Bemnifosbuvir and ruzasvir are potent HCV DAAs with favorable antiviral profiles against major HCV NS5A and NS5B RAVs supporting use in combination

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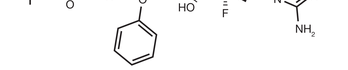
BACKGROUND

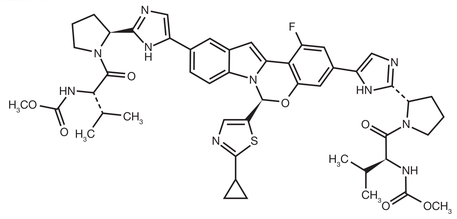
- Bemnifosbuvir (BEM), an oral prodrug of a guanosine nucleotide analog, has demonstrated highly potent, pan-genotypic, best-in-class *in vitro* and clinical antiviral activity against all hepatitis C virus (HCV) genotypes (GTs 1–4) tested[1] **(Figure 1)**
- Ruzasvir (RZR), a potent NS5A inhibitor, has shown broad genotypic *in vitro* antiviral activity (half maximal effective concentration [EC_{50}]≤10 pM) against GTs 1–6[2] **(Figure 1)**
- Viral resistance has emerged as an important consideration for direct-acting antiviral (DAA) drug use since it may impact effectiveness for treatment of HCV infection
- We profiled the antiviral activity of AT-511 (the free base of BEM) and RZR against a panel of previous NS5A Resistance-Associated Variants (RAVs) selected *in vitro,* or hard-to-treat sub-genotypes that have been identified in HCV patients who have failed treatment with currently available DAAs
- In addition, a *de novo* resistance study with AT-511 was performed using HCV GT-1a and -1b replicon cells to identify mutations that confer resistance to its active triphosphate (TP) form, AT-9010

Figure 1. Molecular structure of bemnifosbuvir and ruzasvir

Bemnifosbuvir



Ruzasvir



METHODS

Antiviral activity in transient HCV replicon cells

- Amino acid (AA) substitutions, and NS5A (7-310 AA) from hard-to-treat sub-genotypes, were introduced into the respective wild-type (WT) and GT-1b replicons, respectively
- The linearized plasmid was purified and used for *in vitro* transcription to generate chimeric replicon RNA
- Replicon RNA (10 µg) was electroporated into Lunet cells to test for replication fitness and sensitivity to HCV NS5A or NS5B inhibitors using a 3–4 day transient-transfection assay
- Replication fitness was determined by first normalizing the luciferase expression at 96 hours (h) to expression at 4 h and then dividing the normalized level of luciferase expression of the replicon mutant by that of the wild type

In vitro selection of HCV GT-1a and -1b replicon cells

- GT-1a and -1b replicon cells were cultured in the presence of G418 and increasing concentrations of AT-511 starting at their respective EC_{50} values
- Cells were passaged whenever they reached ~80% confluence and replenished with G418 medium containing fresh compound
- Aliquots of cells at each passage were saved for RNA isolation, cDNA synthesis, and PCR amplification for sequencing analysis

RESULTS

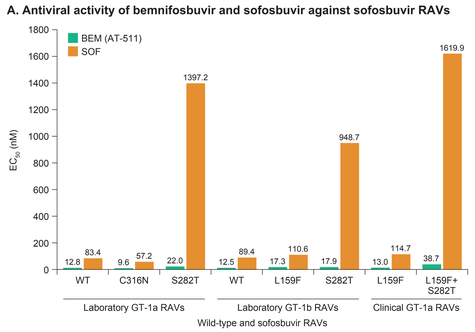
Bemnifosbuvir and ruzasvir antiviral profiles against major HCV NS5A and NS5B RAVs

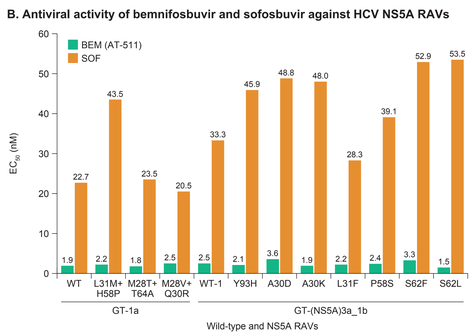
- BEM (AT-511) exhibited >10-fold increased potency vs sofosbuvir across all genotypes tested and is not resistant to known sofosbuvir RAVs such as S282T and L159F/S282T **(Figure 2A)**
- BEM (AT-511) also retained antiviral activity against all GT-1a and GT-3a NS5A RAVs tested **(Figure 2B)**
- In GT-1a, RZR was 10 times more potent than velpatasvir, and retained its single digit pM potency against RAVs (M28V+Q30R, M28T+T64A, L31M+H58P) selected by previous NS5A inhibitors **(Figure 2C)**
- In GT-3a, one of the most difficult-to-treat HCV genotypes, RZR was 6 times more potent vs velpatasvir, and retained sub-nM potency against NS5A RAVs (such as A30K and Y93H), which were treatment-emergent in HCV GT-3 patients failing DAAs **(Figure 2C)**

Ruzasvir antiviral activity against hard-to-treat HCV sub-genotypes

- The efficacy of NS5A inhibitors against several less common subtypes of HCV is poorly characterized
- Some HCV sub-genotypes including GT-3g, and -6v, commonly harbor AA residues in NS5A that may confer resistance to DAAs in other common subtypes; data from patients also suggest that GT-1l and -4r with substitutions at positions from AA 24 to 93 in NS5A are relatively resistant to standard-of-care DAA therapy.[3] AA residues (24 to 93) at RAV positions of the sub-genotype HCV clones are summarized in **Table 1**
- In this study, efficacy of RZR and velpatasvir were tested against these sub-genotypes using the HCV-GT-1b replicon backbone
- Both RZR and velpatasvir maintained pM potency over the majority of these difficult-to-treat sub-genotypes such as GT-1l, -4r, and -6v, but significantly lost potency against GT-3g, which carries a A30K+L31M+S54T+S62L-linked RAV **(Figure 3)**

Figure 2. *In vitro* antiviral activity of bemnifosbuvir, ruzasvir, sofosbuvir, and velpatasvir against HCV strains containing NS5A and NS5B RAVs





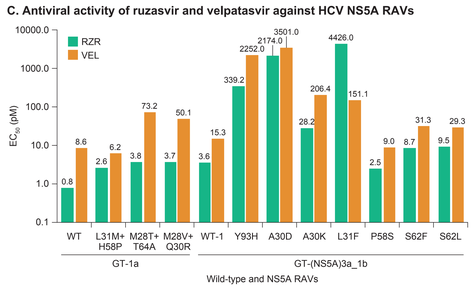
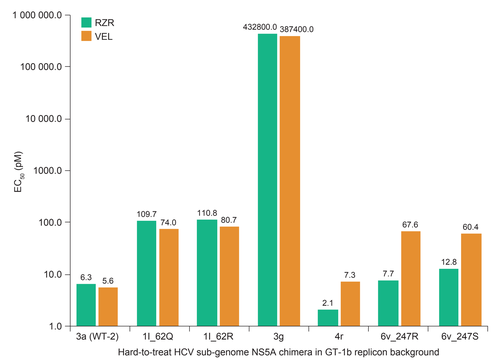


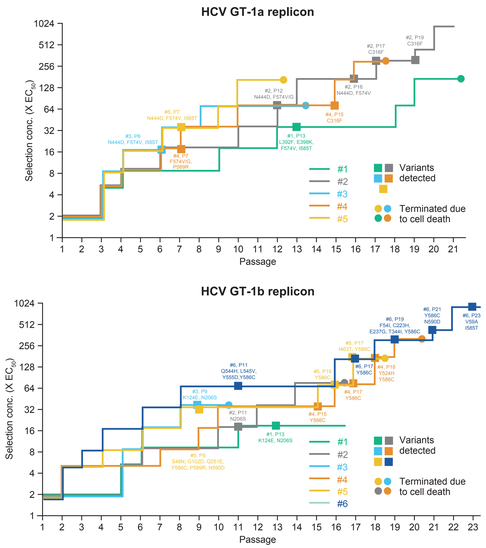
Figure 3. Efficacy of ruzasvir and velpatasvir against uncommon and potentially difficult-to-treat HCV sub-genotypes



BEM *de novo* resistance selection using HCV GT-1a and -1b replicons

- AT-511, like PSI-352938 and PSI-353661, is also a prodrug of β-D-2'-deoxy-2'-α-fluoro-2'-β-C-methylguanosine-5'-monophosphate which serves as an alternative substrate inhibitor of the NS5B RNA-dependent RNA polymerase during HCV replication[1,4]
- In earlier studies using HCV GT-2a (JFH-1) replicon cells to identify resistance mutations, PSI-352938 and PSI-353661 developed resistance to both compounds. A combination of three AA changes, S15G/C223H/V321I, was required to confer a high level of resistance[4]
- AT-511 is also predicted to pose a high barrier to resistance
- AT-511 resistance selections in GT-1a and -1b replicons were recently initiated by starting at concentrations of 2× EC_{50} (7.6 and 9 nM for GT-1a and -1b replicons, respectively) with gradual concentration increases
- Both GT-1a and GT-1b replicon cells survived up to 10,000× EC_{50} concentrations and at >20 passages; genotypic analysis of the NS5B region showed AA changes from different cell passages and phenotyping of previously reported NS5B resistant mutants, such as L314I, C316F, N206S, and C223H (single and linked with other emerged variants), is ongoing **(Figure 4)**

Figure 4. Resistance selection of AT-511 with HCV GT-1a and -1b replicon cells



*Key variants emerged in resistance selection:
GT-1a: C316F
GT-1b: N206S and C223H

Table 1. AA residues at NS5A RAV positions on the sub-genotype HCV clones

Sub-genotype	GenBank #	Amino acid (variants in red)												Fitness, % normalized to 3a (WT-2)
		24	28	29	30	31	32	54	58	62	91	92	93	
1a (WT)	NC004102	K	M	P	Q	L	P	H	H	E	N	A	Y	
1b (WT)	AJ242654	Q	L	P	R	L	P	Q	P	Q	N	A	Y	
3a (WT-1)	GU814263	S	M	P	A	L	P	S	P	S	N	E	Y	
3a (WT-2)	DQ430820	S	M	P	A	L	P	S	P	T	N	E	Y	100
1l_62Q	KC248196	G	M	P	Q	M	P	N	P	Q	N	A	Y	1
1l_62R	KC248196	G	M	P	Q	M	P	N	P	R	N	A	Y	1
3g	KY620876	S	M	P	K	M	P	T	P	L	N	E	Y	1
4r	JX227962	K	M	P	R	L	P	H	P	S	N	A	Y	21
6v_247R	FJ435090	K	V	P	S	L	P	T	P	Q	N	A	S	16
6v_247S	FJ435090	K	V	P	S	L	P	T	P	Q	N	A	S	13

CONCLUSION

Given the highly potent, pan-genotypic antiviral activity of BEM and RZR, the high resistance barrier of BEM, complementary mechanisms of action of BEM and RZR, clinically demonstrated safety and efficacy of each agent when administered individually, and the lack of DDI between the two, combination of BEM and RZR is a promising treatment option for HCV infection and is currently being evaluated in a Phase 2 clinical trial[5]

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Acknowledgements

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Disclosures

Qi Huang, Steven S. Good, Dawei Cai, Nancy G.B. Agrawal, and Jean-Pierre Sommadossi are employees of and may own stock in Atea Pharmaceuticals, Boston, MA, USA.

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