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DRUG EVALUATION

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Bemnifosbuvir (BEM, AT-527), a novel nucleotide analogue inhibitor of the hepatitis C virus NS5B polymerase

Xiao-Jian Zhou, Steven S Good, Keith Pietropaolo, Qi Huang, Adel Moussa, Janet MJ Hammond and Jean-Pierre Sommadossi

Departments of Preclinical and Clinical Development, Atea Pharmaceuticals, Boston, MA, USA

ABSTRACT

Introduction: Chronic hepatitis C virus (HCV) persists as a public health concern worldwide. Consequently, optimizing HCV therapy remains an important objective. While current therapies are generally highly effective, advanced antiviral agents are needed to maximize cure rates with potentially shorter treatment durations in a broader patient population, particularly those patients with advanced diseases who remain difficult to treat.

Areas covered: This review summarizes the in vitro anti-HCV activity, preclinical pharmacological properties of bemnifosbuvir (BEM, AT-527), a novel prodrug that is metabolically converted to AT-9010, the active guanosine triphosphate analogue that potently and selectively inhibits several viral RNA polymerases, including the HCV NS5B polymerase. Results from clinical proof-of-concept and phase 2 combination studies are also discussed.

Expert opinion: BEM exhibits potent pan-genotype activity against HCV, and has favorable safety, and drug interaction profiles. BEM is approximately 10-fold more potent than sofosbuvir against HCV genotypes (GT) tested in vitro. When combined with a potent NS5A inhibitor, BEM is expected to be a promising once-daily oral antiviral for chronic HCV infection of all genotypes and fibrosis stages with potentially short treatment durations.

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Bemnifosbuvir; AT-527; AT-511; antiviral; hepatitis C virus; nucleotide analogue; RNA polymerase

1. Introduction

Following the advent of curative all-oral therapies for chronic hepatitis C virus (HCV) infection in 2013–2014, the global prevalence of chronic HCV infections declined to an estimated 71 million people as of 2015 [1]. Despite this advance, in many countries the overall prevalence of HCV infection has stabilized or increased in recent years, primarily due to a rising incidence of new HCV infections among injection drug users and others engaged in high-risk activities [2].

Current all-oral therapies for chronic HCV infection provide high cure rates with overall good safety profiles [3]. In the US, among approved treatment options, the combination of glecaprevir, a NS3/4A protease inhibitor, and pibrentasvir, a NS5A inhibitor, for 8 weeks, and the combination of sofosbuvir, a nucleotide NS5B polymerase inhibitor and velpatasvir, a NS5A inhibitor, for 12 weeks have emerged as preferred first-line therapeutics for HCV. Despite these therapeutic advances, HCV treatment options remain limited or suboptimal for some patient groups. Disease characteristics and complications such as decompensated cirrhosis, hepatocellular carcinoma, and HCV GT3 infection have proven to be more difficult to treat and can sometimes increase the required duration of therapy or reduce cure rates [4]. Recent data indicate that SVR rates with current regimens are also lower in Sub-Saharan Africans who carry uncommon HCV genotypes [4,5]. Many patients have comorbidities requiring medications that have potentially treatment-limiting drug interactions with HCV therapeutics; NS3 protease inhibitors are particularly problematic in this regard. Regimens containing protease inhibitors are contraindicated for patients with decompensated cirrhosis (moderate or severe hepatic impairment; Child-Pugh B or C) due to an increased risk of hepatotoxicity in this population [6]. Ribavirin, which can increase cure rates when added to some regimens, cannot be used in patients with high risk of anemia or other ribavirin-related adverse events [7]. Patients with compliance issues may benefit from shorter treatment durations, and those who failed to respond to previous HCV therapies may carry HCV strains with resistance mutations that contraindicate other drugs of the same class [8–11].

These considerations suggest that patients with difficult-to-treat disease characteristics would benefit from new HCV therapies that offer improved efficacy, short treatment durations with once-daily dosing, and minimal drug interaction potential. Therapies with these characteristics may also facilitate cost-efficient public health programs for reducing HCV prevalence.

Bemnifosbuvir (BEM, AT-527) (Box 1) is the hemisulfate salt of AT-511, a prodrug of a novel guanosine monophosphate analogue [12]. BEM has potent pan-genotypic activity against HCV *in vitro*, acting through inhibition of the HCV NS5B RNA polymerase after metabolic conversion to its active triphosphate form. In initial clinical trials, BEM has demonstrated

CONTACT Xiao-Jian Zhou zhou.xj@ateapharma.com Departments of Preclinical and Clinical Development, Atea Pharmaceuticals, Inc 225 Franklin Street, Suite 2100, Boston, MA 02110, USA

Article highlights

- Chronic HCV infection continues to be a threat to public health worldwide despite multiple available direct-acting antiviral regimens; novel advanced antiviral agents are still needed to optimize HCV therapy particularly for more difficult to treat populations.
- Bemnifosbuvir is a guanosine nucleotide protide with structural features that facilitate target cell uptake, prevent formation of mutagenic metabolites, and maximize formation of the intracellular active triphosphate AT-9010, a potent and selective inhibitor of several viral RNA polymerases, including the HCV NS5B polymerase.
- Bemnifosbuvir exhibits pan-genotype antiviral activity against all HCV variants tested in vitro and is approximately 10-fold more potent than sofosbuvir; bemnifosbuvir is also active against sofosbuvir-resistant HCV variant S282T.
- As a single agent, bemnifosbuvir demonstrated high and dose-dependent clinical anti-HCV activity across HCV genotypes regardless of cirrhosis status.
- When combined with daclatasvir, a first-generation HCV NS5A inhibitor, a high SVR rate was achieved after mostly 8 weeks of therapy with a satisfactory safety profile.
- Bemnifosbuvir has a low risk for clinical drug-drug interaction.
- Bemnifosbuvir in combination with ruzasvir, a novel pan-genotypic HCV NS5A inhibitor, is under clinical development and is expected to be a convenient oral once daily therapy for chronic HCV infection of all genotypes and fibrosis stages with potentially shorter treatment durations.

Box 1. Drug Summary Box.

Drug name: bemnifosbuvir (AT-511) hemisulfate (AT-527)
Phase: II
Indication: chronic hepatitis C (HCV)
Pharmacology/mechanism of action: inhibition of the RNA-dependent RNA polymerase of flaviviruses including the NS5B polymerase of HCV
Route/frequency of administration: oral/once daily
Notable trials: NCT03219957 (Phase Ib Proof-of-concept as a single agent, completed), NCT04019717 (Phase II bemnifosbuvir and daclatasvir combination, completed), NCT05904470 (Phase II bemnifosbuvir and ruzasvir combination, ongoing).

potent pan-genotypic antiviral activity in participants with chronic HCV infection, including those with cirrhosis, as well as favorable pharmacokinetics (PK) and safety [13,14]. These initial data suggest that BEM may offer a treatment advance for important groups of HCV patients, including those with HCV GT-3 infection or cirrhosis.

Here we summarize the unique structural chemistry supporting BEM clinical development, and preclinical and early clinical findings that define the potential role of BEM in HCV therapy and eradication.

2. Preclinical studies

2.1. Chemistry and metabolic activation

The structure of BEM includes three moieties that facilitate target cell uptake, prevent formation of toxic metabolites, and confer potent and selective inhibition of the viral RNA polymerase (Figure 1). Multistep activation of the prodrug produces its active triphosphate metabolite within infected cells. The 5′-phosphoramidate group increases oral bioavailability, facilitates target cell uptake, and maximizes formation of the intracellular triphosphate active metabolite AT-9010 (Figure 1). Within the target cell, the 5′-phosphoramidate group is removed by cellular enzymes to produce the 5′-monophosphate metabolite. This conversion is believed to entail a pathway similar to that of sofosbuvir, which is a pyrimidine analogue with the same 2′ and 5′ substitutions on the sugar moiety. In this pathway, hydrolysis of the carboxyester moiety of the 5′-phosphoramidate group is catalyzed by human cathepsin A (CatA) and/or carboxylesterase 1 (CES1), yielding an L-alanyl intermediate prodrug (M1; Figure 1). Once inside cells, the amino acid moiety is removed by histidine triad nucleotide-binding protein 1 (Hint1) to produce an intermediate 5′-monophosphate metabolite (M2; Figure 1) that retains the 2′-fluoro-2′-C-methyl sugar substitutions and the N^6-methyl base substitution.

The N^6-methyl group on the base moiety is critical to the safety profile of BEM. This group stabilizes the glycosidic bond and confers resistance to hydrolytic formation of toxic metabolites. BMS-986094 (aka INX-189) and PSI-938 (aka PSI-352938, GS-0938), which are guanosine analogues lacking this critical substitution, failed in clinical development due to toxicity [15–18]. Within target cells, the N^6-methyl group of M2 is converted by adenosine deaminase-like protein 1 (ADALP1) to the natural guanine base linked to the 2′-substituted sugar moiety (M3; Figure 1), which is further phosphorylated to produce the antiviral triphosphate active metabolite AT-9010, the 2′-substituted analogue of GTP. M2 that is not converted to M3 is dephosphorylated to M4 (Figure 1).

The 2′-fluoro-2′-C-methyl substituent confers inhibitory selectivity by preventing binding in the active sites of cellular enzymes but not the HCV polymerase; this substituent is also found in sofosbuvir. As shown in Figure 1, since the nucleoside metabolite 2′-fluoro-2′-C-methylguanosine (AT-273) can only be formed via dephosphorylation of intracellular phosphates, plasma AT-273 has been used as a plasma surrogate marker for PK and pharmacodynamic (PD) assessments of intracellular phosphorylated metabolites, comprising predominantly AT-9010.

2.2. Virology

In vitro replicon assays with BEM demonstrated potent pan-genotypic antiviral activity; EC_{50} (concentrations achieving 50% inhibition) values of 9.2 to 28.5 nM were observed across HCV genotypes [12] The potency of BEM *in vitro* was 5.5- to 11.4-fold greater than that of sofosbuvir against all HCV genotypes tested. Similar potency was demonstrated in cells transiently transfected with constructs derived from HCV clinical isolates.

Resistance to HCV nucleotide NS5B inhibitors is infrequent in clinical practice, but can cause treatment failure when it occurs. Insertion of L159F or C316N, which are known resistance-associated variants (RAVs) for nucleotide NS5B inhibitors [9,10,19] into the NS5B gene of HCV GT1 and 2, had no apparent effect on BEM antiviral activity. Insertion of S282T, the predominant sofosbuvir resistance polymorphism, into the NS5B gene resulted in a 2.5-fold reduction in BEM potency and a 15-fold reduction of sofosbuvir potency. BEM was 8- to 36-fold more potent than sofosbuvir against all GT-1a and GT-3a NS5A RAVs tested including Y93H, A30D, A30K, L31F, P58S, S62F, S62L, L31M +H58P, M28T+T64A, and M28V+T30R [20]. BEM is largely inactive

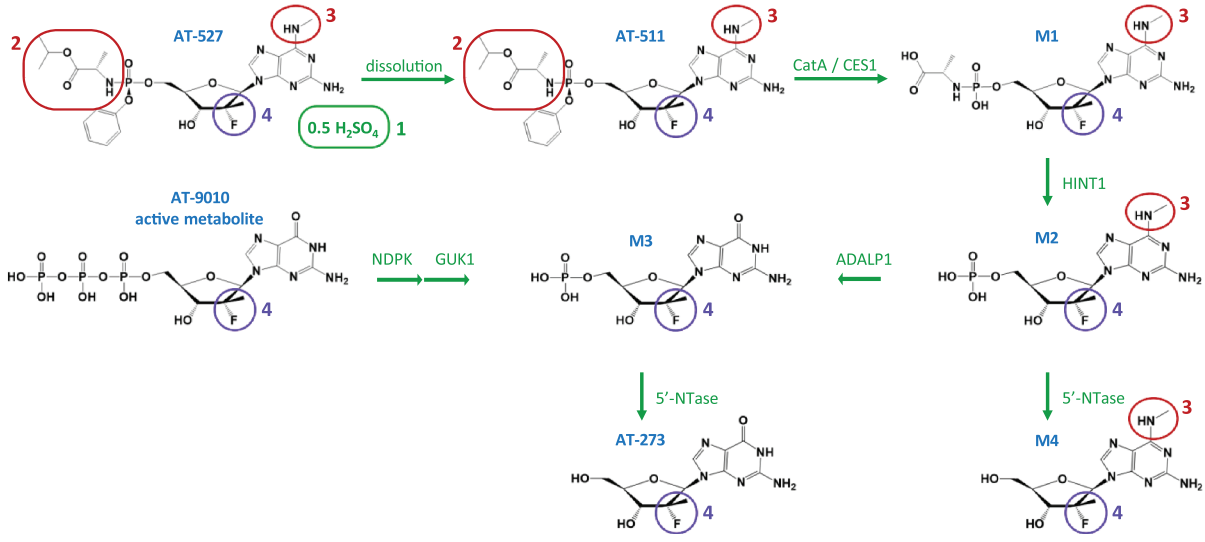

Figure 1. Metabolism and key structural elements of bemnifosbuvir.

The metabolic activation and key elements of bemnifosbuvir are illustrated. Key elements include (1) the hemisulfate (0.5 H_2SO_4) salt which enhances solubility of the orally administered tablet; (2) the 5′-phosphoramidate moiety which facilitates oral bioavailability and target cell uptake; (3) the N^6-methyl group which stabilizes the glycosidic bond, preventing formation of toxic metabolites, increasing target cell update, and enhancing formation of the triphosphate active metabolite AT-9010 through monophosphate intermediates M2 and M3; and (4) the 2′-fluoro-2′-C-methyl substitution which provides selective inhibitory activity against the viral RNA polymerase. As described in the text, after dissolution of AT-527 to release AT-511, multiple enzymatic steps convert AT-511 to AT-9010. ADALP1, adenosine deaminase-like protein 1; CatA, human cathepsin A; CES1, carboxylesterase 1; GUK1, guanylate kinase 1; Hint1, histidine triad nucleotide-binding protein 1; 5′-NTase, 5′- nucleotidase; NDPK, nucleotide diphosphate kinase.

BEM activation processes:

BEM undergoes sequential hydrolysis, catalyzed by human CatA and/or CES1 followed by spontaneous cleavage of the unstable phenolic moiety, produces the L-alanyl intermediate (M1). Removal of the amino acid moiety by HINT1 results in metabolite M2 which is then converted to M3 by ADALP1. M3 is further anabolized sequentially by GUK1 and NDPK to the pharmacologically active triphosphate, AT-9010. Both M2 and M3 can be dephosphorylated by 5′-NTase to the corresponding nucleosides M4 and AT-273.

against most other RNA and DNA viruses in vitro, with EC_{50} values exceeding 100 µM. Notably, however, BEM has demonstrated potent *in vitro* antiviral activity against human seasonal coronaviruses and SARS coronaviruses, including SARS-CoV-1 and SARS-CoV-2, in human airway epithelial cells [21]. BEM has also been shown to inhibit rubella virus in vitro [22]

2.3. Preclinical safety assessments

BEM exhibited no evidence of cytotoxicity *in vitro* [12]. TC_{50}, concentration required to produce a toxic effect on 50% of exposed cells, including animal and human hepatocytes, typically exceeded 100 µM. Assessments of BEM in human-induced, pluripotent stem cell cardiomyocytes, granulocyte macrophages, and erythroid human bone marrow progenitor cells showed no evidence of toxicity (TC_{50} > 100 µM).

AT-9010, the active triphosphate metabolite of bemnifosbuvir, is highly selective against the HCV polymerase (IC_{50}, 50% inhibitory concentration, 0.15 µM), not human DNA polymerases α, β, and γ or human mitochondrial RNA polymerase (PolRMT) (IC_{50} > 100 µM). Inhibition of PolRMT impairs mitochondrial RNA and protein synthesis and is believed to be a primary mechanism of BMS-986094–induced cardiac toxicity [16,23].

Unlike 2′-C-methyl guanosine triphosphate (the active metabolite of BMS-986094) which was efficiently incorporated by PolRMT, the relative efficiencies of incorporation of sofosbuvir triphosphate and AT-9010 into RNA, compared with the natural UTP and GTP substrates, were similar and much lower. BEM also had no discernible effects on mitochondrial function in cultured cells at concentrations up to 20 µM, consistent with findings from enzymology studies [12].

No evidence was found for formation of mutagenic metabolites by BEM *in vitro*. A key reason for the discontinued development of related prodrugs is the metabolic formation of mutagenic O^6-alkylguanine base analogues. Two such compounds, PSI-661 and BMS-986094, have O^6-methyl substitutions and PSI-938 has an O^6-ethyl substitution instead of the N^6-methyl group present in BEM (Figure 1). When incubated with recombinant human cytochrome P450 3A4 in the presence of NADPH, approximately 50% of PSI-661, 30% of PSI-938 and 9% of BMS-986094 were converted to mutagenic O^6-alkylguanine bases. In comparison, BEM released no detectable O^6-substituted bases and low levels of 2,6-diaminopurine and N^6-methyl-2,6- diaminopurine, representing 1.8% of BEM in the medium.

In a liver microsomal assay, BEM had no discernible inhibitory activity (IC_{50} > 30 µM) against seven cytochrome P450 (CYP) isozymes, including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6, and minimal activity (IC_{50} = 25 µM) against CYP3A4, suggesting that BEM will have limited potential for drug-drug interactions (DDI) with drugs that interact with CYP enzymes [12].

2.4. Preclinical pharmacokinetics

After single oral doses of BEM in rats (500 mg/kg) and cynomolgus monkeys (30, 100, and 300 mg/kg), BEM was rapidly absorbed with dose-related plasma exposure [12]. The active triphosphate metabolite AT-9010 was preferentially formed in the liver than the heart: 87-fold and up to 18-fold higher in the liver than heart, in rats and in monkeys, respectively.

3. Clinical studies

BEM has been evaluated in a multiple-part early phase clinical study of first-in-human (FIH) single- and multiple-ascending doses (SAD, MAD) in healthy participants with proof-of-concept (POC) in HCV-infected individuals without or with compensated cirrhosis, and in a phase 2a study in combination with daclatasvir (DCV) in HCV-infected individuals.

3.1. FIM study with POC

The safety, PK, and clinical anti-HCV activity of BEM were assessed in a phase 1 clinical study (NCT03219957) comprising 5 sequential parts [13,23–27]. SAD (BEM 46, 92, 184, and 369 mg; randomized, double-blind, placebo-controlled) was assessed in healthy subjects, followed by open-label, single-dose, POC (BEM 92, 277, 369, and 553 mg sequentially) in non-cirrhotic participants with GT-1b HCV. POC was further carried out as part of MAD (BEM 138, 277 and 553 mg QD x 7 days; randomized, double-blind, placebo-controlled) in treatment-naïve, non-cirrhotic participants with GT-1b HCV [13]. Two additional multiple-dose cohorts were then initiated, evaluating clinical anti-HCV activity with BEM 553 mg QD x 7 days open-label in non-cirrhotic participants with GT-3 HCV as well as participants with compensated cirrhosis (Child-Pugh class A) with GT-1b, 2 or 3 HCV.

BEM was well tolerated with no treatment-emergent serious adverse events (SAE) or treatment-related discontinuations. Most events were of mild or moderate intensity and nonspecific, most frequently headache, back pain, diarrhea, or nausea, with no apparent relationship to BEM dose [13,24]. With respect to clinical laboratory parameters, generally low to modest increases in blood cholesterol and triglycerides were observed in some HCV- infected participants with BEM treatment, but not placebo [13], consistent with previous data showing increases in plasma lipids upon initiation of antiviral therapy of chronic HCV [28,29]. No lipid-related events were observed in healthy volunteers. Levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased in HCV-infected participants during BEM treatment, with the greatest changes in individuals with elevated levels at baseline. ALT and AST returned to baseline levels after completion of dosing [13].

BEM was rapidly absorbed upon oral dosing to reach maximum plasma concentration typically within an hour and was quickly cleared from blood, with a short half-life of approximately 0.5–1 h. PK was mostly comparable in healthy and HCV-infected participants, without or with cirrhosis, and in male and female participants. Plasma levels of 2′-fluoro-2′-methylguanosine (AT-273), a major nucleoside metabolite of BEM that is regarded as a surrogate for intracellular AT-9010 concentrations, were dose-proportional and sustained over time with a long half-life (~24 h) [26]. Plasma PK for AT-273 was similar in the fasted and fed conditions, suggesting similar levels of intracellular AT-9010 are achieved under fed and fasted conditions, supporting once-daily dosing without regard to meal.

With repeat dosing, BEM did not accumulate [13]. Steady-state was mostly achieved by day 3–4, based on trough levels of AT-273.

After single doses of BEM in non-cirrhotic individuals with GT-1b HCV, mean maximum plasma HCV RNA levels were 0.8, 1.7, 2.2, and 2.3 $\log_{10}$ IU/mL after 92 mg, 277 mg, 369 mg, and 553 mg doses, respectively [26,27]. After 7 days of dosing in non-cirrhotic individuals with GT-1b HCV, the 138, 277, and 553 mg daily doses exhibited mean HCV RNA reductions (±SD) of 2.6 ± 1.073, 4.0 ± 0.415, and 4.4 ± 0.712 $\log_{10}$ IU/mL, respectively (Figure 2) [13]. BEM 553 mg QD x 7 days resulted in similar HCV RNA reductions in non-cirrhotic participants with

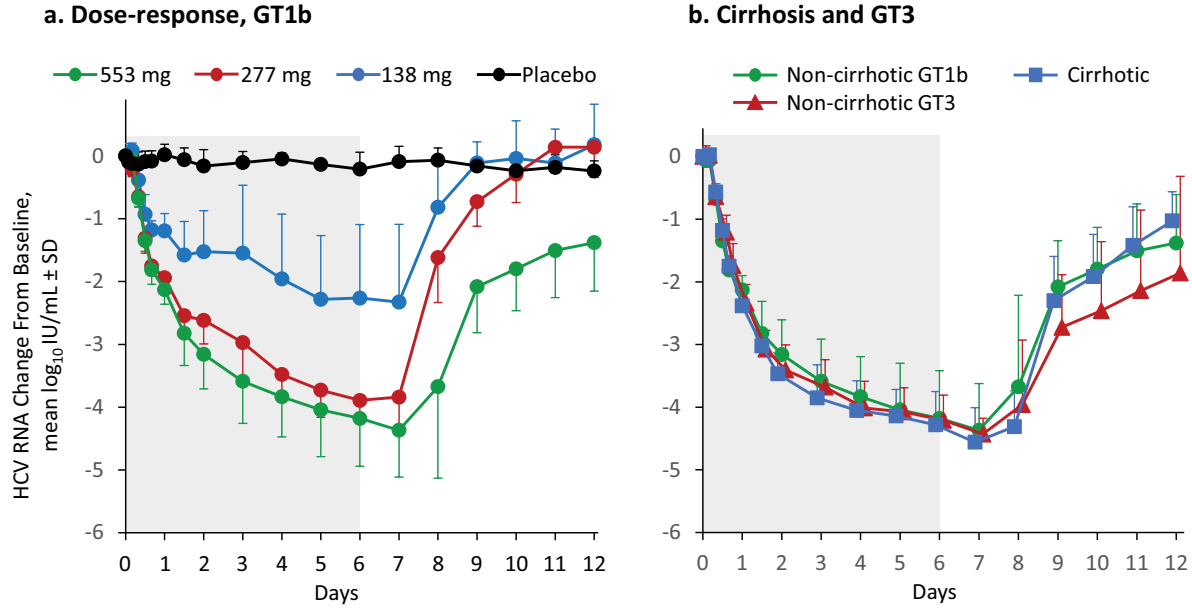

Figure 2. HCV RNA changes over 7 days of bemnifosbuvir monotherapy.

HCV RNA mean changes from baseline with one standard deviations are shown for (a) the bemnifosbuvir dose-response in non-cirrhotic patients with GT1b infection (138, 277, and 553 mg/day), and (b) bemnifosbuvir 550 mg/day in non-cirrhotic patients with GT1b infection, non-cirrhotic patients with GT3 infection and cirrhotic patients with GT1b, 2 or 3 infection. The shaded areas indicate BEM dosing periods.

GT-3 HCV (4.5 ± 0.262 $\log_{10}$ IU/mL) and cirrhotic participants with GT-1b, 2 or 3 HCV (4.6 ± 0.485 $\log_{10}$ IU/mL) as non-cirrhotic participants with GT-1b HCV (above) [13]. Among participants who received BEM 553 mg QD, HCV RNA was below the lower limit of quantitation (15 IU/mL) in 5 participants including 1 cirrhotic participant. BEM 553 mg QD over 7 days showed similar PK/PD relationships between AT-273 plasma concentration and HCV RNA response for participants with GT-1b, 2 or 3 HCV, without or with cirrhosis (Figure 3). From the first dose, AT-273 trough concentrations exceeded the EC_{95} of BEM for inhibition of replicon constructs of clinical isolates for HCV GT-1b (~22 ng/mL), GT-2 (≈12 ng/mL) and GT-3 (~18 ng/mL). A close PK/PD relationship is depicted in Figure 3. Plasma HCV RNA continued to decline as long as plasma AT-273 was maintained above the respective EC_{95} values. At the end of the 7-day dosing, as plasma AT-273 dropped below the threshold, plasma HCV RNA started to rebound, as expected.

BEM exhibited similar anti-HCV activity independent of HCV genotype and cirrhosis status, allowing for dose-response

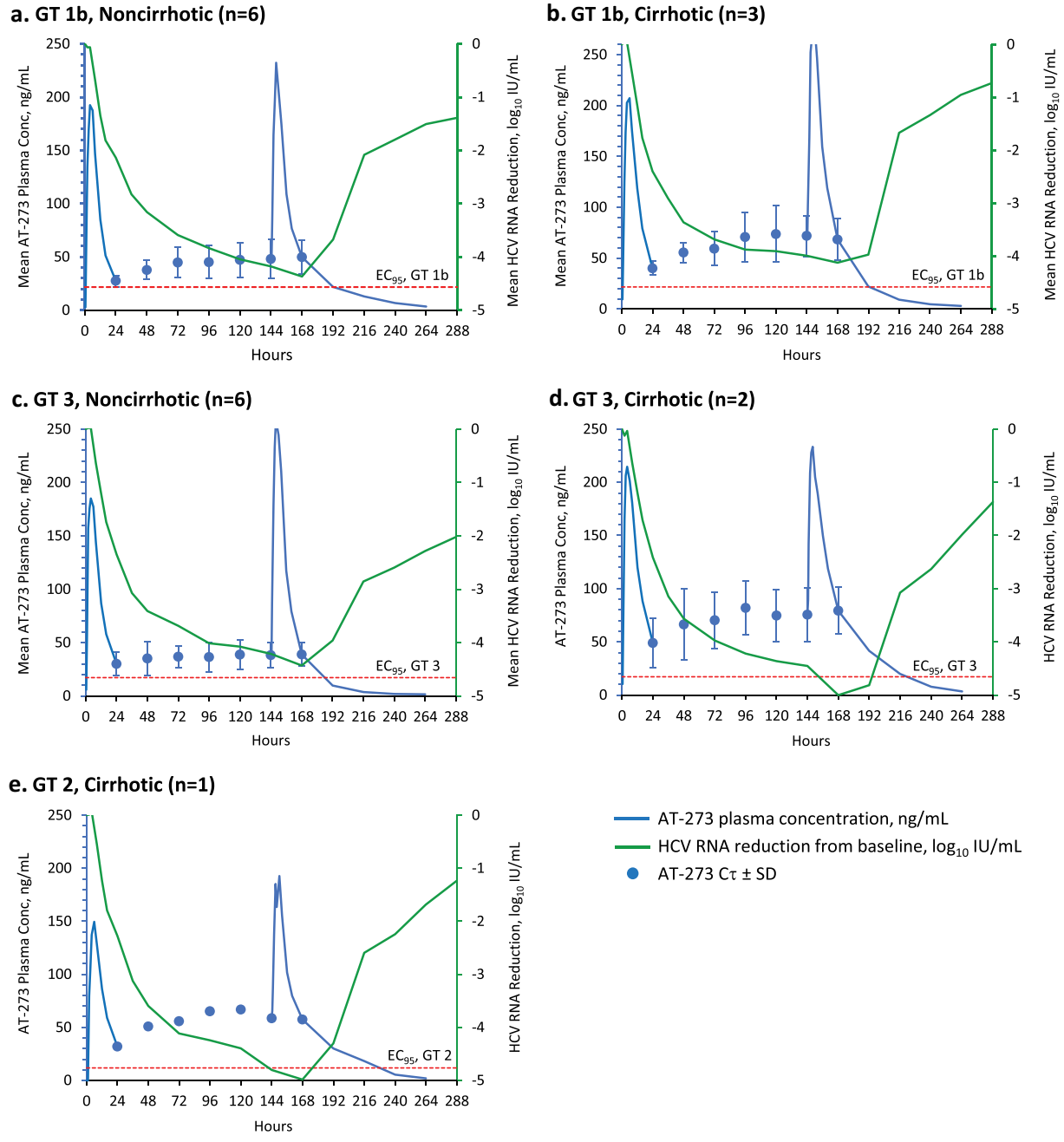

Figure 3. Pharmacokinetic/Pharmacodynamic analysis in patients with or without cirrhosis.

Relationships between AT-273 plasma PK and HCV RNA reductions are shown for (a) non-cirrhotic participants with GT1b HCV; (b) cirrhotic participants with GT1b HCV; (c) non-cirrhotic participants with GT 3 HCV; (d) cirrhotic participants with GT3 HCV; and (e) cirrhotic participant with GT2 HCV. Blue spots indicate mean AT-273 trough concentrations (Cτ) ± standard deviations. Horizontal lines indicate the BEM concentration (expressed as AT-273 equivalent) required to achieve 95% inhibition of the HCV clinical isolates in vitro (EC_{95}) [14]

analysis using pooled HCV RNA reductions from baseline after 7 days of dosing from 138 to 553 mg QD [24]. Steady-state AUC of AT-273 (AUCτ), the guanosine nucleoside metabolite representing intracellular active TP AT-9010, was selected as the PK measure reflecting total exposure for the PK/PD analysis. A standard E_{max} model was used and described the data well (Figure 4). The fitted curve indicates that E_{max} is achieved with AT-273 AUCτ greater than 2000 ng/mL x h. As can be appreciated from Figure 4, only the 553 mg/day dose of AT-527 produced AT-273 AUCτ values that were consistently above the threshold of 2000 ng/mL x h. Therefore, this analysis provided PK/PD support for the selection of the 550 mg/day dose for later phase HCV clinical trials.

An HCV viral kinetics analysis was undertaken to estimate the time required to achieve a virologic cure (plasma HCV RNA < 1 IU/mL) with BEM as a single agent (Figure 5) [25,26] using previously described model [30,31]. Results suggest that 80% and 95% of patients would have plasma HCV RNA below the assay lower limit of quantitation (LLOQ) by weeks 2 and 4, respectively, and 80% and > 90% would achieve a cure by weeks 6 and 8, respectively.

3.2. Phase 2a clinical study

BEM 550 mg QD in combination with DCV 60 mg QD was evaluated in a response-guided trial (NCT04019717) [14]. Ten,

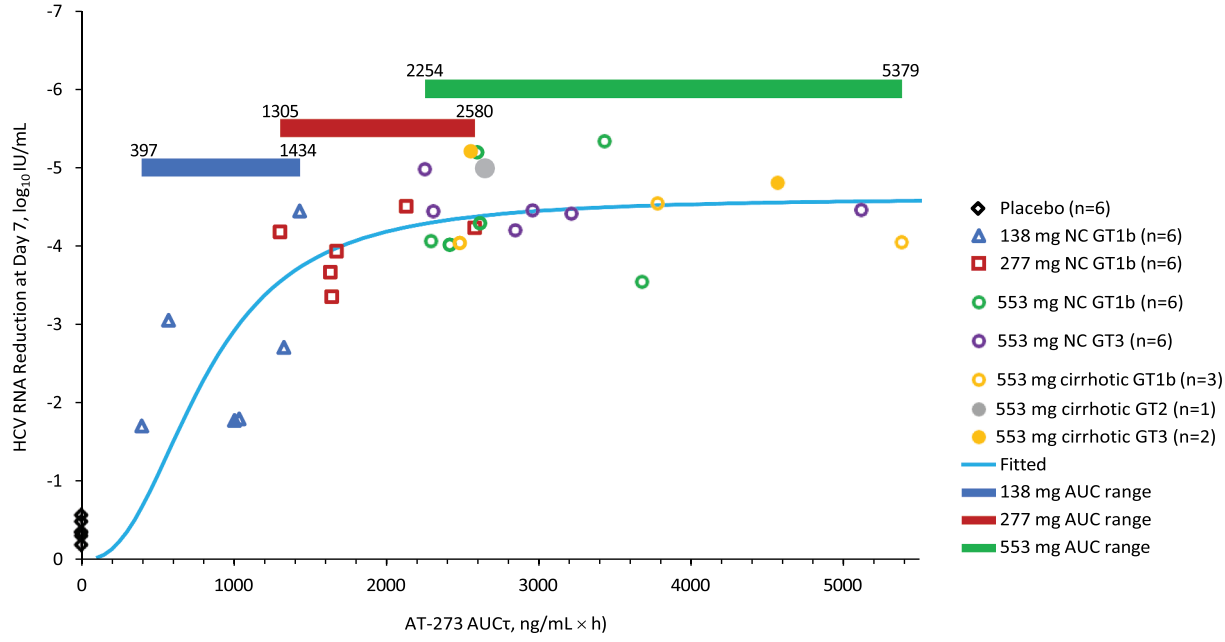

Figure 4. Emax model of bemnifosbuvir dose/anti-HCV response.

Relationship between AT-273 AUCτ and HCV RNA change from baseline after 7 days of BEM treatment is illustrated. The various symbols represent individual values for HCV RNA reductions, with the different symbols identifying HCV genotype, BEM dose, and cirrhosis status as shown.

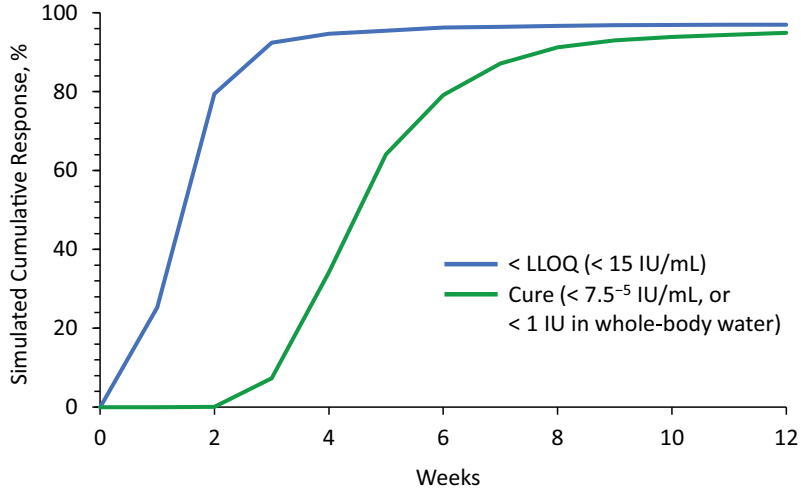

Figure 5. Viral kinetic simulation of bemnifosbuvir 550 mg QD as a single agent.

The blue line indicates the proportions of participants estimated to achieve HCV RNA below the lower limit of quantitation (LLOQ) over time; the green line indicates the proportions achieving HCV RNA less than 1 IU/mL over time, which is estimated to predict a durable cure.

Table 1. Virologic outcomes with bemnifosbuvir + DCV.

	Response at Study Visit, n/N (%)				
Response Measure	Week 2	Week 4	EOT	SVR4	SVR12
< LLOQ	7/10 (70%)	9/10 (90%)	10/10 (100%)	10/10 (100%)	9/10 (90%)
TND	5/10 (50%)	9/10 (90%)	10/10 (100%)	10/10 (100%)	9/10 (90%)

Data indicate response rates at weeks 2 and 4 during treatment, at end of treatment (EOT), and at post-treatment weeks 4 and 12 (SVR4 and SVR12). Post-treatment responses reflect sustained virologic responses at the indicated visits. Response measures include plasma HCV RNA below the lower limit of quantitation (<LLOQ; <15 IU/mL) and target not detected (TND). TND refers to HCV RNA not detected.

treatment-naïve, GT1, non-cirrhotic participants with HCV RNA > 10,000 IU/mL were treated were enrolled. Per protocol, 9 participants with HCV RNA (15 IU/mL) by week 4 stopped treatment at week 8; the remaining participant was treated for 12 weeks.

Five of the 10 participants were of African origin and 5 were of European origin. Plasma HCV RNA declined rapidly from > 800,000 IU/mL to below LLOQ in 7/10, 9/10, and 10/10 participants at week 2, week 4, and at end of treatment, respectively (Table 1) [14]. Nine of 10 participants achieved sustained virological response 12 weeks post treatment (SVR12). One GT1b-infected participant achieved undetectable HCV RNA by week 2, stopped treatment at week 8, and relapsed between post-treatment weeks 4 and 12. Serum ALT concentrations declined in parallel with HCV RNA reductions. There were no SAE or clinically relevant patterns of adverse events, lab abnormalities, electrocardiograms (ECG), or other safety parameters. No lipid-related adverse events were reported. The patient who relapsed was subsequently found to have multiple polymorphisms at baseline including NS5A-R30Q and NS5B-L159F/A218S/C316N [14]. The NS5B polymorphisms had minimal effect on BEM antiviral activity *in vitro*; however, NS5A-R30Q has been associated with virologic failure during combination therapy with DAC and the NS3 protease inhibitor asunaprevir [11], inferring its involvement in the observed viral relapse.

4. Conclusions

The changing demographics of HCV-infected populations and clinical experience with current HCV therapeutic options indicate a need for additional therapies to address deficiencies related to efficacy, safety, convenience, and drug-drug interactions. Nucleoside and nucleotide polymerase inhibitors are the cornerstones of treatment regimens for many viral diseases, most prominently therapies for HIV, herpesviruses, and hepatitis B and C viruses [32]. For HCV, sofosbuvir is the only nucleos(t)ide analogue approved as of 2023, and is used in several multi-drug combination regimens, notably sofosbuvir/velpatasvir [3]. Discovery of alternative nucleos(t)ide analogues to treat HCV has proven difficult due to the limited tolerance of the HCV polymerase for molecular alternatives that can bind in its active site. Several agents of this class have been identified, but failed in clinical development, due to unacceptable toxicity, primarily cardiac or hepatic toxicity, and/or potential for oncogenicity [16,17,33,34].

The double prodrug approach plus salt formation of BEM provides a highly soluble, orally bioavailable drug that maximizes formation of the active triphosphate metabolite AT-9010 in the target organs without producing toxic metabolites [12].

BEM demonstrated pan-genotypic anti-HCV activity against laboratory strains and clinical isolates *in vitro*, with 5- to 10-fold or greater potency than sofosbuvir against HCV genotypes, including GT3, which is among the hardest to treat [12]. BEM further retained activity against sofosbuvir-resistant single and double variants including S282T.

Preclinical safety data indicate that BEM has low toxicity against a broad panel of cell types *in vitro* and in animal studies; correspondingly, early phase clinical data indicate a favorable safety and tolerability profile of BEM for up to 12 weeks [12,13]. Lipid elevations observed with BEM were consistent with previous reports suggesting that these elevations are due to rapid suppression of HCV replication and reversal of lipid perturbations caused by HCV infection, and independent of the antiviral regimens or drug classes received [28,29].

Preclinical and clinical PK data support a QD regimen for BEM against HCV and that BEM can be dosed without regard to food and without adjustments based on hepatic fibrosis status [13,26,27]. Metabolic assessments indicate low interactions with CYP enzymes, suggesting a generally favorable drug interaction profile [12].

The favorable *in vitro* antiviral properties were successfully translated into clinical antiviral activity with BEM as a single agent: short-term treatment of HCV-infected patients in the POC study elicited very rapid virologic suppression, with little interpatient variation and no observable differences, according to HCV genotype (including GT3), baseline viral load, or fibrosis/cirrhosis status [13]. Viral kinetic modeling studies predict that approximately 80% and > 90% of patients with chronic HCV infection would achieve a cure after 6 to 8 weeks of treatment with bemnifosbuvir as a single agent, respectively, regardless of HCV genotype or cirrhosis status [25]. Virologic outcomes in the initial phase 2a study combining BEM with DAC, a first-generation HCV NS5A inhibitor, in participants with GT1 HCV are consistent with these predictions with 50% and 90% of participants having HCV RNA target not detected at week 2 and 4, respectively [14]. As a reference, in previous studies with sofosbuvir plus velpatasvir, 58% and 91% participants having HCV RNA target not detected at weeks 2 and 4, respectively [35,36].

It is of interest that 5 of the study participants in the phase 2a study were of African origin. Sub-Saharan Africans have been reported to experience treatment failure with current HCV therapeutic regimens at a higher rate than other demographic groups due to the presence of unusual HCV genotypes [4,5]. Retreatment with more potent regimens proved successful in most of these cases. Further study of BEM in diverse HCV patient populations will determine whether the high potency and early efficacy advantage observed in initial clinical studies

with BEM will translate to more rapid achievement of SVR or higher rates of SVR in problematic patient groups.

Viral kinetic modeling data coupled with initial clinical data combining BEM with DAC, support the development of BEM as the backbone of a pan-genotypic combination regimen [14,25]. In that context, a strong *in vitro* synergistic anti-HCV activity has been demonstrated for the combination of BEM and ruzasvir (RZR) [37], a novel HCV NS5A inhibitor with potent *in vitro* and clinical pan-genotypic anti-HCV activity [38,39]. Furthermore, there was no clinically meaningful pharmacokinetic DDI between BEM and RZR in healthy participants [40]. BEM and RZR combination is currently being evaluated in an 8-week phase II study in participants with GT1–7 HCV, without or with compensated cirrhosis [NCT05904470].

5. Expert opinion

Chronic HCV infection continues to be a threat to public health worldwide despite multiple available direct-acting antiviral (DAA) regimens. Curative treatments mitigate transmission rates and can prevent HCV-related liver cancer, end-stage liver disease, and death. Despite the availability of approved DAAs there are limitations and gaps to these drugs, highlighting the need for novel, advanced, antiviral agents to optimize HCV therapy, particularly for more difficult to treat populations.

The minimum treatment duration for most approved direct-acting antivirals (DAAs) is 12 weeks, with the exception being the use of glecaprevir/pibrentasvir for 8-weeks. Unfortunately, the use of glecaprevir/pibrentasvir is contraindicated in people with decompensated cirrhosis. In those who have severe HCV, the treatment duration can extend even longer, for up to 24 weeks or require add-on medication such as ribavirin, which has known contraindications and risks associated with its use. Hemolytic anemia is a known risk and can worsen cardiac disease and lead to fatal and nonfatal myocardial infarctions. In addition, ribavirin is contraindicated for pregnant women and in the male partners of women who are pregnant due to its significant teratogenic and embryocidal effects.

A key determinant of treatment success is adherence to the HCV treatment regimen. Treatment noncompliance leads to poor disease control, and treatment adherence is particularly important for SVR in HCV and reducing disease transmission. Shorter course treatments impose less burden on the patient and fewer pills are dispensed. There are presently no short-course, nucleoside inhibitor-free HCV treatments.

BEM is a promising and innovative therapy for chronic HCV infection that exhibits pan-genotype antiviral activity against all HCV variants tested *in vitro*, including found to be resistant to sofosbuvir, and is approximately 10-fold more potent than sofosbuvir. As a single agent, BEM demonstrated high and dose-dependent activity across HCV genotypes regardless of cirrhosis status, as well as a low risk for clinically significant drug-drug interactions (DDI). When BEM was combined with DCV, a high SVR rate was achieved after mostly 8 weeks of therapy with a satisfactory safety profile. Together, these features will support treatment compliance and simplify patient monitoring, and, in turn, are support effective and cost-efficient public health programs for reducing HCV prevalence, when coupled with systemic patient identification, treatment, and risk reduction programs. The combination of BEM and RZR is an important step toward the development of a convenient, oral, once daily, protease inhibitor-free therapy for chronic HCV infection of all genotypes and fibrosis stages with potentially shorter treatment durations.

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Declaration of interest

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ORCID

Xiao-Jian Zhou http://orcid.org/0009-0009-7662-1616

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