Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Boceprevir in the treatment of hepatitis C infection: rationale and clinical data

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An estimated 1.8% of the population in the USA is infected with the hepatitis C virus (HCV). The standard of care treatment for HCV consists of pegIFN and ribavirin. Genotype is considered a strong predictor determining response rates to treatment. Patients infected with genotype 1 are more resistant to treatment with an estimated response rate of approximately 40–50%. The recent discovery of polymorphisms in the *IL28-B* gene has given new insight into response rates and is now being considered the strongest pretreatment predictor of response. Recent insights into the HCV lifecycle and structure have led to the development of drugs that inhibit the NS3 protease in the HCV virus molecule, thereby preventing viral replication and increased degradation, and thus increasing chances of achieving a sustained viral response to therapy. Two drugs – boceprevir and telaprevir – have recently been approved by the US FDA and have been shown to have increased efficacy for genotype 1 patients, increasing the sustained virologic response rates to 75%. These drugs will be the biggest advance in the field since the introduction of pegIFN and ribavirin.

Keywords: boceprevir • genotype 1 • hepatitis C virus • *IL-28B* polymorphism • NS3 protease inhibitor • telaprevir

Omer Khalid & Bruce R Bacon*

Division of Gastroenterology & Hepatology, Saint Louis University Liver Center, Saint Louis University School of Medicine, 3635 Vista Avenue at Grand Blvd, St Louis, MI 63110-0250, USA
*Author for correspondence:
Tel.: +1 314 577 8764
Fax: +1 314 577 8125
E-mail: baconbr@slu.edu

Epidemiology

It has been estimated by the WHO that approximately 170 million people worldwide are infected with the hepatitis C virus (HCV) and these individuals are at risk of subsequently developing liver cirrhosis and/or hepatocellular carcinoma. The prevalence of HCV infection has been noted to be high in certain regions of the world including Africa, the eastern Mediterranean and southeast Asia, as compared with North America and Europe where the prevalence rates are lower. The estimated prevalence rate for positive HCV antibodies in the USA is 1.8% of the population, with an estimated 3.1 million individuals having active HCV infection [1–3]. HCV is a blood-borne infection and injection-drug use is the most common risk factor associated with HCV infection [4,5]. Other identified factors linked to increased risk of infection include poverty, high-risk sexual behavior, having fewer than 12 years education and either being divorced or separated. Many of these risk factors relate to lifestyle choices. Maternal–fetal transmission occurs infrequently and is often associated with maternal HIV co-infection [6,7]. Previously, blood transfusions posed a significant risk of HCV infection in developed countries, but the introduction in 1990 and 1992 of improved screening measures has significantly decreased the risk of HCV transmission via this route [8–10].

History of HCV treatment

The treatment of HCV has undergone significant advances over the past two decades with the arrival of a new era of direct-acting antiviral agents (DAAs). The

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initial treatment of HCV infection was with recombinant human IFN-α-2 given as thrice-weekly injections [11,12]. IFN-α was chosen because of its broad antiviral effects and the belief that it may possess antiviral activity against HCV (then-known as the non-A, non-B hepatitis virus). IFN-α administration reduced serum aminotransferase levels in the era when the HCV had not yet been discovered and improvement in aspartate aminotransferase and alanine aminotransferase levels were the only assessment tools for gauging therapeutic response. With the discovery and subsequent cloning of HCV in 1989, it was noted that IFN-α treatment resulted in a decrease in HCV RNA levels that led to a sustained absence of virus and normalization of aminotransferase levels in a small proportion of patients [12,13]. IFN-α-2b use was first approved in Europe in 1989, with Portugal the first country to approve this therapy for chronic non-A, non-B hepatitis. In 1995 the entire EU approved IFN-α-2b as a treatment for chronic HCV infection. IFN-α-2b and then -α-2a were approved for treatment in the USA in 1991 and 1992, respectively. IFN monotherapy was used from 1991 to 1998 when the US FDA approved the combination of IFN-α-2b and ribavirin. Response rates improved, and then in 2001, pegIFN plus ribavirin combination therapy (PR) was approved. From 2001 to 2011, PR was the mainstay of treatment.

Definitions of treatments

It is important for clinicians to understand the various definitions of virologic response, not only to assess patient response but also to interpret the results of clinical studies. While not all definitions are standardized, the following have been used widely in clinical trials.

Rapid virologic response (RVR) is defined as HCV RNA levels that are undetectable at week 4 of treatment. Early virologic response (EVR) is defined as a ≥2 log10 reduction in HCV RNA level from baseline at week 12 of treatment. EVR can be further categorized as complete EVR, which is the absence of HCV RNA in the serum at week 12 of treatment, or partial EVR, which is a decline of ≥2 log10 in HCV RNA level, but the virus remains detectable at week 12 of treatment. A partial response is when HCV RNA levels decline by ≥2 log10, but are never undetectable at any time during treatment. A nonresponder is a <2 log10 decrease in HCV RNA by week 12. A slow responder is a patient who achieves a partial EVR, followed by delayed viral negativity until week 24 of therapy. End-of-treatment response is undetectable HCV RNA levels at the completion of treatment, which is generally 48 weeks for patients with genotypes 1 or 4, and 24 weeks for patients with genotypes 2 or 3. A sustained virologic response (SVR) is undetectable HCV RNA levels 24 weeks after the completion of treatment and is the best definition of cure at this time. Achieving an SVR is the goal of therapy. Relapse is recurrence of detectable virus during the 24 weeks of follow-up after achieving an end-of-treatment response, and breakthrough is recurrence of detectable virus on therapy after achieving viral negativity [10,14–19].

Factors affecting response

The likelihood of achieving an SVR can be predicted by viral or host factors. The most important viral factors include the genotype, followed by the viral level. There are six major HCV genotypes [20]. The genotype does not predict the natural history of infection; it does, however, predict the likelihood of treatment response and, in many cases, determines the duration of treatment. In most prospective studies genotype is the strongest predictor of response; however, recently it has been shown that RVR is a stronger predictor of SVR than genotype [21]. SVR rates were higher in patients who had genotypes 2 or 3 and lower pretreatment HCV RNA levels. Host factors include sex, age at infection, duration of infection, race or ethnicity, the presence of hepatic steatosis, genetic factors and the patient's immune response. It has also been noted that African–American and Hispanic patients have lower SVR rates than Caucasians [10,12,22–26].

The recent discovery of certain polymorphisms in the *IL-28B* gene has given new insight into on-treatment response rates. Variations in the gene have been linked to better response rates among people with chronic HCV infection. The *IL-28B* gene encodes IL-28, also known as IFN-λ, a cytokine with antiviral activity. Thompson *et al.* evaluated the clinical relevance of on-treatment virologic response and SVR in genotype 1 patients with respect to *IL-28B* polymorphism [27]. A total of 1671 patients were genotyped as CC, CT or TT, viral levels were checked at weeks 2 and 4 to detect ultrarapid virologic response and RVR, and complete EVR at 12 weeks. CC genotypes were observed more frequently in Caucasians (37%), followed by Hispanics (29%), and African–Americans (14%). The TT genotype was more common in African–Americans (37%) as compared with Hispanics (22%) or Caucasians (12%). The CC genotype was associated with improved early viral kinetics and viral suppression such that by week 2 of treatment the median reduction in viral load was ≥2-log. The effect was similar amongst all races. A recent abstract presented at the European Association for the Study of the Liver 2011 by Poordad *et al.* evaluated SVR rates in patients treated with pegIFN, ribavirin and boceprevir [28]. Patients from two Phase III trials, SPRINT-2 and RESPOND-2, who received boceprevir were tested for *IL-28B* polymorphism. Amongst the treatment-naive

patients, SVR rates were 50% higher in the CC-type patients than in the CT- or TT-type patients, while the boceprevir arms were 25% higher in the CC-type as compared with the CT- and TT-types. For treatment-failure patients there was a clear advantage for boceprevir in all categories. In multivariate analysis it was noted that *IL-28B* genotype was a stronger predictor than other baseline variables; however, it was not a stronger predictor when the week 4 virologic response was included in the analysis.

The strongest pretreatment predictor of SVR is considered to be *IL-28B*. However, RVR at week 4 is the best on-treatment predictor of SVR and treatment success regardless of *IL-28B* status [27,28].

Use of viral kinetics for tailoring hepatitis C treatment

Quantitative measurement of HCV RNA has been used to assess HCV treatment response and the likelihood of a SVR [29]. Studies have shown that levels of HCV reach a steady state based on the equilibrium between production and clearance of HCV [30,31]. This level remains relatively stable within a range of 1 log10 until the introduction of antiviral agents to treat the infection, which is aimed at halting the virus production [32]. The decline in HCV RNA then follows a biphasic slope [31,33,34]. The first phase is typically seen within 8 h of IFN administration, with a rapid decline of HCV levels determined by the efficacy of IFN-α at preventing viral production [31,34,35]. The second phase of virus elimination is highly variable, with the rate of decay being based on the two factors noted above, namely the intrinsic viral clearance as well as the continued effectiveness of the therapy in inhibiting viral replication [31]. As previously stated, this model can be used to explain the differences in treatment efficacy between different genotypes and may also be used to explain racial differences in response rates. Until May 2011, the treatment for patients with HCV was a combination of pegIFN and ribavirin for 24–48 weeks. This approach remains highly effective for patients with HCV genotypes 2 or 3 who have SVR rates of approximately 80%, with the majority requiring 24 weeks of treatment. However, this treatment algorithm is less effective for patients with genotype 1, as these patients have SVR rates of 40–50% after standard 48 weeks treatment, with higher SVR rates with 48 weeks rather than with 24 weeks of treatment [10]. The availability of telaprevir and boceprevir has changed our standard of care treatment of HCV genotype 1.

Treatment for genotype 1

Prior to May 2011, the recommended treatment for HCV genotype 1 patients was 48 weeks with pegIFN and ribavirin. This treatment duration could be tailored by viral response using viral kinetics [36]. A recent prospective trial demonstrated that patients with genotype 1 baseline HCV RNA levels ≤600,000 IU/ml who undergo RVR achieve an SVR of up to 90% with either 48 or 24 weeks [37,38]. These studies have shown that a shorter treatment duration of 24 weeks may be sufficient in HCV genotype 1 patients who demonstrate RVR and have low baseline HCV RNA levels, similar to those in genotype 2 or 3 patients exhibiting RVR.

Patients who may need to be considered for a longer treatment duration than 24 weeks include those who have a baseline viral load of >600,000 IU/ml, cirrhosis, co-infection with HIV or are immunosuppressed. Several recent studies have suggested that extending treatment beyond 48 weeks may lead to improved SVR rates in some genotype 1 patients [39–43]. In those who fail to achieve SVR, there is either a pattern of nonresponse (failure to clear virus from the serum), relapse, where HCV RNA is cleared from serum and returns after therapy is discontinued, or breakthrough, where the virus reappears after clearance from the serum. With the use of pegIFN and ribavirin, improving SVR rates is achieved by 'minimizing relapse'. The two large randomized registration trials by Fried *et al.* and Manns *et al.* evaluated combination therapy with pegIFN and ribavirin, and revealed comparable relapse rates of 18 and 19%, respectively, when treatment was continued for 48 weeks [22,23]. Patients with a delayed virologic response to HCV therapy have a lower likelihood of achieving SVR with 48 weeks of treatment than those with a more rapid decline in viral load [25].

Past studies have demonstrated that the probability of attaining SVR is greater with a longer period of undetectable serum HCV RNA during treatment [44]. Two recent studies have helped provide insight into extending treatment. Berg *et al.* compared therapy extension for 72 weeks with standard duration of 48 weeks in genotype 1 HCV patients who received IFN-based therapy, and found no overall difference in SVR (54 vs 53%) or relapse rates (21 vs 29%) between the two groups (n = 53) [41]. However, in a subgroup analysis of patients who were late responders (virus level HCV RNA <6000 IU/ml at 12 weeks, but undetectable virus at 24 weeks), extending the duration of treatment to 72 weeks from 48 significantly decreased the relapse rate. Patients who did not have a serum HCV RNA <6000 IU/ml at 12 weeks had a high rate of relapse regardless of treatment duration. Of note, there was a higher incidence of dropouts in the 72-week treatment arm than the 48-week treatment arm, particularly between weeks 48 and 72, even with the lower ribavirin dose of 800 mg. This possibly explains the lack of difference between treatment groups. Aggressive adherence

measures should be undertaken to maximize response rates when considering extending therapy. Similar results were noted in a study by Sanchez-Tapias *et al.* that examined the role of 72 weeks of therapy compared with 48 weeks in HCV patients of all genotypes who did not achieve an RVR (TeraViC-4 trial) with pegIFN and ribavirin [40,41]. The vast majority of the patients who did not undergo RVR were genotype 1 (291 out of 326). Extension of therapy duration to 72 weeks within these genotype 1 patients improved the SVR rate (44 vs 28%) significantly by decreasing the relapse rate (53 vs 17%).

Finally, a third trial by Pearlman *et al.* compared SVR rates among slow-responder genotype 1 patients with 48 weeks treatment versus extension of treatment to 72 weeks. They noted that SVR rates were superior in those patients who were treated for 72 versus 48 weeks (38 vs 18%, respectively) [44]. A higher dropout rate has been seen in the 72-week treatment group. Nonetheless, extension of therapy to 72 weeks appears to be an option in those who fail to clear virus by week 12, and may be an option in those who do not undergo week 4 RVR. While some of these studies used flat-dose ribavirin, weight-based dosing of ribavirin should be used in an effort to minimize relapse. A recent study by Fried *et al.* studied the efficacy of high-dose pegIFN-α-2a and ribavirin, compared with conventional-dose treatment in genotype 1 patients with features predicting a poor response to treatment [45]. Patients were randomized to double-blind treatment with 180 or 270 µg/week pegIFN-α-2a plus 1200 or 1600 mg/day ribavirin for 48 weeks. Patients randomized to higher doses of pegIFN and ribavirin experienced the highest rates of SVR and the lowest relapse rates (47 and 19%, respectively) [10,37,45].

Anemia during HCV treatment

The relationship between anemia and epoeitin-α has been studied with respect to impact on SVR in patients undergoing IFN-based therapy. Successful treatment of HCV is often limited by adverse events such as anemia. An adequate amount of ribavirin reduces the risk of post-treatment HCV relapse, but the incidence of anemia rises with higher ribavirin doses. Shiffman *et al.* conducted a study to determine whether using erythropoietin (EPo) with or without a higher dose of ribavirin could enhance SVR [37]. They randomized 150 treatment-naive patients with genotype 1 chronic HCV into three treatment groups. Group 1 was treated with pegIFN-α-2b with weight-based ribavirin; group 2 was treated with pegIFN, weight-based ribavirin and EPo; group 3 was treated with pegIFN, high-dose weight-based ribavirin and EPo. They noted that a significantly smaller percentage of patients in group 2 had a decline in hemoglobin to less than 10g/dl and that fewer patients required ribavirin dose reduction. Despite this, SVR rates were not significantly different in groups 1 and 2 (19 vs 29%, respectively). However, the SVR rate was significantly greater in group 3 (49%), resulting from a significantly lower HCV relapse rate: 8% in group 3 versus 38% in groups 1 and 2. This confirms that a higher starting dose of ribavirin is associated with lower relapse rates and higher SVR, and preventing anemia-related side effects can help reduce dropout rates [37]. Sulkowski *et al.* recently investigated the relationship among treatment outcomes, anemia and their management with ribavirin dose reduction and/or the use of an EPo-stimulating agent (ESA) among patients being treated for HCV genotype 1 with IFN and ribavirin. Approximately 3070 patients enrolled in the IDEAL trial from 118 centers from around the USA were randomized and treated. Anemia was observed in 28.6% (n = 865) of patients, of whom 51.9% (n = 449) were prescribed ESAs. Virologic response rates and end-of-treatment response rates were significantly higher in patients who developed anemia. On subanalysis, SVR rates were significantly higher for patients with a hemoglobin decline of >3 g/dl (43.7%) compared with those with a decline of <3 g/dl (29.9%). Anemic patients treated with ESA had higher end-of-treatment response rates than patients who did not receive ESAs (69.9 vs 60.8%). Interestingly, for patients noted to have early-onset anemia (<8 weeks of treatment) ESA use was associated with a higher SVR rate and less discontinuation of treatment compared with those who did not receive ESA (45 vs 25.9%). Use of ESA did not affect SVR or discontinuation rates amongst patients who developed anemia late in the course of their treatment (>8 weeks of treatment). These data support that higher plasma ribavirin concentrations are associated with higher hemoglobin decline and improved virologic response rates in patients treated with IFN and ribavirin [46].

Dawn of a new age: boceprevir & telaprevir

Thus far, treatment for HCV has consisted of therapies to stimulate the immune system and interfere in a non-specific manner with viral replication. As mentioned previously, SVR rates in genotype 1-infected individuals remain suboptimal. With the increased understanding of the HCV lifecycle and of the structural features of the HCV proteins, there has been a shift in investigational focus towards DAA therapy for HCV. This treatment inhibits HCV proteins that are essential for intracellular replication. Newer data have demonstrated promise for two protease inhibitors – boceprevir and telaprevir – both of which improved SVR while decreasing the duration of treatment. These drugs are referred to as DAAs. The remainder of this review will focus

on boceprevir and discuss its mechanism of action and effects on HCV [47,48].

NS3/A protease inhibitors

HCV is a single-stranded RNA molecule approximately 9600 nucleotides in length. Viral protein synthesis is mediated by an internal ribosome entry site that binds directly to ribosomes and RNA is translated into a polyprotein of 3000 amino acids that is proteolytically cleaved into four structural and six nonstructural (NS) proteins. The structural proteins are used to assemble new viral particles and the NS proteins support viral RNA replication (Figure 1) [3,49].

The NS2 metalloprotease and NS3 serine protease are two viral proteolytic enzymes that allow the production of NS proteins from the HCV polyprotein. The NS2 enzyme cleaves itself at its C-terminus, activating the second protease, the NS3 serine protease, which is contained within the N-terminal of the multifunctional NS3 protein. The NS3 protease is responsible for all subsequent downstream cleavages of the polyprotein, such as NS3–NS4A, NS4A–NS4B, NS4B–NS5A and NS5A–NS5B. Much attention has been paid to the NS3 site for antiviral therapeutics. A key feature of the NS3 protease is that one strand of its seven stranded N-terminal β-barrel structure is supplied in

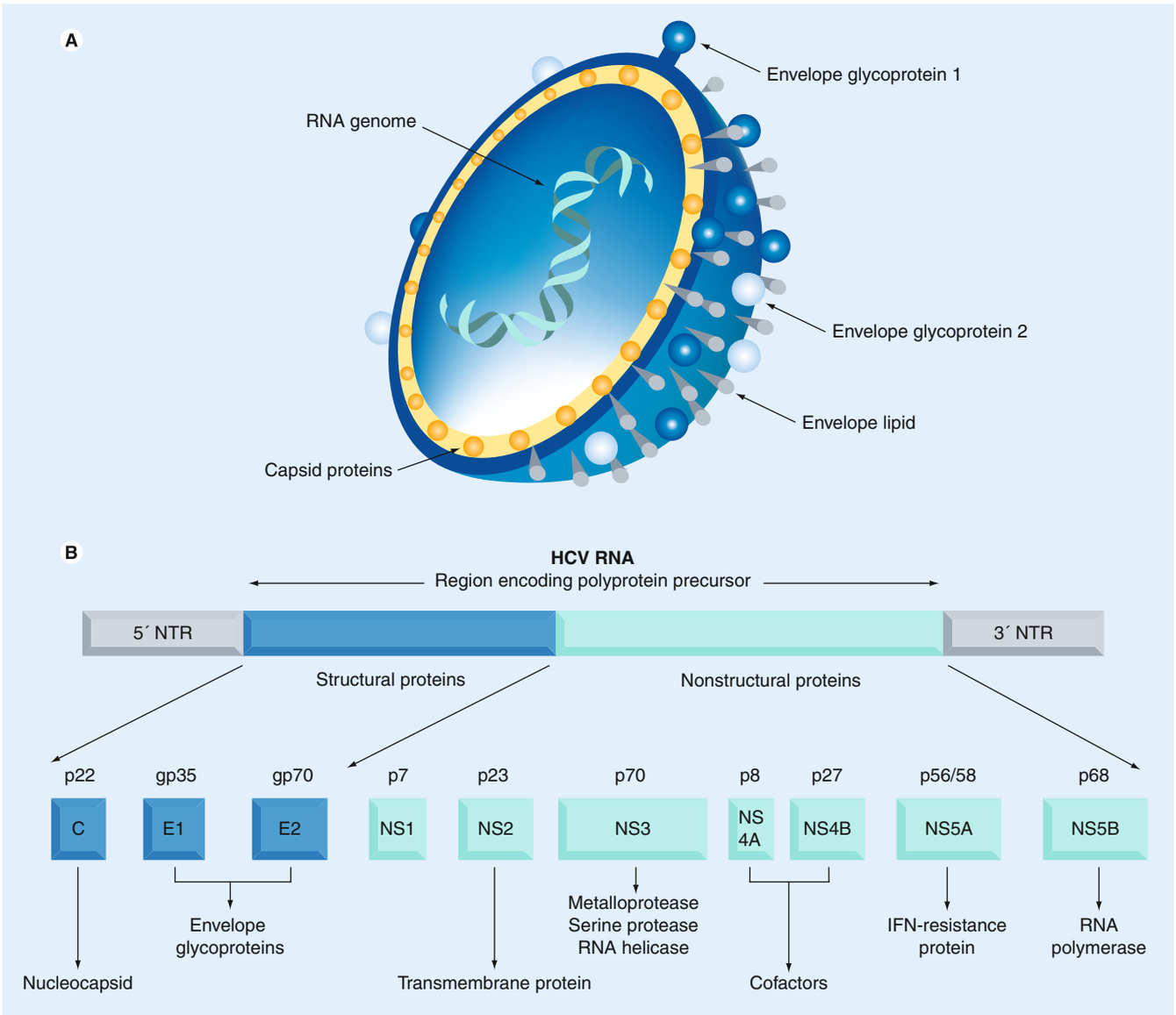


Figure 1. Hepatitis C virus (A) model structure and (B) genome organization.
HCV: Hepatitis C virus.

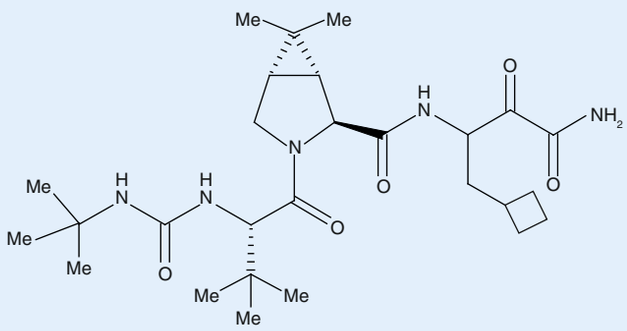
trans by the NS4A cofactor protein or peptide. Kinetic and structural studies have shown that the HCV NS3 serine protease requires intercalation of a strand of NS4A cofactor for proper alignment of the catalytic triad and full proteolytic activity. Without the intercalation of the NS4A strand, the N-terminus remains partially disordered resulting in imperfect alignment of the catalytic triad and a corresponding decrease of approximately 950-fold in catalytic efficiency [50,51].

Boceprevir is a potent, orally administered, serine protease inhibitor that has been specifically designed to inhibit the HCV NS3 protease, thus enabling it to inhibit viral replication in HCV-infected host cells. The mechanism of action involves the formation of stable, but reversible, covalent bonds between the ketoamides of boceprevir and the NS3 protease active site's serine (Figures 2 & 3).

Inhibition of the NS3 protease is rapid and at low nanomolar concentrations, and is shown to have significant inhibitory activity in cell-based HCV replicon systems. There is rapid entry of compound into the cell with loss of replicon RNA, reflecting the inhibition of newly synthesized protease. Boceprevir acts rapidly to suppress proteolytic activity and prevent formation of new replisomes. The ongoing degradation of extant replisomes and replicon RNA has shown to have a first-order decline in replicon RNA levels.

The drug is extensively metabolized and excreted in urine, bile and feces. Other common metabolic pathways for boceprevir include oxidation, oxidative deamination, oxidative deamidization, cleavage, dimerization, or a combination of these processes.

Potent anti-HCV activity has been shown both alone and in combination with PR in patients infected with HCV. Based on *in vitro* data in the HCV replicon, boceprevir monotherapy was limited due to the development of resistance to boceprevir; however, in the presence of IFN-α-2b the development of resistance was decreased 25-fold [52].



(1*R*,5*S*)-*N*-[3-AMINO-1-(CYCLOBUTYLMETHYL)-2,3-DIOXOPROPYL]-3-[2(*S*)-[[[(1,1-DIMETHYLETHYL)AMINO]CARBONYL]AMINO]-3,3-DIMETHYL-1-OXOBUTYL]-6,6-DIMETHYL-3-AZABICYCLC[3.1.0]HEXAN-2(S)-CARBOXAMIDE

Figure 2. SCH 503034 structure and chemical name.
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Malcolm *et al.* demonstrated the antiviral activity of boceprevir using HCV replicons [52]. Treatment resulted in a 1.5–2 log drop in HCV RNA levels at 72 h and a 3.5–4 log drop by day 15. Cells treated with IFN-α had a greater HCV replicon suppression than both agents separately and this effect appeared to be additive, rather than synergistic.

Initial Phase I studies by Susser and Zeuzem *et al.* evaluated the safety of boceprevir as monotherapy in HCV genotype 1 patients who were nonresponders to standard therapy [53,54]. After 14 days of treatment, a mean log10 reduction in HCV RNA of 2.06 was achieved in patients receiving 400 mg daily. The treatment was well tolerated; however, viral breakthrough occurred in some patients in a dose-dependent frequency.

A subsequent study by Sarrazin *et al.* evaluated combination therapy of boceprevir plus PR in genotype 1 nonresponders [55]. This was a multicenter, open-label, two-dose level, three-way crossover, randomized (to crossover sequence) study carried out in three medical centers in Europe. Either boceprevir was administered at doses ranging from 200 to 400 mg every 8 h individually for 1 week, or PegIFN-α-2b as monotherapy for 2 weeks, or combination therapy for 2 weeks, with washout periods between each treatment period. Combination therapy was well tolerated and mean maximum log10 changes in HCV RNA were -2.45 and -2.88 for PegIFN-α-2b plus 200 and 400 mg boceprevir, respectively, compared with -1.08 and -1.61 for boceprevir 200 and 400 mg, respectively, and -1.08 and -1.26 for PegIFN-α-2b alone in the 200 and 400 mg boceprevir groups, respectively. Boceprevir was well tolerated alone or in combination with pegIFN [53–55].

These initial studies set the stage for a Phase II study (SPRINT-1) conducted to assess the safety and efficacy of boceprevir in combination with PR in treatment-naive HCV genotype 1 patients. In part 1, participants were randomly allocated to receive various schedules of boceprevir 800 mg three-times daily + 1.5 mcg/kg once-weekly pegIFN-α-2b + 800–1400 mg/day weight-adjusted ribavirin:

- A 4-week lead-in period of PR, followed by addition of boceprevir for 24 weeks (total treatment duration of 28 weeks);
- A 4-week lead-in period of pegIFN + ribavirin, followed by addition of boceprevir for 44 weeks (total treatment duration of 48 weeks);

- Boceprevir + pegIFN + ribavirin, all started at the same time and continued for 28 weeks;
- Boceprevir + pegIFN + ribavirin, started at the same time and continued for 48 weeks;
- Control arm: standard therapy with pegIFN + ribavirin for 48 weeks (Figure 4).

Patients in all four boceprevir groups had higher rates of SVR than the control group, with 54 and 56% SVR after 28 weeks. After 48 weeks of triple-therapy SVR rates were 67 and 75%, respectively. Low-dose ribavirin was associated with a high rate of viral breakthrough (27%) and a relapse rate of 22%, which was similar to controls of 24%. Boceprevir-based groups had higher rates of anemia (55 vs 34%) and dysgeusia (27 vs 9%) than the control group. In general, the SPRINT-1 trial has proven a higher antiviral efficacy of combination therapy with boceprevir in comparison to the standard of care treatment, with better results after the 4-week lead-in phase. The basis for the potential advantage of a lead-in strategy is based on the fact that both pegIFN and ribavirin reach a steady state concentration in 4 weeks, and with the lead-in strategy patients have a protease inhibitor added when the backbone drug levels have been optimized and the patient's immune system has been optimally activated. This approach may minimize the period of time when there is a period of 'functional monotherapy' with a DAA, possibly reducing the likelihood of the development of resistance. This strategy may also have the potential to reduce the likelihood of development of resistance by identifying patients who are responders to PR before giving them a protease inhibitor or other DAA drug [47,48].

Telaprevir is the other orally bioavailable NS3 protease inhibitor that belongs to the α-ketoamides and reversibly binds to the enzyme covalently. Two landmark Phase II studies, PROVE-1 (conducted in the USA) and PROVE-2 (conducted in Europe) showed an excellent response to therapy in treatment-naive genotype 1 patients. Results showed SVR rates of up to 70% when telaprevir was added to the existing standard of care regimen. Subsequent studies (ADVANCE and ILLUMINATE) have been conducted evaluating SVR rates in telaprevir-based triple therapy in treatment-naive genotype 1 patients. Both the ADVANCE and ILLUMINATE trials evaluated the possibility of tailoring treatment based on achieving RVR, whereby those patients who were able to exhibit such a response would be entitled to have a shorter duration of therapy, thus, helping to minimize unnecessary exposure and development of resistance to these drugs. Thus, all patients who achieved RVR can be safely given 12 weeks of telaprevir-based therapy with an expected SVR rate of 70%, whereas those patients who still exhibit viral response and become negative by week 12 should be considered for 48 weeks of treatment with a similar expected SVR rate [56-59].

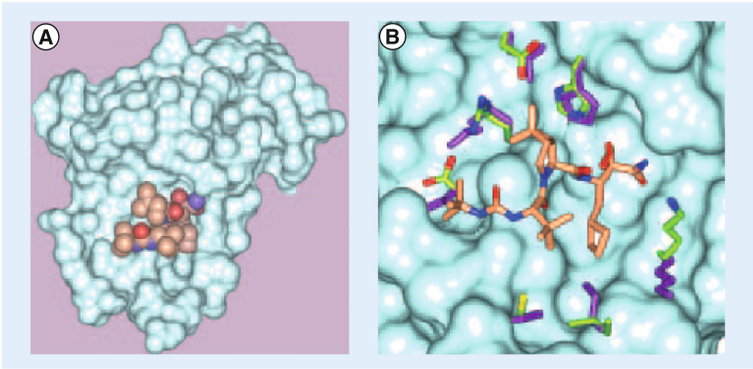

Figure 3. SCH 503034 complexed with the hepatitis C virus NS3 protease.
(A) Connolly surface for the NS3 protease. SCH 503034 is rendered in CPK format (Corey-Pauling-Koltun space-filling model). **(B)** Close-up of the NS3:SCH 503034 complex showing side chains that are perturbed upon binding of SCH 503034. Please see www.future-science.com/doi/full/10.4155/cli.11.121 for color version.
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A Phase III clinical trial (SPRINT-2) evaluating boceprevir in treatment-naive genotype 1 patients has been completed. Given the marked difference in SVR rates amongst black and nonblack patients, the study was designed for two different cohorts evaluating black and nonblack patients. A total of 938 nonblack patients and 159 black patients were enrolled. Patients were randomized to receive either standard therapy with PR plus placebo (arm 1) three-times daily starting at week 5, or to receive 24 weeks of boceprevir after lead-in with PR for a total duration of 28 weeks if the HCV RNA levels were undetectable by week 8 (arm 2, response-guided therapy). If the virus was detectable at week 8, but not at week 24, then patients were to receive PR plus placebo from weeks 28 to 48, or were to receive PR for 4 weeks lead-in, followed by boceprevir 800 mg three-times daily with 44 weeks PR (arm 3, fixed-duration therapy) [60].

Response rates were higher in patients who received boceprevir compared with the control group. SVR rates amongst nonblacks were 40, 67 and 68% in arms 1, 2 and 3, respectively. SVR rates amongst blacks were 23, 42 and 53% in the respective groups. These data confirm that the addition of boceprevir increases SVR rates amongst all treatment-naive patients, regardless of race. In addition, the trial evaluated response-guided therapy allowing treatment to be tailored according to patients achieving a negative HCV RNA level before or

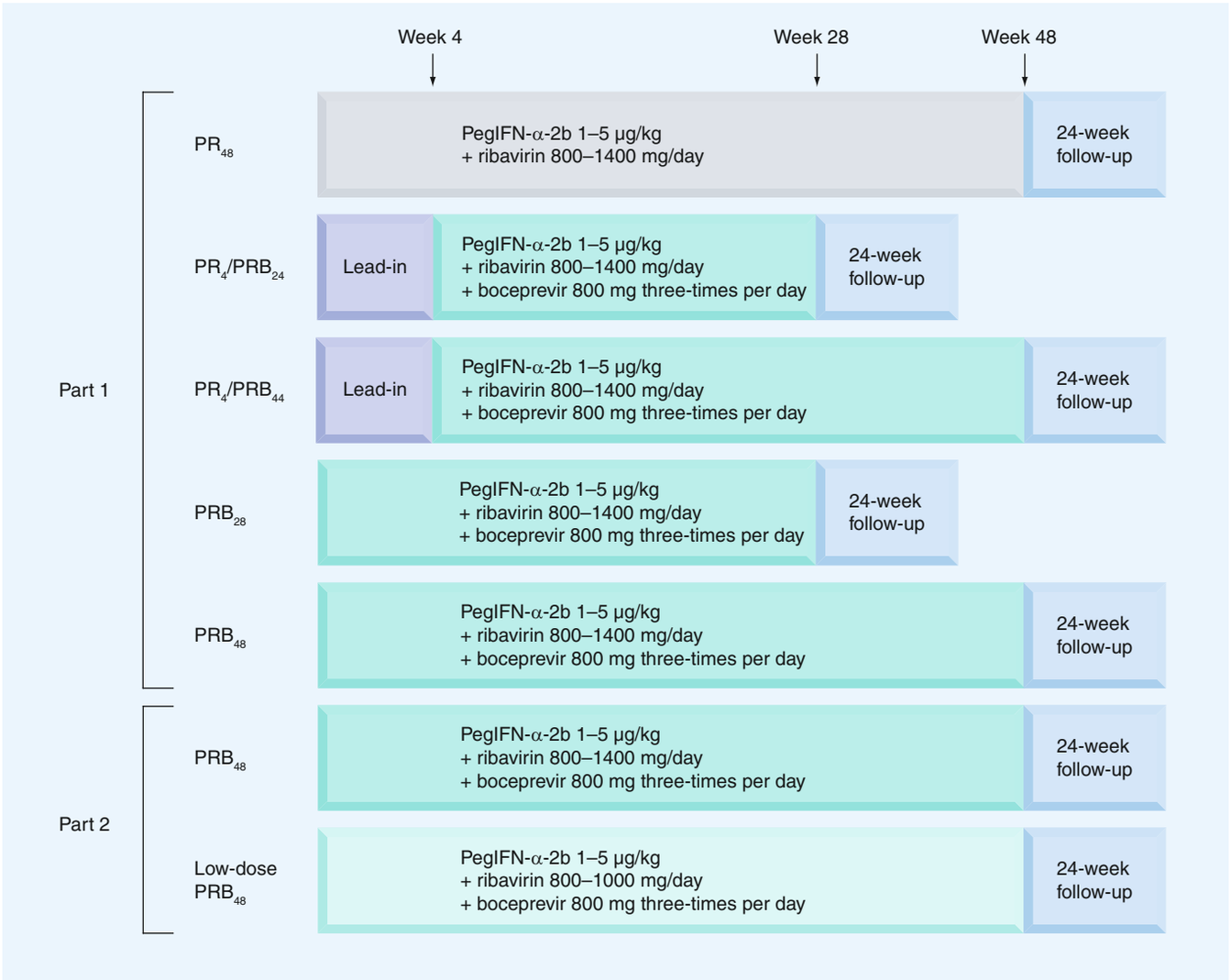

Figure 4. SPRINT-1 trial design. Patients in all groups were followed up for 24 weeks after the end of treatment. PR_4 (lead-in): pegIFN-α-2b 1–5 μg/kg plus ribavirin 800–1400 mg per day for 4 weeks. PR_{48} (control): pegIFN-α-2b 1–5 μg/kg plus ribavirin 800–1400 mg daily for 48 weeks. $PRB_{24/28/44/48}$: pegIFN-α-2b 1–5 μg/kg, ribavirin 800–1400 mg and boceprevir 800 mg three-times daily for 24, 28, 44 or 48 weeks. Low-dose PRB_{48}: pegIFN-α-2b 1–5 μg/kg, ribavirin 400–1000 mg and boceprevir 800 mg three-times daily for 48 weeks. Adapted with permission from [63] © *The New England Journal of Medicine*.

after week 8. This was arm 2 of the trial, where those patients who achieved a negative HCV RNA by week 8 received a total of 28 weeks of therapy with boceprevir after a 4 week lead-in with PR. Those patients who were still virus positive by week 8 were assigned to receive an additional 24 weeks (48 weeks total therapy) of PR and placebo, after a 24-week course of treatment with boceprevir/PR. Anemia was noted to be a common adverse event, being more pronounced amongst those patients receiving boceprevir (43%) compared with controls (29%). However, drop-out rates were extremely low at only 1% in controls and 2% in the boceprevir groups.

With these excellent success rates noted in naive patients the focus was switched to the more important, difficult-to-treat genotype 1 population. The PROVE-3 study with telaprevir enrolled 453 patients who had not achieved SVR (including nonresponders, relapsers and those with breakthrough) after having received a full course of therapy with PR. Patients were assigned to four treatment groups as follows: arm 1 – patients received telaprevir/PR for 12 weeks followed by placebo/PR for another 12 weeks; arm 2 – patients received telaprevir/PR for 24 weeks followed by just PR for an additional 24 weeks; arm 3 – tested the effects of treatment without

ribavirin. Patients received telaprevir and pegIFN for 24 weeks; arm 4 – the control group where patients received placebo/PR for 24 weeks followed by only PR for another 24 weeks. PegIFN was provided as 800 µg/week pegIFN-α-2a 1 by subcutaneous injection and ribavirin was dosed according to a weight-based regimen. Telaprevir was dosed at 750 mg every 8 h with an initial loading dose of 1125 mg. Several stopping rules were implemented in this study, where all those patients who had a viral breakthrough from weeks 4 through week 24 (increase in HCV RNA level of >100 IU/ml after being undetected), or a nonresponse by week 4 (<1 log drop from baseline), or a nonresponse at week 12 (<2 log drop in HCV RNA from baseline), or, for the control arm, detectable HCV RNA level at week 24, were taken off-treatment. SVR rates were quite similar in arms 1 and 2 (51 and 53%, respectively). They were considerably lower in arm 3 with no ribavirin on board, with 24% SVR rates and 14% in the control group. When the data were evaluated by prior response to treatment, those patients who were considered nonresponders exhibited SVR rates of 39, 38, 11 and 9% in arms 1 to 4, respectively. Patients with prior relapse to treatment had a much more successful response to telaprevir-based therapy with 69 and 76% SVR rates in arms 1 and 2, and 42 and 20% in arms 3 and 4, respectively. Patients with cirrhosis also did well with treatment, with similar results compared with those without advanced fibrosis. An SVR rate of 53% in arm 1 and 45% in arm 2 was documented. The Phase III REALIZE study assessed the efficacy of telaprevir-based therapy in HCV genotype 1 nonresponders. Results were similar to those noted with treatment-naive patients and also similar to the RESPOND-2 trial [61,62].

In addition, a boceprevir-based nonresponder Phase III study (RESPOND-2) was conducted to look at the effects of boceprevir on retreatment of genotype 1 patients who had partial response or relapse on previous treatment with PR. Patients were assigned to one of three arms. Arm 1 received placebo plus PR for 44 weeks, arm 2 was response-based therapy where patients received boceprevir plus PR for 32 weeks if they had undetectable virus at week 8, and those patients who had detectable HCV RNA levels at week 8 received an additional 12 weeks of PR. Arm 3 received boceprevir with PR for 44 weeks. In total, 403 patients were randomized and treated. SVR rates were significantly higher amongst patients receiving boceprevir compared with those being treated with PR alone, with overall rates of 21, 59 and 66% in arms 1, 2 and 3, respectively. There was a higher incidence of anemia noted in the boceprevir-containing regimens (43–47%) compared with controls (20%); however, discontinuation rates due to anemia were infrequent (0, 0 and 3.1%), respectively.

These data demonstrate that the addition of boceprevir or telaprevir to PR leads to higher rates of SVR in patients who had previously failed therapy. Furthermore, those patients who had experienced relapse prior to standard therapy experienced an SVR rate of 75%. Likewise, those patients who had a historical partial response to previous standard therapy were noted to have 43–53% SVR rates. In addition, treatment can be tailored according to viral negativity amongst these previously difficult-to-treat patients, as shown by arm 2 of this study. Those patients showing viral negativity by week 8 can be treated with a shorter course of 32 weeks, whereas those who do not become HCV RNA negative by week 8 can be given 44 weeks of therapy with improved success rates [63,64]. Patients who were treated with telaprevir in combination with PR also exhibited similar results, with SVR rates of up to 70%. The rates of SVR did differ amongst patients who were treatment-naive versus those who had a relapse or were previous nonresponders. Studies showed improved SVR rates for patients with prior relapse and partial response to PR therapy as compared with those who had no response. Similarly, treatment can be tailored in those patients who exhibit RVR. Such patients can be safely given 12 weeks of therapy with expected SVR rates of 70%, thus helping minimize drug exposure and potential resistance.

Future perspective

The addition of DAAs such as boceprevir and telaprevir to PR has shown early promise. These drugs will likely be the biggest advance in the field since the introduction of PR. Thus far, trials have shown that there is remarkable improvement in SVR rates along with a shortened duration of treatment. The lead-in strategy, as suggested by the SPRINT-1 study, suggests that this may diminish the chances of resistance and optimize response rates. Anemia was a common occurrence in both boceprevir- and telaprevir-based therapies. Approximately 40% of patients required EPo administration. Results based on the RESPOND-2 study showed approximately 8% of patients in the fixed-duration therapy group with boceprevir had a significant reduction in hemoglobin levels, with at least 9% requiring blood transfusions. In these studies, EPo use was allowed at the investigators discretion. However, in those with ribavirin dose reductions, the SVR rates remained the same as when given EPo (79 vs 76%). Discontinuation of medications because of anemia occurred in 4% of patients in telaprevir versus 1% in boceprevir-based trials. Currently, EPo is used in approximately 28% of patients undergoing therapy for HCV and this practice will likely continue to be used with protease inhibitors. Cost-effective models have not yet been done with either boceprevir or

telaprevir, but the weekly cost of telaprevir is US$4100 ($49,200 for 12 weeks) compared with $1100 per week for boceprevir ($26,400 for 24 weeks or $35,200 for 32 weeks). Duration of therapy with boceprevir will be 24 weeks in approximately half of the treatment-naive patients and 32 weeks in half of previous relapsers/partial responders. Nonresponders and cirrhotics will require 44 weeks of boceprevir [64,65]. The safety and efficacy of protease inhibitors has not yet been established in patients who suffer from coinfections with hepatitis B or HIV, nor has it been studied for patients who have undergone organ transplantation.

As with the emergence of resistance during HIV therapy, important consideration and monitoring will be needed to detect for the emergence of resistant strains, as these newer drugs are infused into the current standard of therapy. Drug-resistant mutants likely preexist in the HCV quasispecies and studies have shown that mutant viral species resistant to these molecules could emerge and be quickly selected, both *in vitro* and *in vivo*. Both drugs are associated with rapid development of resistance mutations when given as monotherapy. It has been noted that the R155K mutant shows reduced susceptibility to protease inhibitors and has significant selective advantage, raising the concern over the potential emergence of R155K as a multidrug resistant, highly fit mutant in HCV patients treated with protease inhibitors [54,66].

Recent Phase Ib studies for new NS3/4A protease inhibitors provide promise that HCV treatment will continue to improve over the next few years and we will likely see the emergence of better therapies in the next 4

Executive summary

Epidemiology

- An estimated 170 million people worldwide are infected with hepatitis C virus (HCV) and are at risk for developing advanced liver disease and, subsequently, complications of portal hypertension.
- Regions such as Africa, the eastern Mediterranean and southeast Asia have a higher prevalence of the disease compared with North America and Europe.
- The estimated prevalence rate for positive HCV antibodies in the USA is 1.8% of the population with an estimated 3.1 million individuals having active HCV infection.

Predictors of response

- Several host and viral factors predict a patient's response to antiviral therapy. HCV genotype is the strongest predictor of response, however, recently it has been shown that rapid virologic response is a stronger predictor of sustained viral response (SVR).
- Recent studies have shown that *IL-28B* genotype is a stronger predictor than other baseline variables, with the CC-genotype showing better favorability to achieving SVR compared with the CT- and TT-genotypes.

Molecular biology

- HCV is a single-stranded RNA molecule approximately 9600 nucleotides in length.
- NS2 metalloprotease and NS3 serine protease are two viral proteolytic enzymes that allow the production of nonstructural proteins from the HCV polyprotein.
- The NS3 protease is responsible for all subsequent downstream cleavages of the polyprotein. Kinetic and structural studies have shown that the HCV NS3 serine protease requires intercalation of a strand of NS4A cofactor for proper alignment of the catalytic triad and full proteolytic activity.
- Without the intercalation of the NS4A strand, the N-terminus remains partially disordered resulting in imperfect alignment of the catalytic triad and a corresponding drop of roughly 950-fold in catalytic efficiency.

Treatment

- Current standard of care treatment with pegIFN and ribavirin have yielded suboptimal SVR rates for patients infected with genotype-1 virus infections.
- With the increased understanding of the HCV life cycle and of the structural features of the HCV proteins there has been a shift in investigational focus towards direct acting antiviral therapy for HCV. This treatment inhibits HCV proteins that are essential for intracellular replication.
- Newer data have demonstrated promise for two protease inhibitors, both of which improved SVR while decreasing the duration of treatment.
- Boceprevir and telaprevir are potent orally administered protease inhibitors that have been specifically designed to inhibit the HCV NS3 protease, thus enabling it to inhibit viral replication in HCV-infected host cells. The mechanism of action involves the formation of stable but reversible covalent bonds between the ketoamides of the drugs and the NS3 protease active site serine.
- Potent anti-HCV activity has been shown both alone and in combination with pegIFN and ribavirin in patients infected with HCV.
- The safety and efficacy of protease inhibitors have not yet been established in patients who suffer from co-infections with hepatitis B or HIV, nor has it been studied yet for patients who have undergone organ transplantation.

to 5 years. Danoprevir is a macrocyclic protease inhibitor that has shown high specificity and high hepatic concentrations. Treatment safety and pharmacokinetics for 14 days of therapy in 40 treatment-naive genotype 1 patients and ten previous nonresponders were evaluated. HCV RNA decline was noted in all patients. Overall viral kinetics showed that 27% of patients experienced viral rebound, 35% plateau and 38% continuous viral decline, including prior nonresponders. Resistance mutations were detected. Those patients who had virologic rebound had R155K or D168V/E/T mutations. The R155K mutations, which may confer crossresistance, persisted following treatment cessation. All these studies with monotherapy, as important as they are for configuring viral kinetics, have shown that monotherapy is not yet a reasonable option and, for now, backbone standard of care therapy with PR will prevail, until such time when oral combination therapies that do not produce resistance will be available. Careful consideration to prior nonresponders will also need to be addressed, given that studies have revealed only a 30% SVR rate despite the addition of protease inhibitors. For now, it might be appropriate that only those patients with advanced liver disease be offered treatment with current protease inhibitors, while those with lower levels of fibrosis may be referred to a clinical trial using at least two DAAs [67]. As with HIV, multiple drugs will need to be developed to rapidly suppress viral replication and prevent virus mutation. None the less, these are exciting times for HCV therapy, as a new chapter is about to be written.

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