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; EC50 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% [27/2007]; 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 (EC50 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.

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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; EC50, half maximal effective concentration; EOS, end of study.
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-naive 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 A0238799) 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 (aa27–192) 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 (EC50) values, and compared with that of a reference GT1b wild-type HCV replicon, as described earlier. Fold changes in EC50 were calculated [12].

### Table 1. Prevalence of NS3 polymorphisms in simeprevir Phase IIb/III studies.

<table>
<thead>
<tr>
<th>NS3 Polymorphism</th>
<th>SMV FC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Patients n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any polymorphism with SMV FC &gt;2.0</td>
<td>-</td>
<td>300 (14.9)</td>
</tr>
<tr>
<td>Q80K</td>
<td>7.7&lt;sup&gt;b&lt;/sup&gt;/9.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>273 (13.6)</td>
</tr>
<tr>
<td>Q80R</td>
<td>6.9&lt;sup&gt;b&lt;/sup&gt;/13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12 (0.6)</td>
</tr>
<tr>
<td>R155K</td>
<td>33&lt;sup&gt;b&lt;/sup&gt;/88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6 (0.3)</td>
</tr>
<tr>
<td>D168E</td>
<td>42&lt;sup&gt;b&lt;/sup&gt;/26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8 (0.4)</td>
</tr>
<tr>
<td>Q80K + D168E</td>
<td>373&lt;sup&gt;b&lt;/sup&gt;/589&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1 (0.05)</td>
</tr>
</tbody>
</table>

<sup>a</sup>EC50 values compared with GT1b wild-type replicon assessed as site-directed mutant in a transient replicon assay. <sup>b</sup>GT1b backbone. <sup>c</sup>GT1a backbone.
Research Article

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 Ib/III treatment-naïve and -experienced studies, 6/9 patients with Q80K, 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 ≤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 Ib/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). 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 23.0; 69.5] ([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 Ib/III studies, 245 did not achieve SVR. N53 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 N53 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 N53 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

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Table 1. Prevalence of baseline Q80K polymorphism in simeprevir Phase Ib/II studies.

<table>
<thead>
<tr>
<th>Region</th>
<th>All HCV GTs</th>
<th>HCV GT1a*</th>
<th>HCV GT1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>274/2007</td>
<td>269/911</td>
<td>5/1096</td>
</tr>
<tr>
<td>Europe</td>
<td>76/1254</td>
<td>73/377</td>
<td>3/877</td>
</tr>
<tr>
<td>North America</td>
<td>185/538</td>
<td>185/385</td>
<td>0/153</td>
</tr>
<tr>
<td>South America</td>
<td>2/60</td>
<td>2/22</td>
<td>0/38</td>
</tr>
</tbody>
</table>

*aIncludes 15 patients with GT non-1a/b.

GT, genotype; HCV, hepatitis C virus.

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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 EC50 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 EC50 values), none of the mutations reduced simeprevir activity 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 (<2.0-fold change in EC50). Clinical isolates obtained at time of failure generally showed decreased susceptibility to simeprevir (median fold change in EC50, 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 EC50 values vs. their respective baseline

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

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

*May include some patients with GT non-1a/b subtypes.

GT, genotype; PR, peginterferon/ribavirin; SVR, sustained virologic response.
GT1a w/o Q80K  GT1a w/ Q80K  GT1b
with D168V (mainly GT1b [17 weeks; range: 12.1–20.1 weeks]) emerging mutations became undetectable was shorter in patients (36 weeks; range: 31.7–40.9 weeks) (Fig. 4A). Median time until (24 weeks; range: 19.6–36.1 weeks) compared with GT1a sequencing was shorter in patients infected with HCV GT1b emerging mutations became undetectable using population baseline; EC50, half maximal effective concentration; FC, fold change; HCV, susceptible to SMV (FC isolates tested as well as wild-type replicon carrying single S122T were fully determined against wild-type GT1b replicon. Isolates from the same patient are investigated in simeprevir/PR-treated patients with failure. after treatment failure until the last available timepoint (EOS) treatment duration or outcome. The persistence of viral variants was no longer observed at EOS with population sequences available at time of failure and the sequences available at time of failure and Q80K (32 weeks; range: 30.1–35.9 weeks) compared with GT1a 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

**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]).

**Fig. 3.** *In vitro* activity of simeprevir against NS3 clinical isolates obtained at baseline and time of failure assessed in a chimeric replicon backbone. The FC in EC50 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). *Compared with GT1b wild-type replicon. BL, baseline; EC50, 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.

**Fig. 4.** Loss of emerging mutations over time. (A) HCV geno/subtype and (B) HCV genotype and emerging mutation. BL, baseline; CI, confidence interval; EOS, end of study; GT, genotype; HCV, hepatitis C virus; wk, weeks.
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 simprevir 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 simprevir by 9.3-fold, but when Q80K was present in combination with R155K (88-fold change in EC₅₀ value of R155K alone in GT1a backbone), simprevir activity was reduced by ~2000-fold. When simprevir 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. Treatment with low doses of simprevir 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.

In conclusion, simprevir 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 simprevir activity in vitro, but might facilitate the emergence of additional mutations in patients treated with simprevir/PR, especially in those with poor response to interferon, ultimately resulting in lower SVR in these patients when treated with simprevir/PR. Treatment failure is 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 simprevir, whereas many other amino acid substitutions, such as Q80L or S122N, had no effect on simprevir activity [12].

Mutations that emerged in patients failing to respond to simprevir 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 simprevir. 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, simprevir 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, simprevir 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 simprevir activity in vitro, but might facilitate the emergence of additional mutations in patients treated with simprevir/PR, especially in those with poor response to interferon, ultimately resulting in lower SVR in these patients when treated with simprevir/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.

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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.

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Supplementary data

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