Research Article

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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.
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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].

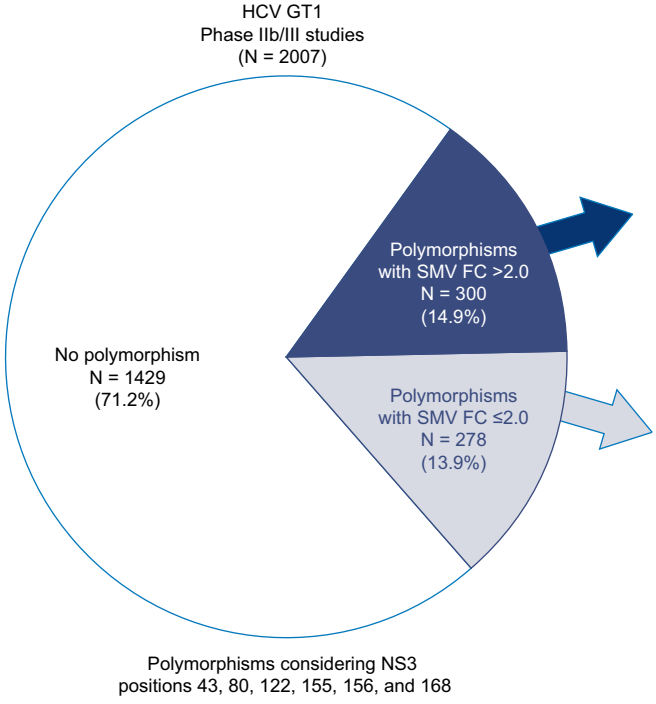
Polymorphisms were defined as amino acid changes from the H77 (GenBank accession number AF009606) or the HCV Con1 (GenBank accession number AJ238799) reference sequences for HCV GT1a/other and GT1b, respectively. Emerging mutations were defined as amino acid changes from patient-specific baseline sequences.

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.