Retrospective analyses of the boceprevir and telaprevir phase 3 trial data demonstrate the clinical relevance of detected but not quantifiable hepatitis C virus (HCV) genotype 1 RNA during treatment. These analyses illustrate the importance of using precise and standard terminology in reporting low-level HCV RNA results for consistent data collection across clinical trials, and to ensure optimal virologic response-guided treatment decision making in clinical practice. In the context of currently available quantitative HCV RNA assays, we clarify that unquantifiable HCV RNA should be classified as target detected or target not detected, as both have been shown to reflect clinically different qualitative HCV RNA levels during treatment. Additionally, use of terms such as “undetectable” or “below limit of detection” should be avoided as such terms are imprecise, not consistently defined, and often misinterpreted.

**Keywords.** direct-acting antiviral (DAA); hepatitis C virus (HCV); lower limit of quantitation (LLOQ).

An estimated 180 million persons are infected worldwide with hepatitis C virus (HCV), of whom 41.4–5.2 million reside in the United States [1, 2]. Chronic HCV infection is a leading cause of liver cirrhosis and hepatocellular carcinoma, a problem compounded by the large majority of HCV-positive individuals unaware of their infection [3, 4]. For HCV genotype 1–infected patients aware of their status, the slow and asymptomatic progression of the disease must be balanced with the <50% success rate and complications associated with pegylated interferon alfa and ribavirin treatment (P/R). Advances in treatment, higher success rates, and promising interferon-free direct-acting antiviral agent (DAA) combinations compel us to identify and potentially treat all HCV-infected individuals, for whom we need better-defined viral RNA thresholds.

During P/R treatment, a validated predictor of virologic cure, known as sustained virologic response (SVR), is achieving a rapid virologic response (RVR), defined as undetectable HCV RNA at week 4 of treatment using a sensitive test with a lower limit of detection of 50 IU/mL [3]. Here, and throughout the paper, we will use the newly proposed nomenclature system, and refer to RVR as W4UTND, where W4U stands for HCV RNA unquantifiable at week 4, and TND refers to “target not detected” (see below) [5]. Several studies of P/R response-guided therapy (RGT) found that patients who achieved W4U_TND could receive a shorter duration of P/R treatment of 24 total weeks compared to 48 weeks, without compromising SVR rates [6–8]. As RGT was borne out of P/R treatment, existing definitions were sufficient to determine SVR [3, 9].

However, the role of P/R as the standard of care for HCV genotype 1–infected patients has been replaced...
by P/R cocktails that include one of the currently approved DAAs: boceprevir and telaprevir [10–17]. As a result, HCV treatment progress has begun to outpace the definitions that many clinicians may have used to determine the duration of treatment for P/R. During post hoc analyses of phase 2 and 3 clinical trials for boceprevir and telaprevir, the accuracy of RGT decisions based on current HCV RNA thresholds came into question owing to poorer treatment outcomes in patients with detected but unquantifiable vs undetected HCV RNA levels during the treatment period [18]. The goal of this commentary is to describe a clear set of definitions for HCV RNA thresholds used during HCV clinical trials and treatment, specifically in the context of currently available HCV RNA assay platforms.

Currently, there are 3 US Food and Drug Administration (FDA)–approved, commercially available, quantitative assays to determine HCV RNA levels during clinical trials and treatment: Siemens (Versant branched DNA [bDNA]), Roche Diagnostics (COBAS Ampliprep/COBAS TaqMan HCV Test, that uses an automated sample preparation or the COBAS TaqMan for use with the High Pure System [HPS] HCV Test, v2.0) and the Abbott RealTime HCV assay [3, 19] (Figure 1). While each assay accurately measures up to 10^7 IU/mL of HCV RNA, this manuscript will focus on the lower limits of each assay’s quantitation and detection due to the importance of low HCV RNA levels during RGT, end of treatment (EOT), and EOT follow-up. Please note that although it is FDA-approved, the Siemens Versant bDNA assay is not recommended for RGT decision making owing to its reduced sensitivity for the detection of HCV RNA [20].

DEFINITIONS AT THE LOWER END

The increasing efficiency of P/R plus boceprevir/telaprevir in reducing HCV RNA levels, coupled with the use of specific HCV RNA thresholds to guide treatment decisions, requires that terminology across drug labels, assay package inserts, and treatment guidelines be accurately defined. The Siemens, Roche, and Abbott assays all have an upper limit of quantitation and a lower limit of quantitation (LLOQ), with values between these 2 limits defined as the linear range of the assay. As assay technology has improved, the LLOQ values have declined, but differ across the available platforms [21, 22]. Complicating the interpretation of results, another value referred to as the limit of detection (LOD)—usually lower than LLOQ—is included in the Siemens and Roche platforms, and in some cases has been reported out by commercial labs to clinicians (Table 1). The LOD equals the LLOQ for the Abbott RealTime HCV assay and the Roche COBAS Ampliprep/COBAS TaqMan HCV Test v2.0. The latter, however, is only commercially available in CE-marked countries (where the

Table 1. Hepatitis C Virus RNA Assay Definitions for Currently Approved Platforms

<table>
<thead>
<tr>
<th>HCV Assay Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLOQ</td>
<td>Lowest amount of HCV RNA that is in the linear range</td>
</tr>
<tr>
<td>LOD</td>
<td>Lowest amount of HCV RNA detected in 95% of samples as a positive signal</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; LLOQ, lower limit of quantitation; LOD, limit of detection.
manufacturer declares that the product conforms to the essential requirements of the applicable European Community directive) and currently for research use only in the United States. Similarly, the American Association for the Study of Liver Diseases (AASLD) guidelines state that for RGT, an undetectable reading of a value ≤10–15 IU/mL is required, based on the LOD of commercially available assays [3, 23, 24]. This poses a problem as LOD is calculated as the lowest amount of HCV RNA detected in ≥95% of samples as a positive signal, meaning that ≤5% of the time the signal would statistically receive a TND result [25, 26]. Furthermore, analysis of the calculated LOD value can vary from experiment and genotype, leading to confusion and differing interpretation of results across testing centers [27].

This is illustrated by a recent European Association for the Study of the Liver 2012 abstract presented by Feyery et al regarding the PILLAR study (TMC435-C205, NCT00882908), demonstrating that some values reported as undetectable by one platform may be reported as detected by another platform [28]. Thus “undetectable” would be more accurately expressed as “target not detected” (TND), as the term “undetectable” connotes that HCV RNA would always be undetectable, when in reality it was not detected in the aliquot of samples tested under the conditions of a specific assay (Table 2) [5]. In contrast, the LLOQ is a quantifiable, reproducible, and generally more reliable lower limit, and its specific International Units per milliliter value can be applied more consistently across different HCV RNA assays [29, 30]. It is also critical to point out that although commercially available assays may or may not have differing values for the LOD and LLOQ, a range of “target detected” (TD) values still exist across all platforms.

The potential for confusion with the use of terms such as “below the LOD” and “undetectable” has been recognized by AASLD and the FDA, based on a recently published response from the AASLD treatment guidelines authors, as well as a recent updates to the telaprevir and boceprevir labels [10, 16, 24]. However, AASLD guidelines and FDA-approved product inserts for boceprevir and telaprevir would be clearest if values below the LLOQ were defined as TD or TND, and the LLOQ the only listed numerical value.

Table 2. Summary of Recommended Terminology and Definitions of Hepatitis C Virus RNA Results Used for Response-Guided Therapy With Telaprevir or Boceprevir

<table>
<thead>
<tr>
<th>HCV RNA Quantitation</th>
<th>Definition for HCV RNA Assay and RGT</th>
<th>Suggested RGT Regimen Based on Results at W4TND and W12TND (telaprevir), or LI4W8 (boceprevir)*</th>
<th>Clinical Outcomes at Follow-up Weeks 12 and 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥LLOQ</td>
<td>HCV RNA above LLOQ, reported as specific IU/mL</td>
<td>Standard</td>
<td>Relapse (if TND at end of treatment)</td>
</tr>
<tr>
<td>TD, &lt;LLOQ</td>
<td>HCV RNA detected but below LLOQ</td>
<td>Standard</td>
<td>SVR, confirmation recommended</td>
</tr>
<tr>
<td>TND</td>
<td>HCV RNA is not detected</td>
<td>Shortened</td>
<td>SVR</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; LI4W4-week pegylated interferon alfa and ribavirin treatment lead-in; LLOQ, lower limit of quantification; RGT, response-guided therapy; SVR, sustained virologic response; TD, target detected; TND, target not detected; W4, week 4; W8, week 8; W12, week 12.

* See current prescribing information for telaprevir and boceprevir for additional details on the recommended patient populations for RGT, as well as other important HCV RNA considerations (eg, treatment futility criteria) [10, 16].

With the previous P/R-only regimen, patient response to treatment was best predicted by HCV on-treatment RNA measurements at week 4 and week 12 (W4 and W12) [31]. For clinical trials with a P/R regimen including telaprevir or boceprevir, virologic responses at W4U_TND, W12U_TND, and LI4W, W8U_TND, LI4W16U_TND, respectively, were still among the most consistent predictors of SVR (U: unquantifiable; LI4W: 4-week P/R lead-in) [5, 11–14]. All boceprevir and telaprevir phase 3 trials were assayed with the Roche COBAS HPS TaqMan HCV Test 2.0, with RGT decisions to shorten treatment based on TND readings.

Reanalysis of the boceprevir (P05216, SPRINT-2) and telaprevir (C216, REALIZE and 108, ADVANCE) phase 3 trial data by Harrington et al addressed whether clinical outcomes based on HCV RNA TD readings were distinct from TND readings [18]. Harrington et al showed that 28% of telaprevir-treated patients registered W4UTD, whereas 17% of boceprevir-treated patients had HCV RNA levels LI4W8U_TD [24]. In both situations, these patients had a 5%–20% lower SVR rate than patients with HCV RNA W4U_TND/LI4W8U_TND, indicating that TD likely reflects a reduced on-treatment virologic response relative to TND.

Harrington et al’s results demonstrate that during treatment, TD but below the LLOQ and TND results are not
equivalent. Thus for optimal treatment efficacy for the approved DAAs, only W4U_{TND} and W12U_{TND} (telaprevir) and L151W,W8U_{TND} (boceprevir) readings should be considered for a shorter duration of treatment, as this is how the RGT approaches were validated in clinical trials (see current prescribing information for telaprevir and boceprevir for additional details on the recommended patient populations for RGT) [10, 16]. Patients with any other HCV RNA readings (TD or >LLOQ) at these timepoints should receive the longer duration regimen to maximize their chances at achieving SVR. Similarly, a confirmed TD reading at EOT is a predictive factor for relapse, and signals likely treatment failure.

However, at follow-up, clinical experience has shown that SVR can be defined as all values below the LLOQ [3]. (Table 1) Viral relapse by follow-up week 12 or 24 is usually obvious and is characterized by a dramatic increase in HCV RNA levels. TD but below the LLOQ readings at follow-up week 12 or later, however, are considered to reflect continued viral suppression or clearance, and not necessarily continued active infection, and are often not reproducible [18, 32]. Such a result would be best confirmed by a repeat or subsequent HCV RNA test.

CONCLUSIONS

Although clinical trials utilizing the Roche COBAS HPS TaqMan HCV Test 2.0 clearly demonstrate that treatment duration decisions for P/R plus boceprevir/telaprevir are ideally based on achieving HCV RNA TND at the prescribed RGT decision timepoints, multiple questions remain. For example, other clinical trials of HCV DAAs (with or without P/R) base RGT decisions on achieving HCV RNA below the LLOQ at early on-treatment timepoints. Will similar SVR and relapse rates be observed for patients with HCV RNA TD but below the LLOQ compared to those with HCV RNA TND at RGT timepoints? Will this be regimen-dependent? Also, if HCV RNA TND/TD is the optimal HCV RNA cutoff for RGT decisions when using a Roche COBAS HPS TaqMan assay, can LLOQ be used as the HCV RNA cutoff for RGT decisions when using the Abbott RealTime assay, as suggested by Fefery et al [28]? Regardless of how this story unfolds, having a thorough understanding of the assay parameters used to report HCV RNA results, while using precise and consistent assay terminology when describing such results, will be critical to ensure optimal RGT approaches in clinical practice.

It is remarkable to imagine that just over a decade ago, SVR for HCV genotype 1 with interferon monotherapy averaged 15%–20%, rose to >50% upon implementation of pegylated interferon and ribavirin, and with the recent introduction of boceprevir and telaprevir, has risen to rates around 70% for certain patient populations [11, 13, 33–35]. To further demonstrate the rapid evolution of the field, many current clinical trials of multiple DAA plus P/R or DAA-only regimens do not employ RGT at all given the consistency of early responses to treatment. Foretelling a future without P/R, current phase 2 and 3 clinical trials determining the efficacy of next-generation DAAs report some DAA-only cocktails achieving SVR rates >90% within even shorter treatment periods [36, 37]. With boceprevir and telaprevir functioning as the bridge to next-generation DAA plus P/R regimens, and ultimately to an interferon-free future, the validated assay thresholds and standard terminology discussed here will serve to solidify the foundation of the current, but hardly final, standard of care for HCV-infected patients.

Notes

Acknowledgments. We thank Rob J. Besaw, Gavin Cloherty, Bryan Cobb, Gabrielle Hellek, Lisa Naeger, Jules O’Rear, and Kathleen Whitaker for helpful manuscript suggestions and discussion.

Disclaimer. The views expressed in this report are those of the authors and do not necessarily represent official policy of the US Food and Drug Administration.

Financial support. Staff work involved in the writing of the manuscript (E. L., N. M., and V. M.) was supported by unrestricted grants received by the Forum for Collaborative HIV Research for the HCV Drug Development Advisory Group Project.

Potential conflicts of interest. All authors: No reported 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