Next generation core inhibitors ABI-H3733 and ABI-4334 have significantly improved potency and target coverage for both antiviral and cccDNA formation activities compared to first-generation core inhibitors

WED-114

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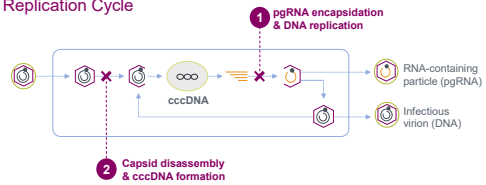
Introduction

- Chronic hepatitis B virus infection (cHBV) is a significant global health problem, with an estimated 296 million infected people worldwide, resulting in about 820,000 deaths each year, mostly due to cirrhosis and hepatocellular carcinoma[1-4]
- Core inhibitors are a novel class of small molecules with the potential to improve cHBV cure rates. These agents:
  - Inhibit multiple steps in the HBV life cycle, including 1) pregenomic (pg)RNA encapsidation, which prevents formation of new viral particles, and 2) disruption of incoming capsids, which prevents de novo formation of covalently closed circular (ccc)DNA from incoming HBV (**Figure 1**)[5]
  - Demonstrated potent antiviral activity in Phase 1 studies[6,7] and enhanced antiviral activity when combined with nucleos(t)ide reverse transcriptase inhibitors (NrtIs) in Phase 2 studies[8,9]
- ABI-H3733 (3733) and ABI-4334 (4334) are novel, next-generation core inhibitors with improved in vitro potency against pgRNA encapsidation and cccDNA formation compared to the first-generation core inhibitor vebicorvir (VBR)[10]

Figure 1. Core Inhibitors Target Multiple Steps of the HBV Replication Cycle



cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; pgRNA, pregenomic RNA.

- First-generation core inhibitors were more optimized for viral replication (1) vs cccDNA formation (2)
- Potent activity against both replication **and** cccDNA formation may be crucial for optimal patient responses

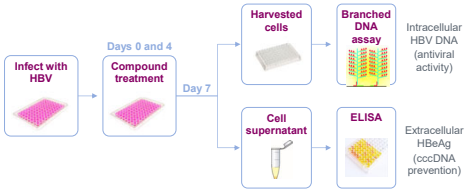
Objective

- To compare human plasma and liver concentrations for VBR, 3733, and 4334 relative to the protein-adjusted half-maximal effective concentrations ($paEC_{50}$s) for each mechanism of action

Methods

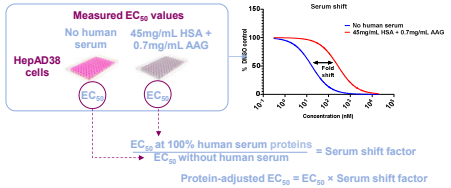
- The antiviral activity of VBR, 3733, and 4334 was measured in primary human hepatocytes (PHH) (**Figure 2**). PHH were infected with HBV and treated with compound on Day 0. Cells were retreated on Day 4, and cultures were harvested on Day 7 or 8. The following measurements were then taken:
  - Intracellular HBV DNA was measured by branched DNA assay (antiviral activity)
  - Extracellular hepatitis B e antigen was measured by enzyme-linked immunosorbent assay (ELISA) (cccDNA prevention)
- $paEC_{50}$ were determined in AD38 cells cultured with tetracycline-free media supplemented with 45 mg/mL human serum albumin (HSA) and 0.7 mg/mL alpha (1)-acid glycoprotein (AAG) (**Figure 3**). Four days following compound treatment, intracellular HBV DNA was measured by quantitative polymerase chain reaction
- Liver concentrations were measured by liquid chromatography mass spectrometry (LC-MS/MS) in liver tissues collected in nonclinical pharmacokinetic (PK) studies
- Concentrations of VBR, 3733, and 4334 in human plasma from clinical study participants were measured by LC-MS/MS, and PK parameters estimated using noncompartmental analysis

Figure 2. Determining EC_{50} in Primary Human Hepatocytes



cccDNA, covalently closed circular DNA; EC_{50}, half-maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

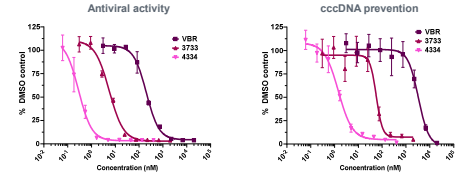
Figure 3. Measuring the Effect of Human Serum on EC_{50} (Serum Shift Assay)



AAG, alpha (1)-acid glycoprotein; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; HSA, human serum albumin.

Results

Figure 4. Potency of VBR, 3733, and 4334 in PHH

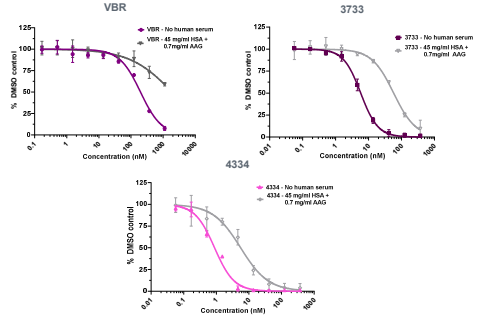


EC_{50} (nM, n=3)	VBR	3733	4334
Antiviral activity	281±61	8.8±3.2	0.5±0.01
cccDNA prevention	3032±948	61±7	2.6±0.5

3733, ABI-H3733; 4334, ABI-4334; cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; PHH, primary human hepatocyte; VBR, vebicorvir.

- Compared with the first-generation core inhibitor VBR, the next-generation core inhibitors 3733 and 4334 more potently inhibit pgRNA encapsidation (antiviral activity) and HBeAg secretion (cccDNA formation) in PHH

Figure 5. Serum Shift Values for VBR, 3733, and 4334 in AD38 cells



Compound	VBR (n=2)	3733 (n=2)	4334 (n=2)
Serum shift factor	8	9.6	5.5
$paEC_{50}$ (antiviral activity)	2248	84.5	2.8
$paEC_{50}$ (cccDNA activity)	24256	585.6	14.3

3733, ABI-H3733; 4334, ABI-4334; AAG, alpha (1)-acid glycoprotein; cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; HSA, human serum albumin; $paEC_{50}$, protein-adjusted half-maximal effective concentration; VBR, vebicorvir.

- In cell-based antiviral assays incorporating HSA and AAG, all core inhibitors demonstrate <10-fold protein shift

Table 1. PK Parameters Determined From Phase 1 Studies

PK parameter	VBR 300 mg QD[6]	3733 300 mg QD[11,a]	4334 300 mg QD[12,a]
C_{min} (nM)	3081	10617	689
C_{max} (nM)	8536	27074	5680
AUC (h·ng/mL)	22700	189000	22705
$T_{1/2}$ (h)	24	24	15

[a]PK data were extrapolated from 50-mg 3733 and 100/200-mg 4334 once daily multiple dose data and assumptions of dose proportionality and similar accumulation.
3733, ABI-H3733; 4334, ABI-4334; AUC, area under the curve; C_{max}, steady-state peak concentration; C_{min}, steady-state minimum (trough) concentration; PK, pharmacokinetics; QD, once daily; $T_{1/2}$, elimination half-life; VBR, vebicorvir.

- PK parameters, including steady-state minimum concentration (C_{min}; 300-mg once-daily [QD] dosing), were estimated from Phase 1 studies
  - VBR: Study ABI-H0731-101 (cHBV patients, NCT02908191)[6]
  - 3733: Study ABI-H3733-102 (cHBV patients, NCT05414981)[11]
  - 4334: Study ABI-4334-101 (healthy volunteers, NCT05569941)[12]
- PK parameters for VBR are from observed data, while PK parameters for 3733 and 4334 are extrapolated from 50-mg and 100/200-mg once-daily multiple dose cohorts, respectively

Table 2. Comparisons between Human Plasma and Liver C_{min} and Protein-Adjusted EC_{50} Values

Mechanism	Parameter	VBR 300 mg QD	3733 300 mg QD[a]	4334 300 mg QD[a]
Liver:plasma ratio[b]		18	6	7
Antiviral activity	Plasma C_{min}/$paEC_{50}$	1.4	126	250
	Liver C_{min}/$paEC_{50}$	25	756	1750
cccDNA activity	Plasma C_{min}/$paEC_{50}$	0.1	18	48
	Liver C_{min}/$paEC_{50}$	1.8	108	336

[a]PK data were extrapolated from 50-mg (3733) and 100/200-mg (4334) once-daily multiple dose data. [b]Liver exposure determined in preclinical animal studies.
3733, ABI-H3733; 4334, ABI-4334; cccDNA, covalently closed circular DNA; C_{min}, steady-state minimum (trough) concentration; $paEC_{50}$, protein-adjusted half-maximal effective concentration; PK, pharmacokinetics; QD, once daily.

- At 300-mg QD dose in humans, 3733 and 4334 are projected to achieve plasma C_{min} concentrations >100-fold over antiviral $paEC_{50}$s, while VBR achieved 1.4-fold coverage
- 3733 and 4334 are projected to achieve plasma C_{min} concentrations 18-48 fold above their cccDNA prevention $paEC_{50}$s, with 4334 having greater-fold coverage relative to 3733
- In preclinical animal studies, all core inhibitors had increased drug exposure in the liver relative to the plasma
- Estimated liver C_{min} concentrations for 3733 and 4334 were >100-fold above both antiviral activity and cccDNA prevention $paEC_{50}$s, with 4334 having the greatest-fold coverage for both mechanisms of action

Conclusions

- Next-generation core inhibitors 3733 and 4334 more potently inhibit both mechanisms of action in vitro, particularly cccDNA prevention, compared with VBR
- 3733 and 4334 have significantly improved human exposure coverage for both antiviral and cccDNA formation activities compared with VBR as demonstrated in recently completed Phase 1b and Phase 1a studies, respectively (Posters SAT-168, SAT-186)[11,12]

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1) European Association for the Study of the Liver. *J Hepatol*. 2017;67:370-98. **2)** World Health Organization. *Global Hepatitis Report 2017*. Geneva: World Health Organization; 2017. **3)** World Health Organization. Key Facts. 2021. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Accessed on March 23, 2023. **4)** El-Serag HB, et al. *Gastroenterology*. 2012;142:1264-73. **5)** Huang Q, et al. *Antimicrob Agents Chemother*. 2020;64:e01463-20. **6)** Yuen MF, et al. *Lancet Gastroenterol Hepatol*. 2020;5:152-66. **7)** Agarwal K, et al. *J Viral Hepat*. 2023;30:209-22. **8)** Yuen MF, et al. *J Hepatol*. 2022;77:642-52. **9)** Sulkowski M, et al. *J Hepatol*. 2022;77:1265-75. **10)** Unchwaniwala N, et al. Poster presentation at AASLD 2022. November 4-8, 2022. **11)** Gane EJ, et al. Presented at the European Association for the Study of the Liver (EASL) 2023. Abstract 1097. **12)** Gane EJ, et al. Presented at the European Association for the Study of the Liver (EASL) 2023. Abstract 1116.

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