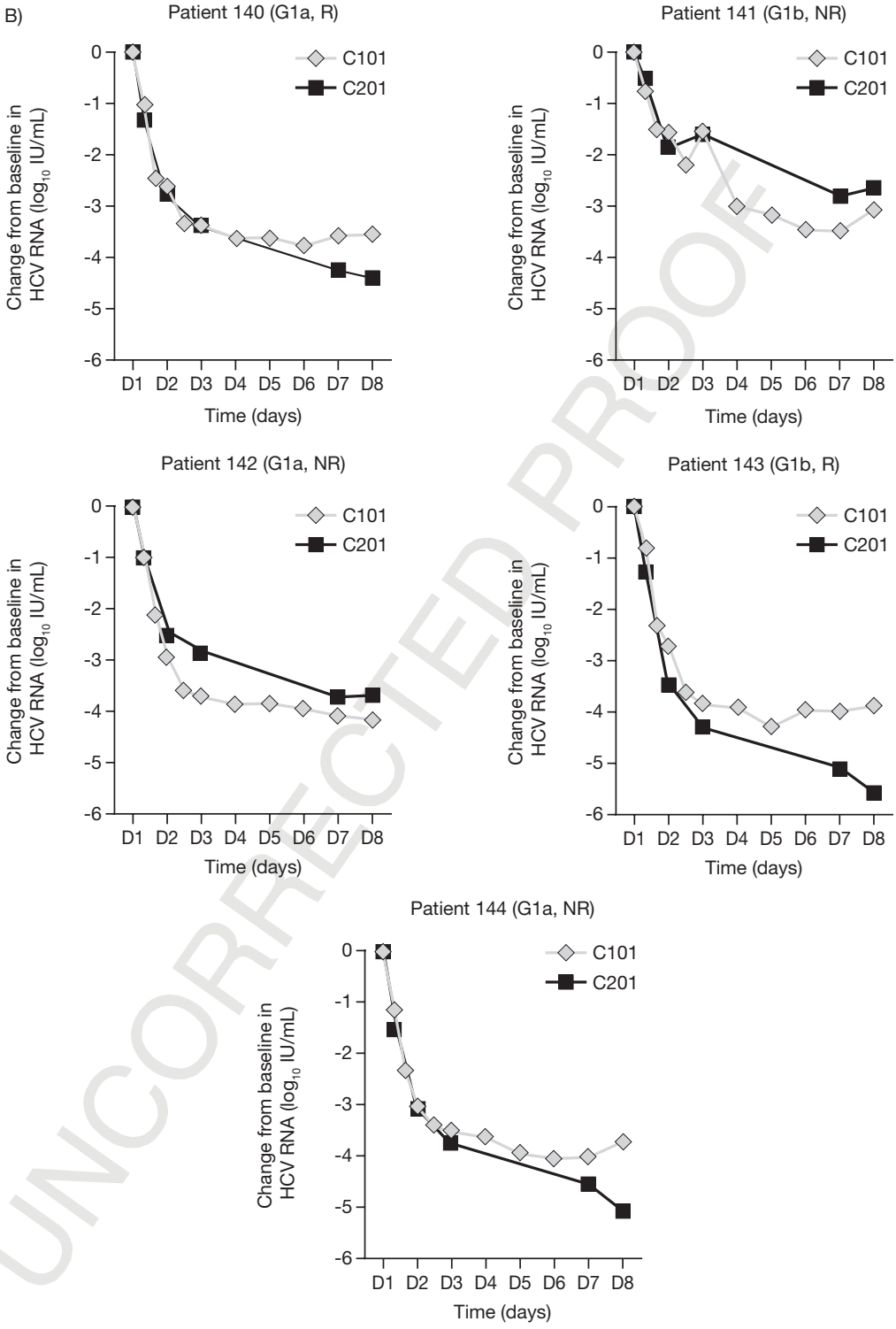
NOTE. Mutations T40A, S91T/A, and L153I were found in all HCV genotype 1b patients at all time points analyzed, and the mutation R26K was found in all HCV genotype 1b patients at all time points analyzed. *Dark grey* indicates NS3 position at which mutations have been shown to reduce TMC435 activity in vitro.
R, relapse; NR, nonresponse.



G, genotype; NR, non-responder; PegIFN, pegylated interferon; R, relapser; RBV, ribavirin; RNA, ribonucleic acid
*Patient discontinued due to hyperbilirubinemia. Treatment stopped at Day 14. Dose reduction to 100 mg TMC435 at Day 10

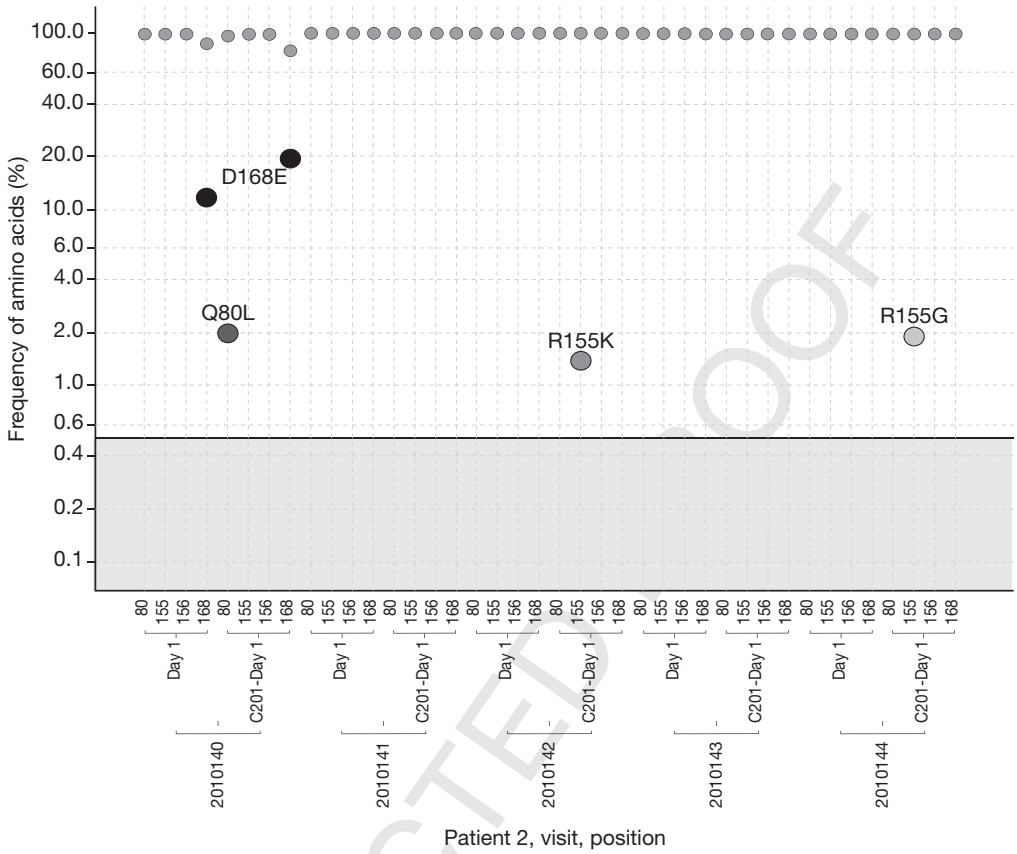
Supplementary Figure 2. (*A*) Individual HCV RNA levels in cohort 5 of OPERA-1 over the entire study period and (*B*) individual changes in HCV RNA levels from baseline to day 8 in study C101 compared with OPERA-1.

B)



D, day; G, genotype; HCV, hepatitis C virus; NR, non-responder; PegIFN, pegylated interferon; R, relapser; RBV, ribavirin; RNA, ribonucleic acid

Supplementary Figure 2. Continued



NS3 amino acid position 80, 155, 156, and 168 are shown. Wild-type amino acids are shown as small circles, mutations defined as changes from reference sequence (H77 for genotype 1a and con1 for genotype 1b) are large circles. Frequencies of individual amino acids were calculated as number of sequence reads per amino acid divided by total number of reads at that position. Amino acid frequency to a level of 0.1% is shown but frequency below 0.5% should be considered below the sensitivity of the assay (data not shown)

Supplementary Figure 3. Presence of viral variants at baseline in studies C101 and OPERA-1 based on 454 deep sequencing.