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

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Background. Population sequencing (PS) has shown that telaprevir-resistant variants are not typically detectable at baseline (prevalence, ≤5% of patients), and most variants present at the time of treatment failure are no longer detectable at the end of the study.

Methods. To gain insight into the evolution of telaprevir-resistant variants, their baseline prevalence and persistence after treatment was investigated using a more sensitive, deep-sequencing (DS) technique in a large number of treatment-experienced patients from the REALIZE study who were infected with hepatitis C virus genotype 1.

Results. Before treatment initiation, telaprevir-resistant variants (T54A, T54S, or R155K in 1%–2% of the viral population) were detected by DS in a fraction (2%) of patients for whom PS failed to detect resistance; these variants were not necessarily detected at the time of treatment failure. Of 49 patients in whom telaprevir-resistant variants were detected by PS at the time of treatment failure but not at the end of the study, DS revealed the presence of variants (V36A/L/M, T54S, or R155K in 1%–36% of the viral population) in 16 patients (33%) at the end of the study.

Conclusions. Similar to PS findings, DS analysis revealed that the frequency of telaprevir-resistant variants before treatment was also low, and variants detected at the time of treatment failure were no longer detectable in the majority of patients during follow-up.

Keywords. deep sequencing technique; Genotype 1; HCV; mutation; population sequencing; resistance; telaprevir.

Telaprevir is a potent and selective inhibitor of the nonstructural 3•4A (NS3•4A) protease of hepatitis C virus (HCV) [1] and is approved in combination with pegylated interferon and ribavirin (PR) for the treatment of chronic HCV genotype 1 (G1) infection in adult patients with compensated liver disease [2, 3]. Telaprevir in combination with PR has demonstrated significantly higher sustained virologic response (SVR) rates over PR alone in both treatment-naive and treatment-experienced patients [4–6]. In patients who did not achieve SVR with telaprevir-based treatment, a consistent, subtype-dependent, resistance profile has been reported [7], wherein NS3 protease variants V36M, R155K, and V36M plus R155K were the most common emerging mutations in HCV G1a–infected patients, and V36A, T54A, and A156S/T were the most common in HCV G1b–infected patients.

Naturally occurring telaprevir-resistant variants likely exist in all HCV-infected patients, albeit at low
immunode resistance upon treatment of viral infections, such as human immunodeficiency virus (HIV) [11, 12]. These more sensitive sequencing techniques, with a LOD below the 20%–25% thresholds of PS, may provide additional information on the evolution and importance of low-level variants for the prediction of viral response to HCV inhibitors.

In this study, both PS and deep sequencing (DS) of viral populations were performed. The association between the prevalence of baseline mutations and treatment outcome and the persistence of telaprevir-resistant variants were tested using a large number of samples from treatment-experienced patients enrolled in the phase 3 REALIZE study.

**METHODS**

**Study Design and Patient Population**

The methods for REALIZE, a randomized, double-blind, placebo-controlled phase 3 study in treatment-experienced HCV-infected patients who did not achieve SVR with prior PR treatment, has been described fully elsewhere [6]. Patients were categorized by their previous response to PR therapy as prior relapers, partial responders, and null responders. Patients received placebo or 750 mg of telaprevir every 8 hours for 12 weeks in combination with PR for 48 weeks, with or without a PR lead in (defined as receipt of PR for 4 weeks before starting telaprevir therapy). Virologic stopping rules were included in the protocol. The protocol was approved by all relevant independent ethics committees, and the study was performed in accordance with the provisions of the Declaration of Helsinki and good clinical practice guidelines. All patients provided written informed consent. The study was registered with ClinicalTrials.gov (NCT00703118).

**Definition of Treatment Outcomes**

In patients who did not achieve SVR, treatment outcomes were categorized as on-treatment virologic failure (defined as viral breakthrough or achievement of a virologic stopping rule), relapse, or other (defined as an HCV RNA level of >25 IU/mL, without viral breakthrough, at the planned end of treatment or as a missing SVR assessment during planned follow-up) [6].

**Selected Patient Samples**

Baseline samples from 185 of 530 patients who received telaprevir in the REALIZE study and did not have telaprevir-resistant variants detected by PS were analyzed. In addition, we analyzed end-of-study samples obtained from a subgroup of 55 patients for whom PS detected telaprevir-resistant variants at the time of treatment failure but not at the end of the study and whose follow-up time was ≥10 months (for patients infected with HCV G1a) or ≥3 months (for those infected with HCV G1b) after the end of treatment.

**HCV RNA Quantification and Subtyping**

Quantification of plasma HCV RNA levels was performed using the High Pure System/COBAS TaqMan assay, version 2.0 (Roche, Switzerland). The lower limit of quantification was 25 IU/mL. Results below the lower limit of quantification were reported as “<25 IU/mL, detected” or “<25 IU/mL, target not detected” (also described as undetectable HCV RNA). HCV genotype/subtype was determined by sequence analysis of the HCV NS3•4A protease region.

**PS Analysis**

PS methods have been presented elsewhere [13]. Briefly, the NS3•4A protease regions were amplified from isolated RNA from plasma virions, using a nested reverse-transcription polymerase chain reaction (PCR) assay. The resulting DNA was purified and sequenced directly; the lower LOD for the sequencing assay was approximately 1000 IU/mL of HCV RNA. PS can typically detect variants present in as low as 20%–25% of the viral population.
Paired-end DS of a fragment spanning the HCV NS3–4A protease regions was performed using Illumina technology [14]. Briefly, viral RNA was reverse transcribed using random hexamers and a high-fidelity reverse transcriptase (AccuScript High Fidelity Reverse Transcriptase, Agilent Technologies). An amplicon encompassing the HCV NS3–4A protease region (2.4 kb) was generated by a 2-round nested PCR. PCR reactions were performed in triplicate and subsequently pooled to reduce possible PCR bias. The purified amplicons (0.1–0.5 µg DNA) were fragmented to an average length of 200 bp in accordance with Illumina’s standard protocol. Following DNA end repair and adenylation, the Illumina compatible adaptors (NEXTflex DNA Barcodes, Bioo Scientific) were ligated. Fragments were enriched during 12 PCR cycles and visualized on a Bioanalyzer (Agilent Technologies) for quality control and quantification. Multiplexed samples were loaded on the Illumina cluster station for cluster generation using the TruSeq PE Cluster Kit v2 (Illumina). A multiplexed, paired-end sequencing run of 147 cycles was executed using the TruSeq SBS Kit v5 (Illumina) on the Genome Analyzer IIx (GAIIx; Illumina). Obtained images were analyzed and base called using GAIIx pipeline software, version 1.8.

As a starting point for subsequent DS data analyses, the viral population consensus sequence was first determined for each sample. This consensus sequence was derived through the mapping of all of the available sequencing reads from a sample against a universal reference sequence (H77 for G1a and con1 for G1b). In a second step, all individual sequence reads per sample were subsequently mapped to their sample-specific consensus sequence, using CLCBio Workbench (CLCBio). All codon variants versus the universal reference were determined per amino acid position (HCV NS3 1–181), and their relative frequencies were calculated as the proportion of reads containing a specific codon divided by the coverage at that position. The relative frequencies of codon variants were calculated separately for the forward and reverse sequencing directions, with only the lowest observed frequency reported. As reported earlier by Nakamura et al [15], the insertion of errors during high-throughput sequencing is highly dependent on the direction of the sequence read. Therefore, bidirectionality is often used as a factor for error correction/filtering.

**HCV and Telaprevir-Resistant Variants**

The following variants with reduced sensitivity to HCV protease inhibitors (PIs) were identified in clinical studies: NS3 substitutions V36A/G/M, T54A/S, R155G/R, I132V (HCV G1a only), R155G/K/M/T, A156F/N/S/T/V, D168A/E/G/H/N/T/V/Y, and V170A/T [7, 16].

**Deep-Sequencing of Telaprevir Resistance**

**Table 1. Characteristics of Patients From Whom Baseline Samples Were Evaluated by Deep Sequencing**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Proportiona (%)</th>
<th>SVR</th>
<th>Virologic Failureb</th>
<th>Relapse</th>
<th>Otherc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>530</td>
<td>185/530 (35)</td>
<td>75</td>
<td>76</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Prior relapser</td>
<td>286</td>
<td>40/286 (14)</td>
<td>29</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Prior partial responder</td>
<td>97</td>
<td>6/97 (6)</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prior null responder</td>
<td>147</td>
<td>139/147 (95)</td>
<td>46</td>
<td>69</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>530</td>
<td>185/530 (35)</td>
<td>75</td>
<td>76</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>HCV G1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior relapser</td>
<td>142</td>
<td>20/142 (14)</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Prior partial responder</td>
<td>55</td>
<td>5/55 (9)</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prior null responder</td>
<td>88</td>
<td>82/88 (93)</td>
<td>25</td>
<td>47d</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>285</td>
<td>107/285 (38)</td>
<td>39</td>
<td>53</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>HCV G1b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior relapser</td>
<td>140</td>
<td>20/140 (14)</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Prior partial responder</td>
<td>40</td>
<td>1/40 (3)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Prior null responder</td>
<td>59</td>
<td>57/59 (97)</td>
<td>21</td>
<td>22</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>78/239 (33)</td>
<td>36</td>
<td>23</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: G, genotype; SVR, sustained virologic response.

a Data are no. of patients in the analysis/no. of patients in the overall study (%).
b Defined as on-treatment virologic failure including patients who discontinued treatment because of a virologic stopping rule and/or patients with viral breakthrough.
c Defined as patients with an HCV RNA level of >25 IU/mL at their planned end of treatment but who did not have viral breakthrough and as patients with a missing SVR assessment during planned follow-up.
d Two patients had a V36L mutation detected by population sequencing at baseline.

**DS Analysis**

Paired-end DS of a fragment spanning the HCV NS3–4A protease regions was performed using Illumina technology [14]. Briefly, viral RNA was reverse transcribed using random hexamers and a high-fidelity reverse transcriptase (AccuScript High Fidelity Reverse Transcriptase, Agilent Technologies). An amplicon encompassing the HCV NS3–4A protease region (2.4 kb) was generated by a 2-round nested PCR. PCR reactions were performed in triplicate and subsequently pooled to reduce possible PCR bias. The purified amplicons (0.1–0.5 µg DNA) were fragmented to an average length of 200 bp in accordance with Illumina’s standard protocol. Following DNA end repair and adenylation, the Illumina compatible adaptors (NEXTflex DNA Barcodes, Bioo Scientific) were ligated. Fragments were enriched during 12 PCR cycles and visualized on a Bioanalyzer (Agilent Technologies) for quality control and quantification. Multiplexed samples were loaded on the Illumina cluster station for cluster generation using the TruSeq PE Cluster Kit v2 (Illumina). A multiplexed, paired-end sequencing run of 147 cycles was executed using the TruSeq SBS Kit v5 (Illumina) on the Genome Analyzer Ix (GAIIx; Illumina). Obtained images were analyzed and base called using GAIIx pipeline software, version 1.8.

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Minority Variant Detection Using Illumina Sequencing

The lower limit for quantitative detection by Illumina sequencing was assessed by amplifying and sequencing the HCV NS3 protease region from a plasmid containing wild-type HCV G1b. Sequencing resulted in an average coverage of 92,481 reads per nucleotide position in NS3 (amino acid positions 1–181). At the codon level, 1963 variants, considered false positives, were observed, with relative frequencies ranging from 0.001% to 0.95% of the viral population. To confidently call codon variants in more-heterogeneous clinical samples, the LOD was set at 1% of the viral population. An empirical validation of this cutoff was obtained by plotting the variant fraction as a function of position for a random subset of clinical samples (analyzed in different runs and different lanes), which enables visual identification of true codon variants versus the false-positive background level. Although there is some variability in background level between different experiments, samples, and sequence positions, it appeared justified to define 1% of the viral population as the LOD for genuine minority variants.

RESULTS

Presence of Telaprevir-Resistant Variants at Baseline

Baseline samples from 185 telaprevir-treated patients from the REALIZE study were randomly selected on the basis of availability and were considered to be representative of the study population for subtype, prior response, and treatment outcome,
with a focus on patients with prior null responses because these individuals are most likely to be impacted by baseline resistance, owing to their intrinsically poor ability to respond to PR (Table 1). Selected samples had no lower- or higher-level telaprevir-resistant variants detected by PS. Two samples had a V36L at baseline, which confers <3-fold resistance to telaprevir in the replicon system.

DS of these 185 samples was performed, and all variants with reduced sensitivity to any HCV PI that were identified in clinical studies were reported when present in ≥1% of sequence reads. In 7 of these 185 samples, an HCV variant was detected by DS that was not detectable by PS (Table 2). These variants mostly composed either 1% or 2% of the total viral population: T54A composed 1% of the viral population (n = 1); T54S, 2% (n = 1); Q80K, 1% (n = 1); R155K, 1% (n = 1); and V170T, 1% (n = 2). In addition, 1 sample carried Q80K at a frequency of 14%. Three of the 5 different variants detected by DS but not by PS are considered telaprevir-resistant variants, indicating that telaprevir-resistant variants at baseline were detectable by DS alone had HCV G1a infection and included 2 prior null responders with T54A or T54S and one prior partial responder with R155K. Both prior null responders (patients 001 and 003 in Figure 1) had viral breakthrough during the telaprevir treatment phase and discontinued telaprevir therapy because they met the week 4 stopping rule. Both patients had the higher-level telaprevir-resistant variants V36M, R155K, and A156T present at time of treatment failure, but the T54A or T54S variant detected by DS at baseline was not detectable by PS or DS at the time of treatment failure or during follow-up (Figure 1).

The prior partial responder (patient 002 in Figure 1) completed 12 weeks of telaprevir treatment but discontinued PR after 22 weeks because of an adverse event and subsequently experienced treatment failure. The R155K variant detected by DS at baseline in the prior partial responder was also detected by PS at the time of failure (Figure 1).

### Persistence of Viral Variants Over Time in Patients Who Did Not Achieve SVR

Of the 185 patients, PS detected lower- or higher-level telaprevir-resistant variants in 55 at the time of failure but not at the end of the study by PS, with a follow-up time of ≥10 or ≥3 months after the end of treatment for patients infected with HCV G1a and HCV G1b, respectively. DS data for the end-of-study samples were generated for 49 of these patients (31 had HCV G1a infection, and 18 had HCV G1b infection; 10 were prior relapsers, 4 were prior partial responders, and 35 were prior null responders; and 35 experienced on-treatment virologic failure, 4 experienced relapse, and 10 had an outcome of other).

DS revealed telaprevir-resistant variants in the end-of-study samples from 16 of 49 patients (33%) who had no variants detected by PS at the end of the study, at a range of 1%–36% of the viral population (Table 3); in 20 of 31 patients (65%) with HCV G1a infection and 13 of 18 (72%) with HCV G1b infection, no telaprevir-resistant variants were detected by DS at the end of the study (follow-up time, 11–16 months and 4–15 months, respectively). Lack of detection of variants by PS in these samples was consistent with the presence of variants at levels below the PS LOD of 20%–25% of the viral population, except for 1 patient, in whom V36L was detected in 36% of the viral population yet was not detected by PS. The follow-up time for patients with telaprevir-resistant variants detected by DS was similar to that for patients without detectable variants, except for HCV G1b-infected patients with a detectable V36A (follow-up time, <4 months for patients with detectable variants, compared with 4, 5, or 12 months for those without detectable variants).

Twenty-two patients had the V36M plus R155K mutations detected by PS at time of treatment failure, the most commonly observed telaprevir-resistant variant in HCV G1a–infected patients with on-treatment telaprevir failure. One additional patient had the R155K mutation detected by PS at the time of treatment failure, but both V36M and R155K mutations were detected at subsequent time points. In 12 of these 23 patients (52%), both V36M and R155K were no longer detected at the end of the study; in 4 (17%), R155K was still detectable by DS (range, 4%–13% of the viral population; follow-up time, 13–16 months); and in 7 (30%), V36M was still detectable by DS (range, 3%–22% of the viral population; follow-up time, 13–16 months). V36M and R155K were not detected simultaneously in any patients at the end of the study. In the 11 patients with the V36M or R155K mutation still detectable by DS at the end of the study, the frequency of these mutations decreased gradually over time after treatment (mutations were no longer detectable by PS at the end of the study, in contrast to the time of treatment failure). Examples of individual HCV RNA profiles and telaprevir-resistance profiles determined by DS for 2 patients infected with HCV G1a with on-treatment virologic failure are shown in Figure 2.

### DISCUSSION

The clinical relevance of preexisting resistance mutations and their role in therapeutic failure has been extensively described in HIV-infected patients [17]. In contrast to HIV, major populations of HCV with decreased sensitivity to direct-acting antivirals (DAAs) are rarely observed in DAA-naive patients before treatment initiation, with the exception of HCV protease variants containing the Q80K polymorphism. However, their rapid appearance after the beginning of HCV PI monotherapy strongly suggests the preexistence of quasispecies harboring resistant protease variants at low frequencies [9, 18]. Sensitive techniques have revealed the presence of drug-resistant HCV...
NS3 protease variants at low frequencies (<1%) among DAA-naive HCV-infected patients [19, 20]. Here, we performed a DS analysis with a variant detection of ≥1% of the viral population to analyze baseline samples from treatment-experienced patients included in the REALIZE study. A threshold of 1% for minority detection was implemented because, below this threshold, true positives cannot be distinguished from the noise of the assay [21]. However, because of the high viral load (>100 000 IU/mL) in baseline samples, this implied that quasispecies at frequencies of <1000 IU/mL could not be assessed using a 1% cutoff.

Our results found that only a limited number of variants with reduced sensitivity to HCV PIs that were not detectable by PS were detected by DS. Our findings are in close agreement with results from a study showing the overall presence of pretreatment mutants at NS3 positions 36, 54, 155, 156, 168, and 170.
Table 3.  Follow-up Evaluation of Telaprevir-Resistant Variants, by Resistance Profile and Genotype

<table>
<thead>
<tr>
<th>Mutation Profile at Time of Failure (by PS)</th>
<th>HCV G1a Variants, by WT Status at EOS(^a)</th>
<th>HCV G1b Variants, by WT Status at EOS(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Not WT</td>
</tr>
<tr>
<td></td>
<td>Proportion (%)(^b)</td>
<td>FU Time, mo</td>
</tr>
<tr>
<td>V36A</td>
<td>1/1 (100)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V36M</td>
<td>3/3 (100)</td>
<td>11–13</td>
</tr>
<tr>
<td>T54A</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T54A/S</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>T54S</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>A156S</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>A156T</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>R155K</td>
<td>1/2(^{\dagger}) (50)(^b)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V36L + R155K</td>
<td>2/2(^{\dagger}) (100)(^f)</td>
<td>13–16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V36M + A156T</td>
<td>1/1 (100)</td>
<td>12</td>
</tr>
<tr>
<td>V36M + R155K</td>
<td>11/20 (55)</td>
<td>13–16</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>V36M + R155K + T54S</td>
<td>0/1</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V36M + R155K + A156S</td>
<td>1/1 (100)</td>
<td>13</td>
</tr>
<tr>
<td>T54S + A156T</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>20/31 (65)</td>
<td>11–16</td>
</tr>
</tbody>
</table>

Abbreviations: DS, deep sequencing; EOS, end of the study; FU, follow-up; PS, population sequencing; WT, wild-type.

\(^a\) WT or same mutation profile as at baseline, as revealed by DS.

\(^b\) Data are no. of samples testing positive by DS/no. of samples with the specified PS-based mutation profile.

\(^c\) Defined as the frequency of each mutation detected by DS.

\(^d\) In 1 patient, V36M + R155K was detected by PS at subsequent time points after treatment failure.

\(^e\) One patient had R155K detected by DS at baseline.

\(^f\) One patient had V36L detected by PS at baseline and EOS.
Figure 2.  A and B, Individual hepatitis C virus (HCV) RNA profiles (A) and telaprevir-resistance profiles (B) determined by DS (deep sequencing) for 2 HCV genotype 1–infected patients with on-treatment virologic failure and follow-up DS data available. In panel A, telaprevir (TVR)-resistant variants detected by PS at time points also analyzed by DS are indicated. TVR-resistant variants V36A/G/I/L/M, T54A/S, I132V, R155G/K/M/T, A156F/N/S/T/V, and D168N detected by PS or DS are shown. Of the other HCV variants, only the Q80K mutation was detected in all samples from patient 004 by PS and DS. Abbreviations: FU, follow-up; LI, lead in; LLOQ, lower limit of HCV RNA quantification (25 IU/mL); Pbo, placebo; PR48, 48-week course of pegylated interferon plus ribavirin therapy; T12, 12-week course of TVR therapy; W, week.
in only 4 of 33 samples (12%), as measured by DS with an assay cutoff of 0.25% [22].

Population sequencing showed previously that the prevalence of telaprevir-resistant variants was uncommon (≤5% of patients) in the REALIZE, treatment-experienced, patient population [9]. Furthermore, the presence of telaprevir-resistant variants at baseline did not necessarily prevent achievement of a SVR among treatment-naive patients. However, it was noted that, in patients with poor PR responsiveness (ie, prior null responders), treatment outcome may be affected by the presence of telaprevir-resistant variants at baseline [7, 9]. Patients included in the current analysis were only those who did not have baseline, telaprevir-resistant variants detectable by PS. In 7 of 185 samples, additional mutations with a frequency of 1%–14% of the viral population were detected by DS, and in 3 of those 7 samples, telaprevir-resistant variants were detected (prevalence, 1% of the viral population, for T54A; 2%, for T54S; and 1%, for R155K). Two of these 3 patients with additional telaprevir-resistant variants detected by DS were prior null responders. In both patients, the baseline variant (T54A or T54S) was not detectable by PS or DS at the time of treatment failure, suggesting no effect of the baseline minority variant on treatment outcome. In the other patient (a prior partial responder) with the telaprevir-resistant variant R155K detected by DS at baseline, the R155K variant was also detected by PS at the time of failure. Therefore, the R155K variant detected at baseline might have affected treatment outcome. Overall, despite the low number of patients with baseline resistance detected either by PS and/or DS, the impact of baseline resistance on treatment outcome cannot be excluded, especially in patients for whom the response to interferon therapy was poor.

In contrast to the sporadic nature of resistant variant detection at baseline, telaprevir-resistant variants were detected by DS in 71% of patients (116 of 161) in the REALIZE population after SVR was not achieved. However, these variants were no longer detectable with the same sequencing method in 58% of patients (60 of 104) with available follow-up data [9]. In the current analysis, DS data were generated for 49 of these 60 patients. Six patients had a limited follow-up time of <10 months and <3 months, for those infected with HCV G1a and HCV G1b, respectively, and were excluded from the analysis. For 5 other patients, no DS data could be generated, owing to PCR amplification failure. Telaprevir-resistant variants (Y36A/L/M, T54S, or R155K) were still detected in 33% of patients (16 of 49), at a range of 1% to 36% of the viral population, but in the majority of patients (65% and 72% of those infected with HCV G1a and HCV G1b, respectively), no telaprevir-resistant variants were detected by DS. These results confirm and extend previous findings of both PS and clonal analysis of an ongoing, 3-year observational study (EXTEND) involving patients treated with telaprevir-based regimens in phase 2 and 3 clinical studies. Interim analyses from the EXTEND study suggest that HCV populations returned to the pretreatment state during long-term follow-up (median duration, 22 months) [22]. In patients in whom mutations were still detectable by DS at the end of the study, the frequency of these mutations gradually decreased over time after treatment was completed. A planned DS analysis of long-term follow-up samples of these patients in the EXTEND study will assess for undetectability (<1%) of these variants. Our study also confirms results from another study that evaluated the frequency of resistant NS3 variants in patients 4 years after 14-day monotherapy with telaprevir, using 454 pyrosequencing. At time of follow-up, frequencies were low and, in general, were not increased, compared with pretreatment levels [23].

For boceprevir, similar findings with PS of a low prevalence (7%) of baseline resistance-associated mutations, with limited impact on treatment outcome and a decline in mutations over time, were reported [24]. Clonal sequence analysis at long-term follow-up in patients who received short-course treatment with telaprevir or boceprevir revealed resistant variants in 2 of 14 telaprevir-treated patients and 4 of 14 boceprevir-treated patients, but the resistance profile could not generally be predicted from variants present at the end of treatment [25]. Although the number of patients (n = 49) included in our analysis was limited, it represents the largest study to date involving a sensitive sequencing technique in which telaprevir resistance was found to decrease over time in the majority of patients. However, additional research is needed to evaluate whether these patients can be successfully retreated with the same DAA class. Recently, proof of concept was provided that a combination of DAs with another mechanism of action may provide effective therapy for HCV-infected patients who do not respond to PI-based therapy [26].

In conclusion, DS techniques detected a minority (prevalence, >1% of the viral population) of telaprevir-resistant variants in only a small proportion (2%) of patients who did not have telaprevir-resistant variants detected by PS at baseline. Overall, pretreatment sequencing, both by PS or more-sensitive DS techniques, fails to identify patients at risk of experiencing failure of telaprevir-based therapy, although there could be an impact of baseline resistance on treatment outcome in prior null responders. Finally, these DS results confirmed that the frequency of telaprevir-resistant variants declines after treatment failure to low or undetectable levels after treatment is stopped.

Notes

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