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The role of triple therapy with protease inhibitors in hepatitis C virus genotype 1 naïve patients

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Keywords
boceprevir – direct-acting antiviral agents – hepatitis C – novel therapies – protease inhibitors – resistant mutations – telaprevir

Abbreviations
BOC, boceprevir; DAA, direct acting antiviral agents; PEG-IFN, pegylated interferon; SOC, standard of care; SVR, sustained virological response; TPR, telaprevir.

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Abstract
With the introduction of direct-acting oral antiviral agents we are on the verge of a new era that will transform the treatment landscape. This review discusses recent developments in drug discovery for hepatitis C protease inhibitors. First generation protease inhibitors will offer higher sustained viral response rates in naïve populations when combined with standard pegylated interferon and ribavirin. However, these dramatic gains will be partially offset by new challenges in viral resistance and increased adverse events.

Hepatitis C virus (HCV) infection is a global problem with an estimated prevalence of 170 million worldwide and 4 million (1.6%) in the US (1–3). Most patients with acute HCV infection become chronically infected, which increases the risk of developing further complications associated with advanced liver disease (4). Given the projected increase in HCV-related cirrhosis (the proportion of chronic HCV infection with cirrhosis is now 25% and projected to reach 45% in 2030) and hepatocellular carcinoma (HCC), optimal treatment of chronic HCV is a high priority (5). Current standard therapy for HCV includes pegylated interferon (PEG-IFN) and ribavirin (RBV), a combination which is effective in approximately 40–50% of genotype 1-infected patients and 80% of genotype 2 and 3-infected patients (6–8). Unfortunately, most patients in the US and Western Europe are infected with HCV genotype 1. The relatively low response rates in treating genotype 1-infected patients, as well as the long treatment durations and adverse side effects has meant that a small minority of patients opt for treatment. Less than 20% of the HCV-infected population in the US is estimated to have been treated. However, the introduction of oral, direct-acting antiviral agents (DAA) is now on the horizon with anticipated higher cure rates, and potentially shorter treatment durations. Approval of the first oral inhibitors is expected by mid-2011 and many patients are awaiting these new therapies.

Direct-acting antiviral agents: protease inhibitors

The class of drugs in the latest stages of development is HCV serine protease NS3–NS4A inhibitors. The NS3/4A protease is essential to viral replication and is responsible for cleaving the HCV polyprotein and releasing most of the nonstructural proteins. The design of NS3/4A inhibitors is complicated because the active site of the NS3/4A protease is located in a very shallow groove composed of three highly conserved amino acid residues. This key concept explains why most NS3/4A protease inhibitors under development display high antiviral efficacy but a low genetic barrier to resistance and will frequently cause the selection of resistant mutants which can lead to viral breakthrough. The most mature protease inhibitors are telaprevir (TVR) and boceprevir (BOC), which have now completed phase II and III trials and will probably be approved in mid 2011. These programmes have yielded some consistent early lessons for the protease inhibitor class. For naïve, genotype 1 patients, higher cure rates and a shorter duration of therapy can be expected, which will be partially offset by new issues of resistance and increased adverse events.

Protease Inhibition for Viral Evaluation 1 and 2: telaprevir trials

The recently published Protease Inhibition for Viral Evaluation (PROVE 1 and 2, evaluating TVR) and Serine Protease Inhibitor Therapy (SPRINT-1, evaluating BOC) studies evaluated protease inhibitors in combination with PEG-IFN/RBV in genotype 1, naïve patients. In PROVE 1, TVR was dosed at 750 mg every 8 h for 12 weeks in combination with PEG-IFN and RBV followed by an additional 12 weeks or 36 weeks of standard of care (SOC). The results were compared with 48 weeks of SOC (see Table 1). The sustained virological response (SVR) rate in SOC was 41%, compared with 61% ($P = 0.02$) in the 24-week treatment group and 67% ($P = 0.002$) in the 48–week treatment group (9). Relapse rates were highest in the control group (23%) compared with the 24- (2%) and 48-week TVR treatment group (6%). However, more patients discontinued therapy in the TVR treatment groups secondary to adverse side effects, with a rash being the most common reason for discontinuation. In the PROVE 2 trial, shorter treatment duration was investigated with treatment groups receiving triple therapy (TVR+PEG-IFN/RBV) for only 12 weeks (with and without RBV) compared with an additional 12 weeks of SOC (See Table 1). SVR was 46% in the control group, compared with 36% in the non-RBV group ($P = 0.20$), 60% in the 12 weeks triple therapy TVR group ($P = 0.12$) and 69% in the 24 weeks triple therapy TVR group ($P = 0.004$) (10). Relapse rates were highest in the non-RBV treated group (48%) compared with the control group (22%), 12 weeks triple therapy group (30%) and 24 weeks triple therapy group (14%). The most important side effects with TVR were rash, gastrointestinal disorders and anaemia. Although severe rash may require treatment discontinuation, moderate forms can be successfully treated with topical steroids. The median decline in blood haemoglobin concentrations with TVR was approximately 1 g/dl.

The PROVE 1 and 2 seem to indicate that TVR can help overcome negative host and viral factors. A recent pooled analysis looked at a subgroup of patients with characteristics associated with low virological response (11). The overall SVR for the pooled TVR treatment groups was 65 vs. 44% in the control group ($P < 0.001$). SVR rates were significantly higher with TVR-based vs. SOC among patients with baseline HCV RNA $\geq 800\,000$ IU/ml ($P < 0.05$), patients with genotype 1a HCV infection ($P < 0.05$), patients with genotype 1b HCV infection ($P < 0.05$), men ($P < 0.05$), patients > 50 years of age ($P < 0.05$) and those with bridging fibrosis ($P < 0.05$). The conclusion of this analysis is that TVR is effective in all subgroups of patients who have traditionally been considered difficult to treat. Another phase II trial with TVR was recently released (Study C208) that suggests that SVR rates in naïve patients may be higher than previously reported, especially when a response-guided duration is followed. In this study, treatment-naïve, genotype 1 patients ($n = 161$) were administered triple therapy for 12 weeks with the subsequent PEG-IFN/RBV treatment duration determined according to a response-guided strategy (12). Patients who achieved a rapid virological response (RVR) received a total of 24 weeks of therapy and those who did not have RVR continued PEG-IFN/RBV to weeks 48. The SVR rates in this study ranged from 81 to 85%, higher than those observed in the phase II PROVE trials. These high overall SVR rates emphasize the potential of the triple therapy approach. Results may be explained in part by experienced study centres with very low discontinuation rates (5%) compared with the PROVE studies. In addition, treatment duration was shortened to 24 weeks in patients who achieved RVR, while the remaining patients received 48 weeks of therapy. Between 80 and 83% of patients treated with PEG-IFN-α2a, and 67–69% of patients treated with PEG-IFN-α2b achieved RVR and could therefore be treated for 24 weeks. This study clearly suggests that response-guided therapy based on RVR at week 4 may optimize SVR and provides a useful guide for determining which patients should be treated for 24 vs. 48 weeks.

Table 1. Lessons learned from Phase II data

Higher SVR (60–70%) in genotype 1
Response-guided therapy
Resistance emergence
Differences in genetic barrier to resistance for subtypes (1a vs. 1b)
PEG-IFN and RBV necessary to maximize efficacy

PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response.

Serine Protease Inhibitor Therapy-1: boceprevir trial

In the phase II SPRINT-1 trial, triple combination therapy with BOC and the current SOC, PEG-IFN and RBV, was found to induce high rates of SVR (54–75%) in genotype 1 treatment-naive patients, depending on the duration of therapy (13). Unlike TVR, BOC was administered for the duration of treatment. The treatment regimens included a control group treated with 48 weeks of SOC compared with five BOC treatment regimens (4 weeks of PEG-IFN/RBV lead-in followed by triple therapy for 24 or 44 weeks; triple therapy for 28 or 48 weeks; triple therapy, but with low-dose RBV for 48 weeks). The ideal duration of therapy appears to depend upon early viral kinetics. Patients who cleared the virus by week 4 of triple therapy had 82 and 94% chances of achieving SVR after 28 and 48 weeks of treatment respectively. If HCV RNA is detectable after week 4, but becomes undetectable by week 12, 48 weeks of treatment resulted in a 79% SVR rate; shortened treatment was significantly inferior, with only 21% of patients achieving SVR after 28 weeks. Clearance after week 12 was associated with a negligible chance of SVR and appears to indicate an early stopping rule at week 12. In addition to the expected side effects associated with the SOC, treatment with a

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BOC-containing regimen was associated with increased dysgeusia and anaemia. Anaemia (defined as a decline in haemoglobin level < 10 g/dl) occurred in 52–56% of patients in the triple-therapy groups despite administration of epoetin-αat the investigator's discretion, compared with 34% in controls. Higher rates of discontinuation secondary to adverse side effects and viral breakthrough occurred in the BOC treatment groups compared with the control group and anaemia appeared to be a significant problem, with up to 50% of patients receiving erythropoietin. Of note, the highest reported viral breakthrough was seen in the low-dose RBV group.

Ribavirin is required to maximize efficacy with protease inhibitors

As shown above, early phase II studies strongly suggest that RBV is needed in protease inhibitor drug regimens. Patients who did not receive RBV in the PROVE trials and those with low-dose RBV (400–1000 mg) in the SPRINT-1 trial had increased viral breakthrough, higher relapse and lower SVR. This data strongly indicates that standard-dose RBV is needed to optimize response to these first generation protease inhibitors by reducing the development of resistance/breakthrough. It is also clear that the initial rapid decrease in HCV viral levels with protease combination therapy is because of inhibition of wild type virus that then leads to the 'uncovering' of pre-existing resistant variants. Resistant variants are present in most patients at very low rates ($< 1\%$) and are usually detected after near complete suppression of the dominant, wild type virus. The continued replication of these variants can then lead to a virological breakthrough. To date, mutations conferring TVR-resistance have been identified at four positions, V36A/M/L, T54A, R155K/M/S/T and A156S//T (14). A detailed kinetic analysis of TVR-resistant variants was performed in genotype 1 patients during 14 days of TVR monotherapy and combination therapy with PEG-IFN. TVR monotherapy initially led to a rapid decline in HCV RNA in all patients as a result of a strong reduction in the wild-type virus. In patients who developed a viral rebound during TVR monotherapy, the single mutation variants R155K/T and A156/T were mainly uncovered by wild-type reduction and became dominant after day 8. These single mutation variants were selected from pre-existing quasispecies. The combination of TVR and PEG-IFN was sufficient to inhibit the breakthrough of resistant mutations in a 14-day study (15). It is important to note that low to medium levels of V36 and R155 variants were still observed in single patients up to 3 years after TVR treatment. Antiviral resistance is also a concern with BOC. Mutations were frequently associated with virological breakthrough, and most substitutions occurred at locations comparable to those with TVR. Of note, the time to revert back to wild type varied with the specific mutation in patients who developed resistance mutations. Another important finding from the TVR Phase II trials is the different rates of viral resistance and breakthrough detected between genotype 1a and 1b (much higher for 1a). This can be explained by a difference in the genetic barrier to resistance between the subtypes. For example, the mutation most frequently associated with resistance to TVR is R155K; changing R to K at position 155 requires one nucleotide change in HCV subtype 1a and 2 nucleotide changes in subtype 1b isolates. Thus HCV subtyping may play an important role in helping to select future treatment regimens and predict the development of resistance.

Phase III data for telaprevir and boceprevir

Phase III clinical trials evaluating TVR in combination with PEG-IFN and RBV have now been completed with top-line SVR data being released. The ADVANCE trial enrolled treatment-naïve HCV genotype 1 patients to evaluate 24 weeks of TVR-based therapy. TVR was dosed at 750 mg every 8 h and given for 8 or 12 weeks in combination with PEG-IFN and RBV followed by PEG-IFN and RBV alone until treatment week 24. Patients who did not achieve RVR were treated with PEG-IFN and RBV until week 48. A significantly greater proportion of patients achieved SVR with 12-week and 8-week TVR-based combination regimens (75 and 69% respectively) than in the SOC arm (44%) (16). Relapse rates were reduced three-fold (9%) compared with SOC (28%). In the ILLUMINATE trial, TVR was given for 12 weeks in combination with PEG-IFN and RBV followed by PEG-IFN and RBV alone until treatment week 24 or 48. The aim of the ILLUMINATE trial was to assess whether extending treatment beyond 24 weeks of total therapy improves SVR rates in patients with RVR or EVR. 72% of all subjects achieved SVR, while those with extended RVR (virus negative from week 4 to week 12) achieved SVR rates of 92 and 88% in randomized 24- and 48-week treatment groups respectively (17). Thus, data from these two phase III trials support the use of 24-week TVR-based therapy in a response-guided regimen for patients with RVR. Of note, treatment discontinuation from adverse events were double that of SOC but were much lower than that in Phase 2 trials. The most common adverse events reported in the ILLUMINATE study, were, in order of frequency, fatigue, pruritus, nausea, anaemia, rash and headache. Most of these adverse events were mild or moderate. Adverse events leading to discontinuation of all study drugs during the 12-week TVR dosing period occurred in 6.9%, while treatment discontinuation of all drugs because of anaemia and rash occurred in 1.1 and 0.6% of people in this study, respectively, during the TVR dosing period (Figs 1 and 2).

The phase III clinical trial (SPRINT-2) evaluating BOC in over 1000 treatment-naïve patients was also recently completed. Equivalent to the SPRINT-1 study design, patients (two separate cohorts were enrolled; one African American and the other non) received 800 mg BOC three

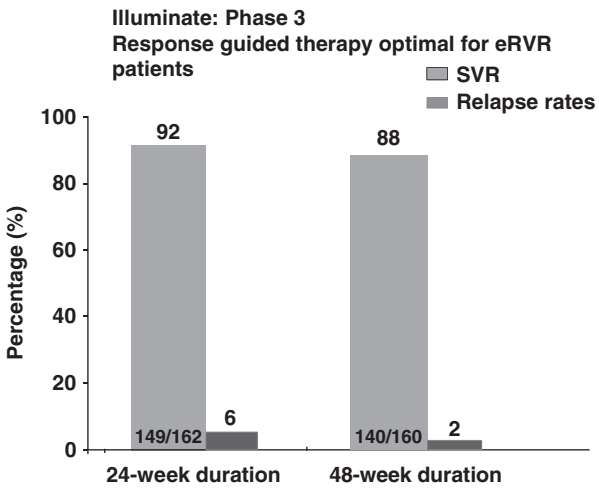

Fig. 1. ILLUMINATE: Phase 3: response-guided therapy optimal for eRVR Patients. eRVR, extended rapid virological response; SVR, sustained virological response.

ADVANCE: Phase 3
12-week duration telaprevir optimal

Treatment regimen	SVR	RVR	Relapse rates	AE-related D/C
TVR 12 weeks + PR	75%	68%	8.6%	6.9%
TVR 8 weeks + PR	69%	66%	9.5%	7.7%
Peg-IFN / RBV	44%	9%	28%	3.6%

Fig. 2. ADVANCE: Phase 3: 12-week duration telaprevir optimal. PEG-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response; TVR, telaprevir.

times daily in combination with PEG-IFN and weight-based RBV for 24 or 48 weeks. A lead-in strategy for 4 weeks with PEG-IFN and RBV was utilized in all investigational arms. In this study, 66% of the patients in the BOC 48-week treatment group and 63% of the patients in the response-guided therapy group achieved SVR respectively, compared with 38% of patients in the control group (18). Among the non-African American patients in the BOC 48-week treatment group, 69% achieved SVR, and 67% achieved SVR in the response-guided therapy, compared with 40% in the control SOC group. Among African American patients, 53% of patients in the 48-week treatment group and 42% of patients in the response-guided therapy group achieved SVR, compared with 23% in the control group. This data is less clear than TVR phase 3 studies on the utility of response-guided therapy in all genotype 1 populations and suggest that extending therapy may be beneficial in African Americans. Further details from this study will help clarify the importance of host factors in response-guided therapy durations. In the HCV SPRINT-2 study, the most common treatment-emergent adverse events reported for the BOC 48-week treatment group, BOC response-guided therapy group and control group, respectively, were: fatigue (57, 53 and 60%), headache (46, 46 and 42%), nausea (43, 48 and 42%), anaemia (49, 49 and 29%) and pyrexia (fever) (32, 33 and 33%). Treatment was discontinued because of anaemia in 2% of each of the BOC groups compared with 1% in the control group, although erythropoietin use was allowed to maintain RBV dosing. Overall treatment discontinuations from adverse events were 16 and 12% for the BOC groups, respectively, compared with 16% for the control group. The utility of erythropoietin in these patients is currently under investigation in another phase 3 trial.

Conclusion

In conclusion, clinical trials have shown that the addition of protease inhibitors to standard therapy results in potent viral suppression and shortened duration of therapy. SVR rates approaching 75% can now be anticipated for genotype 1 patients, which should lead to increased treatment opportunities for many HCV populations. However, new issues of viral resistance and increased adverse events will increase the importance of close medical management. A new era of DAA is upon us and offers new hopes for HCV-infected patients.

Conflicts of interest

David Nelson has received research funding and is on advisory boards or a consultant for Vertex, Merck, Genentech, Pharmassett, Bayer-Onyx and GSK. He has received research funding from BMS, Gilead and Tibotech and is a consultant for Abbott.

References

1. Armstrong GL, Wasley A, Simard EP, *et al.* The prevalence of Hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705–14.
2. Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35–46.
3. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47–56.
4. Verna EC, Brown RS. Hepatitis C and liver transplantation: enhancing outcomes and should patients be retransplanted. *Clin Liver Dis* 2008; **12**: 637–59.
5. Davis GL, Alter MJ, El-Serag H, *et al.* Aging of hepatitis C-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010; **138**: 513–21.
6. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic Hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.
7. Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with Interferon

alfa-2b plus ribavirin for initial treatment of chronic Hepatitis C: a Randomised Trial. *Lancet* 2001; **358**: 958–65.
8. Asselah T, Benhamou Y, Marcellin P. Protease and polymerase inhibitors for the treatment of hepatitis C. *Liver Int* 2009; **29**(Suppl. 1): 57–67.
9. McHutchison JG, Everson GT, Gordon SC, *et al*. Telaprevir with Peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
10. Hézode C, Forestier N, Dusheiko G, *et al*. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839–50.
11. Everson GT, Dusheiko GM, Ferenci P, *et al*. Telaprevir, peginterferon alfa-2a and ribavirin improved rates of sustained virologic response (SVR) in 'difficult-to-cure' patients with chronic Hepatitis C (CHC): a pooled analysis from the PROVE1 and PROVE2 trials. *Hepatology* 2009; **50**: 1565.
12. Marcellin P, Forns X, Goeser T, *et al*. Virologic analysis of patients receiving telaprevir administered q8h or q12h with Peginterferon-alfa-2a or -alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis: study C208. *Hepatology* 2009; **50**: 194.
13. Kwo P, Lawitz E, McCone J, *et al*. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705–16.
14. Sarrazin C, Kieffer TL, Bartels D, *et al*. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroenterology* 2007; **132**: 1767–77.
15. Kieffer TL, Sarrazin C, Miller JS, *et al*. Telaprevir and pegylated interferon-alpha 2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. *Hepatology* 2007; **46**: 631–9.
16. Vertex Pharmaceuticals. 75% of treatment-naive patients with chronic hepatitis C achieve SVR (viral cure) with telaprevir-based treatment in Phase 3 trial. Press release, 25 May 2010.
17. Vertex Pharmaceuticals. Phase 3 ILLUMINATE Study supports 24-week telaprevir-based therapy within a response-guided regimen for people with hepatitis C who had not received prior treatment. Press release, 10 August 2010.
18. Poordad F, McCone J, Bacon BR, *et al*. Boceprevir combined with peginterferon alfa-2b/Ribavirin for treatment-naïve patients with hepatitis C virus genotype. 1: SPRINT-2 final results. *Hepatology* 2010; **52**: 107A.