Analysis of boceprevir resistance associated amino acid variants (RAVs) in two phase 3 boceprevir clinical studies

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A B S T R A C T
Background: We investigated the frequency of RAVs among patients failing to achieve SVR in two clinical trials. We also investigated the impact of interferon responsiveness on RAVs and specific baseline RAVs relationship with boceprevir treatment failure.

Methods: Data are from 1020 patients enrolled into either SPRINT-2 or RESPOND-2; patients received a 4-week PR lead-in prior to receiving boceprevir or placebo. RAVs were analyzed via population-based sequence analysis of the NS3 protease gene (success rate of ≥ 90% at a virus level of ≥ 10,000 IU/mL)

Results: The high SVR rate in patients who received boceprevir resulted in a low rate of RAVs; 7% was detected at baseline in all patients, which rose to 15% after treatment. However, RAVs were detected in 53% of patients that failed to achieve SVR, which declined to 22.8% – 14 months following cessation of boceprevir therapy. Baseline RAVs alone were not predictive of virologic outcome; poor interferon responsiveness was highly predictive of non-SVR. RAVs were more frequently detected in poor interferon responders.

Conclusions: We detected no association between the presence of baseline amino acid variants at boceprevir resistance-associated loci and outcome in the context of good IFN response.

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Introduction

Up to 2–3% of the world’s population is chronically infected with Hepatitis C Virus (HCV) and three to four million people globally become newly infected each year (Shepard et al., 2005). In developed countries, HCV related cirrhosis is one of the most common reasons for liver transplantation. It is estimated that 20% of persons with untreated chronic HCV infections will progress to cirrhosis and 15% of individuals with cirrhosis will develop hepatocellular carcinoma within 5 years (Davis et al., 2010). While peginterferon and ribavirin (PR) therapy of HCV infection allows for a sustained virologic response (SVR) in about 40–50% of genotype 1-infected patients and in 80–90% of those infected with genotypes 2 or 3 (Zeuzem, 2008; McHutchison et al., 2009), other treatments are available. The new standard of care for genotype 1 is combination therapy with PR in combination with boceprevir or telaprevir.

Treatment of HCV infection has historically been based on host-directed therapies that stimulate the immune system. However, recent advances in the field have enabled the development of numerous direct-acting antiviral agents that directly inhibit essential HCV proteins required for viral replication (McGovern et al., 2008). Boceprevir is a peptidomimetic ketoamide protease inhibitor that binds reversibly to the HCV non-structural protein 3 (NS3) active site (Berman and Boceprevir, 2009; Mederacke et al., 2009). Boceprevir has demonstrated antiviral activity in vitro (e.g., HCV replicon models), as monotherapy in Phase 1 studies, and in multiple Phase 2 trials in combination with the standard of care of PR (Malcolm et al., 2006; Sarrazin et al., 2007; Schiff et al., 2008; Kwo et al., 2010) Boceprevir has also been studied in Phase 3 clinical trials in treatment naïve (SPRINT-2) (Poordad et al., 2011) and previously non-responsive (RESPOND-2) (Bacon et al., 2011) HCV infected patients.

While the discovery and development of direct acting antiviral therapies against HCV has advanced treatment significantly, the use of these new agents is not without potential challenges. As with HIV, the potential for rapid emergence of drug-resistant
variants of HCV is of major concern. HCV variants arise from a combination of the inherent poor fidelity (Castro et al., 2005) of the RNA dependent RNA polymerase and the high rate of viral replication (Neumann et al., 1998; Perelson et al., 1996). Amino acid changes in the HCV NS3 protease domain sequence that confer reduced susceptibility to boceprevir (also termed RAVs; resistance associated amino acid variants) have been characterized identified both in replicons selected for boceprevir resistance in vitro and in viruses isolated in vivo from HCV-infected patients failing to achieve SVR with a boceprevir regimen (Tong et al., 2006, 2008; Curry et al., 2008; Susser et al., 2009).

In this report we investigate the frequency of baseline and post-baseline RAVs among patients failing to achieve SVR in SPRINT-2 and RESPOND-2, the registration trials for boceprevir. In addition, we investigate the impact of interferon responsiveness on RAV frequency at failure as well as whether specific baseline RAVs were more commonly associated with boceprevir treatment failure.

Results

Sample accounting

A total of 1020 patients received boceprevir in two phase three studies; SPRINT-2 (Poordad et al., 2011) (PR treatment naïve patients) and RESPOND-2 (Bacon et al., 2011) (patients previously treated with PR). To analyze boceprevir resistance, the NS3/4a region of the HCV genome was amplified from patient plasma samples using standard reverse transcriptase/PCR techniques and subjected to population sequence analysis. Of the 1020 patients enrolled in the SPRINT-2 and RESPOND-2 studies that received boceprevir, baseline samples from 1013 patients were sequenced and data were obtained for 980/1013 subjects (Fig. 1). Sequencing failed for 33 patients and 7/1020 patients did not have adequate baseline samples (available prior to any treatment) for resistance testing. Of the 343 patients who did not achieve SVR, 41/343 patients did not have post-baseline samples available or did not have samples with an adequate viral load for resistance analysis. Viruses from 302/343 patients were sequenced and resistance data was available for 295 of these subjects (Fig. 1).

Sequence analysis from post-baseline samples

The generally high overall SVR rate in patients who received boceprevir in both treatment naïve (SPRINT-2; 63–66%) and treatment experienced (RESPOND-2; 59–66%) resulted in an overall low rate of RAVs. Overall, 15% (157/1020) of all patients treated with boceprevir had post-baseline RAVs detected (Fig. 2A). One patient that achieved SVR had RAVs (T54S) detected at TW8 of the study. Of the patients not achieving SVR with available resistance data (n=295), 53% (156/295) had post-baseline RAVs detected (Table 1). RAVs were more frequently detected among genotype 1a non-SVR patients (55%; 118/214) compared to genotype 1b infected patients (47%; 38/81) (Fig. 2B). In addition SVR rates were also lower in genotype 1a patients (63%) compared to genotype 1b patients (73%) (data not shown). The difference in SVR rates among genotype 1a and 1b patients was similar in the SPRINT-2 (11.2%) and RESPOND-2 (9.2%), respectively.

Among genotype 1a infected patients, the RAVs most frequently detected at virologic failure were V36M (60%), R155K (67%) and T54S (19%) (Fig. 2C). In contrast, the RAVs most commonly detected in patients with genotype 1b virus were T54A (42%), T54S (37%), V55A (24%), A156S (26%) and V170A.

Table 1

Non-SVR patients with post-baseline RAVs detected.

<table>
<thead>
<tr>
<th>Patient Category</th>
<th>Total</th>
<th>Patients with RAVs detected/patients data available</th>
<th>% Patients with RAVs detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-SVR</td>
<td>343</td>
<td>156/295</td>
<td>53%</td>
</tr>
<tr>
<td>Incomplete virological response</td>
<td>61</td>
<td>54/59</td>
<td>92%</td>
</tr>
<tr>
<td>Viral breakthrough</td>
<td>26</td>
<td>18/24</td>
<td>75%</td>
</tr>
<tr>
<td>Relapse</td>
<td>79</td>
<td>33/78</td>
<td>42%</td>
</tr>
<tr>
<td>Nonresponder/Futility</td>
<td>79</td>
<td>34/71</td>
<td>48%</td>
</tr>
<tr>
<td>Nonresponder/Non-futility</td>
<td>98</td>
<td>17/63</td>
<td>27%</td>
</tr>
</tbody>
</table>
Post-baseline RAVs were more frequently detected among non-SVR patients that experienced virologic breakthrough during the boceprevir/PR dosing period, i.e. during the period of maximum boceprevir-exposure. Overall, 92% (54/59) of patients that experienced IVR, 75% of BT subjects (18/24) and 48% of NR FUT patients (34/71) had viruses with RAVs detected post-baseline (Table 1). RAVs were detected less frequently among RL patients (32/78, 41%) that experienced virologic failure after cessation of Boceprevir/PR therapy. RAVs were only detected in 27% (17/63) of NR Other patients that discontinued boceprevir/PR therapy for reasons other than futility (Table 1).

RAVs are rapidly selected during short-term monotherapy dosing studies with first generation HCV protease inhibitors. Therefore, in order to maintain viral suppression of resistant variants, boceprevir is administered in combination with peginterferon/ribavirin. To investigate the impact of interferon responsiveness on the development of RAVs, interferon response was assessed based on the decrease in HCV viral load at the end of the
4-week PR lead-in or by assessment of host IL-28b genotype (Sarrazin et al., 2007; Kieffer et al., 2007). All patients in the SPRINT-2 and RESPOND-2 studies received PR alone for 4-weeks, prior to boceprevir/PR dosing, allowing an on-study assessment of interferon responsiveness in patients. Interferon responses in patients were classified based on the decline in HCV RNA at the end of the week 4 PR lead in (TW4). Patients that experienced a < 1 log₁₀ decline in HCV RNA levels at TW4 were classified as poor interferon responders and patients with a decline in HCV RNA of ≥1 log₁₀ at TW4 were classified as interferon responders.

Poorly interferon responsive non-SVR patients were more likely to fail treatment with detectable RAVs than those who were classified as IFN responders (Fig. 3A); 68% (115/169) of poorly interferon responsive patients had detectable RAVs, compared to 31% of interferon responders (41/128) (Fig. 3A). The higher frequency of RAVs detected among poorly interferon responsive patients is partly explained by the higher proportion of patients that experienced virologic failure during boceprevir dosing (BT, IVR and NR³⁵⁴) 63% (118/186) compared to interferon responsive patients 31% (47/149) (Supplementary Table S1).

Genome-wide association studies have identified genetic variations near the IL-28b gene, encoding interferon-λ, that can inform on the interferon/ribavirin responsiveness of patients (Ge et al., 2009). Interferon responsiveness has been linked to a single nucleotide polymorphism in the IL-28b gene (http://www.ncbi.nlm.nih.gov/pubmed/19684573). Approximately 60% of patients in the SPRINT-2 and RESPOND-2 studies consented to have IL-28b genotypic testing, allowing another independent measure of the association of RAVs to interferon responsiveness. Patients with a ’CC’ rs12979860 IL-28b genotype have been associated with favorable IFN responsiveness, whereas patients with ’TC’ or ’TT’ rs12979860 genotypes have less favorable interferon responsiveness. Consistent with this, SVR rates among ’CC’ patients was higher (80%) than that of ’TC’ (68%) or ’TT’ patients (59%) (Poordad et al., 2012). The data from this analysis corroborate previous results indicating a higher frequency of RAVs in patients who are poorly IFN responsive (Fig. 3B). Roughly 29% of ’CC’ IFN responsive patients had a post-baseline RAV detected. However RAVs were detected more frequently among poorly IFN-responsive patients, with 51% of ’TC’ and 62% of ’TT’ poorly IFN-responsive subjects harboring post-baseline RAVs, respectively.

Consistent with reported lower responsiveness to interferon among black patients, 59% of black patients had post-baseline RAVs detected, compared to 45% for non-black patients (Supplementary Fig. S1).

Baseline RAVs

Due to the poor fidelity of the HCV RNA-dependent RNA polymerase (NS5B), it is likely that many boceprevir RAVs pre-exist prior to initiation of therapy (Colson et al., 2008; Bartels et al., 2008). Baseline sequence data were available for 980/1020 patients; 628/656 patients with genotype 1a virus, 352/359 with genotype 1b virus (Table 2). Amino acid variants were detected at one or more of the 11 boceprevir resistance-associated loci (RAVs)

![Fig. 3](image-url)

(A) RAVs detected in non-SVR patients by interferon responsiveness at TW4 and (B) RAVs detected in non-SVR patients by IL-28b genotype. CC, IFN responsive; CT, less IFN responsive; TT, not IFN responsive.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Subjects Sequenced</th>
<th>Samples Sequenced</th>
<th>Samples Sequenced and RAVs Detected, n/N (%)</th>
<th>Number of subjects with samples sequenced and RAVs detected, n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>V36I</td>
<td>V36L</td>
</tr>
<tr>
<td>All Subjects</td>
<td>1020</td>
<td>980</td>
<td>66/980(6.7)</td>
<td>2/66(3.0)</td>
</tr>
<tr>
<td>1a</td>
<td>656</td>
<td>628</td>
<td>53/628(8.5)</td>
<td>1/53(1.9)</td>
</tr>
<tr>
<td>1b</td>
<td>359</td>
<td>352</td>
<td>13/352(3.7)</td>
<td>1/13(8.0)</td>
</tr>
</tbody>
</table>

RAV=resistance associated amino acid variants.
Two patients had an indeterminate genotype 1 subtype and 3 patients infected with non-genotype 1 viruses were excluded from the above analysis.

(a) Number of subjects with samples sequenced and RAVs detected/total number of subjects with samples sequenced.

(b) Number of subjects with samples sequenced and RAVs detected for each RAV/total number of subjects with samples sequenced and RAVs detected. Subjects may have more than one RAV and appear in the more than one RAV count.
variants were shown to have resistance to boceprevir virologic failure samples from non-SVR patients; however, these infrequent post-baseline variants V36I/L, Q41H, V55I, V170M interferon responsive (as de
and/or V55A, 36 were interferon responsive and 7 were poorly not shown). Of the 43 patients who had V36M, R155K, T54A/S, rates among interferon responsive patients with V36I/L, Q41H, V55A RAVs present at baseline (78%; 28/36) was similar to the SVR rate among interferon responsive patients with V36M, R155K, T54A/S, and/or V55A was 0% (0/7), compared to SVR rates of 50% (3/6) among poorly interferon responsive patients with RAVs V36M, R155K, T54A/S, and/or V55A RAVs detected at baseline by 454-sequencing at baseline and after the 4-week PR lead in or those without baseline RAVs detected (79%, 513/648) (Table 3). In contrast, the SVR rate among poorly interferon responsive patients with RAVs V36M, R155K, T54A/S, and/or V55A was 0% (0/7), compared to SVR rates of 50% (3/6) among poorly interferon responsive patients with baseline RAVs V36M, R155K, V55A, V170M and/or M175L or 34% (86/254) with no baseline RAVs detected (Table 3). Although the numbers of patients are small, the combination of poor interferon responsiveness and V36M, R155K, T54A/S, and/or V55A RAVs at baseline appeared to correspond with a poor treatment outcome. The numbers of patients with a combination of poor interferon responsiveness and baseline V36M, R155K, T54A/S, and/or V55A RAVs represented 1% (7/1020) of patients treated with boceprevir. It is important to remember that these data are from population sequencing and therefore RAVs present in low number (<20%) may not be detected due to the relatively low assay sensitivity.

RAVs detected after the 4-week PR lead-in

As RAVs do not confer resistance to PEG IFN or ribavirin, one of the putative benefits of the 4-week PR lead in would be to reduce the viral burden of pre-existing RAVs, prior to the addition of boceprevir to the combination therapy. To test this hypothesis, RAVs were quantified by 454-sequencing at baseline and after the 4-week lead-in period. As viruses that harbor RAVs often make up a very minor part of the circulating population, six patients were selected that had viruses with RAVs detected at baseline by population sequencing. This guaranteed that a significant proportion of RAVs in the subsequent pyrosequence analysis. Four of the six patients experienced <1 log_{10} decline in HCV RNA levels by TW4 and were classified as poor-interferon responders. Two patients experienced >1 log_{10} decline in HCV RNA levels and were classified as interferon responders (Table 4). Patients were selected with high viral loads to mitigate re-sampling errors in the pyrosequence analysis.

RAVs identified by population sequencing at baseline were detected in >95% of all viral sequences in the pyrosequence analysis. These included V55A, T54A or T54S. In three patients, RAVs were encoded by two different codons, indicating the

Table 3
SVR rates by treatment week 4 response among patients with or without baseline RAVs detected.

<table>
<thead>
<tr>
<th>Total</th>
<th>SVR (%)</th>
<th>Interferon responders</th>
<th>Poor interferon responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patient (n)</td>
<td>SVR (%)</td>
</tr>
<tr>
<td>Patients without baseline RAVs</td>
<td>902</td>
<td>67%</td>
<td>648</td>
</tr>
<tr>
<td>Patients with baseline RAVs</td>
<td>64</td>
<td>65%</td>
<td>51</td>
</tr>
<tr>
<td>V36M, R155K, T54A/S and V55A</td>
<td>43</td>
<td>63%</td>
<td>36</td>
</tr>
<tr>
<td>Other baseline RAVs (V36L, V36I, Q41H, V55L, V170M, M175L)</td>
<td>21</td>
<td>67%</td>
<td>15</td>
</tr>
</tbody>
</table>

* Total with Week 4 vial load available (treatment week 4 data not available for 12 patients without baseline RAVs and 2 with baseline RAVs).

Table 4
454-Sequence analysis quantification of RAVs during the 4-week lead-in.

<table>
<thead>
<tr>
<th>Patient</th>
<th>HCV RNA baseline (IU/ml)</th>
<th>HCV RNA treatment Week 4 (IU/ml)</th>
<th>Log10 decline in HCV RNA</th>
<th>Treatment response</th>
<th>RAV (Codon)</th>
<th>Baseline RAV %</th>
<th>Treatment Week 4 %</th>
<th>% Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11,700,000</td>
<td>5,030,000</td>
<td>0.37</td>
<td>Poor IFN responder</td>
<td>V55A (GCC)</td>
<td>97.92%</td>
<td>97.65%</td>
<td>0.27%</td>
</tr>
<tr>
<td>2</td>
<td>20,900,000</td>
<td>3,380,000</td>
<td>0.79</td>
<td>Poor IFN responder</td>
<td>V55A (GCC)</td>
<td>93.17%</td>
<td>96.16%</td>
<td>2.99%</td>
</tr>
<tr>
<td>3</td>
<td>4,030,000</td>
<td>3,660,000</td>
<td>0.04</td>
<td>Poor IFN responder</td>
<td>V55A (GCT)</td>
<td>6.43%</td>
<td>3.75%</td>
<td>2.68%</td>
</tr>
<tr>
<td>4</td>
<td>3,810,000</td>
<td>449,000</td>
<td>0.93</td>
<td>Poor IFN responder</td>
<td>T54A (GCC)</td>
<td>64.62%</td>
<td>57.32%</td>
<td>7.30%</td>
</tr>
<tr>
<td>5</td>
<td>399,000</td>
<td>690</td>
<td>1.76</td>
<td>IFN responder</td>
<td>V55A (GCC)</td>
<td>83.38%</td>
<td>81.13%</td>
<td>2.25%</td>
</tr>
<tr>
<td>6</td>
<td>2,450,000</td>
<td>175,000</td>
<td>1.15</td>
<td>IFN responder</td>
<td>V54S (GCC)</td>
<td>15.84%</td>
<td>18.33%</td>
<td>2.49%</td>
</tr>
</tbody>
</table>

in 7% (66/977) of patients with samples sequenced; 53/628 (8%) patients with genotype 1a virus and 13/352 (4%) patients with genotype 1b virus.

SVR rates among patients with baseline RAVs detected (65%, 43/66) were similar to those without baseline RAVs detectable (67%, 599/902) (Table 3). Overall, baseline RAVs alone were not predictive of virologic outcome.

Of the 66 boceprevir-treated patients with RAVs detected at baseline, 43 had V36M, R155K, T54A/S, and/or V55A (Table 3) which were frequently detected in non-SVR patients at virologic failure (Supplementary Fig. S2). 21 patients at baseline had infrequent post-baseline variants V36I/L, Q41H, V55I, V170M and/or M175L. These 5 variants were not commonly detected in virologic failure samples from non-SVR patients; however, these variants were shown to have resistance to boceprevir in vitro (data not shown). Of the 43 patients who had V36M, R155K, T54A/S, and/or V55A, 36 were interferon responsive and 7 were poorly interferon responsive (as defined at TW4). The SVR rate among interferon responsive patients with RAVs V36M, R155K, T54A/S, and/or V55A present at baseline (78%; 28/36) was similar to the SVR rate among interferon responsive patients with V36I/L, Q41H, V55I, V170M and/or M175L at baseline (73%; 11/15) or those without baseline RAVs detected (79%, 513/648) (Table 3).
presence of at least two independent viral quasi-species with RAVs that existed prior to treatment (Table 4). As all six patients had high viral loads (> 400,000 IU/ml) at baseline, these viral variants did not appear to have impaired replication capacity. The frequency with which these RAVS were detected in the overall population at baseline and at treatment week 4 is listed in Table 4. In all six patients, the frequency of viruses harboring RAVs at treatment week 4 was similar to the frequencies identified at baseline.

The viral load of viruses harboring each RAV can be estimated at each time point by multiplying the frequency of each RAV by the total viral load. As the viral load declined during the 4 week PR lead-in in all six patients, the viral load of all RAVs also declined from their baseline values (Table 4). Importantly, boceprevir RAVs at other loci (positions 36, 155, 156, 170 or 175) were not detected at the end of the 4 week P/R lead-in phase, indicating that there was no significant enrichment of other RAVs.

RAVs were present in over 95% of the sequences at baseline and remained at these levels at the end of the 4 week lead-in phase. RAVs declined in viral load in all six patients during the 4-week lead in, suggesting that viruses that harbor these RAVs were sensitive to P/R therapy and that the viral burden of RAVs declines during the lead-in phase.

**RAVs detected during follow-up**

Among non-SVR patients, resistance sequencing was performed at two time points; virologic failure and at what would have been the SVR24 time point (i.e. at both the virologic failure time point and the end of study time point). An assessment of the longevity of RAVs during follow-up could not be made as patients discontinued all treatment at virologic failure. As patients experienced virologic failure at different times; the length of follow-up differed, ranging from 6 to 14 months with a median of 10 months. Data were available at both these time points for 127/156 of the non-SVR patients with detectable RAVs. Fig. 4 shows the frequency of patients that experienced virologic failure with specific RAVs that were no longer detected at the end of the second ‘end of study’ time point. The incidence of RAVs at the second time point differed among different variants. RAVs T54A and A156S declined to undetectability in >83% of genotype 1a and 1b infected patients. However, only 67–69% of patients with T54S or R155K no longer had these RAVs detected at the second time point. There was little difference in the speed of decline based on the genotype (differences with V55A were due to small numbers of this variant detected). Given the low sensitivity of population sequencing (detection limit ~10–20% of minor variants), it is possible that RAVs not detected at the second time point are still present.

Population sequence data were compared for 506 subjects in the response guided therapy arms and 514 subjects in the boceprevir/P/R 48 week arms of SPRINT-2 and RESPOND-2 (Supplementary Table S2). Post-baseline sequence data were available for 158/506 patients in the response guided therapy arms and 142/514 patients in boceprevir/P/R 48 week arms. The percentages of patients with samples sequenced and post-baseline RAVs were identical between the two arms (52%, 82/158 and 52%, 74/142). The most common RAVs detected in both arms were V36M, R155K, T54A, and T54S.

**Discussion**

In the current report we investigated the frequency of baseline and post-baseline RAVs among non-SVR patients enrolled in SPRINT-2 and RESPOND-2 by population sequencing. Given the high error rate of the NS5B RNA-dependent RNA polymerase and the high rate of HCV replication, it has been hypothesized that all single- and double-amino acid variants that can lead to a decrease susceptibility to direct acting antiviral agents pre-exist in the viral population prior to the initiation of therapy (Rong et al., 2010). Consistent with this hypothesis, RAVs were detected in 7% of patients (by population sequencing), prior to the treatment with boceprevir (Supplementary Fig. S2). It is possible that many RAVs exist prior to the treatment below the level of detection of population sequence analysis (detection limit ~20% of circulating viruses). RAVs with a low replicative capacity/fitness might not be detected using this technology; therefore, studies that seek to identify these variants at baseline using deep-sequence technology are ongoing. Although RAVs were detected by population sequence analysis, in most cases the presence of these variants did not preclude the ability of patients to achieve SVR. As viruses harboring boceprevir RAVs are sensitive to PR treatment (Table 4) the responsiveness of patients to interferon likely plays a key role in the clearance of pre-existing variants which harbor resistance to boceprevir. This would be consistent with the observation that patients who had baseline RAVs commonly associated with virologic failure (V36M, T54A/S, V55A and R155K) and who were also poor interferon responders all failed to achieve SVR. However, this accounts for only a small number of the non-SVR patients and given the heterogeneous responses of patients to PR, the ability to clear viruses containing RAVs will differ making it hard to establish a viral load threshold for RAVs at baseline that could aid in predicting the treatment outcome.

In a recent telaprevir publication based on phase III data, resistance associated variants were observed in 77% (299/388) of patients who did not achieve SVR (Sullivan et al., 2013). This compares to 47% in the current study (143/300). Telaprevir RAVs occurred more commonly in subtype 1a (86%; 232/269) than subtype 1b infections (56%; 67/119), compared to the current study where boceprevir RAVs were found in 55% (118/214) and 47% (38/81) of genotype 1a and 1b infected non-SVR patients, respectively.

RAVs detected in both the telaprevir study and the current study were similar. The majority (~91%) of 1a-infected patients in the telaprevir study had resistant variants carrying V36M (12%), R155K (22%), or the combination of V36M and R155K (56%). The majority (84%) of 1b-infected patients in the telaprevir study had V36A (28%), T54A (39%), or A156S/T (22%). In the current study the RAVs most frequently detected at virologic failure among genotype 1a infected patients were V36M (60%), R155K (67%) and T54S (19%). RAVs most commonly detected in patients with genotype 1b virus in the current study were T54A (42%), T54S (37%), V55A (24%), A156S (26%) and V170A (32%).
Interferon responsiveness was highly predictive for development of RAVs. The incidence in patients poorly responsive (≤ 1 log₁₀ decrease in HCV-RNA at TW 4 from baseline) to IFN was twofold higher than in those with good response to IFN (≥ 1 log₁₀ decrease in HCV-RNA at TW 4 from baseline). Among all subjects with samples analyzed, 68% of poorly IFN responsive subjects had RAVs compared to 32% of those with better IFN response. This finding is partly explained by the fact that poorly interferon responsive patients were more likely to experience virologic failure during dosing than interferon responsive patients (Table 3). This is consistent with data obtained when patient IL-28B genotype is used as a marker of interferon responsiveness (Fig. 3B).

Further analysis by race category demonstrated that Black patients had a higher percentage of RAVs detected post-baseline compared to non-black patients. Detection of RAVs after the initiation of treatment in Black patients was similar for both boceprevir/PR treatment strategies (response guided therapy vs. 48 weeks). This is likely due to the poorer responsiveness to interferon in these patients (Brau et al., 2006).

The differences in the detected RAVs between genotype 1a and 1b are likely to at least in part be influenced by the differences in genetic barrier of the more common Gt1a and Gt1b RAVs. Variants V36M or R155K require only a single nucleotide change in genotype 1a viruses, but require two nucleotide changes in genotype 1b viruses. Conversely, V170A variants require only a single nucleotide change in genotype 1b viruses but two nucleotides in genotype 1a virions.

Following treatment, RAVs frequently declined to below the level of detection in population sequence assays, reflecting a decrease in abundance of viruses harboring RAVs over time in patients. The number of patients with RAVs detected at the second time point differed for the different RAVs, likely reflecting differences in the viral fitness of each RAV. Indeed, the in vitro fitness of RAVs that declined most rapidly (A156T) have been demonstrated to have a lower replicative capacity than variants such as R155K that declined at a lower rate in vivo (Shimakami et al., 2011). The clinical significance of these findings on the re-treatment of patients with HCV protease inhibitors with overlapping resistance profiles remains to be determined.

In conclusion, it is important to note that the potential presence of baseline RAVs should not preclude therapy with PEG/RBV and boceprevir. The likelihood of developing RAVs relates to PEG IFN responsiveness of the hepatitis C infected individual. The use of lead-in as well as the determination of IL-28 B status can help guide clinicians in assessing the risk for developing RAVs when considering boceprevir based therapy.

Methodology

Subjects

Data reported herein are from patients enrolled into either SPRINT-2 or RESPOND-2 (the registration trials for boceprevir). Detailed methodologies for both of these studies have been published (Poordad et al., 2011; Bacon et al., 2011). For the SPRINT-2 and RESPOND-2 Studies, HCV-RNA was quantified using the Roche High Pure System/Cobas Taqman assay, v2.0 with an overall limit of detection of 25 IU/mL. LOD by 95% hit rate Probit analysis for genotype 1 isolates in plasma was 9.3 IU/mL. Briefly, both trials were double-blinded for boceprevir, and all patients received a 4-week lead-in with PR prior to receiving either boceprevir or placebo. The primary endpoint of each trial was SVR, compared to the placebo control. In both SPRINT-2 and RESPOND-2 clinical trials, non-SVR patients were categorized based on the following 5 treatment responses; Breakthrough (BT; patients on treatment whose HCV-RNA became detectable after having achieved an undetectable level of HCV-RNA), Incomplete Virologic Response (IVR; patients that experienced ≥1 log₁₀ IU/mL increase in HCV-RNA from nadir during treatment), Relapsers (RL; patients that had undetectable HCV-RNA at the end of treatment but subsequently had detectable HCV-RNA at end of the 6 month follow-up period), Non-Responder Futility (NR Fut; patients that had detectable HCV RNA at treatment week 12 (SPRINT-2) or week 24 (RESPOND-2) and met the futility discontinuation rule) and Non-Responder Other (NR Other; patients that discontinued for reasons other than meeting a futility rule, such as the occurrence of an adverse event or withdrawal of consent).

Study procedures

There were a total of 1058 patients randomized to receive boceprevir in the SPRINT-2 (735 patients) and RESPOND-2 (324 patients) studies. However, 38/1058 of these patients (30 from SPRINT-2 and 8 from RESPOND-2) discontinued the study during the lead-in phase, prior to receiving a dose of study drug. Therefore, 1020 patients received study drug in SPRINT-2 and RESPOND-2 and were the focus of the resistance analysis. To detect RAVs prior to study treatments in SPRINT-2 and RESPOND-2, plasma samples were collected for NS3 sequence analysis in all patients (n = 1020) at baseline (or from screening sample if day 1 was missing). In addition, samples were taken at or near the time of virologic failure in patients who did not achieve SVR during SPRINT-2 or RESPOND-2 (n = 343). Population sequencing of the entire NS3 region was performed by Janssen Diagnostics (NJ).

Sequence analysis

In both the SPRINT-2 and RESPOND-2 clinical studies, sequence analysis was performed on plasma samples obtained at the following time points; at baseline (all patients), at treatment week 8 (if the viral load ≥ 1000 IU/mL), at virologic failure (all non-SVR patients). For technical reasons, sequence analysis was performed only on patient plasma samples with a viral load of > 1000 IU/mL. Plasma samples were evaluated by population sequencing and analyzed to detect amino acid variants in the NS3/4A protease known or suspected to be associated with reduced susceptibility to boceprevir. The population sequence analysis was performed at Janssen Diagnostics BVBA (Belgium). Where possible, the entire NS3/4a region of the HCV genome was amplified for using genotype-specific primers by nested RT-PCR. However, if amplions could not be generated for the full NS3/4a region, a smaller amplicon encompassing just the NS3 region (amino acids 1–180 of the HCV NS3 protease) was amplified. The NS3/4a or NS3 regions were then subjected to population sequence analysis. The success rate of the assay was > 90% at a virus level of ≥ 10,000 IU/mL but decreases with declining viral loads to approximately 71% success rate at 1000 IU/mL.

Resistance analyses focused on 11 positions (“loci”) in the amino acid sequence of the NS3/4A protease domain at which substitutions (“RAVs”) had been identified and shown to confer reduced susceptibility to boceprevir. The amino acids found at the 11 loci of NS3 in the genotype 1a strain H77 used in the analysis were V36, Q41, F43, T54, V55, R155, A156, V158, D168, I/V170, and L175. The amino acids found at the 11 loci of NS3 in the genotype 1b strain Con1 used in the analysis of samples with genotype 1b were V36, Q41, F43, T54, V55, R155, A156, V158, D168, I/V170, and M175. Samples with genotype 1a1b, genotype 1 (subtype not specified) and non-1 genotypes were aligned to the genotype 1a H77 reference sequence.
Pyrosequencing

Pyrosequence analysis was performed by Jansen Diagnostics (Belgium). Viral RNA was isolated from plasma samples collected at baseline and treatment week 4 for the six patients enrolled in the boceprevir phase 2/3 clinical studies. Amino acids 1–181 of the HCV NS3 gene were amplified by two separate amplicons using standard RT-PCR techniques and subjected to pyrosequence analysis. Sequence analysis was performed using the Roche 454 Genome Sequencer FLX using titanium plates. Processing of the raw sequence data was analyzed using the Roche 454 software package (Sequencing System Software). GS Amplicon Variant Analyzer (AVA) software (Roche) was used for mapping and variant calling. No filtering or trimming on Phred quality values (QVs) was performed other than those dictated by the Roche Software. Variants were determined at each codon (position 3) using proprietary software developed by Jansen Infectious Disease-Diagnostics.

The number of independent sequences for each HCV NS3 codon varied for each amplicon, with >95% of codons being represented by at least 1800 sequences and >75% represented by 5000 sequences. In order to separate real variants from sequencing artifacts, codons had to be represented by >50 sequences, according to manufacturer’s instructions. This led to a lower limit of sensitivity of this assay that ranged from 0.13 to 10.92%, depending on the number of independent sequences.

Disclosures


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Appendix A. Supporting information

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References