No Impact of RASs on the High Efficacy of BEM and RZR in Combination: Resistance Analysis from a Phase 2 Study in HCV-infected Patients

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BACKGROUND

Bemnifosbuvir (BEM, AT-527) is an investigational oral, potent, pan genotypic inhibitor of the HCV nonstructural protein 5B (NS5B) RNA-dependent RNA polymerase (RdRp).[1,2] Ruzasvir (RZR) is an investigational oral, potent, pan genotypic inhibitor of the HCV nonstructural protein 5A (NS5A), an essential component of the viral replication complex.[3,4] The AT-01B-004 Phase 2 study investigated BEM 550 mg QD in combination with RZR 180 mg QD for 8 weeks in chronic HCV-infected patients with or without compensated cirrhosis. This was an open-label single-arm trial, and a total of 275 treatment-naïve subjects were enrolled.[5] The overall SVR12 rate in the primary analysis population was 98%. Very high SVR12 rates (99%) were observed in non-cirrhotic subjects with the 8-week treatment regimen. Lower SVR12 rates (88%) were observed in compensated cirrhotic subjects. There were 14 subjects in the overall study population who experienced virological failure (VF).

OBJECTIVES

A Phase 2 open-label study evaluated the efficacy and safety of BEM+RZR for 8 weeks in 275 HCV-infected patients (NCT05904470). Detailed analyses were conducted on Next-Generation Sequencing (NGS) data to determine the prevalence of NS5A and NS5B resistance-associated substitutions (RASs) at baseline and the impact of these RASs on virological responses. In addition, for the 14 patients in the overall study population who experienced VF, sequencing data in conjunction with *in vitro* phenotyping data from baseline and on-treatment samples were examined for correlation with VF to establish clinical resistance profiles.

METHODS

Clinical resistance study: NGS was performed at baseline for all treated patients and at failure for the 14 VF patients to determine BEM and RZR resistant substitutions in the Phase 2 study. Baseline RASs and on-treatment NS5A and NS5B substitution analyses were conducted using DDL Athena NGS analysis pipeline, with a detection limit of 1%. Substitutions were reported as differences compared with the following genotype-specific reference strains: genotype 1a H77 (NC004102); genotype 1b Con1 (AJ238799); genotype 1c HC-G9 (D14853); genotype 2a JFH-1 (AB047639); genotype 2b HC-J8 (D10988); genotype 3a S52 (GU814263); genotype 3b Tr Kj (D49374); genotype 4a ED43 (GU814265).

***In vitro* phenotyping study:** Selected RASs that were pre-existing at baseline in the HCV-infected patients or emerged during combination treatment were engineered back to HCV replicon plasmids. Huh7 cells were transiently transfected with replicon RNA by electroporation and cultured and treated with the compounds at 37°C and 5% CO_2 for 4 days. HCV replication was measured by Nano-Luciferase expression.

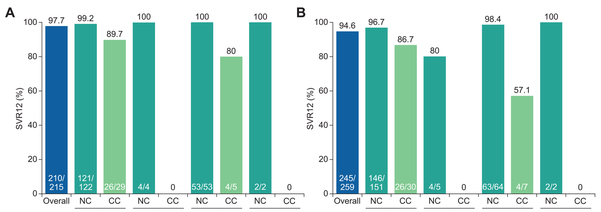
Table 1. Phase 2 Enrollment Summary

	1a	1b	1c	2a	2b	3a	3b	4a	Total
Cirrhotic	4	26				6	1		37
Non-cirrhotic	45	113	1	2	5	66	4	2	238
	49	**139**	**1**	**2**	**5**	**72**	**5**	**2**	**275**

RESULTS

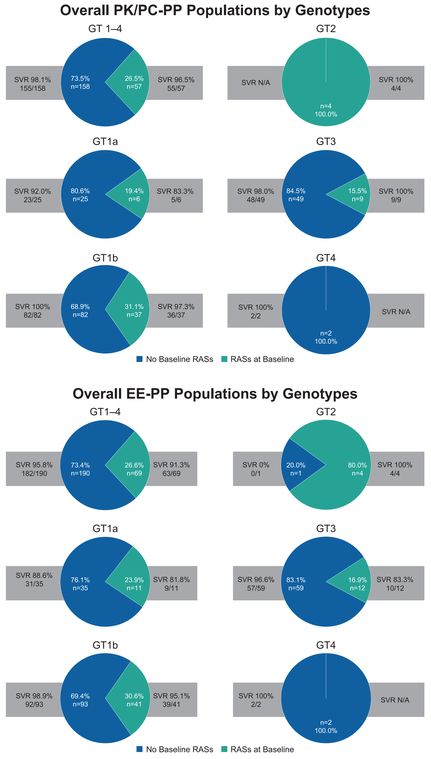
Patients who met all eligibility criteria, completed treatment (defined as being ≥90% compliant with the study drug regimen), had outcomes at post-treatment week 12, and had adequate study-drug exposure corroborated by pill counts and plasma drug levels adjudicated by an independent clinical pharmacologist, were included in the **Pharmacokinetic and Pill Compliant Per-Protocol** (PK/PC-PP) population (primary analysis). A secondary, **Efficacy Evaluable Per-Protocol** (EE-PP) population included patients who met all eligibility criteria, received at least one dose of study drug, and had outcomes at post-treatment week 12, regardless of drug adherence. SVR12 rates for each analysis population are summarized in Figure 1 (by genotype and cirrhosis status) and Figure 2 (by baseline NS5A RAS status).

Figure 1: High SVR rates in PK/PC-PP (A) and EE-PP (B) population



CC, compensated cirrhosis; GT, genotype; NC, no cirrhosis; SVR12, sustained virologic response at 12 weeks post-treatment

Figure 2: SVR12 rates in participants with and without baseline NS5A RASs



NS5A RAS positions: 24, 28, 30, 31, 32, 38, 58, 92, 93 (data reported above 15% threshold)

- There was no apparent impact of baseline NS5A RASs on SVR12 rates in PK/PC-PP population. However, PK compliance may have impacted SVR12 rates, especially in GT3 patients

Table 2. Baseline NS5A RASs associated with Virologic Failure

GT	NS5A RASs by Amino Acid Position at Baseline								
	24	28	30	31	32	38	58	92	93
1a	R	L/T/V	H(H)/P/R			Y	D/N/ P(P)/Q/Y		D/H(H)/ N(N)
1b	K/R	M	H/K/Q	I/M(M)/V			L/Q/R/S (S)	E/T/V	H(H)
1c		V							Y
2a				M					
2b		P		L					
3a	A/P	I/L/V	K(K)/S/T			Y	A(A)/S		H(H)
3b	A/T		M	M					
4			R						

Black = Achieved SVR12; Red = Viral Failure

- Major NS5A RASs pre-existed in some patients across GTs
- No apparent impact of baseline NS5A RASs on SVR12 rate

Table 3. NS5A and NS5B RASs associated with Virologic Failure

GT	VF Type	Time	PK/ PC-PP	NS5A RAS Baseline	NS5A RAS at Fx	NS5B RAS Baseline	NS5B RAS at Fx
Subjects with Cirrhosis							
3a	BK	W8	No	S62I/M/R/V/T (80/2/2/3/12%) Y93H (42%)	S62I/M/V/T (89/2/7/2%) Y93H (100%)	K206N (99%)	K206D/N (1/98%)
3a	BK	W2	No	A30K (53%) S62T (99%)	A30K (67%) L31V/I (2/4%) S62A/T (2/97%) Y93H (91%)	S15R (100%) K206E (99%)	Failed amplification
1a	Relapse	SVR4	Yes	Q30H (8%) Y93H/N (20/22%)	M28T (100%) Q30H (100%) Y93N/H (2/98%)	None	None
1a	Relapse	SVR4	Yes	None	S38Y (1%) H58N (1%)	None	None
1b	Relapse	SVR4	Yes	L31M (35%)	L31M (99%)	None	N206K (44%)
1b	Relapse	SVR12	No	P58S (99%) Y93H (1%)	P58S (99%)	N206D (48%)	N206D (100%)
3a	Relapse	SVR4	Yes	None	Y93H (100%)	K206E (1%)	None
Subjects without Cirrhosis							
1a	BK	W8	No	H58P (99%)	M28T (100%) Q30H (100%) H58P (100%)	Failed amplification	Failed amplification
3a	BK	W4	No	P58A (6%) S62T (10%) Y93H (1%)	L31I (5%) P58A (27%) Y93H (93%)	K206Q (100%)	K206Q (100%)
2b	Relapse	SVR4	No	None	None	None	None
1a	Relapse	SVR4	Yes	None	None	None	None
1b	Relapse	SVR12	No	None	None	V321I (100%)	V321I (97%)
1a	Relapse	SVR12	No	None	L31V (100%) Y93H (100%)	None	None
1a	BK	W4	No	None	M28T (5%) Q30K/R (5/51%) L31V (56%)	Q206K (20%)	Q206K (41%)

NS5A RASs monitored: 24, 28, 30, 31, 32, 38, 58, 92, 93 and 62 (GT-3 only) positions; NS5B RASs monitored (with 1% threshold): 15, 142, 159, 206, 223, 237, 282, 289, 314, 321 positions (with 1% threshold); GT, genotype; VF Type, Viral Failure Type; PK/PC-PP, Pharmacokinetic and pill- compliant per protocol; Fx, Failure; BK, Breakthrough on-treatment; SVR, Sustained virologic response; W, week

- Of the 14 patients who did not achieve SVR12, 5 experienced viral breakthrough on-treatment (BK), and 9 experienced relapse post-treatment
- 5/5 of BK patients and 4/9 relapse patients had drug levels consistent with non-adherence

Table 4. Phenotypic analysis of selected NS5A RASs observed in VF patients

GT	Resistance Level (EC_{50} fold shift)					
	>10,000X	>1,000X	>100X	>10X	>2.5X	>0.5X
1a	L31V+Y93H, M28T+Q30H+ Y93H/N	M28T+Y93H /N, Q30R+L31V	M28T+Q30H, M28T+Q30H +H58P	Q30H+Y93 H/N, M28T+Q30R	Y93H/ N, L31V	Q30H, H58P, P58A, S62T
1b					P58S, P58S+Y93H	L31M, Y93H
3a	A30K+L31I, A30K+Y93H, L31I+Y93H	S61I/T/M/V+ Y93H, L31I, P58A+Y93H	Y93H, S62R+Y93H	A30K, A30K+S62T		S62I/M/R/V/T

Black = Baseline; Red = Viral Failure

- Baseline NS5A RASs conferred no/low levels of resistance to RZR
- NS5A RASs detected at viral failure are likely linked and often conferred higher levels of resistance to RZR

Table 5. Phenotypic analysis of selected NS5B RASs observed at baseline and enriched in VF patients

GT	NS5B RASs	Fitness (% of GT-1b WT)	BEM		Sofosbuvir (SOF)	
			EC_{50} ± SD (nM)	EC_{50} fold-change	EC_{50} ± SD (nM)	EC_{50} fold-change
1b	N206S	143.6	3.5 ± 0.4	1	87 ± 0.3	1.2
1b	K124E+A218S+C316H+V321I	35.2	3.7 ± 0.5	1.4	77.5 ± 5.4	0.7
1b	K124E+A218S+C316H+V321I	40.3	4.5 ± 0.2	1.7	65.5 ± 7.3	0.6
2a	T150A	2.1	1.7 ± 0.04	0.7	92.3 ± 13.7	0.8
3a	A150V+K206E	ND	20.3 ± 1.6	1.5	198.5 ± 1.1	1.4
3a	A150V	ND	19.8 ± 0.2	15	216.6 ± 0.2	1.6

- BEM has a high resistance barrier in HCV patients
  - C223H, the primary RAS for BEM, was not detected at baseline or at viral failure
  - N206K/D, the only NS5B substitution that emerged or was enriched in two GT-1b VF patients, did not confer resistance to BEM *in vitro*
  - Other HCV NS5B RASs such as A150V, K206E and C316H conferred no or low resistance to BEM (<1.7-fold)

CONCLUSIONS

- BEM and RZR 8-week combination treatment can achieve high SVR rates in HCV genotype 1, 2, 3 and 4 infected patients
- NS5A RASs: VF occurred in the presence (7/14) or absence of pre-existing primary RASs. These RASs could emerge, be enriched, or linked to other RASs acquired during the study. Phenotyping results showed that the linked RASs conferred very high resistance to RZR. NS5A resistance to RZR was observed in most (8/14) of the VF patients
- NS5B RASs: No BEM C223 site substitution or SOF S282T was detected at baseline or VF. Substitutions at aa position 206 emerged or were enriched but did not confer significant resistance to BEM or SOF, either alone or linked with other NS5B substitutions. **These results indicate that BEM viral resistance is not developing rapidly**
- Viral kinetics and PK analyses showed that most of the viral failures were due to treatment non-adherence. In VF subjects without cirrhosis, 6/7 were not PK/PC-compliant

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