

Clinical Implications of Detectable Baseline Hepatitis C Virus-Genotype 1 NS3/4A-Protease Variants on the Efficacy of Boceprevir Combined With Peginterferon/Ribavirin

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Background. We analyzed the impact of pretreatment variants conferring boceprevir-resistance on sustained virologic response (SVR) rates achieved with boceprevir plus peginterferon- α /ribavirin (P/R) for hepatitis C virus (HCV)-genotype-1 infection.

Methods. NS3-protease-polymorphisms emerging coincident with virologic failure on boceprevir/P/R regimens were identified as resistance-associated variants (RAVs). Baseline samples pooled from 6 phase II or phase III clinical trials were analyzed for RAVs by population sequencing. Interferon (IFN)-responsiveness was predefined as >1 log reduction in HCV-RNA level during the initial 4-week lead-in treatment with P/R before boceprevir was added. The effective boceprevir-concentration inhibiting RAV growth by 50% (EC₅₀) was determined using a replicon assay relative to the wild-type referent.

Results. Sequencing was performed in 2241 of 2353 patients (95.2%) treated with boceprevir. At baseline, RAVs were detected in 178 patients (7.9%), including 153 of 1498 genotype-1a infections (10.2%) and 25 of 742 genotype-1b infections (3.4%) (relative risk, 3.03; 95% confidence interval [CI], [2.01, 4.58]). For IFN-responders, SVR₂₄ (SVR assessed 24 weeks after discontinuation of all study medications) rates were 78% and 76% with or without RAVs detected at baseline, respectively. For the 510 poor IFN-responders, SVR₂₄ rates were 8 of 36 subjects (22.2% [11.7%, 38.1%]) when baseline RAVs were detected vs 174 of 474 subjects (36.7% [32.5%, 41.1%]) when baseline RAVs were not detected (relative likelihood of SVR₂₄ [95% CI], 0.61 [0.32, 1.05]). Sustained virologic response was achieved in 7 of 8 (87.5%) IFN-nonresponders with baseline variants exhibiting ≤ 2 -fold increased EC₅₀ for boceprevir in a replicon assay, whereas only 1 of 15 (7%) IFN-nonresponders with baseline RAVs associated with ≥ 3 -fold increased EC₅₀ achieved SVR.

Conclusions. Baseline protease-variants appear to negatively impact SVR rates for boceprevir/P/R regimens only when associated with decreased boceprevir susceptibility in vitro after a poor IFN-response during the lead-in period.

Keywords. boceprevir; hepatitis C-genotype 1; RAVs; resistance-associated variants.

Drug resistance represents a new challenge to the fast-evolving therapy of chronic hepatitis C virus

(HCV) [1–4]. Virologic failure during treatment with directly acting antiviral agents is often accompanied by the emergence of resistance-associated variants (RAVs) [5, 6]. The error-prone HCV RNA-polymerase generates quasi-species of genetically related viruses harboring minor genomic differences [7]. Polymorphic variants associated with decreased susceptibility to some drugs are likely already circulating at low levels in many patients, even before exposure to directly acting antiviral agents [8–11]. Resistance-associated variants may then become the dominant species under selective drug pressure when viral replication is inadequately suppressed [12, 13].

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Table 1. Baseline Characteristics and Study Outcomes by Presence or Absence of Baseline RAVs

	Baseline RAVs N = 178	No Baseline RAVs N = 2063	Not Sequenced N = 111
Median age, years [range]	50 [22, 68]	51 [18, 76]	50 [21, 70]
Gender, n (%)			
Female	81 (45.5)	923 (44.7)	49 (44.1)
Male	97 (54.5)	1140 (55.3)	62 (55.9)
Self-identified Race, n (%)			
White	158 (88.8)	1662 (80.6)	96 (86.5)
Black	14 (7.9)	337 (16.3)	8 (7.2)
Asian	3 (1.7)	30 (1.5)	6 (5.4)
Other or mixed	3 (1.7)	34 (1.6)	1 (0.9)
Region, n (%)			
Europe	23 (12.9)	421 (20.4)	27 (24.3)
North America	153 (86.0)	1617 (78.4)	84 (75.7)
South America	2 (1.1)	25 (1.2)	0 (0.0)
HCV subtype, n (%)			
1a	153 (86.0)	1345 (65.2)	73 (65.8)
1b	25 (14.0)	717 (34.8)	14 (12.6)
Other	0 (0.0)	1 (0.0)	24 (21.6)
HCV-RNA level at entry, n (%)			
≤400 000 IU/mL	20 (11.2)	153 (7.4)	17 (15.3)
>400 000 to ≤800 000 IU/mL	16 (9.0)	142 (6.9)	14 (12.6)
>800 000 IU/mL	142 (79.8)	1768 (85.7)	80 (72.1)
METAVIR score, n (%)			
F0, F1 or F2 (or unknown)	155 (87.1)	1831 (88.8)	102 (88.8)
F3 or F4	23 (8.7)	232 (11.2)	9 (8.1)
IL28B genotype, n (%)			
CC	48 (27.0)	361 (17.5)	20 (18.0)
CT	52 (29.2)	672 (32.6)	28 (25.2)
TT	13 (7.3)	232 (11.2)	8 (7.2)
Unknown	65 (36.5)	798 (38.7)	55 (49.5)
Prior treatment history ^a , n (%)			
Naive	159 (89.3)	1652 (80.1)	95 (85.6)
Incomplete response to P/R	11 (6.2)	322 (16.1)	12 (10.8)
Relapse after P/R	8 (4.5)	79 (3.8)	4 (3.6)
Study endpoint			
SVR ₂₄	115 (64.6)	1326 (64.3)	84 (75.7)
All-cause failure (non-SVR ₂₄)	63 (35.4)	737 (35.7)	27 (24.3)
Virologic failure ^b	39 (21.9)	501 (24.3)	16 (14.4)
Nonvirologic failure ^c	24 (12.4)	236 (11.4)	11 (9.9)

Abbreviations: HCV, hepatitis C virus; P/R, peginterferon alfa plus ribavirin; RAV, resistance-associated variant; SVR₂₄, sustained virologic response assessed 24 weeks after discontinuation of all study medications.

^a No patient had received a directly acting antiviral agent of any class before enrollment.

^b Virologic failure encompasses incomplete response (including discontinuation for futility and lack of efficacy), breakthrough, and relapse.

^c Nonvirologic failure includes all non-SVR₂₄ not due to virologic failure.

Boceprevir is a NS3/4A-protease inhibitor approved for treatment of HCV genotype-1 infection in combination with peginterferon- α /ribavirin (P/R). Boceprevir RAVs were (1) initially discovered in the NS3-protease gene after in vitro selection using HCV-replicon cell lines and (2) presumptively confirmed by sequencing the protease gene from viruses emerging in HCV-infected patients coincident with virologic failure on triple therapy with boceprevir plus P/R [4, 5]. These variants were subsequently shown to confer varying degrees of decreased susceptibility to boceprevir when introduced as amino acid substitutions into the recombinant NS3-protease enzyme or wild-type replicons. The common mutants have been mapped to their positions in the 3-dimensional structure of the NS3-protease active site and correlated with their effects on the enzymatic properties of the NS3/4A-protease and the magnitude of resistance conferred in replicon assays. Although RAVs have been well characterized in vitro, their full therapeutic ramifications are still inadequately understood [1, 3, 13–15].

The objective of the current pooled analysis of the clinical trial database was to assess the impact of preexisting variants on treatment outcomes in subjects treated with boceprevir plus P/R. The primary question was if or when did detection of RAVs at baseline predict a high likelihood of treatment failure. In particular, we sought to determine whether baseline RAVs at levels detectable by population sequencing (~20% of circulating quasi-species) were associated with treatment failure and whether interferon-responsiveness was a critical cofactor in any relationship between baseline RAVs and SVR. Although the current analysis focused on boceprevir, these data can help inform how baseline variants might impact the efficacy of newer antiviral regimens, including or exclusively consisting of directly acting agents against HCV.

METHODS

Study Design

Data were gathered from 6 clinical trials (including 1 phase II study [P03523] and 5 phase III studies [P05101, P05216, P05411, P05685, and P06086]) conducted in treatment-naive and treatment-experienced patients with HCV-genotype 1 infection, comparing the then standard P/R therapy to treatment regimens that added boceprevir after an initial 4-week lead-in period with P/R alone [16–21]. Interferon responsiveness was defined per protocol as >1 log reduction in HCV-RNA level during the initial 4-week treatment with P/R before boceprevir was added. Patients exposed to a directly acting antiviral agent were ineligible. Futility rules were prespecified per the protocols; patients in whom study therapy was stopped for futility were considered virologic failures. Circulating viral quasi-species in plasma specimens obtained at baseline and at the time of virologic failure were to undergo population sequencing with a

detection limit for variants of ~20% prevalence [22]. IL28B genotype (r12979860) was determined using the Illumina Bead-Chip Technology (San Diego, CA) in 4 studies. Only patients treated with ≥ 1 dose of boceprevir were eligible for the current analyses.

Viral and Resistance Assays

Plasma HCV-RNA levels were measured by the TaqMan 2.0 assay (Roche Diagnostics, Branchburg, NJ) with lower limits of quantification of 25 IU/mL and lower limits of detection of 9.3 IU/mL. To assess genotypic variation at baseline or at virologic failure, the NS3/4A gene was amplified from samples with RNA levels ≥ 1000 IU/mL using reverse transcription-polymerase chain reaction, followed by population sequencing of the NS3-protease region (amino acid residues 1–181) [23]. Resultant amino acid sequences were compared with wild-type HCV genotype 1a (H77) or 1b (Con1) reference sequences. Amino acid substitutions at 11 loci (V36A/M, T54A/C/G/S, V55A/I, V107I, R155C/K/T, A156S/T/V, V158I, D168N, I170A/F/T/V, V170A/F/T, and M175L) alone or in combination were considered to represent RAVs irrespective of genotype-1 subtype because these variants commonly emerged coincident with virologic failure in patients treated with boceprevir/P/R in the pivotal trials.

In phenotypic analyses to measure the antiviral potency of boceprevir against RAVs, genotype-1a (H77) and genotype-1b (Con 1) replicon and enzyme constructs were engineered to incorporate NS3-mutations. Stable subgenomic replicons expressing NS3 mutants were constructed in Huh-7 cells. Fold shift in boceprevir susceptibility for each variant replicon was expressed relative to the effective concentration inhibiting viral growth by 50% (EC_{50}) for the subtype-specific wild-type

replicon. Boceprevir inhibitory activity against recombinant NS3-enzymes was tested with a chromogenic assay. Inhibition constants from 4 to 8 experiments using a single clone for each variant (K_i^*) were averaged, and the fold change in boceprevir susceptibility was expressed relative to the wild-type K_i^* .

RESULTS

Subject Accounting and Baseline Characteristics

Population NS3-sequence data were obtained at baseline from 2241 of 2352 (95.3%) boceprevir recipients, including 1498 of 1571 (95.4%) with genotype-1a infections and 742 of 756 (98.1%) with genotype-1b infections. Baseline characteristics were generally similar in patients with or without baseline RAVs, except that genotype 1a infections were disproportionately represented among the RAVs (Table 1). At baseline, no RAVs were detected by population sequencing in 2063 of 2241 (92%) of patients. Baseline RAVs were identified in 178 of 2241 (7.9%) patients at 8 positions (V36, T54, V55, V107, R155, V158, I170, and M175) of the 11 defined boceprevir resistance-associated NS3-loci. Resistance-associated variants were found in 153 of 1498 (10.2%) patients with genotype-1a virus and in 25 of 742 (3.4%) patients with genotype-1b virus (relative risk of detected RAV at baseline [95% confidence interval] = 3.03 [2.01, 4.58]). In genotype-1a infections, the most common substitutions were I170V, V55A, T54S, and V55I; in genotype-1b infections, the most common substitutions were T54S, V55A, and V107I (Figure 1). No other substitution occurred in $\geq 10\%$ of cases. The large majority of boceprevir recipients included in our analysis population were treatment-naive at study entry: 1906 of 2352 patients (81.0%) overall, including 159 of 178 patients (89.3%) with RAVs detected at baseline, and 1652 of 2063 patients (80.13%) without RAVs detected at baseline. No patient had received a direct-acting antiviral drug before enrollment.

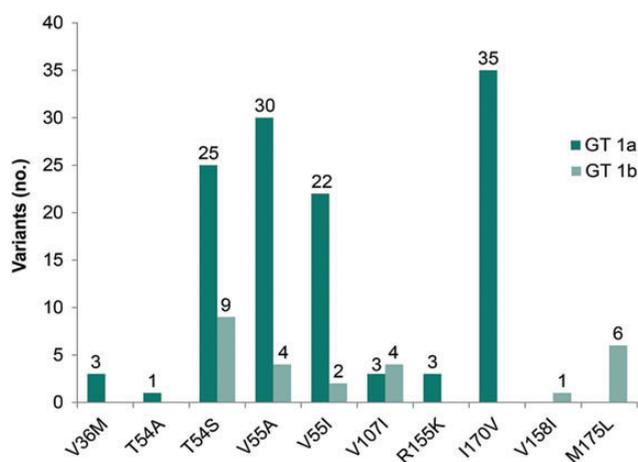


Figure 1. Frequency and distribution of specific amino acid substitutions among the 178 patients with RAVs detected by population sequencing at baseline. Results are shown separately for genotype-1a and genotype-1b variants. Abbreviations: GT, genotype; RAV, resistance-associated variant.

Phenotypic Susceptibilities of Detected Boceprevir Resistance-Associated Variants

The effects of RAVs identified at baseline on boceprevir activity were tested in genotype-1a and genotype-1b replicon cell lines, as well as using recombinant NS3-enzymes containing the substituted amino acid, and expressed relative to the wild-type referent (Table 2). Inhibition of enzyme activity was sometimes discordant with the replicon susceptibility for several variants. V55I and V107I in genotype-1a replicons did not result in decreased boceprevir sensitivity, but these substitutions caused 12-fold and 2-fold reductions, respectively, in the enzyme inhibition. V107I in the genotype 1b replicon and I170V in the genotype 1a replicon decreased boceprevir susceptibility by 2-fold but did not reduce enzyme inhibition. Boceprevir activity against all other tested variants was ≥ 2 -fold less than against the wild-type comparator in both assay systems. Genotype-1a or genotype-1b replicons harboring V36M, T54A/S, R155K,

Table 2. In Vitro Activity of Boceprevir Against Variants Detected at Baseline Relative to Wild-Type Virus Using Replicon and Enzyme Assays

	V36M	T54A	T54S	V55A	V55I	V107I	R155K	V158I	I170V	M175L
Fold Shift (RAV EC ₅₀ /Wild-Type EC ₅₀)										
Replicon										
GT 1a	4	3	3	4	1	1	6	2	2	na
GT 1b	4	6	5	4	2	2	5	5	na	3
Fold Shift (RAV Ki*/Wild-Type Ki*)										
Enzyme										
GT 1a	2	7	3	2	12	2	4	2	1	na
GT 1b	2	4	2	4	7	1	4	nd	na	nd

Abbreviations: EC₅₀, effective concentration 50%; GT, genotype; Ki*, inhibition constant from 4 to 8 experiments using a single clone for each variant; na, not applicable because the specified substitution is not commonly found in that particular HCV-genotype; nd, not done; RAV, resistance-associated variant.

or V55A were ≥ 3 -fold less susceptible to boceprevir than wild-type virus, as were genotype-1b replicons with V158I or M175L.

Pretreatment Variants and Subsequent Virologic Failure

Among boceprevir recipients, SVR₂₄ (SVR assessed 24 weeks after discontinuation of all study medications) rates were 64.3% (1326 of 2063) when baseline RAVs were not detected and 64.6% (115 of 178) when baseline RAVs were detected. A total of 39 (61.9%) of the 63 patients with baseline RAVs who did not achieve SVR₂₄ experienced virologic failure, including 15 patients who also had RAVs at the time of failure and during follow-up (Table 3). In 13 of the 15 patients, a single RAV containing T54S ($n = 1$), V55A ($n = 5$), V55I ($n = 1$), R155K ($n = 1$), or I170V ($n = 5$) was found after virologic failure. Multiple polymorphisms containing V36M, R155K, and I170V ($n = 1$) or T54S and V55I ($n = 1$) were detected in the other 2 patients after virologic failure. Among the 5 patients with the single V55A RAV detected at baseline, 3 patients had only V55A detected subsequent to virologic failure, whereas V55A with V36M or V55A with V36L and V158I were identified in 1 patient each after virologic failure. Of the 5 patients with I170V at baseline, none had this variant detected after virologic failure, although V55A ($n = 1$), A156S ($n = 1$), A156T ($n = 1$), or V36M and R155K ($n = 2$) were found at failure. The patients with either V55I or T54S variants at baseline failed with V36M and V55I or Q41H and T54S, respectively. The only patient with an isolated baseline R155K variant failed with this variant alone, but V36M with R155K was later detected during follow-up.

Interaction of Pretreatment Variant With IL28B Genotype and Interferon Responsiveness

In our analysis, 78% of boceprevir recipients with the CC genotype achieved SVR₂₄ compared with 64% for patients with either the CT or TT genotype. The SVR₂₄ rates were generally similar for CC and non-CC subjects irrespective of the presence of detected RAVs at baseline (Table 4).

For interferon-responders during the lead-in treatment period with P/R before the addition of boceprevir, respective SVR₂₄ rates with boceprevir regimens were 76% and 78% for patients with or without RAVs detected at baseline (Table 5). Of the 510 subjects with poor interferon-responses, SVR₂₄ rates were 8 of 36 patients (22.2% [11.7%, 38.1%]) when baseline RAVs were detected vs 174 of 474 patients (36.7% [32.5%, 41.1%]) when baseline RAVs were not detected (relative likelihood of SVR₂₄ [95% confidence interval, 0.61; 0.32, 1.05]). SVR₂₄ was achieved in 7 of 8 (87.5%) interferon-nonresponders with baseline variants exhibiting ≤ 2 -fold increased EC₉₀ for boceprevir in a replicon assay. In contrast, only 1 of 15 (6.7%) interferon-nonresponders with a baseline RAV associated with ≥ 3 -fold increased EC₅₀, which contained an isolated V55A substitution, achieved SVR.

DISCUSSION

In this pooled analysis of 6 phase II and III studies, baseline RAVs were detected by standard population sequencing in $\sim 8\%$ of patients overall and were 3-fold more prevalent in genotype 1a than genotype 1b infections. Hepatitis C virus genotype 1b has a higher genetic barrier to drug resistance than genotype 1a [9, 13, 24, 25]. The most common baseline RAVs were I170V, V55A, T54S, and V55I in genotype-1a infections and T54S, V55A, and V107I in genotype-1b infections.

Our findings regarding the frequencies and implications of baseline variants with reduced susceptibility to protease inhibitors are generally consistent with earlier reports in the literature [10, 11]. The overall SVR rate of $\sim 65\%$ was not compromised among boceprevir recipients with RAVs detected at baseline. For interferon-responders, SVR₂₄ rates were 78% with RAVs and 76% without RAVs at baseline. In contrast, for the 510 subjects with poor interferon-responses, SVR₂₄ rates were 22% when baseline RAVs were detected vs 37% when baseline RAVs were not detected. Only 1 of 8 interferon-nonresponders with baseline RAVs conferring ≥ 3 -fold decrease in boceprevir

Table 3. Comparison of RAVs at Baseline With RAVs After Virologic Failure in Individual Patients^a

Patient	HCV Genotype	Lead-in P/R Response	Type of Virologic Failure	Sample day	Boceprevir RAVs
1	1a	No	Nonresponse	0	V36M, R155K, I170V
				57	V36M, R155K
				92	V36M, R155K
				113	V36M, R155K
				506	V36M, R155K
2	1a	No	Nonresponse	0	T54S, V55I
				56	T54S, V55I
				502	T54S, V55I
3	1a	Yes	Relapse	0	V55A
				508	V55A
4	1a	No	Nonresponse	0	V55A
				57	V55A
				169	V36M, V55A
				508	V36M, V55A
5	1a	Yes	Nonresponse	0	V55A
				203	V36L, V55A, V158I
				511	V36L, V55A
6	1a	No	Incomplete response	0	V55A
				137	V55A, R155K
				523	V55A, R155K
7	1a	No	Nonresponse	0	V55A
				59	V55A
				109	V55A, R155K/T
				549	V55A
8	1a	Yes	Relapse	0	V55I
				414	V36M, V55I
				512	V36M, V55I
9	1a	No	Relapse	0	R155K
				71	R155K
				365	V36M, R155K
				505	V36M, R155K
10	1a	No	Nonresponse	0	I170V
				225	A156S
				526	A156S
11	1a	No	Nonresponse	0	I170V
				225	V36M, R155K
12	1a	No	Relapse	0	I170V
				505	V55A
13	1a	No	Incomplete response	0	I170V
				71	V36M, R155K
				99	V36M, R155K
				265	V36M, R155K
14	1a	No	Incomplete response	0	I170V
				56	A156T
				84	V36M, R155K, A156S, V158I
				95	V36M, R155K, A156S, V158I
15	1b	Yes	Relapse	0	T54S
				445	Q41H, T54S

Abbreviations: HCV, hepatitis C virus; P/R, peginterferon alfa plus ribavirin; RAV, resistance-associated variant.

^a Day 0 indicates samples obtained at baseline. Lead-in refers to the first 4 weeks of P/R therapy before boceprevir was added.

Table 4. SVR₂₄ Rates in Patients With and Without Baseline RAVs by (A) IL28B Genotype (CC vs CT/TT) at the End of the 4-Week Lead-in Treatment Period With P/R Before Boceprevir Was Added

	CC (N = 409)		Non-CC (N = 969)	
	Patients (n/m)	SVR Rate	Patients (n/m)	SVR Rate
IL28B Genotype				
Subjects without baseline RAVs (N = 1265)	282/361	78%	571/904	63%
Subjects with baseline RAVs (N = 113)	36/48	75%	43/65	66%

Abbreviations: N, number of patients in the category evaluable for the SVR analysis of RAVs x IL28B genotype; n/m, number of patients with SVR/number of patients satisfying the characteristics for the specified cell; P/R, peginterferon alfa plus ribavirin; RAV, resistance-associated variant; SVR, sustained virologic response. SVR₂₄, sustained virologic response assessed 24 weeks after discontinuation of all study medications.

susceptibility in vitro achieved SVR. In contrast to the empiric demonstration of interferon-responsiveness during the lead-in period, the IL28B genotype (a marker of interferon responsiveness) did not influence SVR results as a function of the presence or absence of baseline RAVs.

Not all baseline and emergent HCV-protease variants will actually confer clinically meaningful drug resistance. Before interpretive guidelines for genotypic resistance testing can be established for a given drug, RAVs must be distinguished from therapeutically inconsequential polymorphisms based on extensive clinical correlation. Phenotypic resistance testing using our replicon and enzyme assays did not always yield concordant results. For the purposes of the current analysis, NS3-protease polymorphisms emerging coincident with virologic failure in patients treated with boceprevir/P/R regimens were considered to represent RAVs. When accompanied by poor responses to P/R, the baseline polymorphisms among these variants conferring relatively high levels of boceprevir resistance in vitro predicted failure to achieve SVR, presumably because boceprevir plus P/R approximates functional monotherapy under these conditions.

Our pooled analysis has several noteworthy limitations. The study was retrospective and encompassed 6 different protocols. By missing minor variants, population-based sequencing as used here likely underestimated the frequency of potentially relevant RAVs [22]. Although the lessons may be generalizable to other treatment paradigms, interferon-based regimens are being phased out [26, 27]. At least in the developed world, boceprevir use is rapidly diminishing in favor of other directly acting antiviral drug combinations that achieve SVR rates exceeding 90% [26–28].

The prognostic utility of baseline resistance testing requires continued scrutiny as the use of different classes of directly

Table 5. Interferon Response (>1 Log Reduction From Baseline HCV-RNA Level) at the End of the 4-Week Lead-in Treatment Period With P/R Before Boceprevir Was Added

	Interferon Responders (N = 1447)		Interferon Nonresponders (N = 510)	
	Patients (n/m)	SVR Rate	Patients (n/m)	SVR Rate
Interferon Responsiveness				
Subjects without baseline RAVs (N = 1822)	1009/1327	76%	174/474	37%
Subjects with baseline RAVs (N = 158)	94/120	78%	8/36	22%

Abbreviations: HCV, hepatitis C virus; N, number of patients in the category evaluable for the SVR analysis of RAVs x interferon responsiveness; n/m, number of patients with SVR/number of patients satisfying the characteristics for the specified cell; P/R, peginterferon alfa plus ribavirin; RAV, resistance-associated variant; SVR, sustained virologic response. SVR₂₄, sustained virologic response assessed 24 weeks after discontinuation of all study medications.

acting antiviral agents for chronic HCV infection becomes increasingly widespread. In addition to the infecting HCV-genotype, a recent history of failure on an interferon-sparing regimen may affect the probability of detecting drug-specific or class-wide RAVs at a given point in time. Because combination therapy is universally recommended, baseline variants might not impact outcome unless abundant RAVs, high-level resistance, poor interferon-responsiveness, cross-resistance to other coadministered antiviral agents, and/or erratic compliance with an unforgiving regimen are concurrently present. In our pooled analysis using clinically relevant outcomes, baseline NS3-protease-variants negatively impacted SVR rates in patients treated with boceprevir/P/R regimens most when associated with decreased susceptibility to boceprevir in vitro coupled with a poor interferon response during the lead-in period. As the number of directly acting antiviral agents expands, these data may instruct clinicians about general principles underlying the interpretation of resistance testing in selecting combination regimens to treat individual patients.

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full access to any pertinent data upon request. Each coauthor approved an essentially final version of the manuscript. A penultimate version of the paper was reviewed by the sponsor.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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