#411 The Combination of Bemnifosbuvir (BEM) and Ruzasvir (RZR), the HCV NS5B and NS5A Inhibitors, Demonstrates Potent *In Vitro* Synergistic Antiviral Activity and *In Vivo* Preclinical Safety Without Adverse Interactions

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ABSTRACT

Bemnifosbuvir (BEM), the orally available prodrug of a GTP analog, has demonstrated highly potent and selective pan-genotypic *in vitro* activity and best-in-class clinical efficacy against all hepatitis C virus (HCV) genotypes tested. The combination of this NS5B inhibitor with the potent, pan-genotypic NS5A inhibitor ruzasvir (RZR) is being developed for improved treatment of HCV infections.

The antiviral effects of 9 two-fold dilutions of BEM combined with 5 two-fold dilutions of RZR, each starting at twice their EC_{50} values, were determined in triplicate in HCV GT1b Huh-7 replicons. Analysis of two independent evaluations by Pritchard and Shipman MacSynergy II software provided synergy volumes at the 95% confidence interval (103 and 255 $\mu M^2\%$) that exceeded the 100 $\mu M^2\%$ limit indicative of highly synergistic antiviral activity. Antagonistic or synergistic cytotoxicity was not observed. In a GLP-compliant rat toxicity study, BEM and RZR were orally administered independently and in combination at 500 mg/kg once daily for 13 weeks. All treatments were well tolerated, and this dose was determined to be a no observable adverse effect level for both sexes. Systemic exposures of BEM, its metabolites, and RZR were similar when dosed alone or in combination, suggesting no drug-drug interactions (DDI) that affected their pharmacokinetics even at this high dose.

These data make the BEM/RZR combination treatment for HCV infections highly attractive, and it is being evaluated in clinical trials.

INTRODUCTION

- Approximately 58 million people globally are living with chronic hepatitis C virus (HCV) infection, with an incidence of 1.5 million cases per year. The WHO estimated 290,000 deaths from hepatitis C in 2019[1]
- New HCV treatment regimens with direct-acting antivirals (DAA) have become the standard of care, with sustained virological response (SVR) rates exceeding 95% and treatment duration reduced to 8–12 weeks, depending on the regimen and patient population[2–4]
- Despite high efficacy rates with existing therapies, better treatment options are needed for certain patient populations that include those with severe liver decompensation,[5] active hepatocellular carcinoma (HCC),[6] genotype 3 HCV infection,[7] treatment failure due to resistance requiring at least 12 weeks of treatment, often with adjunctive ribavirin treatment, and those with comorbid conditions receiving concomitant medications leading to drug-drug interactions (DDI)[2]
- Atea Pharmaceuticals, Inc. is in Phase 2 development with bemnifosbuvir (BEM) in combination with ruzasvir (RZR) for the treatment of HCV
- BEM is a novel, oral NS5B polymerase inhibitor; RZR is a novel, oral NS5A phosphoprotein inhibitor. Both have individually demonstrated potent, pan-genotypic, antiviral activity against HCV[8,9]
- The combination of BEM/RZR, which has demonstrated a substantially greater inhibition of HCV replication *in vitro* than the sum of the activities of both agents alone, has the potential to offer a differentiated, short duration, pan-genotypic, protease inhibitor-sparing regimen for HCV-infected patients with or without cirrhosis
- For HCV patients with decompensated cirrhosis, the combination of BEM/RZR may have the additional potential for treatment without the co-administration of ribavirin, which can cause a wide range of serious side effects

METHODS

Antiviral activity in HCV GT1b replicon cells

- The reporter cell line Huh-luc/neo-ET, grown in DMEM supplemented with 10% FCS and 1 mg/mL G418, was seeded in 96-well plates at $7.5x10^3$ cells and incubated at 37ºC 5% CO_2 for 24 h
- Media was replaced without G418, and AT-511 (free base of BEM) and RZR added alone or together, then incubated 72 h before measuring HCV replication by luciferase activity using britelite plus luminescence reporter gene kit (Perkin Elmer)
- Cellular toxicity was assessed by XTT staining. RZR (0.008 nM) and AT-511 (40 nM) were serial two-fold diluted for five and nine concentrations, respectively

Animal study

- Four groups of Sprague Dawley (SD) rats (15/sex/group) were orally administered vehicles, BEM and RZR separately and in combination at 500 mg/kg once daily for 13 weeks
- More animals (4-13/sex/group) were dosed with the same protocol to collect blood samples for a toxicokinetics (TK) study of RZR, BEM's free base AT-511, and its metabolites AT-551, AT-229 and AT-273
- Parameters evaluated in the study included survival, TK, clinical observations, body weights, food consumption, ophthalmologic examinations, clinical pathology evaluations, organ weights, and gross and microscopic pathology analyses

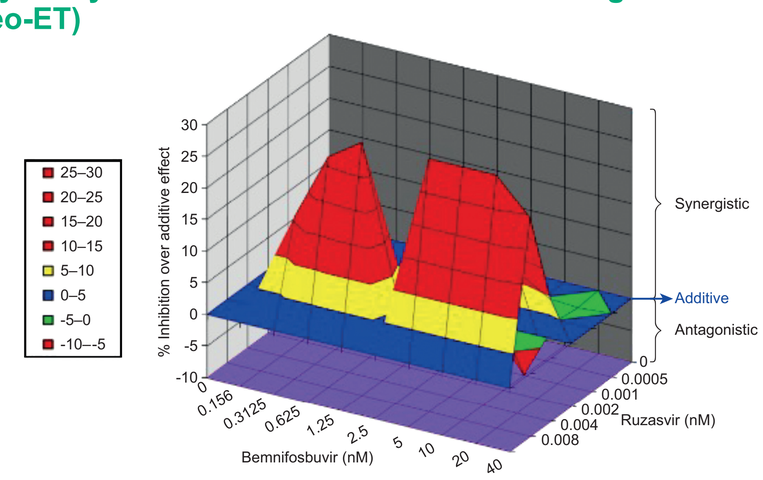
RESULTS

In Vitro Comparison of HCV NS5A Inhibitors

	HCV Replicon EC_{50} (pM)							
Inhibitor	**GT1a**	**GT1b**	**GT2a**	**GT2b**	**GT3a**	**GT4a**	**GT5a**	**GT6a**
pibrentasvir – ABBV[10]	2	4	2	2	2	2	1	3
velpatasvir – GILD[11]	12	15	9	8	12	9	75	6
daclatasvir – BMS[12]	50	9	71		146	12	33	
ruzasvir – MRK (Atea)[9]	1	2	1	4	3	1	2	1
ravidasvir - Presidio[13]	~110	~20	~120		~1100	~50	~40	~400

- RZR has potent pan-genotypic *in vitro* activity against HCV similar to pibrentasvir and more favorable when compared to other NS5A inhibitors

MacSynergy Analysis of the BEM/RZR Combination Against HCV Replicon (Huh-luc/neo-ET)

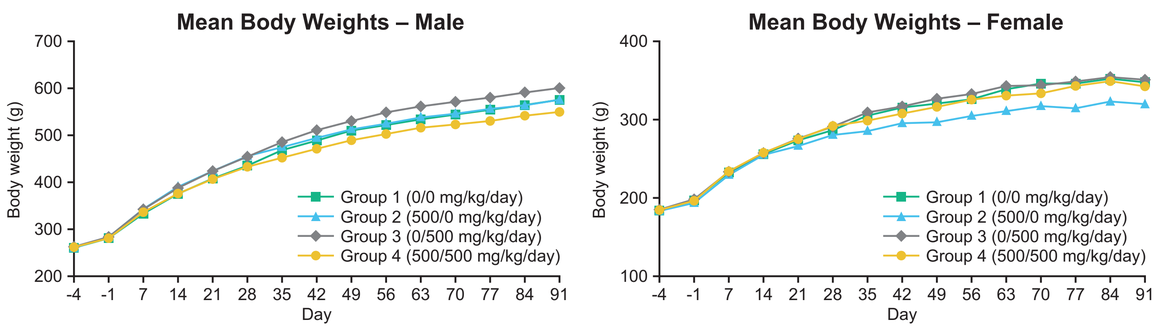


Anti-HCV activities were measured in replicon cells treated with up to 40 nM AT-511, free base of BEM, and up to 0.008 nM RZR, either alone or in combination. The data used Prichard and Shipman MacSynergy II software to determine synergy (above) or antagonism (below the plane of additivity).

- The BEM/RZR combination had synergy volumes greater than 100 $\mu M^2\%$, which is indicative of highly synergistic antiviral activity
- Neither antagonistic nor synergistic cytotoxicity was observed

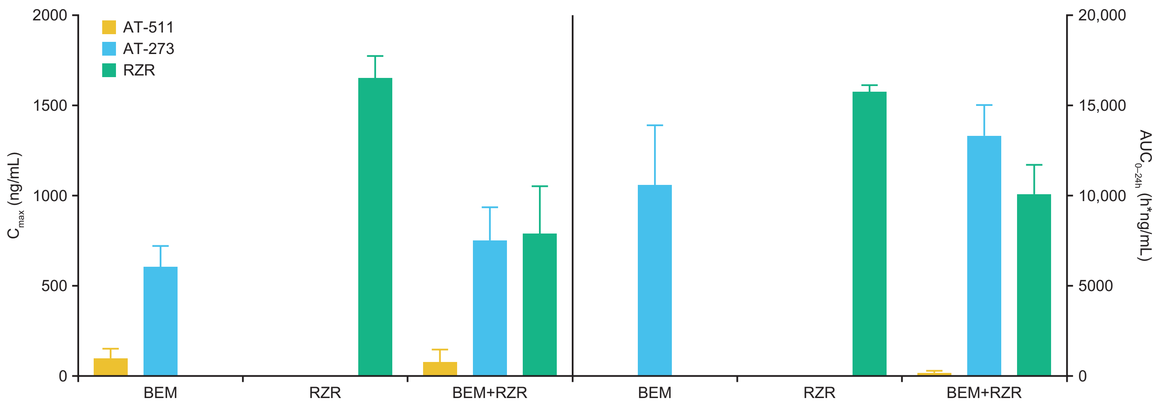
No Adverse Effects on Rats Given Combination for 13 weeks

- There were no notable test article related effects in mortality, clinical signs, food consumption, ophthalmic findings, urinalysis parameters or macroscopic observations
- From Day 35 to the end of the dosing period, females treated with BEM alone had a slight decrease in body weight (up to 8% compared to untreated controls) not observed in male animals
- There were minor test article related changes in some of the clinical pathology values (WBC, RBC count, fibrinogen, triglycerides, total protein, bilirubin and cholesterol) and organ weights (liver, adrenal, thymus, heart), but none were considered adverse
- The no-observed-adverse-effect-level (NOAEL) of BEM and RZR was considered to be 500 mg/kg/day for both male and female animals



BEM and RZR were orally administered to male and female SD rats separately and in combination at 500 mg/kg once daily for 13 weeks.

Systemic Exposures of RZR, BEM and Metabolites on Day 84 of Treatment in Male and Female SD Rats



Male and female values, mean ± standard deviation

Rats were orally administered 500 mg/kg BEM and RZR daily for 13 weeks, alone or in combination. On Day 84, blood was collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 h and plasma separated. RZR, AT-511, free base of BEM, and its metabolites AT-551, AT-229 and AT-273 were determined by LC-MS/MS for TK analysis. Concentrations of AT-511 are low in the BEM-treated groups due to the high levels of esterases in rodent plasma.

- Systemic exposures for RZR, AT-511 (BEM's free base) and AT-273 (plasma metabolite of BEM's intracellular active triphosphate), as well as for the two other main metabolites of BEM (data not shown) were similar whether the drugs were given alone or in combination

CONCLUSIONS

- Separately, BEM and RZR have potent, pan-genotypic, antiviral activity against HCV
- *In vitro*, the combination of BEM and RZR had greater inhibition of HCV replication than the sum of both compounds alone, suggesting a synergistic antiviral effect between the two inhibitors
- Treatment of 500 mg/kg/day BEM and RZR, alone or in combination to SD rats for 13 weeks, was well tolerated and this high dose was determined to be a NOAEL for both sexes
- Systemic exposures of BEM, its metabolites, and RZR were similar when dosed alone or in combination, suggesting no significant DDI between the two drugs

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Disclosures

All the authors are current or former employees of Atea Pharmaceuticals.

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