Genotypic and Phenotypic Analysis of HCV NS5A Inhibitor Resistance Variants: Correlations Between In Vitro and In Vivo Observations

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ABSTRACT

Background: NS5A plays a central role in replication of hepatitis C virus (HCV). BMS-790052 is a first-in-class and potent NS5A inhibitor with broad genotypic coverage. The in vitro picomolar potency seen with BMS-790052 has translated to in vivo effects in clinical studies.

Methods: We assessed the genotypic and phenotypic HCV resistance observed in phase 1 (14-day monotherapy) and phase 2a (BMS-790052 plus pegylated interferon alpha and ribavirin [pegIFNα/RBV]) studies using an HCV replicon system.

Results: In phase 1, HCV-infected subjects generally experienced rapid and marked viral load declines, and in several subjects HCV RNA dropped below the lower limit of quantitation (LLOQ, 25 IU/mL). Viral breakthrough (VBT) was, however, observed on treatment in the majority of genotype 1a-infected subjects, but less often in subjects infected with genotype 1b. In the first 12 weeks of phase 2a, VBT was observed in 2 of 12 subjects who received the lowest dose of BMS-790052 (3 mg) in combination with pegIFNα/RBV and was associated with resistance substitutions previously identified in vitro. Twenty-three of 24 subjects who received BMS-790052 (10 mg or 60 mg) in combination with pegIFNα/RBV achieved HCV RNA levels below detection (LLOD, <10 IU/mL) through week 12, and VBT was not observed, demonstrating antiviral activity of BMS-790052. Baseline variants with resistance substitutions were observed in 3 of the 36 subjects, but were suppressed below LLOQ at week 4 when BMS-790052 was combined with pegIFNα/RBV, demonstrating the impact of combination therapy.

Conclusion: Our results indicate that (i) the majority of resistance substitutions observed in vivo have been previously identified in vitro, demonstrating the relevance of the in vitro replicon system; (ii) resistant variants may preexist at baseline, but the rapid and marked suppression of HCV RNA (viral kinetic analysis) suggests these variants were inhibited by BMS-790052 when combined with pegIFNα/RBV; and (iii) since BMS-790052 belongs to a novel class of HCV inhibitor with a potent antiviral effect in the clinic, it is an excellent candidate for combination therapy, which should reduce the selection of resistant variants.

BACKGROUND AND OBJECTIVE

- NS5A is a multifunctional protein required for in vivo and in vitro HCV replication; it has no known human homologues, making it an attractive target for therapeutic intervention
- BMS-790052 is a potent (picomolar) and highly selective inhibitor of HCV NS5A, with broad genotype coverage; in vitro results suggest small molecule combinations with BMS-790052 can minimize the development of resistance
- BMS-790052 has been evaluated in a phase 1, 14-day monotherapy study and a phase 2a study in combination with pegylated interferon alpha and ribavirin (pegIFNα/RBV)[1]
- **The objective of the current study was to describe the genotypic and phenotypic HCV resistance observed in these studies**

1. Pol S, et al. Poster presented at: 45th Annual Meeting of the European Association for the Study of the Liver. April 14-18, 2010; Vienna, Austria.

NS5A Inhibitor Profile

- The most potent anti-HCV agent reported to date[2]

Replicon or Virus	EC_{50} (pmol/L)
Virus Genotype	
1a	50
1b	9
2a (JFH)	63
2a (JFH) virus	28
3a*	127
4a*	12
5a*	33
Replicon	
BVDV replicon (μmol/L)	>10^7
CPIV, HRV, COX, Polio, CoV, Flu, HIV, HSV-1, 2 (μmol/L)	>10^7

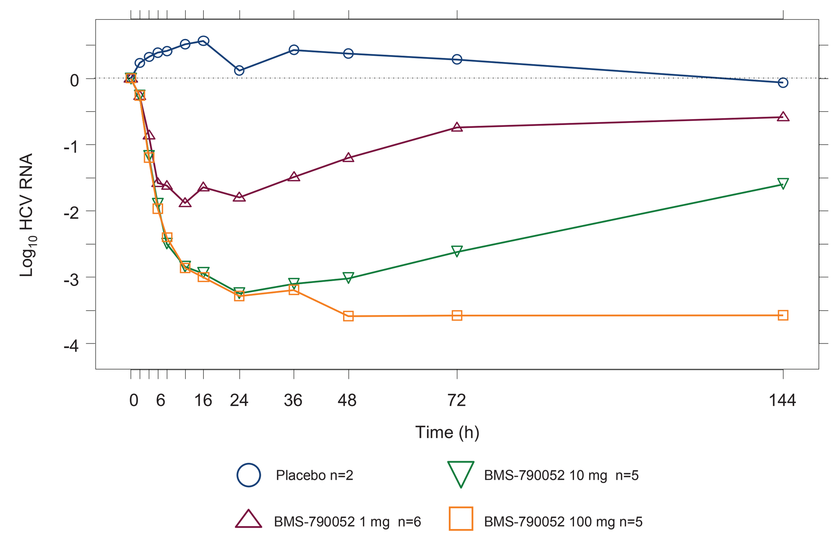
*Derived from hybrid replicons; EC_{50} = 50% effective concentration.

2. Gao M, et al. *Nature*. 2010;465:96-100.

BACKGROUND AND OBJECTIVES (cont'd)

NS5A Inhibitor Profile (cont'd)

BMS-790052 Single Ascending Dose (SAD)

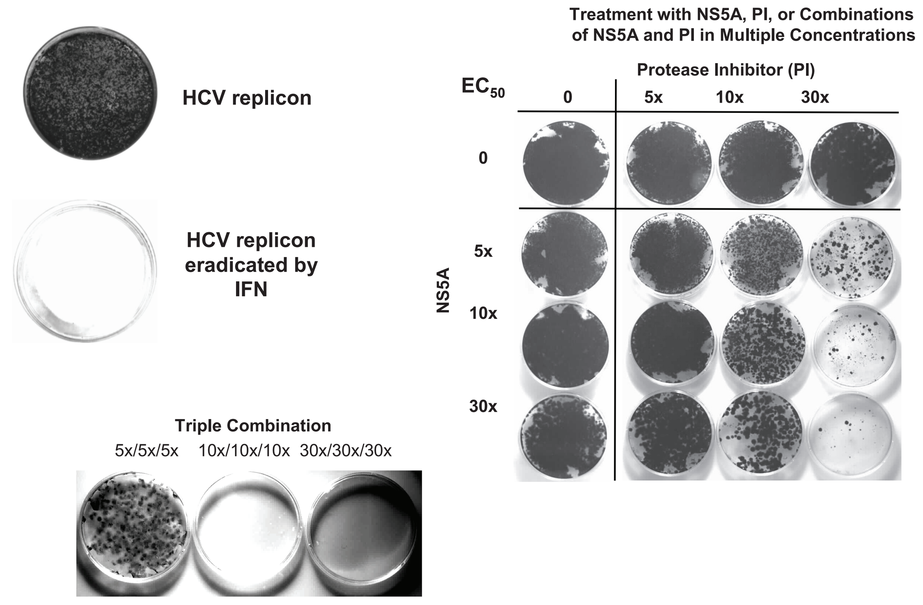


HCV Replicon Combination Studies With BMS-790052

HCV Replicon Combination Studies With BMS-790052				
	Interferon-α2b + BMS-790052	NS3 protease inhibitor + BMS-790052	Nucleoside NS5B polymerase inhibitor NM-107 + BMS-790052	Non-nucleoside NS5B polymerase inhibitor + BMS-790052
Combined activity	Synergistic	Additive/synergistic	Additive/synergistic	Additive/synergistic

- In vitro results suggest small molecule combinations can minimize the development of resistance and offer an option to treatment with pegylated interferon and ribavirin

DAA Combinations Enhance Resistance Barrier In Vitro



- Similar results observed with NS3 + NS5B and NS5A + NS5B
- Triple- > Dual- > Mono-therapy
- BMS-790052 is a good candidate for use in combination therapy

METHODS

- Blood samples for viral resistance analysis were obtained from subjects participating in 2 clinical studies:
  - Phase 1b, 14-day, multiple ascending-dose (MAD) study
  - Phase 2a study of BMS-790052 in combination with pegIFNα/RBV
- Viral resistance was evaluated by genotypic and phenotypic analysis. NS5A sequence traces were examined for possible variations. Viral variants identified were compared with those previously reported for in vitro replicon studies of BMS-790052 resistant viral variants[1,2]

1. Gao M, et al. *Nature*. 2010;465:96-100.
2. Fridell RA, et al. *Antimicrob Agents Chemother*. 2010;54:3541-3650.

RESULTS

Phase 1 MAD study

- Population sequencing revealed that viral breakthrough (VBT) was associated with amino acid substitutions in NS5A that had been previously implicated in resistance development in the in vitro replicon system
- These included the viral variants with single or double mutations that conferred a high level of resistance in the replicon system: M28T, Q30R, Q30H, Q30E, H58D, Y93C, Y93H, L31M, L31V, and L31V-Y93H
- The presence of viral variants was not invariably associated with VBT. One patient in the 100 mg QD cohort demonstrated continued viral decline during the dosing period despite the appearance of M28T, Q30E, and H58D variants at day 3

Phase 2a study

- Three of 36 subjects had known NS5A resistance substitutions at baseline, but achieved HCV RNA levels <25 IU/mL at week 4 and below LLOD at week 6. Preexisting NS5A resistance variants appeared to have minimal impact in subjects treated with BMS-790052 plus pegIFNα/RBV
- VBT was observed in 2 subjects treated with 3 mg BMS-790052 plus pegIFNα/RBV but in no subjects treated with 10 mg or 60 mg BMS-790052
- VBT was associated with the emergence of resistance variants
- BMS-790052 in combination with pegIFNα/RBV suppressed viral variants (eg, Q54H-Y93H) that were not suppressed with BMS-790052 treatment alone

Phase 2a Study Baseline (BL) Analysis

BL analysis: 3 of 36 subjects had known NS5A resistance substitutions, but achieved HCV RNA levels <25 IU/mL at week 4 and <LLOD at week 6

Subject	GT	Dose of BMS-790052 + P/R	v-RNA at BL (IU/mL)	Substitutions Detected at BL[a]	EC_{50} (nmol/L)[b]		Comment
1	1a	60 mg	6.11E + 06	Q30R, Y93F each in 1 amplicon, both <20%	Q30R ~7.3	Y93F ~0.35	
2	1b	10 mg	9.5E + 06	Y93H (50% 1 amplicon)	Y93H ~0.049		
3	1b	10 mg	1.10E + 07	Y93H (~100%), Q54H	Y93H ~0.049	Q54H ~0.0032	EC_{50} of Q54H-Y93H ~0.025 nmol/L
		WT replicon EC_{50} (nmol/L)			1a ~0.006		
					1b ~0.0026		

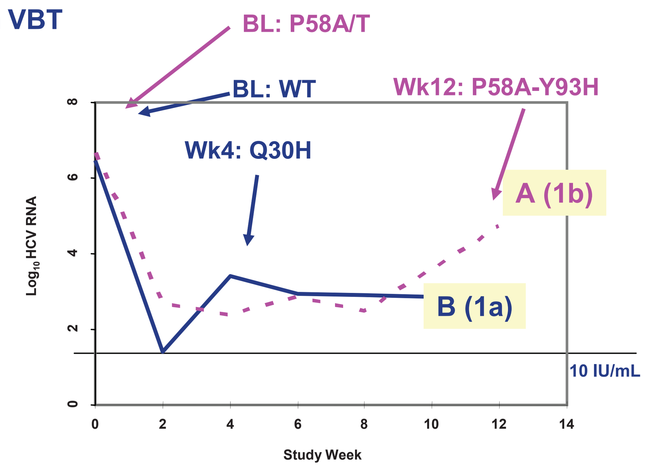
GT = genotype; P/R = pegIFNα/RBV.
[a]Determined by population sequencing.
[b]Determined by transient replication assays.

- Preexisting NS5A resistance variants have minimal impact in subjects treated with BMS-790052 plus pegIFNα/RBV

RESULTS (cont'd)

Impact of Dosage on VBT

- VBT observed in 2 subjects (A and B) treated with 3 mg BMS-790052 plus pegIFNα/RBV
- No VBT in subjects treated with 10 or 60 mg BMS-790052 plus pegIFNα/RBV: v-RNA <10 IU/mL



aa Substitution	Parental (1a, H77C)	Q30H	Parental (1b, Con 1)	P58A	P58T	Y93H	P58A-Y93H
Replication level	100	75	100	140	194	23	48
Potency of BMS-790052 EC_{50} (nmol/L)	0.0059	8.7	0.0023	0.0025	0.0029	0.058	1.9
Fold resistance	1	1474	1	1.1	1.3	25	826

Impact of Combination Therapy on VBT

Subject	GT	Dose	BL	T4	T8	T24	Week 1	Week 2
1	1b	100 mg BMS-790052	19,000,000	3,800,000	799,000	3200	592	840,000
aa substitution(s) detected			Q54H-Y93H	-	-	-	-	L31V-Q54H-Y93H

- A Q54H-Y93H variant present at baseline in a phase 1 study subject was <u>not</u> fully suppressed by 100 mg BMS-790052

Subject	GT	Dose	Viral RNA (IU/mL)						
			BL	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16
3	1b	10 mg BMS-790052 + P/R	11,000,000	30	<10	<10	<10	<10	<10
aa substitution(s) detected			Q54H-Y93H	-	-	-	-	-	-

- A Q54H-Y93H variant present at baseline in a phase 2a study subject <u>was</u> fully suppressed by a combination of BMS-790052 at 10-fold lower dose plus pegIFNα/RBV

P/R = pegIFNα/RBV.

CONCLUSIONS

- In general, the impact of viral resistance on BMS-790052 potency in vitro correlates with the effects observed in vivo
- Preexisting NS5A resistance variants have minimal impact in subjects treated with BMS-790052 plus pegIFNα/RBV
- No VBT was observed with 10 mg or 60 mg BMS-790052 combined with pegIFNα/RBV
- Viral breakthrough was observed in the 14-day study of monotherapy. However, the preliminary clinical study results indicate that the combination of BMS-790052 with pegIFNα/RBV can suppress the emergence of viral resistance and correlate with in vitro study results, indicating that BMS-790052 in combination with interferon or direct-acting antivirals can suppress the emergence of resistance.
- Analysis of resistance data from clinical studies is in progress

FINAL