Bemnifosbuvir Poses High Barrier for Resistance in Both Preclinical and Phase 1b Monotherapy Studies

#1501

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BACKGROUND

- Viral resistance has emerged as an important consideration for direct-acting antiviral (DAA) treatment since it may impact effectiveness
- Bemnifosbuvir (BEM, AT-527), an oral prodrug of a guanosine analog, has potent pan-genotypic, best-in-class *in vitro* and clinical antiviral activities against all HCV genotypes tested
- The potency of AT-511 (free base of BEM) to inhibit viral replication of HCV has been demonstrated in replicon based cellular assays
- In HCV laboratory strains and clinical isolates with genotypes 1–5, the EC_{50} of AT-511 ranged from 6.2–28.5 nM across the genotypes, which is approximately 10- to 20-fold more active than sofosbuvir (SOF)[1]
- Earlier *de novo* resistance studies in HCV GT-1a, -1b and -2a replicons identified C223H as the primary BEM resistance-associated substitution (RAS) that required two nucleotide changes
- More importantly, multiple substitutions at other NS5B regions were required to confer meaningful resistance, suggesting a very high barrier to resistance for BEM *in vitro*[2]

OBJECTIVES

We conducted *in vitro* and clinical BEM studies to understand what mutations might arise and confer viral resistance in the development of this treatment for HCV infection. Our objectives were:

- Identify NS5B RASs potentially impacting BEM antiviral activity *in vitro*
- Analyze Next Generation Sequencing (NGS) of both baseline (screening or Day 1 predose) and on-treatment samples of HCV subjects from Part B, C, D, E of the BEM monotherapy phase I study (NCT03219957)[3] to identify clinical BEM-associated NS5B substitutions and their potential impact on antiviral activities

METHODS

Clinical resistance study

- NGS was performed for 42 HCV-infected patients treated with BEM for 1 or 7 days to determine BEM resistant substitutions in the Phase I study
- Baseline RASs and on-treatment 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 1b Con1 (AJ238799); genotype 2a JFH-1 (AB047639); genotype 3a S52 (GU814263)

In vitro Phenotyping study

- Selected BEM-associated substitutions that pre-existed at baseline in HCV subjects or emerged during monotherapy were engineered back to HCV replicon plasmids
- Huh-7 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

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RESULTS

Table 1. Key NS5B RASs from earlier studies with HCV nucleoside analog inhibitors (NIs)

aa#	Substitutions (GT)	Inhibitors Affected
15	S15G (GT2a)	BEM (NI, *in vitro*)
96	S96T (GT1)	R1479 (NI)
142	N142T (GT3a)	R1479 (NI), SOF (NI)
150	A150V (GT3)	SOF (NI)
159	L159F (GT1,2,3)	SOF (NI)
162	I162F	SOF (NI, *in vitro*)
179	I179M	SOF (NI, *in vitro*)
206	K206E (GT3), N206S (GT1b)	SOF (NI), BEM (NI, *in vitro*)
223	C223H (GT1a, 2a)	BEM (NI, *in vitro*)
282	S282T/R/G/C (GT1-6)	SOF (NI), mericitabine (RG7128, NI), PSI-6130 (NI), VX-135 (NI)
289	E/M289I/L	SOF (NI)
293	M293I	SOF (NI, *in vitro*)
316	C316F/N/Y/H (GT1a/1b)	SOF (NI), BEM (NI), Dasabuvir (ABT-333, NNI), HCV-796 (NNI), Tegobuvir (NNI), IDX375 (NNI)
320	L320F/I/V (GT1a, GT2a)	BEM (NI, *in vitro*), PSI-6130 (NI), mericitabine (RG7128, NI), PSI-6130 (NI, *in vitro*)
321	V321A/G/I (GT1,3,4)	SOF (NI), BEM (NI)
344	T344I/A (GT1b)	BEM (NI, *in vitro*), SOF (NI, *in vitro*)

- *In vitro* GT1a, 1b and 2a resistance studies indicated C223H was the primary BEM-associated resistance substitution

Table 2. Key NS5B NI substitutions detected at baseline in non-cirrhotic HCV-infected subjects (Part B, single dose)

Dose (mg)[a]	Subject #	Genotype		NS5B NI Key RAS[b]	HCV RNA Reduction[c]
		LiPA	NGS		
100	002-001	1b	1b	None	-0.93
	002-002	1b	1b	N206D (17.6%)	-0.63
	002-003	1b	1b	None	-0.82
300	002-005	1b	1b	None	-1.78
	002-008	1b	1b	None	-1.09
	002-012	1b	1b	N206K (99.2%) C316N (99.5%)	-2.21
400	002-006	1b	1b	C316N (99.6%)	-2.55
	002-007	1b	1b	L159F (99.2%) N206T/K (89.3/9.3%) C316N (99.5%)	-2.22
	002-018	1b	1b	None	-1.77
600	002-053	1b	1b	None	-2.07
	002-055	1b	1b	None	-2.57
	002-056	1b	1b	None	-2.29

a, Dose expressed as AT-527 salt form; 600 mg salt equivalent to 553mg free base
b, Only Key NI RASs listed in Table 1 reported
c, Antiviral activity measured as max. HCV RNA reduction (log10 IU/mL)

- After single doses of BEM, a greater mean HCV RNA reduction was observed with increasing doses
- 4/12 subjects carried known NS5B NI RASs such as L159F and C316N; N206D emerged in BEM *in vitro* resistance selection
- Pre-existing NI RASs did not impact antiviral activity in each dosing group

Table 3. Key NS5B drug-associated substitutions detected at baseline and emerged during treatment (Part C, D, E)

PART	Dose (mg)*	Subject #	GT	NS5B NI RAS	Maximum HCV RNA Reduction (log)	Plasma exposure (AT-273, AUC_{tau} mg)
C	300 (no cirrhosis)	002-037	1b	L159F (99.3%) C316N (99.4%)	-3.35	1644
		002-039	1b	None	-3.67	1635
		002-040	1b	None	-4.24	2580
		002-044	1b	N206K (97.2%)	-4.17	1305
		002-047	1b	N206K (99.3%)	-3.93	1675
		002-051	1b	C316N (99.4%)	-4.50	2131
	600 (non cirrhosis)	002-057	1b	None	-4.06	2294
		002-058	1b	L159F (99.1%) C316N (99.1%)	-5.33	3433
		002-060	1b	F162L (1.2%)	-5.20	2596
		002-065	1b	C316N (99.5%)	-4.29	2615
		002-066 (Sanger)	1b	N206K	-3.54	3677
		002-067	1b	E150T/S (19.5/79.0%) L159F (99.4%) N206K (1.5%) C316N (99.5%)	-4.01	2418
D	600 (no cirrhosis)	002-059	3a	A150V (99.3%) K206E (99.4%)	-4.98	2254
		002-073	3a	A150V (99.1%)	-4.41	3215
		002-077	3a	A150I/V (21.3/78.5%)	-4.44	2309
		002-081	3a	None	-4.46	5114
		002-087	3a	A150V (99.5%)	-4.19	2847
		002-088	3a	K206D/N (2.6/97.0%)	-4.45	2959
E	600 (cirrhosis)	002-072	1b	None	-4.03	2483
		002-082	1b	None	-4.05	5379
		002-085	1b	None	-4.55	3780
		002-074	3a	A150T(93.8%)	-4.80	4567
		002-090	3a	Failed amplification	-5.20	2555
		002-091	2a	A150T (93.7%) Y162F (99.5%)	-4.99	2648

* Dose expressed as AT-572 salt form; 600 mg salt equivalent to 553mg free base

Pre-existing HCV NS5B NI RASs

- 16/24 baseline samples from the 300 mg and 600 mg 7 days dosing group (shown above) carried at least one of the NI RASs listed in **Table 1**; No subject possessed SOF key RAS S282T/R/G/C or BEM key RAS C223H at baseline
- Other NI RASs (such as C316N, L159F, K206E, N206K, F162L, Y162F) pre-existed at baseline

Impact of pre-existing RASs on viral response

- Overall, HCV RNA reduction was dose-dependent. Baseline NS5B RASs did not seem to directly correlate with BEM antiviral activity, indicating that most HCV viruses carrying these substitutions or polymorphic variants, especially known SOF RASs, remained sensitive to BEM
- For each individual subject, the impact of baseline RASs and the plasma exposure (**see Table 3**) on HCV RNA reduction remain unclear

Emergent and Enriched HCV NS5B NI RASs

- 24/42 subjects had at least one post Day-1-predose full length NS5B NGS done; Comprehensive analysis showed that the changes of amino acid percentage were generally minimal, most within 1-2% compared with baseline samples
- Neither S282T (SOF key RAS) nor C223H (BEM key *in vitro* RAS) emerged on-treatment
- HCV NI RASs such as S15R, N142S, E150D, N206D or V321I emerged within 16 to 36h in a few subjects at very low levels
- Additional low-level substitutions also emerged/increased on-treatment, such as V421A, A442T, Y448H, Q514R, N117H, D61G, D62N, which were selected *in vitro* or were treatment emergent substitutions in SOF virological failure subjects
- These treatment emergent substitutions occurred at single digit percentages, indicating that they existed at undetectable levels at baseline and are likely associated with poor fitness. This may confer minimal resistance with no significant impact on clinical efficacy at the early phase of treatment. However, it is possible that additional substitutions may be acquired during longer term treatment which may confer higher level of drug resistance

Table 4. Phenotypic analysis of selected NS5B substitutions observed with BEM monotherapy

Mutants (GT1b)	Replication capacity*	EC_{50} Fold Change		
		AT-511 (BEM)	GS-7977 (SOF)	ABT-333 (Dasabuvir)
C316F	20.0	1.2	1.7	~2500
C316N	200.5	0.9	0.7	3.5
L159F+A218S +C316N	18.0	1.2	1.0	1.6
D62N+L159F+A218S +C316N+V322I	18.3	1.1	0.5	1.4
K124E+A218S+C316H +V321I	35.2	1.4	0.7	298.5
K124E+L159F+N206K +A218S+C316N	100.0	2.0	0.8	4.1
S46G+C316H+V321I	43.6	1.1	0.8	312.6
F162Y+C316H+V321I	42.9	1.5	1.0	471.5
N206S	143.6	1.0	1.2	0.8
K124E	119.5	1.2	1.4	1.5
K124E+N206S	143.4	1.2	1.4	1.5

* % relative to GT1b wildtype

- Phenotypic characterization of C316F/N, single and linked with other NS5B substitutions identified in this phase 1 study, showed minimal change in susceptibility to AT-511 and SOF while some conferred high resistance to NNI such as ABT-333. Nonetheless, these substitutions cannot be ruled out as BEM clinical RASs
- Cumulative data suggest that for HCV NS5B NIs, phenotypic data from transient replicon assays may not conclusively determine if these treatment-emergent substitutions in clinical studies are resistance-associated, as NS5B substitutions may impact NS5B replicative capacity or have a direct effect on NI potency[4]

CONCLUSIONS

- ❖ The clinical virological data from this phase 1 study demonstrated that BEM (AT-527) is capable of significantly reducing HCV RNA in genotype 1b, 2a and GT-3a infected patients when used as a monotherapy. Time-related, dose-related, and exposure-related decreases were observed after multiple doses of BEM.
- ❖ The pre-existing NS5B NI RASs at baseline did not correlate to BEM antiviral activity based on the maximum HCV RNA reduction of each subject.
- ❖ The changes of amino acid percentage in on-treatment samples were generally minimal, within 1-2% compared with baseline samples, indicating no rapid development of viral resistance.
- ❖ Given the highly potent pan-genotypic antiviral activity and high resistance barrier of BEM, it is currently being developed in combination with ruzasvir, a picomolar potent pan-genotypic NS5A inhibitor, for the treatment of HCV infection in a Phase 2 clinical trial (NCT05904470).

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