Utility of Hepatitis C Viral Load Monitoring on Direct-Acting Antiviral Therapy

Sreetha Sidharthan,1 Anita Kohli,1 Zayani Sims,1 Amy Nelson,2,3 Anu Osinusi,3,4 Henry Masur,1 and Shyam Kottilil2,3

1Critical Care Medicine Department, National Institutes of Health Clinical Center, National Institutes of Health, Bethesda, 2Institute of Human Virology, University of Maryland, Baltimore, 3Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and 4Gilead Sciences Inc, Foster City, California

Background. Hepatitis C virus (HCV) RNA loads serve as predictors of treatment response during interferon-based therapy. We evaluated the predictive ability of HCV RNA levels at end of treatment (EOT) for sustained virologic response (SVR12) during interferon-sparing direct-acting antiviral therapies.

Methods. HCV genotype 1–infected, treatment-naive patients were treated with sofosbuvir and ribavirin for 24 weeks (n = 55), sofosbuvir and ledipasvir for 12 weeks (n = 20), sofosbuvir, ledipasvir, and GS-9669 for 6 weeks (n = 20), or sofosbuvir, ledipasvir, and GS-9451 for 6 weeks (n = 19). Measurements of HCV RNA were performed using the Roche COBAS TaqMan HCV test and the Abbott RealTime HCV assay. Positive predictive value (PPV) and negative predictive value (NPV) of HCV RNA less than the lower limit of quantification (<LLOQ) at EOT for SVR12 were calculated.

Results. All 55 patients treated with sofosbuvir and ribavirin had HCV RNA <LLOQ at EOT by the Roche and Abbott assays, but only 38 achieved SVR12 (PPV, 69%). Among patients treated with sofosbuvir and ledipasvir with or without GS-9669 or GS-9451, 100% (59/59) had HCV RNA <LLOQ by the Roche assay and 1 relapsed (PPV, 98%). By the Abbott assay, 90% (53/59) had HCV RNA <LLOQ, of whom 1 patient relapsed (PPV, 98%). Notably, 6 patients with HCV RNA ≥LLOQ at EOT (range, 14–64 IU/mL) achieved SVR12 (NPV, 0%). Quantifiable HCV RNA (range, 15–57 IU/mL) was measured 2 weeks posttreatment in 4 individuals, and 4 weeks posttreatment in 1 patient (14 IU/mL).

Conclusions. Contrary to past experience with interferon-containing treatments, low levels of quantifiable HCV RNA at EOT do not preclude treatment success.

Keywords. viral load; direct-acting antiviral; HCV RNA; hepatitis C.

Chronic hepatitis C (CHC) infection affects an estimated 170 million people worldwide [1]. Until recently, the standard of care for treatment of CHC was pegylated interferon, ribavirin, and a direct-acting antiviral (DAA) for up to 1 year, a challenging regimen due to high pill burden and associated adverse effects [2]. Recent clinical trials have demonstrated that treatment regimens utilizing combinations of DAAs allow for shorter therapies of 6–24 weeks with high rates of sustained virologic response (SVR) and improved tolerability [3–8].

Historically, hepatitis C virus (HCV) RNA levels have been a predictive on-therapy marker of treatment outcome [9]. HCV RNA threshold values at select time points have been used to guide decisions regarding the continuation or halting of interferon-containing therapy, based on the likelihood of treatment success [10, 11]. Interferon-free, DAA-only treatment regimens are replacing interferon-containing regimens as the standard of care. As a result, it has become essential to reevaluate the utility of HCV RNA levels in predicting treatment outcome and in guiding clinical decision making. The objective of this study was to determine the ability of HCV RNA levels to predict treatment response in patients treated with novel interferon-sparing regimens consisting of sofosbuvir (nucleoside NS5B inhibitor) with ribavirin for 24 weeks, sofosbuvir and ledipasvir (NS5A inhibitor) for 12 weeks, or sofosbuvir and ledipasvir with GS-9669 (nonnucleoside NS5B inhibitor) or...
GS-9451 (NS3/4 protease inhibitor) for 6 weeks (Supplementary Figure 1). Based on previous studies with interferon-containing regimens [9–11], we hypothesized that quantifiable and detectable HCV RNA at week 4 and end of treatment (EOT) during DAA therapy would be predictive of viral rebound and treatment failure.

**METHODS**

**Patients and Study Design**

One hundred twenty HCV-monoinfected, treatment-naive patients were enrolled into 1 of 2 clinical trials at a single center, the Clinical Research Center of the National Institutes of Health (Bethesda, Maryland). Eligible participants were men and women, aged ≥18 years, and infected with chronic HCV genotype 1 infection (serum HCV RNA ≥2000 IU/mL). Full eligibility criteria were previously published [6, 7]. In the first study (ClinicalTrials.gov number NCT01441180), patients received sofosbuvir (400 mg/day) with weight-based ribavirin (1000 mg/day for participants weighing <75 kg and 1200 mg/day for participants weighing ≥75 kg) for 24 weeks (n = 35) or sofosbuvir with low-dose ribavirin (600 mg/day) for 24 weeks (n = 25) [7]. One patient discontinued therapy after only 12 weeks but had viral load data from week 4, EOT, and 12 weeks posttreatment, and therefore was included in this analysis. Five patients did not have evaluable outcome data available and were excluded. During the second study, (ClinicalTrials.gov number NCT01805882), patients received sofosbuvir and ledipasvir (1 tablet, 400 mg/90 mg daily) for 12 weeks (n = 20), sofosbuvir, ledipasvir, and GS-9669 (500 mg/day) for 6 weeks (n = 20), or sofosbuvir, ledipasvir, and GS-9451 (80 mg/day) for 6 weeks (n = 20) [6]. One patient treated with sofosbuvir, ledipasvir, and GS-9451 did not have evaluable outcome data available and was excluded from this analysis. Overall, 114 patients were included in this substudy.

**Study Oversight**

Both studies were approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases (NIAID) and were conducted in compliance with the Good Clinical Practice guidelines, the Declaration of Helsinki, and regulatory requirements. The Regulatory Compliance and Human Participants Protection Branch of NIAID served as the study sponsor and medical monitor. Gilead Sciences Inc provided study drugs and scientific advice.

**HCV RNA Measurements**

HCV RNA levels were measured using the Roche COBAS TaqMan HCV test, version 1.0, and the Abbott RealTime HCV assay as previously described [6]. Both assays have a lower limit of quantification (LLOQ), defined as the lowest HCV RNA concentration that can be accurately quantified, of 43 IU/mL (Roche) and 12 IU/mL (Abbott) [12]. Below the LLOQ, HCV RNA is said to be “unquantifiable” and may be further qualified as either target detected (TD) or target not detected (TND). The lower limit of detection (LLOD) is not used in this study because it is an extrapolated estimate that can vary for the same assay in different conditions [13]. Additionally, for any given assay, HCV RNA levels between zero and LLOD can still result in target detection with a statistical frequency. Table 1 presents the accepted nomenclature used throughout this article [14, 15], as well as the corresponding readouts for the Roche and Abbott assays.

**Statistical Analysis**

Baseline demographics were compared by 1-way analysis of variance for continuous outcomes and χ² analysis for binary outcomes using PRISM 6.0. The positive predictive value (PPV) and negative predictive value (NPV) of HCV RNA levels at week 4 and EOT for SVR12 were calculated for each treatment regimen and both the Roche and Abbott HCV RNA assays. SVR12 was defined as HCV RNA <LLOQ 12 weeks after treatment completion.

The PPV of unquantifiable HCV RNA is the proportion of patients with HCV RNA <LLOQ who achieve SVR12. The PPV of undetectable HCV RNA is defined as the proportion of patients with HCV RNA TND <LLOQ who achieve SVR12.

**Table 1. Hepatitis C Virus RNA Assay Readouts and Nomenclature**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assay Readout</th>
<th>Definition</th>
<th>Abbreviation Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Roche COBAS TaqMan HCV test</strong></td>
<td>43 IU/mL–69 million IU/mL</td>
<td>Quantifiable</td>
<td>≥LLOQ</td>
</tr>
<tr>
<td></td>
<td>&lt;43</td>
<td>Unquantifiable but detectable</td>
<td>TD &lt;LLOQ</td>
</tr>
<tr>
<td></td>
<td>None detected</td>
<td>Undetectable</td>
<td>TND &lt;LLOQ</td>
</tr>
<tr>
<td><strong>Abbott RealTime HCV assay</strong></td>
<td>12 IU/mL–100 million IU/mL</td>
<td>Quantifiable</td>
<td>≥LLOQ</td>
</tr>
<tr>
<td></td>
<td>&lt;12 detected</td>
<td>Unquantifiable but detectable</td>
<td>TD &lt;LLOQ</td>
</tr>
<tr>
<td></td>
<td>&lt;12 not detected</td>
<td>Undetectable</td>
<td>TND &lt;LLOQ</td>
</tr>
</tbody>
</table>

Abbreviations: HCV; hepatitis C virus; LLOQ, lower limit of quantification; TD, target detected; TND, target not detected.
\[
\text{PPV} = \frac{\text{No. of patients with HCV RNA } < \text{LLOQ who achieve SVR}_{12}}{\text{No. of patients with HCV RNA } < \text{LLOQ}} \times 100 \%
\]

\[
\text{NPV} = \frac{\text{No. of patients with HCV RNA TND } < \text{LLOQ who achieve SVR}_{12}}{\text{No. of patients with HCV RNA TND } < \text{LLOQ}} \times 100 \%.
\]

The NPV of quantifiable HCV RNA is the proportion of patients with HCV RNA \( \geq \text{LLOQ} \) who fail treatment. The NPV of detectable HCV RNA is defined as the proportion of patients with HCV RNA \( \geq \text{LLOQ} \) or HCV RNA TD \(<\text{LLOQ}\) who fail treatment.

\[
\text{NPV} = \frac{\text{No. of patients with HCV RNA } \geq \text{LLOQ who fail treatment}}{\text{No. of patients with HCV RNA } \geq \text{LLOQ}} \times 100 \%
\]

\[
\text{NPV} = \frac{\text{No. of patients with HCV RNA } \geq \text{LLOQ or HCV RNA TD } < \text{LLOQ who fail treatment}}{\text{No. of patients with HCV RNA } \geq \text{LLOQ or HCV RNA TD } < \text{LLOQ}} \times 100 \%.
\]

### RESULTS

#### Study Population

Demographic and clinical characteristics of the study populations are shown in Table 2. Participants were all treatment naive, predominantly of black race (86%), and infected with HCV genotype 1a (70%), and had high baseline plasma HCV RNA levels >800 000 IU/mL (65%). Baseline characteristics were similar among different treatment groups in each study.

#### Predictive Ability of HCV RNA at Week 4 for SVR\(_{12}\)

In patients treated with 24 weeks of sofosbuvir and ribavirin, 96% (50/52) had week 4 HCV RNA \(<\text{LLOQ}\) and 58% (30/52) had HCV RNA TND \(<\text{LLOQ}\) by the Roche assay. Three patients did not have a Roche HCV RNA assay completed at week 4. Among the 50 patients with HCV RNA \(<\text{LLOQ}\), 35 achieved SVR\(_{12}\) (PPV, 70%), and of the 30 patients with HCV RNA TND \(<\text{LLOQ}\), 21 achieved SVR\(_{12}\) (PPV, 70%). Two patients had HCV RNA \(\geq\text{LLOQ}\) at week 4, 1 of whom relapsed (NPV, 50%) and 22

### Table 2. Baseline Demographics and Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sofosbuvir + Low-Dose Ribavirin (n = 22)</th>
<th>Sofosbuvir + Weight-Based Ribavirin (n = 33)</th>
<th>Sofosbuvir + Ledipasvir (n = 20)</th>
<th>Sofosbuvir + Ledipasvir + GS-9691 (n = 20)</th>
<th>Sofosbuvir + Ledipasvir + GS-9451 (n = 19)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment duration</td>
<td>24 wk</td>
<td>24 wk</td>
<td>12 wk</td>
<td>6 wk</td>
<td>6 wk</td>
<td></td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>54 ± 11</td>
<td>54 ± 6</td>
<td>57 ± 8</td>
<td>54 ± 7</td>
<td>54 ± 9</td>
<td>.77</td>
</tr>
<tr>
<td>Male sex</td>
<td>12 (55)</td>
<td>22 (67)</td>
<td>14 (70)</td>
<td>13 (65)</td>
<td>15 (79)</td>
<td>.58</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>20 (91)</td>
<td>26 (79)</td>
<td>16 (80)</td>
<td>19 (95)</td>
<td>17 (89)</td>
<td>.60</td>
</tr>
<tr>
<td>White</td>
<td>2 (9)</td>
<td>5 (15)</td>
<td>4 (20)</td>
<td>1 (5)</td>
<td>2 (11)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m², mean ± SD</td>
<td>32 ± 8</td>
<td>29 ± 5</td>
<td>25 ± 4</td>
<td>28 ± 7</td>
<td>29 ± 6</td>
<td>.03</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>15 (68)</td>
<td>24 (73)</td>
<td>11 (55)</td>
<td>14 (70)</td>
<td>16 (84)</td>
<td>.39</td>
</tr>
<tr>
<td>1b</td>
<td>7 (32)</td>
<td>9 (27)</td>
<td>9 (45)</td>
<td>6 (30)</td>
<td>3 (16)</td>
<td></td>
</tr>
<tr>
<td>HCV RNA &gt;800 000 IU/mL</td>
<td>11 (50)</td>
<td>22 (67)</td>
<td>15 (75)</td>
<td>13 (65)</td>
<td>13 (68)</td>
<td>.53</td>
</tr>
<tr>
<td>IL28B genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3 (14)</td>
<td>6 (18)</td>
<td>5 (25)</td>
<td>2 (10)</td>
<td>4 (21)</td>
<td>.74</td>
</tr>
<tr>
<td>CT/TT</td>
<td>19 (86)</td>
<td>27 (82)</td>
<td>15 (75)</td>
<td>18 (90)</td>
<td>15 (79)</td>
<td></td>
</tr>
<tr>
<td>IFNL4 genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT/TT</td>
<td>2 (9)</td>
<td>6 (22)</td>
<td>3 (15)</td>
<td>3 (15)</td>
<td>4 (21)</td>
<td>.86</td>
</tr>
<tr>
<td>ΔG/TT, ΔG/ΔG</td>
<td>20 (91)</td>
<td>27 (82)</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>15 (79)</td>
<td></td>
</tr>
<tr>
<td>Knodell HAI, Metavir, or Fibrosure fibrosis score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>15 (68)</td>
<td>27 (82)</td>
<td>12 (60)</td>
<td>15 (75)</td>
<td>12 (63)</td>
<td>.42</td>
</tr>
<tr>
<td>3–4</td>
<td>7 (32)</td>
<td>6 (18)</td>
<td>8 (40)</td>
<td>5 (25)</td>
<td>7 (37)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as No. (%) unless otherwise specified.

Abbreviations: BMI, body mass index; HAI, histologic activity index; HCV, hepatitis C virus; SD, standard deviation.
patients had HCV RNA ≥LLOQ or HCV RNA TD <LLOQ, only 7 of whom relapsed (NPV, 32%) (Figure 1A and 1B). Values used to calculate PPV and NPV are presented in detail in Supplementary Table 1.

The predictive ability of HCV RNA at week 4 as measured by the Abbott assay was comparable to that by the Roche assay in this cohort of patients treated with 24 weeks of sofosbuvir and ribavirin (Figure 1A and 1B). The majority (62% [34/55]) of patients had HCV RNA <LLOQ, of whom 24 achieved SVR12 (PPV, 71%). Twenty-nine percent (16/55) of patients had HCV RNA TD <LLOQ at week 4, and of these, 14 achieved SVR12 (PPV, 88%). Only 7 of 21 patients with HCV RNA >LLOQ at week 4 by the Abbott assay relapsed (NPV, 33%). Similarly, of the 39 patients with HCV RNA ≥LLOQ or HCV RNA TD <LLOQ, only 15 relapsed (NPV, 39%).

The high PPV and low NPV values after 4 weeks of DAA therapy observed in patients treated with 24 weeks of sofosbuvir and ribavirin were similarly true of patients treated with 6–12 weeks of sofosbuvir and ledipasvir with or without GS-9669 or GS-9451. In total, for these 3 treatment regimens, 97% (56/58) of patients had HCV RNA <LLOQ by the Roche assay, and of these, 55 patients achieved SVR12 (PPV, 98%). Additionally, 66% (38/58) had HCV RNA TD <LLOQ at week 4, and of these, 37 achieved SVR12 (PPV, 97%). The 2 patients with HCV RNA ≥LLOQ and all 20 patients with HCV RNA ≥LLOQ or HCV RNA TD <LLOQ achieved SVR12 (NPV, 0%). One patient treated with sofosbuvir and ledipasvir for 12 weeks did not have a Roche assay completed at week 4. These data are presented by individual treatment regimens in Figure 1C–H.

Likewise, by the Abbott assay, 63% (37/59) of patients treated with 6–12 weeks of sofosbuvir and ledipasvir with or without GS-9669 or GS-9451 had HCV RNA <LLOQ at week 4, and 27% (16/59) had HCV RNA TD <LLOQ, all of whom achieved SVR12 (PPV, 100%). Of the 22 patients with HCV RNA ≥LLOQ and the 43 patients with HCV RNA ≥LLOQ or HCV RNA TD <LLOQ at week 4, 1 individual relapsed, corresponding to low NPV values of 4.5% and 2.3%, respectively.

Predictive Ability of HCV RNA at EOT for SVR12
All 55 patients receiving sofosbuvir and ribavirin had HCV RNA TD <LLOQ by both the Roche and Abbott assays at EOT (Figure 2A and 2B). However, only 38 patients achieved SVR12 (PPV, 69%) and the remaining 31% (17/55) of patients experienced viral relapse. All (59/59) patients treated with sofosbuvir and ledipasvir with or without GS-9669 or GS-9451 had HCV RNA <LLOQ by the Roche assay at EOT, among whom 1 patient relapsed (PPV, 98%). However, unlike those who received sofosbuvir and ribavirin, not all patients had HCV RNA TD <LLOQ at EOT on the shorter duration regimens of 12 or 6 weeks. Only 93% (55/59) had HCV RNA TD <LLOQ at EOT, all of whom achieved SVR12 (PPV, 100%; Figure 2C–H). Notably, of the 4 patients with HCV RNA TD <LLOQ by the Roche assay at EOT, only 1 relapsed (NPV, 25%, treated with sofosbuvir, ledipasvir, and GS-9669; Figure 2E and 2F).

Compared to the Roche assay, fewer patients treated with sofosbuvir and ledipasvir with or without GS-9669 or GS-9451 had HCV RNA <LLOQ at EOT as measured by the more sensitive Abbott assay. In fact, only 90% (53/59) had HCV RNA <LLOQ at EOT, and of these, 52 patients achieved SVR12 (PPV, 98%; Figure 2C–H). Additionally, only 51% (30/59) had HCV RNA ≥LLOQ at EOT, among whom 6 patients had HCV RNA TD ≥LLOQ at EOT, all of whom achieved SVR12 (PPV, 100%). Notably, 6 patients had HCV RNA ≥LLOQ at EOT (range, 14–64 IU/mL), and none of these patients relapsed (NPV, 0%). Only 1 of the 29 patients with HCV RNA ≥LLOQ or HCV RNA TD <LLOQ relapsed (NPV, 3.4%).

Patients With Quantifiable HCV RNA at EOT by the Abbott Assay Who Achieved SVR12
On the shorter, 6- to 12-week regimens of sofosbuvir and ledipasvir with or without GS-9669 or GS-9451, 23 individuals had HCV RNA TD <LLOQ at another 6 patients had HCV RNA ≥LLOQ at EOT. All 6 participants with quantifiable HCV RNA at EOT were taking the 6-week regimen of sofosbuvir, ledipasvir, and either GS-9669 (n = 5) or GS-9451 (n = 1). Two patients attained HCV RNA TD <LLOQ at 4 weeks posttreatment, 2 patients at 8 weeks posttreatment, and 2 patients at 12 weeks posttreatment. Notably, HCV RNA ≥LLOQ was measured 2 weeks after completion of therapy in 4 patients (range, 15–57 IU/mL) and 4 weeks after completion of therapy in 1 patient (14 IU/mL). Despite the presence of quantifiable virus posttreatment, all 5 of these patients achieved SVR12.

Decline of HCV RNA levels in patients with quantifiable and detectable HCV RNA as measured by the Abbott assay is depicted in Figure 3. Twenty-two patients with HCV RNA TD <LLOQ at EOT achieved SVR12. Only 1 patient treated with sofosbuvir, ledipasvir, and GS-9669 experienced viral relapse.

---

**Figure 1.** Predictive ability of hepatitis C virus (HCV) RNA at week 4 for sustained virologic response after 12 weeks of treatment (SVR12). Left: Percentage of patients with HCV RNA less than the lower limit of quantification (<LLOQ) at week 4 is presented in patients treated with sofosbuvir and ribavirin for 24 weeks (A), sofosbuvir and ledipasvir for 12 weeks (C), sofosbuvir, ledipasvir, and GS-9669 for 6 weeks (E), and sofosbuvir, ledipasvir, and GS-9451 for 6 weeks (G) by both the Roche and Abbott assays. Similarly, the percentage of patients with HCV RNA target not detected (TND) <LLOQ at week 4 is presented for each treatment regimen (B, D, F, and H). The positive predictive value (PPV) and negative predictive value (NPV) for the assays are presented below the corresponding bars on each graph. Right: Percentage of patients who achieved SVR12 for each treatment regimen. Abbreviation: N/A, not applicable.
This patient had HCV RNA levels TD <LLOQ at EOT, a low viral load of 75 IU/mL at week 2 posttreatment, and viral relapse by week 4 posttreatment.

**DISCUSSION**

HCV RNA measurements on treatment and at EOT were not clinically useful for predicting SVR12 in this study of sofosbuvir-containing, interferon-free regimens of DAAs. Low NPVs for HCV RNA at week 4 suggest that the majority of patients with quantifiable or detectable HCV RNA during treatment achieve SVR12. In contrast to what has been observed with interferon-containing therapy [12, 13], low levels of quantifiable virus after treatment completion were not predictive of relapse with these DAA regimens.

Measurements of HCV RNA during interferon-containing regimens have been useful in guiding duration of therapy and predicting treatment efficacy [9–11]. However, data from this study suggest a limited role for viral load monitoring during DAA-based therapy, particularly in trials of short duration [6, 16]. Low NPVs for HCV RNA at week 4 in these trials underscore the importance of continued therapy for patients who fail to achieve undetectable viral loads during treatment, as likelihood of SVR12 is still high. This observation is in accordance with data from a larger phase 3 trial, where patients with HCV genotype 1 were treated with ledipasvir and sofosbuvir with or without ribavirin for 8–24 weeks [17]. However, in that study, the subpopulation of cirrhotic patients with quantifiable HCV RNA at weeks 1 and 2 had significantly higher SVR12 rates after 24 weeks of therapy vs 12 weeks of therapy [17], suggesting a potential role for viral load monitoring in select populations.

In our cohort of patients treated with 6 weeks of ledipasvir, sofosbuvir, and either GS-9669 or GS-9451, more than half had detectable HCV RNA at EOT by the Abbott assay, the majority of whom achieved SVR12. This included 6 patients with low levels of quantifiable HCV RNA <100 IU/mL. Notably, low-level viremia was also detected 2 weeks after treatment completion in 4 of these patients and 4 weeks after treatment completion in 1 patient. These data indicate that viral eradication may be attained even when HCV RNA is suboptimally suppressed by DAA therapy. We offer 2 possible explanations for this novel observation. The presence of quantifiable or detectable virus at EOT in patients who achieve SVR12 may be explained by a role of the host immune system that persists after cessation of therapy. It may also be explained by the detection of noninfectious viral particles synthesized in the presence of agents that disrupt HCV replication complex formation. Recent studies in patients receiving DAA-only therapy have suggested an increase in innate [18] and adaptive [19, 20] immune responses associated with SVR12. In vitro assays have demonstrated that in the presence of HCV NS5A inhibitors, such as ledipasvir, noninfectious virions may be produced by infected hepatocytes [16]. Therefore, practitioners may not need to be overly concerned if detectable or low-level viremia is present at the end of therapy when utilizing the Abbott HCV RNA assay, as this does not necessarily signal therapeutic failure. Patients with low-level detectable viremia should continue to be followed for determination of treatment outcome.

One limitation of this study is our small and selective sample size. All participants were treatment naive and only 5 patients had cirrhosis. Therefore, the clinical utility of viral load monitoring during short-duration DAA therapy needs to be further evaluated through larger studies, and in particular, in patients with cirrhosis and/or past treatment experience. Additionally, due to the high potency of DAA regimens and the few treatment failures, it is difficult to determine viral load predictors for relapse vs SVR12. It should also be noted that the clinical use of HCV RNA monitoring extends beyond response-guided therapy. Rather, it is also a strong tool to measure patient adherence in real-world scenarios and may be valuable to providers on an individual basis. HCV RNA levels are also used to detect viral breakthroughs on therapy. Although sofosbuvir has a high barrier to resistance and no viral breakthroughs in adherent patients to date [21], breakthroughs do occur at low rates with other regimens [5, 22, 23].

The potency of combination DAA therapies permits shorter treatment duration and high SVR12 rates. As the paradigm of HCV treatment continues to shift, clinicians should be aware that frequent monitoring of HCV RNA levels may have limited clinical utility in predicting treatment outcome and guiding treatment duration with DAA therapy. Furthermore, contrary to past interferon-based treatments, low levels of HCV RNA detected at the end of short-duration DAA therapy, and even after therapy, do not signify treatment failure.

**Figure 2.** Predictive ability of hepatitis C virus (HCV) RNA at end of treatment (EOT) for sustained virologic response after 12 weeks of treatment (SVR12). Left: Percentage of patients with HCV RNA less than the lower limit of quantification (<LLOQ) at EOT is presented in patients treated with sofosbuvir and ribavirin for 24 weeks (A), sofosbuvir and ledipasvir for 12 weeks (C), sofosbuvir, ledipasvir, and GS-9669 for 6 weeks (E), and sofosbuvir, ledipasvir, and GS-9451 for 6 weeks (G) by both the Roche and Abbott assays. Similarly, the percentage of patients with HCV RNA target not detected (TND) <LLOQ at EOT is also presented for each treatment regimen (B, D, F, and H). The positive predictive value (PPV) and negative predictive value (NPV) for the assays are presented below the corresponding bars on each graph. Right: Percentage of patients who achieved SVR12 for each treatment regimen. Abbreviation: N/A, not applicable.
Figure 3. Patients with quantifiable hepatitis C virus (HCV) RNA at end of treatment (EOT) achieve sustained virologic response after 12 weeks of treatment (SVR12). Of the 59 patients treated with sofosbuvir and ledipasvir with or without GS-9669 or GS-9451, 6 patients had HCV RNA equal to or greater than the lower limit of quantification (LLOQ), and 23 patients had HCV RNA target detected (TD) less than the LLOQ at EOT. The decline in HCV RNA levels from start of treatment to the SVR12 timepoint is shown for each of these individuals. One patient treated with sofosbuvir, ledipasvir, and GS-9669 relapsed. Abbreviation: TND, target not detected.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.
Notes

Disclaimer. The content of this publication does not necessarily reflect the views of policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the US government.

Financial support. This work was supported in whole or in part with federal funds from the National Cancer Institute at the National Institutes of Health (contract number HHSN261200800001E). This research was supported in part by the National Institute of Allergy and Infectious Diseases.

Potential conflicts of interest. A. O. is an employee of Gilead Sciences Inc. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


