Frequencies of Resistance-Associated Amino Acid Variants Following Combination Treatment with Boceprevir Plus PEGINTRON (PegInterferon Alfa-2b)/Ribavirin in Patients With Chronic Hepatitis C (CHC), Genotype 1 (G1)

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Background

- Chronic Hepatitis C Virus (HCV) Infection and Therapy:
  - Nearly 180 million people are chronically infected with hepatitis C virus (HCV) worldwide.[1]
  - Standard-of-Care treatment of HCV is combination therapy with pegylated interferon and ribavirin.[2-4]
  - Of the six major HCV genotypes, genotype 1 is the least responsive to currently approved therapies, with sustained virologic response rates of less than 50%.[2,5-7]
- Direct Acting Antiviral Therapy:
  - The investigational focus for treatment of HCV has shifted toward direct acting antiviral therapy (**Figures 1 and 2**).[8-11]
  - Boceprevir (SCH503034) is a structurally novel, peptidomimetic ketoamide protease inhibitor that binds reversibly to the HCV NS3 active site (**Figure 3**).[8]
- HCV SPRINT-1 (Serine Protease Inhibitor Therapy-1) Clinical Trial
  - A Phase 2 study conducted in patients with chronic hepatitis C genotype 1 who were treatment-naïve. The primary objective was to determine the safety and efficacy of boceprevir when added to peginterferon and ribavirin.[12]
  - The SPRINT-1 study included an analysis of amino acid variants associated with differential response to boceprevir

Purpose

- To determine factors associated with a differential frequency of resistance associated variants (RAVs) in the SPRINT-1 study

Hypotheses

1) Specific baseline amino acid variants in NS3/4A are associated with differential antiviral activity of boceprevir
2) Specific post-baseline amino acid variants in NS3/4A are associated with differential antiviral activity of boceprevir

Figure 1. Life Cycle of HCV and Potential Direct Acting Antiviral (DAA) Targets

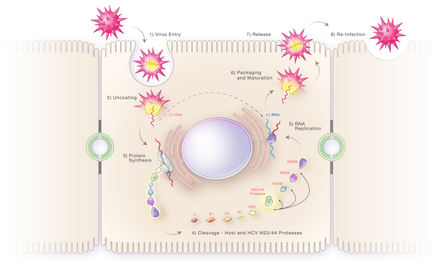
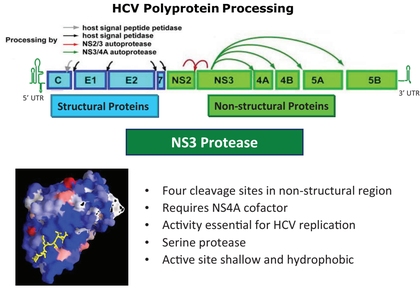
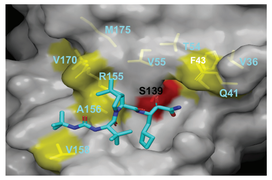


Figure 2. Direct Acting Anti-viral Therapy Targeting the Viral NS3 Protease



- Four cleavage sites in non-structural region
- Requires NS4A cofactor
- Activity essential for HCV replication
- Serine protease
- Active site shallow and hydrophobic

Figure 3. HCV NS3/4A Protease Residues Associated with Boceprevir Resistance



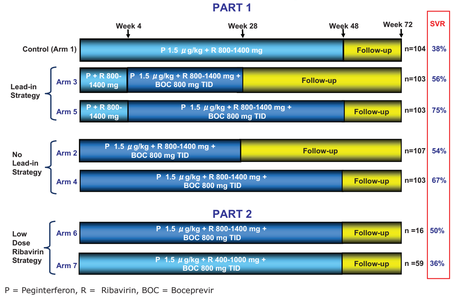
Adapted from Taremi et al. *Protein Science* (1998)

3D crystallographic model of boceprevir binding in the HCV NS3/4A protease binding pocket. Boceprevir resistance associated variants (RAVs) are highlighted by amino acid number. RAVs that appear at the surface of the binding pocket are highlighted in yellow. Boceprevir binds covalently to amino acid S139, highlighted in red.

Methods

- SPRINT-1 Study
  - Previously untreated adults with genotype 1 HCV were enrolled across the United States, Canada, and European Union
  - Two-part, open-label trial (**Figure 4**)
    - **Part 1** - 520 patients randomized to receive peginterferon plus ribavirin for 48 weeks (control) or one of four boceprevir regimens
    - **Part 2** - 75 patients randomized to receive peginterferon and low-dose ribavirin to evaluate the possibility of using a lower dose of ribavirin to reduce complications associated with anemia
  - Arms 2 and 3 of Part 1 received a 4 week lead-in with peginterferon/ribavirin prior to the addition of boceprevir to:
    - Achieve steady state of peginterferon/ribavirin prior to adding 3rd drug
    - Upregulate immune response elements
    - Decrease viral load and quasispecies, thereby decreasing resistance
- Virological Testing:
  - HCV-RNA was detected using Roche Taqman (LLD <15 IU/mL)
  - Amino acid variants at boceprevir resistance loci in the NS3/4A protease were detected using population sequencing
  - Baseline samples - any sample obtained prior to receiving any study medication - were obtained from all subjects
- Definitions
  - Sustained Virologic Response (SVR): Plasma HCV-RNA level below the lower limit of detection at follow-up week 12
  - Incomplete Virologic Response (IVR): A ≥2 $\log_{10}$ increase in HCV-RNA viral load compared with the previous two visits and HCV viral load ≥50,000 IU/mL
  - Viral Breakthrough (BT): Undetectable HCV-RNA and subsequent HCV-RNA ≥2 $\log_{10}$ elevation during therapy
  - Relapser (RL): Undetectable HCV-RNA at end of treatment and detectable HCV-RNA at follow-up week 24
  - Nonresponder (NR) (treatment failure):
    - Subjects in Arm 1 with detectable HCV-RNA at treatment week 24 who crossed over to boceprevir
    - Subjects in any of the seven treatment arms with detectable HCV-RNA at end of therapy and at follow-up week 24
    - Subjects in any of the seven treatment arms with missing HCV-RNA values at follow-up week 24 and do not have an undetectable HCV-RNA at follow-up week 12

Figure 4. SPRINT-1 Study Design and Sustained Virologic Response[12]



P = Peginterferon, R = Ribavirin, BOC = Boceprevir

Results

- Genotype 1 subtype in 595 treated subjects:
  - 401 HCV genotype 1a
  - 188 genotype 1b
  - 3 with genotype 1 subtype either not reported or reported as 1a/1b
- SVR was significantly higher in all boceprevir arms (54-75%) compared to control (38%) (**Figure 4**)
- Patients with RAV
  - **Table 1** - Patients with baseline RAVs previously shown to confer reduced susceptibility to boceprevir in vitro
  - Of the 24 patients who had RAVs detected at baseline, most (17 of 24; 71%) achieved SVR
  - Post-baseline samples were sequenced for 37% (219/595) of subjects
  - Of 109 patients who developed RAVs on-study (i.e. those without RAVs at baseline), 78 were G1a, 30 were G1b and 1 could not be subtyped (**Figure 5**)
- **Figure 6** - Specific on-study variants detected for genotypes 1a and 1b
  - Genotype 1a patients - most frequently detected RAVs were R155K (77%), V36M (68%), and T54S (37%)
  - Genotype 1b patients - most frequently detected RAVs were T54S (57%), T54A (37%), A156S (43%), and I170A (43%)
- The frequency of boceprevir RAVs by response to treatment (**Figure 7**)
  - Among 109 subjects developing RAVs during the study, 2 achieved SVR (both from boceprevir arms)
  - 256 subjects did not achieve SVR (non-SVR group)
  - In 212 non-SVR subjects with available resistance data, RAVs detected in:
    - 90% (57/63) of subjects with viral breakthrough on-treatment
    - 27% (19/70) subjects who relapsed
    - 37% (27/73) of nonresponders
    - 67% (4/6) of subjects with incomplete virologic responses
  - Highest frequency of on-study RAVs (24/59 patients; 41%) in subjects receiving low dose ribavirin (Arm 7; **Figure 8**)
  - Lowest frequency (11/103 patients; 11%) in subjects receiving 4 weeks peginterferon/ribavirin lead-in followed by 44 weeks boceprevir + peginterferon/ribavirin (Arm 5; **Figure 8**)
  - Among the 3 most frequent on-study RAVs, T54S was observed less frequently in patients receiving lead-in therapy (**Figure 9**)

Table 1. Subjects with Baseline RAVs Previously Demonstrated to Confer Reduced Susceptibility to Boceprevir *in vitro*

The treatment response of each subject is listed:

V36 Variants			T54 Variants			V55 Variants			R155K Variants		
Subject #	Variants	Treatment Response	Subject #	Variants	Treatment Response	Subject #	Variants	Treatment Response	Subject #	Variants	Treatment Response
1	V36M	SVR	3	T54S	SVR	11	V55A	SVR	22	R155K	SVR
2	V36M, V55A	SVR	4	T54S	SVR	12	V55A	SVR	23	R155K	NR
			5	T54S	SVR	13	V55A	BT	24	*Q41H, R155K, **I170V	NR
			6	T54S	SVR	14	V55A	RL			
			7	T54S	SVR	15	V55A	SVR			
			8	T54S	SVR	16	V55A	NR			
			9	T54S, V55I	SVR	17	V55A	SVR			
			10	T54S, V55I	SVR	18	V55A	RL			
						19	V55A, **L175M	SVR			
						20	*Q41H, V55A	SVR			
						21	V55I	RL			

*Q41H variant did not confer reduced susceptibility to boceprevir in vitro
**I170V, L175M are subtype-associated variants (polymorphisms) not conferring reduced susceptibility to boceprevir in vitro

Figure 5. Number of RAVs Detected by HCV Genotype

Population Sequence Analysis – Post Baseline

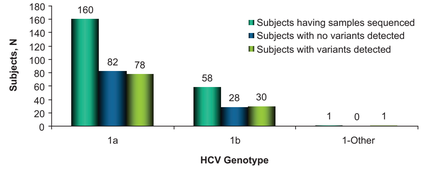
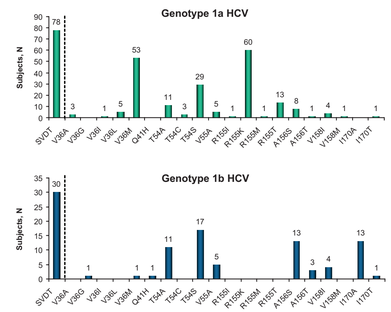


Figure 6. Number of Subjects Having RAVs Detected Post-baseline, Including All Patients Treated with Boceprevir

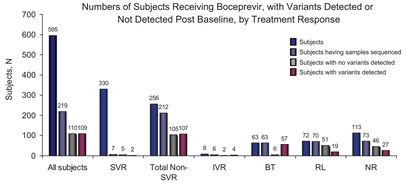
Subjects Having Variants Detected at Boceprevir Resistance Loci



All sequences were aligned to H77 (gt1a) for resistance analysis.
HCV gt1b has a valine at position 170.

Figure 7. Frequency of RAVs for Different Treatment Response Categories

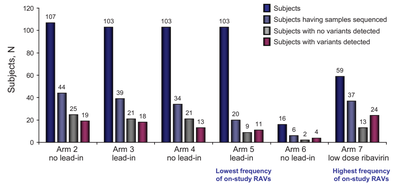
Numbers of Subjects Receiving Boceprevir, with Variants Detected or Not Detected Post Baseline, by Treatment Response



At the failure time point (and subsequent time points), many of the subjects did not have a VL that was high enough for testing (>1000 IU/mL). NR included patients who discontinued.

Figure 8. Frequency of RAVs by Study Arm

Numbers of Subjects with RAVs Detected Post-Baseline, by Study Arm



P = Peginterferon, R = Ribavirin, BOC = Boceprevir

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Disclosure

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Figure 9A. RAVs Detected with No Lead-in (Arm 2) vs. Lead-in (Arm 3) Study Arms

(i.e. Arms where Patients Received a Shorter Duration of Treatment with Boceprevir)

Number of Subjects Having Variants Detected at Boceprevir Resistance Loci

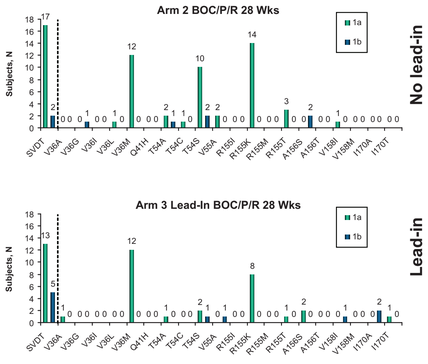
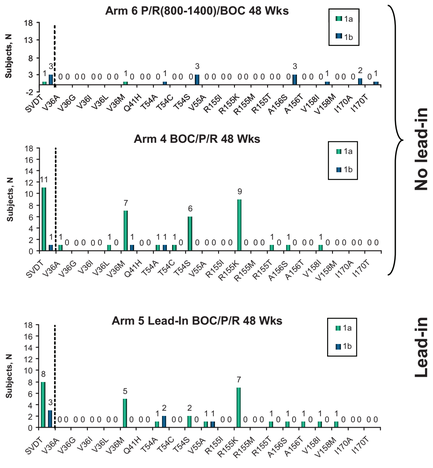


Figure 9B. RAVs Detected with No Lead-in (Arms 4 and 6) vs. Lead-in (Arm 5) Study Arms

(i.e. Arms where Patients Received a Longer Duration of Treatment with Boceprevir)

Number of Subjects Having Variants Detected at Boceprevir Resistance Loci



Conclusions

- In SPRINT-1, combination therapy with boceprevir and peginterferon plus ribavirin increased SVR rates with shorter treatment durations.
- The most common RAVs in genotype 1a patients not achieving SVR were V36M, T54S, and R155K.
- The most common RAVs in genotype 1b patients not achieving SVR were T54A, T54S, A156S, and V170A.
- In subjects with detectable RAVs at baseline, the majority achieved SVR.
- Lead-in therapy may reduce the frequency of on-study RAVs, especially T54S.