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EASL JOURNAL OF HEPATOLOGY

Virology analyses of HCV isolates from genotype 1-infected patients treated with simeprevir plus peginterferon/ribavirin in Phase IIb/III studies

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Background & Aims: Simeprevir is an oral hepatitis C virus (HCV) NS3/4A protease inhibitor approved for treatment of chronic HCV infection. Baseline NS3 polymorphisms in all patients and emerging mutations in patients who failed to achieve sustained virologic response (SVR) with simeprevir plus peginterferon/ribavirin (PR) in Phase IIb/III studies are described.
Methods: Baseline sequencing data were available for 2007 genotype 1 (GT1)-infected patients. Post-baseline data were available for 197/245 simeprevir-treated patients who did not achieve SVR. *In vitro* simeprevir susceptibility was assessed in a transient replicon assay as site-directed mutants or in chimeric replicons with patient-derived NS3 protease sequences.
Results: Baseline NS3 polymorphisms at positions associated with reduced *in vitro* susceptibility to simeprevir (43, 80, 122, 155, 156, and/or 168; EC_{50} fold change >2.0) were uncommon (1.3% [26/2007]), with the exception of Q80K, which confers ~10-fold reduction in simeprevir activity *in vitro* (13.7% [274/2007]; GT1a 29.5% [269/911], GT1b 0.5% [5/1096]). Baseline Q80K had minor effect on initial response to simeprevir/PR, but resulted in lower SVR rates. Overall, 91.4% of simeprevir-treated patients [180/197] without SVR had emerging mutations at NS3 positions 80, 122, 155, and/or 168 at failure (mainly R155K in GT1a with and without Q80K, and D168V in GT1b), conferring high-level resistance *in vitro* (EC_{50} fold change >50). Emerging mutations were no longer detectable by population sequencing at study end in 50% [90/180] of patients (median follow-up 28.4 weeks).
Conclusions: Simeprevir treatment failure was usually associated with emerging high-level resistance mutations, which became undetectable over time in half of the patients.

Keywords: Hepatitis C virus; Genotype 1; Once-daily; HCV NS3/4A protease inhibitor; Simeprevir; Peginterferon; Ribavirin; Q80K; Polymorphism.
Received 14 August 2014; received in revised form 19 November 2014; accepted 21 November 2014; available online 28 November 2014
ClinicalTrials.gov numbers: ASPIRE: NCT00980330; PILLAR: NCT00882908; QUEST-1: NCT01289782; QUEST-2: NCT01290679; PROMISE: NCT01281839.
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Abbreviations: HCV, hepatitis C virus; PR, peginterferon and ribavirin; QD, once-daily; GT, genotype; SVR, sustained virologic response; DAA, direct-acting antiviral agent; EC_{50}, half maximal effective concentration; EOS, end of study.

Introduction

Hepatitis C virus (HCV) infection represents a major public health concern, with approximately 150 million individuals infected worldwide and 3–4 million new infections annually [1]. HCV infection is the leading cause of liver cirrhosis, hepatocellular carcinoma, and liver transplantation.

Simeprevir (TMC435) is a recently approved, one-pill, once-daily (QD), oral HCV NS3/4A protease inhibitor. The anti-HCV activity of simeprevir plus peginterferon/ribavirin (PR) in patients with chronic HCV genotype 1 (GT1) infection has been demonstrated in five multicenter, Phase IIb/III studies [2–6]. In Phase IIb studies (PILLAR and ASPIRE), sustained virologic response (SVR) rates in patients treated with simeprevir/PR were 81–86% in treatment-naïve patients [2], 77–89% in prior relapsers, and 41–86% in prior null and partial responders [3]. In Phase III studies (QUEST-1, QUEST-2, and PROMISE), SVR rates were significantly higher in patients who received simeprevir/PR compared with PR control (80% *vs.* 50% in treatment-naïve patients and 79% *vs.* 37% in prior relapsers) [4–6]. In these studies, approximately 90% of patients met response-guided treatment criteria and were eligible for 24 weeks of PR treatment; SVR rates in these patients ranged from 83% to 91%. Moreover, simeprevir is generally well tolerated (~3800 patients treated in clinical trials to date).

Treatment failure in HCV-infected patients receiving a direct-acting antiviral agent (DAA)-based regimen has been associated with the emergence of resistance mutations in the target region of these agents [7,8]. In addition, naturally occurring amino acid substitutions in NS3 – also referred to as polymorphisms – that can reduce the antiviral activity of DAAs have been reported [9]. In this paper, we describe NS3 baseline polymorphisms in HCV GT1-infected patients enrolled in the simeprevir

Phase IIb/III studies. We also describe the effect of baseline NS3 Q80K polymorphism on antiviral activity and efficacy of simeprevir/PR, and characterize emerging mutations in patients who received simeprevir/PR and did not achieve SVR.

Patients and methods

Study design

NS3 sequence data were analyzed from five Phase IIb/III studies of simeprevir/PR (PILLAR, ASPIRE, QUEST-1, QUEST-2, and PROMISE) (Supplementary Table 1) [2–6]. The prevalence of baseline NS3 polymorphisms was analyzed in all patients enrolled in these studies (N = 2026 in total; n = 2007 patients with sequence data). Paired baseline and post-baseline sequences at the time of failure were available from 197 patients treated with 150 mg simeprevir/PR who did not achieve SVR. The effect of the NS3 Q80K polymorphism on outcome of treatment with simeprevir 150 mg QD in combination with PR therapy was assessed by study and in a pooled analysis of the two Phase III studies in treatment-naïve patients (QUEST-1 and QUEST-2; n = 515 patients treated with simeprevir/PR and sequencing data available) [10].

All studies were conducted in full compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent before participating in any study-related activity.

HCV NS3/4A sequence analysis and subtype determination

HCV geno/subtypes were determined at screening by Trugene or Versant LiPA v2 assay (Siemens Healthcare Diagnostics, IL, USA). HCV GT subtypes were also determined at baseline by sequencing a 329 bp region within *NS5B* followed by basic local alignment search tool (BLAST) analysis. The results of the *NS5B*-based assay were used for efficacy and virology analyses. According to the *NS5B*-based assay, 15 of the 2026 patients enrolled in the five Phase IIb/III studies had non-GT1a/1b subtypes (GT1: n = 4; GT1e: n = 4; GT1d: n = 2; GT1 g, 1i, 1l, 6e, and 6p: each n = 1). Data for patients with non-GT1a/1b subtypes were analyzed together with that for GT1a patients.

NS3/4A sequencing was performed at baseline for all patients and post-baseline for simeprevir/PR-treated patients who did not achieve SVR for any reason. Samples from patients not achieving SVR were selected for sequencing based on the timepoint of failure, availability of samples until end of study (EOS), and the sensitivity of the sequencing assay. The HCV *NS3/4A* region or the NS3 protease domain was sequenced using standard Sanger population sequencing [11].

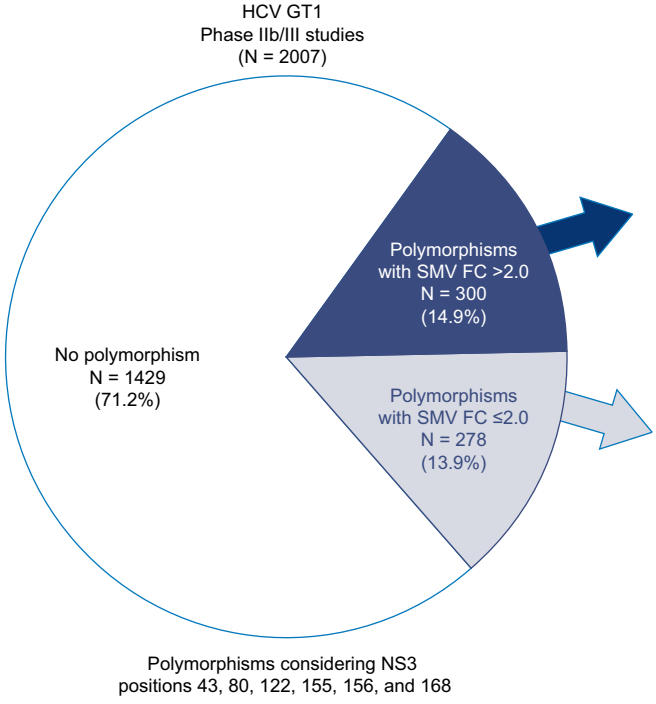
Polymorphisms were defined as amino acid changes from the H77 (GenBank accession number AF009606) or the HCV Con1 (GenBank accession number AJ238799) reference sequences for HCV GT1a/other and GT1b, respectively. Emerging mutations were defined as amino acid changes from patient-specific baseline sequences.

Two lists of NS3 amino acid positions of interest were defined to guide the analyses. The first list comprised six NS3 amino acid positions: 43, 80, 122, 155, 156, and 168; specific amino acid changes at one or more of these positions are known to confer reduced susceptibility to simeprevir *in vitro* [12,13]. The second list also included NS3 positions that have been associated with resistance to other HCV NS3/4A protease inhibitors or that were considered of interest based on observations in *in vitro* or *in vivo* studies with simeprevir [12–15]. This list comprised 18 NS3 amino acid positions: 36, 41, 43, 54, 55, 80, 107, 122, 132, 138, 155, 156, 158, 168, 169, 170, 174, and 175.

In addition, statistical analyses were performed to identify emerging mutations associated with simeprevir treatment failure (Supplementary Table 2).

Phenotypic characterization using a transient replicon assay

Mutations were engineered in a GT1b or GT1a replicon; for the chimeric replicon assay, sequences of the NS3 protease domain (aa7-192) derived from patient isolates were introduced into a GT1b replicon backbone generating chimeric replicons. Antiviral activity of simeprevir against the mutants or chimeric replicons was assessed in a transient replicon assay using luciferase read-out, quantified by the half maximal effective concentration (EC_{50}) values, and compared with that of a reference GT1b wild-type HCV replicon, as described earlier. Fold changes in EC_{50} were calculated [12].



NS3 polymorphism	SMV FC[a]	Patients n (%)
Any polymorphism with SMV FC >2.0	-	300 (14.9)
Q80K	7.7[b]/9.3[c]	273 (13.6)
Q80R	6.9[b]/13[c]	12 (0.6)
R155K	33[b]/88[c]	6 (0.3)
D168E	43[b]/26[c]	8 (0.4)
Q80K + D168E	373[b]/589[c]	1 (0.05)

1.3% other than Q80K

NS3 polymorphism	SMV FC[b]	Patients n (%)
Any polymorphism with SMV FC ≤2.0	-	278 (13.9)
Q80G	1.7	1 (0.05)
Q80L	1.1	39 (1.9)
Q80N	0.9	1 (0.05)
S122C	1.1	1 (0.05)
S122G	0.4	111 (5.5)
S122N	1.1	47 (2.3)
S122T	0.5	71 (3.5)
S122N/T	-	3 (0.1)
Q80L + S122G	n.a.	3 (0.1)
Q80L + S122N	n.a.	1 (0.05)

Fig. 1. Prevalence of NS3 polymorphisms in simeprevir Phase IIb/III studies. [a]FC in EC_{50} values compared with GT1b wild-type replicon assessed as site-directed mutant in a transient replicon assay. [b]In GT1b backbone. [c]In GT1a backbone. EC_{50}, half maximal effective concentration; FC, fold change; GT, genotype; n.a., not applicable; SMV, simeprevir.

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Results

Baseline NS3 polymorphisms

With the exception of the low-level resistance polymorphism Q80K, baseline NS3 polymorphisms associated with reduced *in vitro* susceptibility to simeprevir (at positions 43, 80, 122, 155, 156, and 168; fold change in EC_{50} value >2.0 as site-directed mutant in transient replicon assay) were generally uncommon (1.3%; 26/2007) (Fig. 1). Among patients treated with 150 mg simeprevir/PR in the Phase IIb/III treatment-naïve and -experienced studies, 6/9 patients with Q80R, 3/4 with R155K, and 3/6 with D168E polymorphisms achieved SVR. Given the low prevalence of these baseline polymorphisms, no conclusion can be made concerning their possible impact on the outcome of simeprevir/PR therapy. NS3 polymorphisms at any of the six positions of interest that did not reduce simeprevir activity *in vitro* (fold change in EC_{50} value $\leqslant$2.0) were observed in 13.9% of patients (Fig. 1). Consistent with the *in vitro* activity of simeprevir against these mutants, no impact on treatment outcome was observed (data not shown). Of note, no polymorphisms were observed at NS3 amino acid positions 43 and 156.

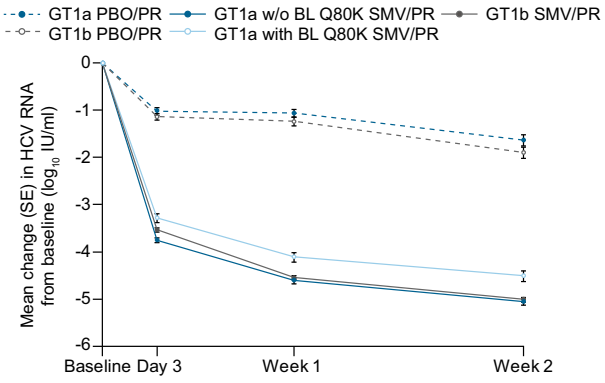
Overall, the NS3 Q80K polymorphism was present in 13.7% of patients (274/2007): 29.5% (269/911) of GT1a and 0.5% (5/1096) of GT1b patients. Large regional differences in Q80K polymorphism prevalence were noted with the highest prevalence in North America (Table 1).

A Q80K amino acid substitution reduced simeprevir activity *in vitro*, with a 7.7- and 9.3-fold change in EC_{50} when tested as site-directed mutant in GT1b and GT1a replicon backbones, respectively (Fig. 1). Baseline HCV GT1a isolates carrying Q80K displayed a median 11-fold change in simeprevir EC_{50} (n = 33) (data not shown). Patients with Q80K at baseline experienced a rapid and pronounced initial decline in HCV RNA from baseline during treatment with simeprevir/PR in Phase IIb/III studies, consistent with the limited effect of Q80K on simeprevir activity observed *in vitro*. In the pooled, treatment-naïve, Phase III studies (QUEST-1/QUEST-2) [10], treatment with simeprevir/PR resulted in a mean change in HCV RNA from baseline to week 1 of −4.1 and −4.6 $\log_{10}$ IU/ml in GT1a patients with and without Q80K, respectively, compared with −1.1 $\log_{10}$ IU/ml in patients treated with placebo/PR (Fig. 2). However, following the initial decline in HCV RNA levels, treatment failure occurred more frequently in simeprevir/PR-treated GT1a patients with baseline Q80K than in those without this polymorphism. On-treatment failure occurred in 19.0% (16/84) *vs.* 7.9% (13/165) of HCV GT1a patients with and without Q80K, respectively, and viral relapse in 22.2% (14/63) *vs.* 6.0% (9/149), respectively. Almost all patients with baseline Q80K who failed simeprevir had emerging mutations that, in combination with Q80K, conferred high-level resistance (Table 2).

Table 1. Prevalence of baseline Q80K polymorphism in simeprevir Phase IIb/III studies.

Region	n/N (%)		
	All HCV GTs	HCV GT1a[a]	HCV GT1b
Overall	274/2007 (13.7)	269/911 (29.5)	5/1096 (0.5)
Europe	76/1254 (6.1)	73/377 (19.4)	3/877 (0.3)
North America	185/538 (34.4)	185/385 (48.1)	0/153 (0)
South America	2/60 (3.3)	2/22 (9.1)	0/38 (0)

[a]Includes 15 patients with GT non-1a/b.
GT, genotype; HCV, hepatitis C virus.



	Mean change (SE) in HCV RNA from baseline at week 1
GT1a without BL Q80K SMV/PR (n = 162)	-4.6 (0.06) $\log_{10}$ IU/ml
GT1a with BL Q80K SMV/PR (n = 83)	-4.1 (0.10) $\log_{10}$ IU/ml
GT1a PBO/PR (n = 131)	-1.1 (0.09) $\log_{10}$ IU/ml
GT1b SMV/PR (n = 262)	-4.5 (0.04) $\log_{10}$ IU/ml
GT1b PBO/PR (n = 131)	-1.2 (0.09) $\log_{10}$ IU/ml

Fig. 2. Mean change in HCV RNA levels from baseline by HCV GT subtype and presence of Q80K in HCV treatment-naïve patients (QUEST-1 and QUEST-2). BL, baseline; GT, genotype; HCV, hepatitis C virus; PBO, placebo; PR, peginterferon/ribavirin; SE, standard error; SMV, simeprevir; w/o, without.

The SVR rates achieved in HCV GT1a patients with baseline Q80K were numerically higher in the simeprevir group than in the placebo/PR group in the pooled QUEST-1/QUEST-2 studies (58.3% [95% confidence interval {CI} 46.9; 72.4] *vs.* 52.3% [95% CI 31.4; 68.1]); however, the difference was not statistically significant. Lower SVR rates were observed in the simeprevir group among HCV GT1a patients with baseline Q80K than in those without this polymorphism (58.3% [95% CI 46.9; 72.4] *vs.* 83.6% [95% CI 82.7; 93.0]). The SVR rate in GT1a patients without Q80K in the placebo/PR group was 43.4% [95% CI 25.0; 49.5] ([10] and Supplementary Table 3).

Emerging mutations in patients with treatment failure

Of the 1136 treatment-naïve and treatment-experienced patients (including null and partial responders) treated with simeprevir/PR in Phase IIb/III studies, 245 did not achieve SVR. *NS3* sequencing data were available for 197 of these 245 patients. Of these 197 patients, 91.4% (180/197; GT1a: 110/116 [94.8%] and GT1b: 70/81 [86.4%]) had emerging mutations at NS3 positions 80, 122, 155, and/or 168 (Table 2).

Although the frequency of emerging mutations in patients with treatment failure was similar for GT1a and GT1b, different mutations emerged (Table 2). An emerging mutation at time of failure was observed in 61/63 GT1a patients (96.8%) without baseline Q80K. The majority of these patients had emerging R155K alone (25 patients, 41.0%) or R155K in combination with other mutations at NS3 positions 80, 122, and/or 168 (22 patients, 36.1%). In 49/53 GT1a patients (92.5%) with baseline Q80K, an emerging mutation at time of failure was observed. The majority of these patients (41 patients, 83.7%) had an

Table 2. Emerging NS3 mutations in patients failing simeprevir/PR treatment (considering NS3 amino acid positions 43, 80, 122, 155, 156, and 168).

GT	Patients not achieving SVR with emerging mutations at time of failure	Emerging mutations at time of failure	
		Overview	Details
Overall	91.4% (180/197)	-	-
GT1a[a] with Q80K	92.5% (49/53)	R155K: 83.7% (41/49)	
		D168E: 12.2% (6/49)	
		Other: 4.1% (2/49)	R155K + D168E (n = 1) S122R (n = 1)
GT1a[a] without Q80K	96.8% (61/63)	R155K: 41.0% (25/61)	
		R155K in combination with mutations at positions 80, 122 and/or 168: 36.1% (22/61)	R155K + D168E (n = 7) R155K + D168A, A/V or V (n = 3) Q80K or R + R155K (n = 4) S122R + R155K (n = 4) Q80R or K + R155K + D168E or V (n = 4)
		Other: 23.0% (14/61)	D168V (n = 6) D168A, A/V, E, H (n = 6) Q80R + D168E (n = 1) S122G + D168A (n = 1)
GT1b	86.4% (70/81)	D168V: 60.0% (42/70)	
		D168A, E, H, T, A/V or E/V: 17.1% (12/70)	D168A or A/V (n = 5) D168E or E/V (n = 3) D168H (n = 2) D168T (n = 2)
		Other: 22.9% (16/70)	Q80R or K + D168E or E/V (n = 9) Q80R + S122A or T + D168E (n = 2) R155Q + D168V (n = 2) R155Q + D168A (n = 1) S122I or T + D168V or F (n = 2)

[a]May include some patients with GT non-1a/b subtypes.
GT, genotype; PR, peginterferon/ribavirin; SVR, sustained virologic response.

emerging R155K mutation at the time of failure, with D168E also seen (6 patients, 12.2%). In the 70 GT1b patients with emerging mutations at the time of failure, a mutation at NS3 position 168 emerged alone (D168V in 42 patients [60.0%]; A, A/V, E, E/V, H or T in 12 patients [17.1%]) or in combination with other mutations at NS3 positions 80, 122, and/or 155 (16 patients [22.9%]). Of note, emerging R155K was not observed in patients with GT1b, while some mutations at position 168 were also observed in patients with GT1a.

Considering the 18 NS3 positions of interest, two additional patients had emerging mutations (I170T in two GT1a patients with baseline Q80K). I170T (15-fold change in EC_{50} values as single mutant in GT1a replicon) was also observed in combination with R155K in three GT1a patients. In addition, in 11 of 197 patients with treatment failure and sequence data available, mutations at NS3 positions 36 (V36M, n = 1), 107 (V107I, n = 2), 132 (I132L, n = 3), 170 (V170I, n = 1), 174 (S or N174F, or S, n = 4), and V132L+N174G (n = 1) were observed in combination with emerging mutations at positions 80, 155, and/or 168. With the exception of I132L (4.5-fold change in EC_{50} values), none of the mutations reduced simeprevir activity ($\leqslant$2.0-fold change in EC_{50} values) when tested as a single mutant in a GT1b or 1a replicon backbone (data not shown). No emerging mutations at the time of failure were observed at NS3 positions 41, 43, 54, 55, 138, 156, 158, 169, and 175.

Emerging mutations in the NS3 protease domain outside of the 18 positions of interest were rarely observed (i.e. none in more than three patients [1.5%] with failure) and almost always in combination with mutations at positions of interest.

Statistical analyses comparing sequence data from simeprevir/PR-treated patients not achieving SVR against the respective baseline samples (paired Liddell test) or a reference sequence set (unpaired Fisher exact test) identified emerging mutations Q80R, S122R, R155K, D168A, D168E, D168V, and V/I170T as being significantly associated with simeprevir/PR treatment failure (Supplementary Table 2).

Of note, although proportions of patients not achieving SVR differed by prior response to peginterferon treatment, the proportions of patients with emerging mutations and type of emerging mutations among the patients failing treatment were similar irrespective of prior treatment history.

Phenotypic characterization

NS3 sequences derived from clinical isolates obtained at baseline and time of failure were introduced in a chimeric GT1b replicon, and susceptibility to simeprevir was assessed. Among the tested baseline isolates, those carrying a Q80K or R155K amino acid substitution showed approximately 10- and 90-fold reduction in simeprevir activity compared with the reference wild-type replicon, respectively (Fig. 3). Most of the remaining baseline isolates tested were fully susceptible to simeprevir *in vitro* ($\leqslant$2.0-fold change in EC_{50}). Clinical isolates obtained at time of failure generally showed decreased susceptibility to simeprevir (median fold change in EC_{50}, 370 [range: 0.2–3110] *vs.* the reference wild-type GT1b replicon). All isolates carrying emerging mutations at positions 80, 122, 155, and/or 168 at time of failure showed an increase in EC_{50} values *vs.* their respective baseline

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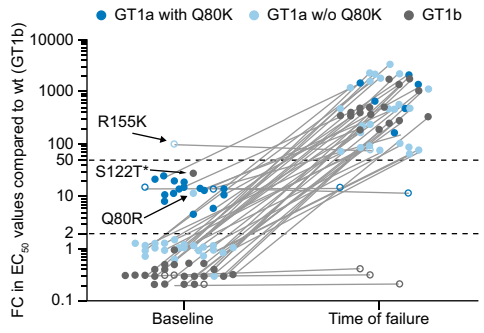


SMV FC in EC_{50}[a]	GT1a with Q80K		GT1a without Q80K		GT1b		Overall	
	BL N = 17	TOF N = 8	BL N = 24	TOF N = 24	BL N = 25	TOF N = 20	BL N = 66	TOF N = 52
Median	13	526	1.0	439	0.3	335	1.0	370
Q1-Q3	11-16	48-1340	0.9-1.2	80-1378	0.3-0.5	196-469	0.3-10	91-1088
Range	4.3-23	11-1970	0.3-95	62-3110	0.2-26	0.2-1640	0.2-95	0.2-3110

Fig. 3. *In vitro* activity of simeprevir against NS3 clinical isolates obtained at baseline and time of failure assessed in a chimeric replicon assay using a HCV GT1b replicon backbone. The FC in EC_{50} values of the chimeric replicon was determined against wild-type GT1b replicon. Isolates from the same patient are connected with a solid line. Filled and open circles present patients with and without emerging mutation at time of failure, respectively. Horizontal dotted lines represent FC values of 2.0 (biological cut-off) and 50 (to differentiate between low- and high-level *in vitro* resistance to SMV). *Isolate only contained S122T considering position 43, 80, 122, 155, 156, and 168. The four other clinical isolates tested as well as wild-type replicon carrying single S122T were fully susceptible to SMV (FC ⩽2.0). [a]Compared with GT1b wild-type replicon. BL, baseline; EC_{50}, half maximal effective concentration; FC, fold change; HCV, hepatitis C virus; N, number of clinical isolates analyzed; Q1-Q3, range of values between quartile 1 and 3; SMV, simeprevir; TOF, time of failure; w/o, without.

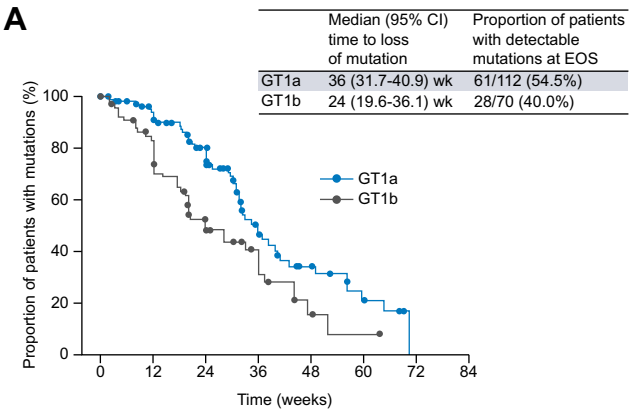
isolates (median fold change ⩾62 *vs.* the reference wild-type GT1b replicon). Of the isolates analyzed, 6 failure isolates did not carry emerging mutations: one carried an R155K and two a Q80K amino acid substitution (all GT1a), which were already present at baseline. No reduction was observed in susceptibility to simeprevir between baseline and time of failure, consistent with the absence of emerging mutations (Fig. 3).

Persistence of emerging mutations

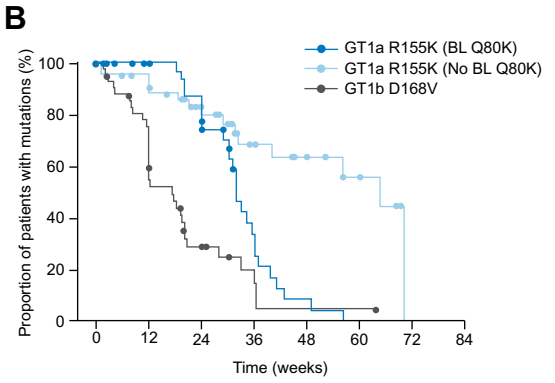
Patients enrolled in the simeprevir Phase IIb/III studies were followed for 72 weeks after treatment initiation, irrespective of treatment duration or outcome. The persistence of viral variants after treatment failure until the last available timepoint (EOS) was investigated in simeprevir/PR-treated patients with failure.

Sequences at EOS in patients with treatment failure were compared against the sequences available at time of failure and baseline. Median time of the EOS visit after the time of failure was 28.4 weeks (range: 0–69.9 weeks). For 90/180 patients (50.0%) with emerging mutations at time of failure, emerging mutations were no longer observed at EOS with population sequencing (GT1a: 46/100 [41.8%] and GT1b: 44/70 [62.9%]).

Kaplan-Meier analyses were performed to evaluate the persistence of emerging mutations over time. Median time until the emerging mutations became undetectable using population sequencing was shorter in patients infected with HCV GT1b (24 weeks; range: 19.6–36.1 weeks) compared with GT1a (36 weeks; range: 31.7–40.9 weeks) (Fig. 4A). Median time until emerging mutations became undetectable was shorter in patients with D168V (mainly GT1b [17 weeks; range: 12.1–20.1 weeks])



	Median (95% CI) time to loss of mutation	Proportion of patients with detectable mutations at EOS
GT1a	36 (31.7-40.9) wk	61/112 (54.5%)
GT1b	24 (19.6-36.1) wk	28/70 (40.0%)



	Median (95% CI) time to loss of mutation	Proportion of patients with detectable mutations at EOS
GT1a R155K (BL Q80K)	32 (30.1-35.9) wk	16/42 (38.1%)
GT1a R155K (No BL Q80K)	64 (40.1-70.0) wk	32/47 (68.1%)
GT1b D168V	17 (12.1-20.1) wk	13/45 (28.9%)

Fig. 4. Loss of emerging mutations over time. (A) HCV geno/subtype and (B) HCV geno/subtype and emerging mutation. BL, baseline; CI, confidence interval; EOS, end of study; GT, genotype; HCV, hepatitis C virus; wk, weeks.

and in GT1a patients with emerging R155K who had baseline Q80K (32 weeks; range: 30.1–35.9 weeks) compared with GT1a patients who had emerging R155K without baseline Q80K (64 weeks; range: 40.1–70.0 weeks) (Fig. 4B).

Discussion

Analyses of the HCV NS3/4A sequence at baseline in patients enrolled in the simeprevir Phase IIb/III studies identified naturally occurring sequence polymorphisms. However, with the exception of Q80K, baseline polymorphisms reducing the *in vitro* activity of simeprevir were uncommon. Overall, the Q80K polymorphism was present in 13.7% of GT1 patients at baseline and was almost exclusively found with GT1a (29.5%). The prevalence of Q80K polymorphism within GT1a differs by region. Recent studies suggest that two clades of GT1a circulate worldwide, one carrying a lysine (K) with a high prevalence at position 80 and the other carrying a glutamine (Q) [16,17]; for reasons that are not fully understood, the K80 clade disseminated more effectively in North America than the Q80 clade. In addition, the ratio of GT1a to GT1b also differs across regions. For example, GT1b is

almost exclusively present in the HCV GT1 population of large parts of Eastern Europe and Asia, while in other regions, such as North America, GT1a is more prevalent than GT1b. The combination of the high prevalence of GT1a and the higher prevalence of Q80K within GT1a resulted in the highest Q80K prevalence in the GT1 population of North America (34.4%), while the GT1 Q80K prevalence in Europe was 6.1%.

The Q80K polymorphism is located in the S2 binding site of NS3 and stabilizes the R155 residue, which is relocated during simeprevir binding to form the extended S2 subsite. Consistent with these structural features, Q80K as a single mutation in GT1a backbone reduced the *in vitro* activity of simeprevir by 9.3-fold, but when Q80K was present in combination with R155K (88-fold change in EC_{50} value of R155K alone in GT1a backbone), simeprevir activity was reduced by ~2000-fold. When simeprevir was given at lower doses, such as 25 mg or 75 mg, the presence of a Q80K variant at baseline reduced the decline in HCV RNA during the first few days of treatment, a parameter that is an indicator of the intrinsic potency of the drug on baseline virus without the confounding effects of the host factors [2,18]. In contrast, in patients receiving simeprevir 150 mg, the initial reduction in HCV RNA was minimally affected by the presence of Q80K.

The initial antiviral activity in simeprevir/PR-treated HCV GT1a-infected patients with Q80K at baseline was followed by higher on-treatment failure and relapse rates, resulting in lower SVR rates at 12 weeks *vs.* simeprevir/PR-treated HCV GT1a-infected patients without this polymorphism. This higher rate of failure was accompanied by the emergence of additional mutations (mainly R155K), which in combination with Q80K resulted in high-level resistance to simeprevir that could not be contained by the simeprevir drug levels achieved with the 150 mg dose. Based on this observation, it can be speculated that as a whole, Q80K-containing virus populations might display a lower resistance barrier, therefore facilitating the emergence of resistance mutations if the antiviral activity of the PR component is insufficient to suppress these emerging variants. Of note, SVR rates with simeprevir/PR regimens in patients with Q80K also depended on factors associated with response to PR (e.g. *IL28B* GT, METAVIR score; Supplementary Table 4). In an interferon-free regimen of simeprevir combined with sofosbuvir, high SVR rates at 12 weeks (88%) were achieved in traditionally difficult-to-cure GT1a-infected patients with Q80K [19].

The majority of patients who failed to respond to simeprevir/PR treatment had emerging mutations at NS3 positions 80, 122, 155, and/or 168. These positions are located in, or close to, the extended S2 binding pocket necessary for simeprevir binding [12,20]. The mutations present in patients who failed treatment generally conferred high-level resistance to simeprevir *in vitro*, with >50-fold changes in EC_{50} values compared with the wild-type reference replicon, with the majority of patients displaying substantially higher levels of resistance. Additional analyses were performed to assess if mutations beyond the positions of interest were associated with treatment failure; the results confirmed that these were either very infrequent and/or did not affect the *in vitro* activity of simeprevir [12].

Emerging mutations differed between patients with GT1a and GT1b infection, but were consistent within each HCV GT subtype. In GT1a patients with Q80K, a single emerging R155K mutation was predominantly observed, while in GT1a patients without Q80K, double or triple mutations of R155K in combination with mutations at position 80, 122, and/or 168 were frequently present. In GT1b patients, R155K was not observed, and treatment failure was typically associated with emerging mutations at position 168, mainly D168V. Of note, only one nucleotide change is needed for an R155K mutation in GT1a, while two are needed for GT1b [21]. Although emerging mutations at position 80 and 122 were observed, only some amino acid substitutions at these positions (Q80K, Q80R, and S122R) reduced the *in vitro* activity of simeprevir, whereas many other amino acid substitutions, such as Q80L or S122N, had no effect on simeprevir activity [12].

Mutations that emerged in patients failing to respond to simeprevir treatment became undetectable over time in many patients, based on population sequencing. The follow-up times in the Phase IIb/III studies were limited and differed substantially between patients, which could explain the presence of mutations at the last study-related visit. Kaplan-Meier analyses showed that the median time until an emerging mutation became undetectable was the shortest for GT1b patients with emerging D168V. Among GT1a patients, those with Q80K at baseline and emerging R155K had a shorter time until the emerging R155K mutation became undetectable, compared with patients with emerging R155K who did not have Q80K at baseline, suggesting that emerging variants with R155K in the presence of a Q80K amino acid substitution are less fit in the absence of simeprevir. Of note, patients with Q80K polymorphism at baseline who failed treatment retained this variant throughout the whole study. Similar results showing that emerging *NS3* mutations become undetectable over time have been described for other DAAs, such as telaprevir and boceprevir, suggesting lower fitness of these mutant viral strains [8,22]. Recent studies showed that emerging mutations that became undetectable by population sequencing could also not be detected using more sensitive sequencing technologies [23,24]. However, in the absence of robust re-treatment data with a protease inhibitor-containing regimen, it is premature to conclude whether the decline in frequency of the emerging mutations is clinically relevant. Importantly, simeprevir resistance mutations remained susceptible to DAAs with other mechanisms of action *in vitro*, and recent data with sofosbuvir/daclatasvir and sofosbuvir/ledipasvir showed successful re-treatment of patients who failed to respond to protease inhibitor-based treatment with emerging NS3 mutations [25,26].

In conclusion, simeprevir in combination with PR results in high SVR rates in HCV treatment-naïve and -experienced patients with HCV GT1 infection. The GT1a NS3 polymorphism Q80K has a modest impact on simeprevir activity *in vitro*, but might facilitate the emergence of additional mutations in patients treated with simeprevir/PR, especially in those with poor response to interferon, ultimately resulting in lower SVR in these patients when treated with simeprevir/PR. Treatment failure is typically associated with emerging high-level resistance mutations in the NS3 region that decline and become undetectable over time in many patients after treatment is stopped. Recent data suggest that emerging mutations do not preclude successful treatment outcome following subsequent treatment with DAAs with other mechanisms of action.

Financial support

This study was funded by Janssen Research & Development. Editorial support was funded by Janssen Research & Development.

Research Article

Conflict of interest

All authors are employees of Janssen Pharmaceuticals, Inc.

Authors' contributions

All authors were involved in data acquisition, analysis and interpretation, as well as in critical revision of the manuscript for important intellectual content. OL contributed to the writing of the manuscript. All authors have contributed to the development of the manuscript, have approved the final content and have agreed to proceed with submission for publication.

Acknowledgements

Special thanks to the patients participating in these studies and their families. Thanks also to all colleagues from Janssen who contributed to this work. Editorial support was provided by Jennifer Coward on behalf of Complete Medical Communications.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.11.032.

References

[1] World Health Organization. Hepatitis C fact sheet No. 164. Available from: <http://www.who.int/mediacentre/factsheets/fs164/en/>; 2014 [accessed 30.04.14].
[2] Fried MW, Buti M, Dore GJ, Flisiak R, Ferenci P, Jacobson I, et al. Once-daily simeprevir (TMC435) with pegylated interferon and ribavirin in treatment-naive genotype 1 hepatitis C: the randomized PILLAR study. Hepatology 2013;58:1918–1929.
[3] Zeuzem S, Berg T, Gane E, Ferenci P, Foster GR, Fried MW, et al. Simeprevir increases rate of sustained virologic response among treatment-experienced patients with HCV genotype-1 infection: a Phase IIb trial. Gastroenterology 2014;146:430–441.
[4] Manns M, Marcellin P, Poordad F, Affonso de Araujo ES, Buti M, Horsmans Y, et al. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2014;384:414–426.
[5] Jacobson IM, Dore GJ, Foster GR, Fried MW, Radu M, Rafalskiy VV, et al. Simeprevir (TMC435) with pegylated interferon alfa 2a plus ribavirin in treatment-naive patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet 2014;384:403–413.
[6] Forns X, Lawitz E, Zeuzem S, Gane E, Bronowicki JP, Andreone P, et al. Simeprevir with peginterferon and ribavirin leads to high rates of SVR in patients with HCV genotype 1 who relapsed after previous therapy: a phase 3 trial. Gastroenterology 2014;146:1669–1679.
[7] Kieffer TL, De Meyer S, Bartels DJ, Sullivan JC, Zhang EZ, Tigges A, et al. Hepatitis C viral evolution in genotype 1 treatment-naive and treatment-experienced patients receiving telaprevir-based therapy in clinical trials. PLoS One 2012;7:e34372.
[8] Barnard RJ, Howe JA, Ogert RA, Zeuzem S, Poordad F, Gordon SC, et al. Analysis of boceprevir resistance associated amino acid variants (RAVs) in two phase 3 boceprevir clinical studies. Virology 2013;444:329–336.
[9] Bartels DJ, Sullivan JC, Zhang EZ, Tigges AM, Dorrian JL, De Meyer S, et al. Hepatitis C virus variants with decreased sensitivity to direct-acting antivirals (DAAs) were rarely observed in DAA-naive patients prior to treatment. J Virol 2013;87:1544–1553.
[10] Jacobson IM, Dore GJ, Foster GR, Fried M, Manns M, Marcellin P, et al. Simeprevir (TMC435) with peginterferon/ribavirin for treatment of chronic HCV genotype 1 infection in treatment-naïve patients: efficacy in difficult-to-treat patient sub-populations in the QUEST-1 and 2 Phase III trials. In: Poster 1122 presented at the Annual Meeting of the American Association for the Study of Liver Diseases; 2013.
[11] Koletzki D, Pattery T, Fevery B, Vanhooren L, Stuyver LJ. Amplification and sequencing of the hepatitis C virus NS3/4A protease and the NS5B polymerase regions for genotypic resistance detection of clinical isolates of subtypes 1a and 1b. Methods Mol Biol 2013;1030:137–149.
[12] Lenz O, Verbinnen T, Lin T-I, Vijgen L, Cummings MD, Lindberg J, et al. *In vitro* resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010;54:1878–1887.
[13] Lenz O, Vijgen L, Berke JM, Cummings MD, Fevery B, Peeters M, et al. Virologic response and characterisation of HCV genotype 2–6 in patients receiving TMC435 monotherapy (study TMC435-C202). J Hepatol 2013;58:445–451.
[14] Vertex Pharmaceuticals Incorporated. Incivek™ (telaprevir) film coated tablets, for oral use. Prescribing Information. Available from: <http://pi.vrtx.com/files/uspi_telaprevir.pdf>; 2013 [accessed 11.10.13].
[15] Merck Sharp & Dohme Corp. Victrelis (boceprevir). Highlights of prescribing information. Available from: <http://www.merck.com/product/usa/pi_circulars/v/victrelis/victrelis_pi.pdf>; 2014 [accessed 25.02.14].
[16] De Luca A, Giambenedetto S, Prosperi M, Lo Presti A, Torti C, Vicenti I, et al. Two distinct HCV genotype 1a clades: geographical distribution and association with natural resistance mutations to HCV NS3/4A inhibitors. Antivir Ther 2013;18:A47, abs 39.
[17] Poon AF, Liang RH, Joy JB, Nguyen T, McCloskey RM, Krajden M, et al. Global origin of HCV NS3 substitution Q80K that is associated with lower simeprevir susceptibility. (Abstract 656LB). In: Conference on Retroviruses and Opportunistic Infections, 2014.
[18] Manns M, Reesink H, Berg T, Dusheiko G, Flisiak R, Marcellin P, et al. Rapid viral response of once-daily TMC435 plus pegylated interferon/ribavirin in hepatitis C genotype-1 patients: a randomized trial. Antivir Ther 2011;16:1021–1033.
[19] Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, et al. Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet 2014;384:1756–1765.
[20] Cummings MD, Lindberg J, Lin TI, de Kock H, Lenz O, Lilja E, et al. Induced-fit binding of the macrocyclic noncovalent inhibitor TMC435 to its HCV NS3/NS4A protease target. Angew Chem Int Ed Engl 2010;49:1652–1655.
[21] Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology 2007;132:1767–1777.
[22] Sullivan JC, De Meyer S, Bartels DJ, Dierynck I, Zhang EZ, Spanks J, et al. Evolution of treatment-emergent resistant variants in telaprevir phase 3 clinical trials. Clin Infect Dis 2013;57:221–229.
[23] Dierynck I, Thys K, Ghys A, Sullivan JC, Kieffer TL, Aerssens J, et al. Deep-sequencing analysis of the gene encoding the hepatitis C virus nonstructural 3–4A protease confirms a low prevalence of telaprevir-resistant variants at baseline and the end of the REALIZE study. J Infect Dis 2014;210:1871–1880.
[24] Fevery B, Thys K, Van Eygen V, Verbinnen T, Van Rossem E, Picchio G, et al. Deep sequencing analyses of minority baseline polymorphisms and persistence of emerging mutations in HCV genotype 1-infected patients treated with simeprevir. J Hepatol 2014;60:S139.
[25] Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, et al. Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 2014;370:211–221.
[26] Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med 2014;370:1483–1493.