Diagnostics in hepatitis C: The end of response-guided therapy?

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Summary

On-treatment hepatitis C virus (HCV) RNA has been used to predict response to interferon (IFN)-based therapy. The concept of response-guided treatment (RGT) was established to determine optimal treatment duration and to early identify patients not responding to futile therapies. RGT helped to improve sustained virologic response (SVR) rates and lower the rates of adverse effects. RGT was of particular importance for telaprevir- and boceprevir-based triple therapies. RGT strategies are dependent on highly sensitive and reproducible HCV RNA quantification. However, different HCV RNA assays are used in routine clinical practice and these differ significantly in their performance characteristics. The development of IFN-free therapies has fundamentally changed the role of on-treatment HCV RNA for SVR prediction. Given the high efficacy and excellent tolerability of IFN-free regimens, the interest in treatment individualization has decreased. However, shorter treatment durations may still be desirable, particularly with respect to the high costs of current IFN-free direct-acting antiviral agents (DAAs). Moreover, some difficult-to-treat patients remain, e.g., those infected with HCV genotype 3 in whom the current standard of care may not always be sufficient to achieve SVR, especially in treatment-experienced patients with cirrhosis. Here, a RGT extension may be feasible. However, current data on the predictive value of on-treatment HCV RNA are limited and have shown conflicting results. As more potent DAAs become available, the role of response prediction may diminish further. Currently, shorter treatment duration is only based on baseline HCV RNA whereas no RGT strategy is recommended for any of the approved DAA regimens available.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that can progress to cirrhosis and hepatocellular carcinoma [1]. Chronic HCV infection accounts for approximately 500,000 deaths each year [2]. Following the discovery of HCV in the late 1980s, development of molecular methods for the detection of nucleic acids was a milestone towards successful treatment of HCV infection [3]. Until today, direct detection and quantification of viral nucleic acid (HCV RNA) is generally regarded as the definite diagnostic criterion to document active HCV infection, regardless of the presence of anti-HCV antibodies and/or elevated liver enzymes [4,5]. Moreover, the primary goal of HCV therapy is the achievement of a sustained virologic response (SVR), defined as undetectable HCV RNA by a sensitive assay 12 or 24 weeks after treatment completion [5].

Qualitative assays were the first nucleic acid assays available. However, with these assays only the presence or absence of active HCV infection can be confirmed. Development of quantitative assays also allowed for on-treatment response monitoring and SVR prediction. This became an integral part of pegylated interferon (PegIFN)-based treatment, which was the mainstay of antiviral therapy until 2013 [6]. On-treatment HCV RNA levels were used to determine optimal treatment duration and to early identify patients not responding to antiviral therapy who were advised to stop treatment due to futility [7]. The paradigm of response-guided treatment (RGT) also became a key concept of HCV therapy following the approval of first generation HCV protease inhibitors (PIs) in 2011. With PI-based triple therapies, higher SVR rates could be achieved using shorter treatment durations compared with PegIFN/RBV alone [8–11]. RGT was further facilitated by the increase in sensitivity and accuracy of real-time PCR-based assays. Today, highly sensitive assays with a limit of detection (LOD) ≤15
International Units (IU)/ml are universally recommended for monitoring treatment response [5]. However, several quantitative nucleic acid tests with different sensitivities and different ranges of quantification are commercially available [12]. These differences in performance characteristics must be addressed when comparing assay results in a given clinical setting.

PegIFN-based dual and triple therapies are associated with severe side effects that can sometimes even be fatal [12–15]. The concept of RGT was implemented not only to increase SVR but also to shorten treatment, which may be associated with better tolerability and patient compliance. In 2014, the first IFN-free regimens became available. Currently, IFN-free treatment with direct-acting antiviral agents (DAAs) is the standard of care in many countries. These treatments are generally safe and well tolerated, and viral eradication can be achieved in the vast majority of patients [16]. DAA treatment durations are mostly short, ranging from 8–24 weeks only, with most patients achieving undetectable HCV RNA relatively early during antiviral therapy. This has fundamentally changed the role of on-treatment HCV RNA monitoring. Indeed, the original concept of RGT is now very much in question as on-treatment HCV RNA may no longer be used to increase SVR significantly. Instead, RGT may still be useful for other reasons, in particular for its cost-saving potential.

In this review we discuss the role of on-treatment HCV RNA quantification to guide treatment duration in light of the therapeutic advances that have evolved over the past years.

**Virologic tools for HCV RNA quantification**

Molecular assays for HCV RNA detection and quantification are routinely used to diagnose and monitor treatment of patients with chronic HCV infection. These assays have evolved over the past 20 years in parallel with the tremendous advances in the therapeutic field. Currently, several HCV RNA assays are commercially available that use different combinations of amplification and detection methods (Table 1). These include signal amplification techniques, such as branched DNA amplification, and target amplification techniques, such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) [12,17].

**Real-time PCR technology**

The classic PCR technique involves thermal cycling and a thermostable DNA polymerase that has both reverse transcriptase and polymerase activity. During the process, HCV RNA is transcribed into complimentary DNA, which serves as a template in the PCR reaction. The number of DNA copies is doubled with each PCR cycle. However, the number of amplicons can only be analysed at the end of the PCR reaction. Quantification is based on competitive amplification of the HCV target sequence and a known amount of a quantification standard that is added to each reaction tube.

The limitations of classic end-point PCR assays, such as relatively low sensitivity, narrow dynamic range and lack of automation have been largely overcome with the advent of real-time PCR technology [18–20]. Real-time PCR relies on the detection and quantification of a fluorescent reporter that is linked to a quencher and annealed to the target sequence. The reporter signal is released during each PCR cycle and increases in direct proportion to the amount of the PCR product. By measuring the amount of fluorescence emission at each cycle, it is possible to collect the PCR data during the exponential growth phase in real-time as opposed to end-point detection.

**Commercially available real-time HCV RNA assays**

Several real-time PCR assays are commercially available. The two most widely used assays are the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM; Roche Diagnostics, Rotkreuz, Switzerland) and the Abbott RealTime HCV assay (ART; Abbott Molecular, Des Plaines, IL, USA) as well as the COBAS TaqMan for use with the High Pure System (HPS/CTM; Roche Diagnostics), which is used in most clinical trials, are discussed herein. All three assays have received Conformité Européenne (CE) marking and United States Food and Drug Administration (FDA) approval for monitoring HCV RNA during antiviral therapy.

For assay standardization purposes, a WHO international standard for HCV was established. The standard comprises genotype 1a HCV RNA positive plasma and has been calibrated in IU/ml [21]. All assays are calibrated against this standard, which is currently available in its 5th version (https://www.nibsc.org/documents/ifu/14-150.pdf). However, despite calibration to the standard material, significant differences between assays have been reported, i.e., lower quantification by ART and higher quantification by CAP/CTM [22,23]. This may result from different reasons, including interindividual and genotype-specific sequence variability, even within the highly conserved 5'-UTR which commonly serves as the primer binding region, and differences in assay properties, such as the internal control [23,24].

The COBAS TaqMan HCV assay was the first real-time PCR assay to be approved for the guidance of antiviral therapy. The assay is highly sensitive and linear over a broad dynamic range [25,26]. A common misconception is that there is only one COBAS assay available. However, for HCV RNA extraction either the manual HPS viral nucleic acid kit that uses glass fibre columns or the fully automated COBAS AmpliPrep (CAP) instrument that uses magnetic
microparticles for the purification of nucleic acids are combined with the COBAS TaqMan assay (HPS/CTM and CAP/CTM, respectively). Each assay has distinct performance characteristics with regard to the LOD and dynamic range of HCV RNA quantification (Table 1). The HPS/CTM assay has been commonly used in clinical trials, particularly in most approval studies involving DAAs. In these studies, an HCV RNA result <25 IU/ml has been used to determine on-treatment and post-treatment response [8–11, 27–35]. With the first version of the fully automated CAP/CTM assay, significant underestimation of clinical genotype 4 samples was reported [36]. However, with use of a new dual probe design, the following second version has been shown to accurately quantify all 6 genotypes over the full dynamic range [24, 26, 37].

The ART assay is also highly sensitive and shows a linear quantification range across all 6 genotypes [23, 38]. Interestingly, the assay is more sensitive around the lower limit of quantification (LLOQ) when compared to the CAP/CTM and HPS/CTM assays. This has led to the observation that many on-treatment or even post-treatment specimens that had results reported as “target not detected” by HPS/CTM or CAP/CTM showed detectable/not quantifiable or even quantifiable results by the ART assay [22, 39–42].

**Lower limit of quantification and limit of detection**

As mentioned above, HCV RNA <25 IU/ml assessed with HPS/CTM has been widely used to determine on-treatment and post-treatment response in clinical trials. However, while 25 IU/ml represents the lowest HCV RNA level that is within the linear range of the HPS/CTM assay (LLOQ), it is not the lowest possible HCV RNA result that can be detected by this assay. Any positive PCR signal below the LLOQ will be reported as “detectable, <25 IU/ml” or detectable/not quantifiable and this also holds true for the other available assays with their respective cut-offs (Table 2). Thus, HCV RNA results can be reported in three different ways with regard to the lower end of quantification: i) quantifiable, showing a specific number in IU/ml within the linear range of the respective assay; ii) detectable/not quantifiable, representing any detectable result that lies below the LLOQ (i.e., <25 IU/ml for HPS/CTM; <15 IU/ml for CAP/CTM; <12 IU/ml for ART), and iii) an undetectable HCV RNA result, also known as “target not detected”, with no PCR amplification possible (Fig. 1).

The lower limit of detection (LOD) represents a statistical threshold. That is, the lowest HCV RNA level detected >95% of the time as a positive PCR signal. It is important to note that HCV RNA levels below the LOD may therefore still be detectable in <95% of the time. Thus, in samples with HCV RNA levels below the respective assay LOD it can simply be a matter of chance, whether or not HCV RNA is reported to be detectable or undetectable. CAP/CTM, for example, has a LOD of 15 IU/ml but it has been shown to detect HCV RNA levels of 2.5 IU/ml in approximately 50% of the time, which will then be reported as “detectable/not quantifiable (<15 IU/ml)” [26, 43]. Similarly, in one study that assessed on-treatment HCV RNA of patients undergoing telaprevir-based triple therapy, samples were tested in triplicate by the first and second version of

**Table 1. Commercially available real-time HCV RNA assays.**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Extraction device</th>
<th>Amplification device</th>
<th>IVD approval status</th>
<th>Limit of detection (LOD) in IU/ml</th>
<th>Range of quantification in IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>COBAS TaqMan HCV Test v2.0</td>
<td>Roche molecular diagnostics</td>
<td>High pure system (manual)</td>
<td>COBAS TaqMan</td>
<td>CE (Europe) FDA (USA)</td>
<td>8.8–9.3 (GT1; Europe) 20 (all GTS; USA)</td>
<td>25–3.91×10⁸ (GT1; Europe) 25–3×10⁹ (all GTS; USA)</td>
</tr>
<tr>
<td>COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 (CAP/CTM)</td>
<td>Roche molecular diagnostics</td>
<td>COBAS AmpliPrep</td>
<td>COBAS TaqMan</td>
<td>CE (Europe) FDA (USA)</td>
<td>15</td>
<td>15–1.0×10⁸</td>
</tr>
<tr>
<td>RealTime HCV</td>
<td>Abbott molecular</td>
<td>m2000SP</td>
<td>m2000RT</td>
<td>CE (Europe) FDA (USA)</td>
<td>12</td>
<td>12–1.0×10⁹</td>
</tr>
<tr>
<td>Artus HCV QS-RGQ assay</td>
<td>Qiagen</td>
<td>QiAsymphony SP/AS</td>
<td>Rotor-Gene Q</td>
<td>CE (Europe)</td>
<td>36.2</td>
<td>67.6–17.7×10⁶</td>
</tr>
<tr>
<td>Versant HCV 1.0 kPCR Assay</td>
<td>Siemens healthcare</td>
<td>kPCR sample Prep</td>
<td>kPCR amplification and detection (AD) module</td>
<td>CE (Europe)</td>
<td>15</td>
<td>15–1.0×10⁸</td>
</tr>
<tr>
<td>Xpert HCV Viral Load</td>
<td>Cepheid</td>
<td>GeneXpert system</td>
<td>CE (Europe)</td>
<td>4–6.1</td>
<td>10–1.0×10⁹</td>
<td></td>
</tr>
<tr>
<td>Aptima HCV Quant Dx Assay</td>
<td>Hologic</td>
<td>Panther system</td>
<td>CE (Europe)</td>
<td>3.9–4.3</td>
<td>10–1.0×10⁹</td>
<td></td>
</tr>
<tr>
<td>VERIS HCV Assay</td>
<td>Beckman coulter</td>
<td>DrN VERIS molecular diagnostics system</td>
<td>CE (Europe)</td>
<td>12</td>
<td>12–1.0×10⁹</td>
<td></td>
</tr>
<tr>
<td>COBAS HCV test</td>
<td>Roche molecular diagnostics</td>
<td>COBAS 6800/8800 systems</td>
<td>CE (Europe)</td>
<td>10</td>
<td>15–1.0×10⁹</td>
<td></td>
</tr>
</tbody>
</table>

CE, Conformité européenne; FDA, Food and Drug Administration; GT, genotype; IVD, in vitro diagnostics.
The majority of treatment-naive patients with HCV genotype 1 were eligible to receive a shorter course of protease inhibitor-based therapy based on attaining an extended rapid virologic response. The likelihood of achieving SVR to treatment with PegIFN/RBV is directly related to when the patient becomes HCV RNA undetectable during therapy [7]. Patients with HCV genotype 1, 2 or 3 infection, who became HCV RNA undetectable within 4 weeks after initiating treatment (defined as a rapid virologic response; RVR) had high SVR rates in the range of 86–88% compared to patients who only cleared the virus at treatment week 12 (SVR: 54–68%) [45]. Based on a combination of low baseline viral load (<600–800,000 IU/ml) and attainment of RVR, patients can be identified who qualify for shorter-than-standard treatment durations: Patients who meet both criteria and who do not have any negative response predictors at baseline (e.g., cirrhosis or diabetes) can receive 16 or 24 weeks of treatment in HCV genotypes 2/3 and 1, respectively, instead of the standard durations of 24 and 48 weeks, without compromising SVR rates [46–50]. Measurement of on-treatment HCV RNA at later time points during treatment has been mostly used to predict futility [51,52]. In contrast, it was also shown that patients with a >2 log decline at treatment week 12 but detectable HCV RNA benefit from treatment extension to 72 weeks [53–55].

The role of HCV RNA quantification during protease inhibitor-based triple therapy

Following the approval of the first two HCV PIs, telaprevir (TVR) and boceprevir (BOC) in 2011, the concept of RGT was readily incorporated as a central element in treating patients with HCV genotype 1 infection. With these new therapies, SVR rates were significantly higher compared to PegIFN/RBV alone (67–75% vs. 40–44% in treatment-naive patients) [9,10]. The TVR/BOC-specific RGT paradigm was based on phase III data that had shown in treatment-naive patients and those who had experienced relapse following a prior course of PegIFN/RBV therapy that duration of therapy could be safely shortened to 24 weeks (TVR) or 28 weeks (BOC) provided the patient achieved undetectable HCV RNA at week 4 and 8 through week 12 and 24 of triple therapy with TVR and BOC, respectively [6,10,56]. Given the relatively high antiviral activity of the new PIs, shorter treatment durations are applicable in 44–65% of patients [10,56]. In a retrospective analysis of the TVR and BOC registration trials, patients who had detectable/ <25 IU/ml HCV RNA measured by HPS/CTM at week 4, had an approximately 20% lower SVR rate compared to patients with undetectable HCV RNA. Thus, eligibility for shortened therapy should be based on attainment of undetectable HCV RNA at week 4 only, whereas patients with detectable/not quantifiable (<25 IU/ml) HCV RNA should receive a full course of therapy [43]. However, the HPS/CTM assay that was used in these trials has been shown to be less sensitive compared to CAP/CTM and ART, which are mostly used in clinical routine [39,44,57]. Indeed, some data suggest that assay-specific RGT criteria may be required given the significant performance differences among HCV RNA assays. In one study, week 4 samples of patients undergoing response-guided triple therapy with TVR using the CAP/CTM assay were re-tested with ART. In this study, 58% of patients who received shortened treatment based on RGT criteria using the CAP/CTM assay had detectable HCV RNA according to the ART assay and all but one of these patients achieved SVR. Thus, an undetectable HCV RNA result obtained with the ART assay should not be a requirement for shortened treatment durations as this will likely lead to overtreatment in a significant portion of patients, and an HCV RNA result <12 IU/ml at week 4 may therefore be the better threshold [42].

By implementing RGT guidelines and stopping rules to patients who are treated with TVR- or BOC-based triple therapies, potentially serious adverse effects and high costs of futile treatments can be avoided. However, correct use of such criteria requires close patient monitoring and knowledge of the performance characteristics and limitations of the assay in use. Not surprisingly, adherence to RGT guidelines was less than 33%, and adherence to stopping rules was less than 50% in a recent retrospective real-world analysis [58].

In 2014, the second-generation NS3 PI simeprevir (SMV) was approved for the treatment of patients with HCV genotype 1 or 4 infection. In patients treated with SMV and PegIFN/RBV, all medication should be stopped if HCV RNA levels are >25 IU/ml at week 4 [59–61]. As seen with the first generation PIs, SVR rates were approximately 30% lower in patients who had detectable/ <25 IU/ml HCV RNA at week 4 when compared to those with an undetectable viral load [60–62]. In a retrospective analysis of week 4 results, substantially
different RVR rates were observed among the three widely used assays, HPS/CTM, CAP/CTM, and ART. However, patients with undetectable HCV RNA at week 4 had SVR rates >90%, regardless of the assay in use [63].

The IFN-free era: Is there a need for individualized treatments or does one size fit all?

The approval of IFN-free therapies has fundamentally changed the role of RGT. An important step towards HCV eradication is the overall high antiviral efficacy of these new regimens. In the respective phase 3 studies, almost all patients achieved HCV RNA <LLOQ at some point during treatment, often within the first 4 weeks of therapy [27,28,30,32,34,64–69]. SVR rates >90% have now become reality for the majority of patient subgroups. As virological failures during therapy are rare, no stopping criteria have yet been established. Moreover, these regimens are far better tolerated than any of the former IFN-containing treatments [14,33,70,71]. Thus, there seems to be no major interest in shortened treatment durations with respect to drug safety. However, despite the good tolerability and high efficacy, individualized treatment durations may still be beneficial for various reasons (Fig. 2):

1) Reducing treatment cost
   From an economic perspective, short DAA treatments are highly desirable. Indeed, the high costs of DAA treatments and their potential impact on the access to HCV care have sparked an intensive debate. Unlike IFN-based therapies, there are rarely absolute contraindications to the currently approved DAAAs [5]. Thus, the number of patients eligible to receive HCV treatment has dramatically increased in the past two years [71]. In order to address this economic challenge, access to DAA therapy has been restricted to patients with advanced liver diseases or significant extrahepatic manifestations in many countries. However, different epidemiological models have clearly shown that only a significant increase in treatment uptake can achieve a meaningful reduction in HCV prevalence and HCV associated morbidity and mortality. Thus, treatment of all HCV patients should be considered but may only be realistic if treatments costs can be reduced significantly [72,73].

2) Preventing DAA side effects:
   Although DAA-related side effects are infrequent and usually mild to moderate, this does not mean that they are entirely absent. Thus, shorter DAA treatments may even be desirable with regard to treatment safety, particularly in the presence of advanced cirrhosis. There is at least some evidence that SOF-based treatments may be associated with increased adverse effects in patients with advanced liver disease [74]. Reports of hepatic decompensations during treatment with omibitasvir (NS5A inhibitor; OBT), paritaprevir (NS3 PI; PTV/ritonavir (r)) and dasabuvir (non-nucleoside NS5B inhibitor; DSV) in patients with advanced cirrhosis have even led to a post-marketing label change that restricts this regimen to patients with compensated liver disease only [5,75].

3) Limiting the risk of drug-drug-interactions:
   The challenge of drug-drug interactions (DDIs) should be addressed while considering treatment safety. Recent data show that up to 66% of HCV patients can be at considerable risk for DDIs if treated with one of the available IFN-free regimens [76]. The risks for DDIs may increase with longer treatment durations, in particular if due to the good tolerability treatment access is expanded to patients with several co-morbidities.

Thus, even with current DAA regimens, treatment duration should be as short as possible to avoid unnecessary drug exposure. With current DAAs the standard treatment duration is 12 weeks for the majority of patients. For PegIFN-based regimens the optimal treatment duration as well as the concept of a RGT was widely based on viral kinetics modeling estimating the time until viral eradication could be reached [77]. In contrast, there is no scientific base to assume that a 12-week treatment duration is necessary using IFN-free DAA regimens. Indeed, there is increasing evidence that a 12-week regimen with two or more DAA represents overtreatment in a significant number of patients. Easy-to-treat patients, i.e., those without cirrhosis and no previous treatment failure, may require only 8 weeks of treatment without compromising SVR rates [32,65] and even shorter treatment durations may be possible in well-selected patients.
At present, only an eight-week regimen of ledipasvir (NS5A inhibitor; LDV) in combination with sofosbuvir (NS5B polymerase inhibitor; SOF) is approved. However, this treatment was not established based on RGT criteria. Eight weeks of LDV/SOF is recommended for treatment-naive, non-cirrhotic patients with genotype 1 infection provided they have a viral load <6 million IU/ml at baseline [78]. The label was based on a post-hoc analysis of patients treated for 8 weeks in the ION-3 study which showed that only an HCV RNA concentration below 6 million IU/ml at baseline was associated with a low rate of relapse that was comparable to the overall study population [32]. This label has been controversially discussed as it lacks a scientific basis [80]. Moreover, the cut-off may show significant variation depending on the assay that is used [79]. Interestingly, “Real-world” data suggest that in patients who receive 8 weeks of LDV/SOF without consideration of assay differences and without strict adherence to the cut-off show comparable SVR rates to the SVR seen in the ION-3 study in the subgroup of patients with baseline HCV RNA <6 million IU/ml [81]. Robust criteria to select patients that qualify for shorter treatment still need to be better defined. However, these data also clearly show that shorter treatment regimens are certainly possible in a large proportion of the patient population.

On the other hand, there are still some patients who may benefit from extension of treatment duration or addition of ribavirin. This seems to be particularly true in patients with HCV genotype 3 infection and those with decompensated cirrhosis [82–84]. For example, in cirrhotic patients with HCV genotype 3 who also failed a prior course of PegIFN/RBV therapy, triple therapy with daclatasvir (NS5A inhibitor, DCV) and SOF plus addition of RBV for 24-week is recommended [5]. Achieving SVR in these patients with the first course of IFN-free treatment is of particular importance as patients who fail a first course of DAA therapy are at high risk of developing resistance-associated variants (RAVs). This holds particularly true for patients who fail an NS5A inhibitor-containing regimen as these drugs have a relatively low barrier to resistance. NS5A RAVs can be found in the majority of treatment failures and these RAVs may persist for several years thereafter, thereby limiting re-treatment options [85,86]. While the presence of baseline RAVs may not have a significant influence on overall SVR chances to a first course of DAA therapy, few data exist on their impact on re-treatment in patients who failed a DAA treatment [87,88]. Thus, failure to DDAs should be avoided by choosing the best regimen and treatment duration available for each individual patient, particularly in the presence of additional negative response predictors such as cirrhosis and prior failure to PegIFN-based therapy.

In conclusion, a fixed treatment duration (“one size fits all”) may either lead to overtreatment in a significant proportion of easy-to-treat patients whereas the risk of treatment failure may be increased in more difficult-to-treat patients (Fig. 3). Individualized treatment durations have the potential to reduce treatment costs and increase SVR rates. However, at present, the level of treatment individualization is relatively low with respect to IFN-free DAA therapies. On-treatment HCV RNA may have the potential to add an important aspect towards more individualized treatment durations.

**HCV RNA quantification for the prediction of SVR during IFN-free therapy**

RGT strategies have not (yet) been established for IFN-free DAA therapies. Accordingly, on-treatment HCV RNA measurements are not recommended in the respective DAA package inserts. Despite this, the European Association for the Study of the Liver (EASL) treatment guidelines recommend quantitative HCV RNA testing at treatment weeks 2, 4 and at the end of antiviral therapy in order to ensure patient compliance and treatment efficacy [5]. However, these guidelines neither provide stopping rules nor criteria for a response-guided modification of treatment duration based on results generated at the respective on-treatment time points [5]. It could be argued that viral load testing may be too cost intensive to simply document treatment adherence and/or on-treatment efficacy, particularly if it is of no consequence to the patient. For this purpose, the cheaper HCV core antigen (HCV Ag) test may be of clinical use [89,90]. However, as the sensitivity of the HCV Ag assay is comparably low, RGT strategies may not be performed with this assay [90]. For this purpose, HCV RNA is likely to be more useful. Implementation of RGT algorithms to further individualize and optimize DAA treatments would, however, require a meaningful predictive value of on-treatment response (reflected by HCV RNA levels).
for SVR. Overall, data regarding the association between on-treatment HCV RNA levels during IFN-free therapies and SVR are still relatively scarce and viral kinetics that were assessed in many of the DAA approval studies were often incompletely studied. In the following, available data on on-treatment HCV RNA for SVR prediction in DAA regimens will be summarized.

HCV RNA quantification for the prediction of SVR during sofosbuvir/ribavirin treatment

The combination of SOF and RBV was the first IFN-free therapy that was approved for the treatment of HCV infection. On-treatment HCV RNA levels seem to be predictive of SVR with this regimen as long as there is a significant risk of treatment failure in a given patient cohort resulting in a considerable number of relapers.

SOF/RBV given for 24 weeks was used directly after SOF-approval in patients with chronic HCV genotype 1 infection and urgent need for immediate therapy. This regimen is now considered too ineffective for genotype 1 patients as SVR could be achieved in only 50–70% [40,41,91,92]. However, we found that on-treatment HCV RNA levels at weeks 1, 2 and 4 were significantly higher in relapers compared to patients with SVR. The greatest difference in HCV RNA levels between patients with relapse and those who achieved SVR was found at treatment week 2 [40] (Table 3). In another study, patients with an undetectable HCV RNA with the ART assay or HCV RNA <LLOQ with the CAP/CTM assay at treatment week 4 had higher chances to achieve SVR (88% vs. 61% and 70 vs. 50%, respectively). However, this was not statistically significant. HCV RNA results at week 2 were not investigated in this study [41] (Table 3).

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In the era of IFN-free treatments, HCV genotype 3 has evolved as the most difficult genotype to cure [93]. In the VALENCE study, genotype 3 patients were treated with SOF/RBV for 24 weeks and achieved an overall SVR rate of 85%. However, among those who were treatment-experienced and also had cirrhosis, the SVR rate was only 62% [35]. A post-hoc analysis found that SVR rates varied for SVR. Overall, data regarding the association between on-treatment HCV RNA levels during IFN-free therapies and SVR are still relatively scarce and viral kinetics that were assessed in many of the DAA approval studies were often incompletely studied. In the following, available data on on-treatment HCV RNA for SVR prediction in DAA regimens will be summarized.

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SOF/RBV given for 24 weeks was used directly after SOF-approval in patients with chronic HCV genotype 1 infection and urgent need for immediate therapy. This regimen is now considered too ineffective for genotype 1 patients as SVR could be achieved in only 50–70% [40,41,91,92]. However, we found that on-treatment HCV RNA levels at weeks 1, 2 and 4 were significantly higher in relapers compared to patients with SVR. The greatest difference in HCV RNA levels between patients with relapse and those who achieved SVR was found at treatment week 2 [40] (Table 3). In another study, patients with an undetectable HCV RNA with the ART assay or HCV RNA <LLOQ with the CAP/CTM assay at treatment week 4 had higher chances to achieve SVR (88% vs. 61% and 70 vs. 50%, respectively). However, this was not statistically significant. HCV RNA results at week 2 were not investigated in this study [41] (Table 3).

In the era of IFN-free treatments, HCV genotype 3 has evolved as the most difficult genotype to cure [93]. In the VALENCE study, genotype 3 patients were treated with SOF/RBV for 24 weeks and achieved an overall SVR rate of 85%. However, among those who were treatment-experienced and also had cirrhosis, the SVR rate was only 62% [35]. A post-hoc analysis found that SVR rates varied for SVR. Overall, data regarding the association between on-treatment HCV RNA levels during IFN-free therapies and SVR are still relatively scarce and viral kinetics that were assessed in many of the DAA approval studies were often incompletely studied. In the following, available data on on-treatment HCV RNA for SVR prediction in DAA regimens will be summarized.

HCV RNA quantification for the prediction of SVR during sofosbuvir/ribavirin treatment

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Table 3. Predictive value of on-treatment HCV RNA results during IFN-free therapy.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Genotype</th>
<th>Treatment duration</th>
<th>Study/author</th>
<th>Study population</th>
<th>Overall SVR rate</th>
<th>SVR prediction by on-treatment HCV RNA response (selected cut-offs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>24 weeks</td>
<td>Steinebrunner et al. [92]</td>
<td>All patients (n = 12)</td>
<td>50%</td>
<td>Week 4: &lt;LLOQ/≥LLOQ: 67%/33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sidharthan et al. [41]</td>
<td>All patients (n = 55)</td>
<td>69%</td>
<td>Week 4: CTM v.1.0: &lt;LLOQ/≥LLOQ: 70%/50% TND vs. detectable: 70%/68% ART: &lt;LLOQ/≥LLOQ: 71%/67% TND vs. detectable: 88%/61%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maasoumy et al. [40]</td>
<td>All patients (n = 31)</td>
<td>52%</td>
<td>Week 1: CTM: &lt;225 IU/ml vs. ≥225 IU/ml: 93%/8% ART: &lt;270 IU/ml vs. ≥270 IU/ml: 93%/8% Week 2: CTM: &lt;35 IU/ml vs. ≥35 IU/ml: 93%/13% ART: &lt;70 IU/ml vs. ≥70 IU/ml: 93%/13% Week 4: CTM: TND vs. detectable: 77%/35% ART: &lt;LLOQ vs. ≥LLOQ: 92%/28%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12 weeks</td>
<td>FISSION + POSITRON, Wyles et al. [96]</td>
<td>All patients (n = 176)</td>
<td>96%</td>
<td>Week 1: TND/detectable: 100%/96% Week 2: &lt;LLOQ/≥LLOQ: 97%/85% Week 4: TND/detectable: 98%/83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FUSION, Wyles et al. [96]</td>
<td>Prior PegIFN/RBV failure (n = 36)</td>
<td>86%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 90%/85% Week 2: &lt;LLOQ/≥LLOQ: 87%/83%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VALENCE, Zeuzem et al. [94]</td>
<td>All patients (n = 73)</td>
<td>93%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 88%/96% Week 2: &lt;LLOQ/≥LLOQ: 90%/100% Week 4: TND/detectable: 93%/92%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 weeks</td>
<td>Maasoumy et al. [40]</td>
<td>All patients (n = 32)</td>
<td>88%</td>
<td>Week 1: CTM: &lt;70 IU/ml vs. ≥70 IU/ml: 93%/63% ART: &lt;70 IU/ml vs. ≥70 IU/ml: 92%/67% Week 2: CTM: &lt;LLOQ vs. ≥LLOQ: 90%/83% ART: &lt;LLOQ vs. ≥LLOQ: 94%/80% Week 4: CTM: TND vs. detectable: 91%/75% ART: TND vs. detectable: 88%/86%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FUSION, Wyles et al. [96]</td>
<td>Prior PegIFN/RBV failure (n = 32)</td>
<td>94%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 100%/93% Week 2: &lt;LLOQ/≥LLOQ: 96%/75% Week 4: TND/detectable: 96%/83%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12 weeks</td>
<td>FISSION + POSITRON, Wyles et al. [96]</td>
<td>All patients (n = 272)</td>
<td>60%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 72%/50% Week 2: &lt;LLOQ/≥LLOQ: 62%/38% Week 4: TND/detectable: 62%/48%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FUSION, Wyles et al. [96]</td>
<td>Prior PegIFN/RBV failure (n = 61)</td>
<td>31%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 38%/29% Week 2: &lt;LLOQ/≥LLOQ: 37%/8% Week 4: TND/detectable: 38%/9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 weeks</td>
<td>FUSION, Wyles et al. [96]</td>
<td>Prior PegIFN/RBV failure (n = 63)</td>
<td>62%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 70%/58% Week 2: TND/detectable: 72%/55% Week 4: TND/detectable: 65%/44%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maasoumy et al. [40]</td>
<td>All patients (n = 33)</td>
<td>64%</td>
<td>Week 1: CTM: &lt;270 IU/ml vs. ≥270 IU/ml: 88%/40% ART: &lt;190 IU/ml vs. ≥190 IU/ml: 89%/33% Week 2: CTM: &lt;45 IU/ml vs. ≥45 IU/ml: 100%/33% ART: &lt;60 IU/ml vs. ≥60 IU/ml: 100%/29% Week 4: CTM: TND vs. detectable: 79%/53% ART: &lt;LLOQ vs. ≥LLOQ: 82%/44%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VALENCE, Zeuzem et al. [94]</td>
<td>All patients (n = 248)</td>
<td>86%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 99%/80% Week 2: &lt;LLOQ/≥LLOQ: 90%/61% Week 4: TND/detectable: 91%/67%</td>
</tr>
</tbody>
</table>
significantly depending on the week 1 or week 2 HCV RNA result. Overall, only seven patients (3%) were HCV RNA undetectable at week 1 and all of these patients achieved SVR. However, 76 patients (30%) achieved HCV RNA <LLOQ, while 172 (69%) had a result ≥LLOQ, and SVR rates were 99% (n = 75/76) and 80% (n = 138/172), respectively. Similarly, SVR rates among patients with week 2 HCV RNA <LLOQ achieved SVR in 90% (n = 191/212) but in only 61% (n = 22/38) if HCV RNA was ≥LLOQ [94]. We have reported similar results in a “real-world” cohort. In 33 genotype 3 patients treated with SOF/RBV, HCV RNA levels at week 1 and 2 were significantly lower in patients achieving SVR compared to those with treatment failure. Again, the largest difference was found at week 2. We retrospectively identified optimal week 1 and 2 HCV RNA cut-offs to predict treatment outcome. All patients with HCV RNA <45 IU/ml according to the ART assay achieved SVR, while patients with a week 2 HCV RNA above the respective cut-offs achieved SVR in 33% and 29% only [40] (Table 3).

SOF/RBV is widely considered as standard of care in genotype 2 patients and high SVR rates exceeding 88% have been reported [35,95]. As a result, on-treatment HCV RNA was not predictive of SVR in genotype 2 patients treated with SOF/RBV for 12 weeks in the VALANCE study [94]. This was recently confirmed in a “real-world” study [40]. Data from the FISSION + POSTRON trials suggest that patients with a slower HCV RNA decline during treatment have response rates that are 12–15% lower compared to those with faster HCV RNA elimination [90] (Table 3). However, the overall number of relapers was relatively low in these studies, which limits the impact of HCV RNA decline on SVR prediction.

**HCV RNA quantification for the prediction of SVR during simprevir/sofosbuvir treatment**

In the era of IFN-based treatments, patients with cirrhosis were considered the most difficult-to-cure and treatment was even contraindicated in those with advanced disease. In the OPTIMIST-2 study, patients with HCV genotype 1 infection and cirrhosis were treated with SMV and SOF for 12 weeks and only 83% achieved SVR. The considerable number of virologic failures in this study sparked the interest in on-treatment SVR prediction. However, patients with undetectable HCV RNA at week 4 had only slightly higher SVR compared to those with detectable HCV RNA at this time point (86% vs. 76%) [67] (Table 3). Other studies support the notion that on-treatment HCV RNA alone may not be a good predictor of SVR with this DAA regimen [40,97–99].

**HCV RNA quantification for the prediction of SVR during daclatasvir/sofosbuvir and ledipasvir/sofosbuvir treatment**

Combination of a NSSA inhibitor with SOF has been proven to be a highly efficient treatment option. The fixed combination of LDV/SOF is currently one of the most widely used regimens for HCV genotype 1 infection and high SVR rates can be achieved, even in patients with advanced cirrhosis [100]. In a retrospective analysis of the LDV/SOF ION-1 and ION-2 phase III trials, 224 patients with compen-

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**Table 3 (Continued)**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Genotype</th>
<th>Treatment duration</th>
<th>Study/author</th>
<th>Study population</th>
<th>Overall SVR rate</th>
<th>SVR prediction by on-treatment HCV RNA response (selected cut-offs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOF/RBV</td>
<td>1</td>
<td>12 weeks</td>
<td>OPTIMIST-2, Lawitz et al. [67]</td>
<td>Cirrhosis (n = 103)</td>
<td>83%</td>
<td>Week 4: TND/detectable: 86%/76%</td>
</tr>
<tr>
<td>SOF/RBV + daclatasvir + ribavirin</td>
<td>1, 2, 3 and 4</td>
<td>12 weeks</td>
<td>ALLY-1, Poordad et al. [83]</td>
<td>Advanced cirrhosis (CHILD A-C)</td>
<td>83%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 40%/67%; Week 2: &lt;LLOQ/≥LLOQ: 70%/50%; Week 4: &lt;LLOQ/≥LLOQ: 66%/33%</td>
</tr>
<tr>
<td>SOF/RBV</td>
<td>3</td>
<td>12 weeks</td>
<td>ALLY-3, Kowdle et al. [104]</td>
<td>Cirrhosis (n = 32)</td>
<td>63%</td>
<td>Week 1: &lt;LLOQ/≥LLOQ: 96%/96%; Week 2: &lt;LLOQ/≥LLOQ: 98%/91%; Week 4: TND/detectable: 98%/91%</td>
</tr>
<tr>
<td>SOF/RBV + ledipasvir &amp; ribavirin</td>
<td>1</td>
<td>12 or 24 weeks</td>
<td>ION 1 + 2, Welzel et al. [101]</td>
<td>Only patients with cirrhosis (n = 224)</td>
<td>96%</td>
<td>Week 2: TND/detectable: 96%/96%; &lt;LLOQ/≥LLOQ: 98%/94%; Week 4: TND/detectable: 98%/94%; &lt;LLOQ/≥LLOQ: 96%/98%</td>
</tr>
<tr>
<td>Ombitasvir + paritaprevir + ritonavir + dasabuvir &amp; ribavirin</td>
<td>1</td>
<td>12 or 24 weeks</td>
<td>SAPPHIRE I + II, PEARL II + III + IV, TURQUOISE II, Sulkowski et al. [105]</td>
<td>Without cirrhosis (n = 1650)</td>
<td>98%</td>
<td>SVR by first time HCV RNA TND Week 1: 98%; Week 2: 98%; Week 4: 98%; Week 8: 100%</td>
</tr>
<tr>
<td>Ombitasvir + paritaprevir + ritonavir</td>
<td>1</td>
<td>12 or 24 weeks</td>
<td>SAPPHIRE I + II, PEARL II + III + IV, TURQUOISE II, Sulkowski et al. [105]</td>
<td>Cirrhosis (n = 372)</td>
<td>96%</td>
<td>SVR by first time HCV RNA TND Week 1: 100%; Week 2: 94%; Week 4: 97%; Week 8: 100%</td>
</tr>
</tbody>
</table>
HCV RNA >cut-off
Sensitivity: 70%
Specificity: 70%

Fig. 4. Impact of SVR rates on predictive value of on-treatment HCV RNA. The value of SVR predictors diminishes with the proportion of patients achieving SVR. In this model an on-treatment HCV RNA cut-off is able to identify SVR patients with a sensitivity of 70% and a specificity of 70% if the overall SVR rate is 50%, patients with an on-treatment HCV RNA result below the cut-off would achieve SVR in 70% compared to only 30% among those with an HCV RNA result above the selected cut-off. The difference between the two groups is 40% (relative risk 2.3). This difference diminishes to 32% (relative risk 1.6) and 9% (relative risk 1.1) if the overall SVR rate is 75% and 95%, respectively.

HCV RNA quantification for the prediction of SVR during paritaprevir/ombitasvir + dasabuvir treatment

The combination of ritonavir-boosted PTV/OTB and DSV + RBV is the only approved DAA triple therapy that is intended for patients with HCV genotype 1 infection without decompensated cirrhosis. In a retrospective analysis of the six phase 3 studies comprising more than 2000 patients, time to first undetectable HCV RNA (week 1, 2, 4, 6 or 8) was not associated with SVR. Overall, SVR was achieved in 96% and 98% of patients with and without cirrhosis, respectively [105].

Approach towards a response-guided treatment strategy in the DAA era

The predictive value of on-treatment HCV RNA seems to be limited in the setting of currently available DAA regimens. Importantly, lower SVR rates were only observed in patients with a significant risk for relapse either because they had genotype 3 infection or in case a less effective DAA regimen was used. The value of on-treatment HCV RNA for the prediction of SVR is clearly lower compared to what was seen with PegIFN-containing regimens, mainly because these regimens are so efficacious. Even for some less effective DAA regimens such as SMV in combination with SOF, on-treatment HCV RNA does not seem to be significantly associated with SVR [67]. For any DAA regimen that is given in a fixed duration and which shows SVR rates exceeding 95%, HCV RNA is of no use for response prediction. However, if higher relapse rates do occur, on-treatment HCV RNA may still play a considerable role (Fig. 4). This may be the case if much shorter treatment durations are used (e.g., ≤6 weeks) or if RBV is not added to current DAA regimens in difficult-to-cure patient populations. However, response prediction has not been studied in these patients. Interestingly, recent modeling data suggest that the use of early HCV RNA kinetics may be feasible to individualize treatment duration. Dahari and co-workers retrospectively studied a cohort of 58 patients who had been treated with DCV/SOF, LDV/SOF or SMV/SOF. In this cohort, only one patient relapsed after 12 weeks of LDV/SOF. Based on a modeling analysis, relapse could have been prevented in this patient by one additional...
week of therapy. More interestingly, the authors estimated that treatment could have been shortened in the majority of patients without compromising SVR. Based on the use of early viral kinetics they predicted that 43%, 30% and 13% of patients would have required only 6, 8, and 10 weeks of treatment, respectively [106].

Ferenci and co-workers recently suggested a RGT concept for all patients with cirrhosis treated with SOF and either DCV, SMV or LDV but without the addition of RBV. According to preliminary data from their own center, all cirrhotic patients were treated with only 12 weeks of therapy if HCV RNA at week 8 was undetectable with the ART assay. The remaining patients were treated for 16 to 24 weeks and all patients achieved SVR. However, this was not a randomized study and data have not yet been fully published [107]. In our own study, detectable/<12 IU/ml HCV RNA was frequently seen with the ART assay even at the end of therapy. However, SVR rates were still very high in these patients, which argues against treatment extension due to detectable HCV RNA at late stages of antiviral therapy [40]. Other groups have published similar data [41,108]. It has been speculated that the detection of HCV RNA at the end of therapy may not be infectious virus but residual RNA particles [40,41]. Another possible explanation would be that any remaining virus is cleared by the host’s restored immune system after treatment cessation [109,110]. However, this requires further investigation.

Given the high antiviral efficacy of current DAAs, response prediction should most likely never be based on week 4 viral load, as the majority of patients is already HCV RNA negative at this stage. Instead, earlier time points such as week 2 or even after only a few days of therapy may be more useful for response prediction. Indeed, in our study the highest predictive value for SVR was documented for the HCV RNA result at week 2 of SOF/RBV treatment [40]. In a pilot study from China, viral response was assessed after only 2 days of therapy. Treatment-naïve, non-cirrhotic Chinese patients with HCV genotype 1b infection were assigned to one of 3 highly potent regimens involving 3 DAAs. Patients were treated with either LDV/SOF + asunaprevir (ASV), DCV/SOF + SMV or DCV/SOF + ASV. In patients who achieved a pre-defined cut-off of <500 IU/ml at day 2, all treatment was stopped after only 3 weeks of therapy. This pre-defined goal was achieved by 18/26 (69%) patients and all of these patients achieved SVR. Of note, according to a modeling analysis from the same study, no patient should have been cured in this study as treatment was predicted to be too short to hit the viral cure boundary, defined as <1 virion in extracellular body fluid [111]. Such a RGT strategy should certainly be explored in a larger prospective trial. Treatment duration could be significantly shortened in easy-to-treat patients who have an ultrarapid on-treatment response within the first one to two weeks of therapy. Alternatively, treatment could be started with a cheap and simple DAA regimen, which is later further escalated by longer treatment duration or the addition of more DAAs in case of a suboptimal early treatment response (Fig. 5). However, given the difficulties of response prediction with current viral kinetics models there is still a considerable risk for treatment failure, which may be associated with the emer-

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**Fig. 5. Possible algorithms for response-guided strategies in the DAA era.** Among the easy-to-treat group patients are identified who achieve SVR in >70% if they are treated for 4 or 6 weeks with a potent IFN-free regimen. Selection of patients is based on different baseline parameters like the presence of resistant-associated variants (RAVs), gender or baseline viral load. At day 2 of therapy on-treatment HCV RNA is used to predict SVR. Only patients with a predicted SVR rate >90% continue in the RGT arm. The remaining patients are treated for the standard duration (A). In difficult-to-treat patients a similar approach is used. Patients that may not need RBV or a second DAA or those who might be cured with the standard treatment duration are selected using baseline predictors. At day 2 and week 2 on-treatment HCV RNA is used as response predictor. The respective HCV RNA cut-offs need to identify patients with lower SVR chances in whom a second DAA or RBV can be added or treatment duration needs to be expanded (B).

**Key point**

Preliminary data suggest that very early on-treatment HCV RNA may be used to individualize treatment duration with DAAs.
gance of multidrug-resistant variants. Based on current knowledge, RGT is not recommended with currently approved DAAs and treatment durations should not be altered based on on-treatment HCV RNA levels in a given patient.

**Conclusion – Is this the end of response-guided therapy?**

RGT was a cornerstone of HCV therapy in the era of PegIFN-based therapy. RGT resulted in a remarkable improvement in SVR rates and reduction of adverse effects. However, with the approval of IFN-free DAA regimens, the role of on-treatment HCV RNA for SVR prediction has diminished significantly given the high efficacy and overall excellent tolerability of these regimens.

Given the perspective of approval of even more potent regimens over the next few years, RGT strategies with the aim to increase SVR may no longer be desirable. Despite this, individualized treatment durations may still have considerable benefits, in particular with respect to cost and possibly some difficult-to-treat patient subgroups. However, due to the lack of sufficient data, RGT concepts are currently not recommended for any of the approved DAA regimens. Future studies need to address whether additional response predictors may be combined with on-treatment HCV RNA to predict SVR and whether these can be incorporated into RGT algorithms and lead to shorter and less cost-intensive therapies for some patient subgroups.

**Conflict of interest**

BM received speaker and/or consulting fees from Abbott Molecular, AbbVie, Bristol-Myers Squibb, Fujirebio, Janssen-Cilag, Merck/MSD, and Roche. He also received research support from Abbott Molecular and Roche.

JV received speaker and/or consulting fees from Abbott Molecular, AbbVie, Bristol-Myers Squibb, Gilead, and Medtronic.

**Authors’ contributions**

BM and JV wrote and approved the final version of the manuscript.

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Review


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