# ABI-4334, a novel inhibitor of hepatitis B virus core protein, promotes formation of empty capsids and prevents cccDNA formation by disruption of incoming capsids

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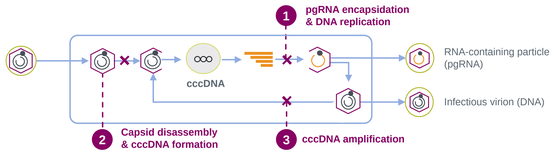
## Background

- Chronic hepatitis B virus (cHBV) infection is a significant global health problem
  - Worldwide, an estimated 296 million people have cHBV infection, resulting in about 887,000 deaths each year, mostly due to cirrhosis and hepatocellular carcinoma[1–4]
- In most patients, nucleos(t)ide reverse transcriptase inhibitors (NrtI) are well tolerated, and while suppression of HBV DNA is achieved in most patients, low-level viremia remains, and treatment duration is indefinite[5–7]
- Novel combination approaches incorporating agents with complementary mechanisms of action (MOAs) may be required to further suppress viral replication and establish finite-duration regimens
- Core inhibitors are a novel class of antivirals with the potential to increase on-treatment responses and cure rates after finite treatment in cHBV patients. These agents:
  - Have multiple MOAs, including inhibition of pregenomic RNA (pgRNA) encapsidation, preventing assembly and release of infectious viral particles, disruption of incoming capsids, and preventing covalently closed circular (ccc)DNA formation[8] (**Figure 1**)
  - Vebicorvir, a first-generation core inhibitor, demonstrated potent antiviral activity in Phase 1 studies[9,10] and additive antiviral activity when combined with NrtIs in Phase 2 studies[11–13]
- ABI-4334 is a novel, next-generation core inhibitor with single-digit nM potency against pgRNA encapsidation and cccDNA formation[14]

### Figure 1. HBV core inhibitor mechanisms of action




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

## Objective of this analysis

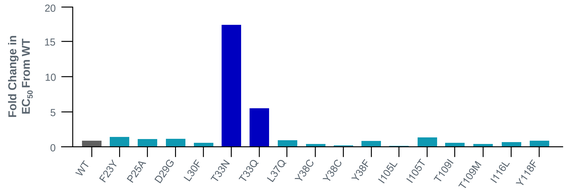
- To further characterize the preclinical properties of ABI-4334

## Methods

- A panel of core inhibitor binding pocket variants and NrtI-resistant mutants was assessed for ABI-4334 sensitivity in HepG2 cells. Constructs were transiently transfected on Day 1, and cells were treated with ABI-4334 on Days 2–8 (drug was refreshed on Day 5). On Day 8, intracellular HBV DNA was measured by quantitative polymerase chain reaction (qPCR)
- HepAD38 cells were induced and simultaneously treated with drug. For entecavir (ETV) and ABI-4334, single concentrations representing 10× half-maximal effective concentrations ($EC_{50}$) for HBV DNA synthesis inhibition in HepAD38 cells were chosen. At Day 4, total cell lysates were used for analyzing core protein levels via denaturing sodium dodecyl-sulfate polyacrylamide gel electrophoresis and western blotting
- For capsid analysis, cells were lysed with 1% NP-40 lysis buffer, and cytoplasmic fractions were electrophoresed on native agarose gels and blotted onto a polyvinylidene difluoride membrane for core immunostaining and nylon membrane for probing against HBV DNA. Viral replicative intermediates (VRIs) were extracted from the cytoplasmic fraction by micrococcal nuclease digestion followed by nucleic acid extraction. VRIs were electrophoresed and transferred to nylon blots for Southern and northern blotting analyses for detection of HBV DNA and pgRNA, respectively. For northern blotting, total RNA was extracted from cells using a total RNA isolation kit and electrophoresed along with the VRIs. Lastly, extracellular levels of DNA and RNA in the culture supernatant were measured by qPCR and reverse transcriptase-qPCR, respectively
- Specificity of ABI-4334 for HBV inhibition was assessed using a panel of 7 different viruses. Inhibition of adenovirus, herpes simplex virus type 1, influenza virus, respiratory syncytial virus, human rhinovirus, and human immunodeficiency virus type 1 was assessed by measuring the ability of ABI-4334 to prevent virus-induced cytopathic effects in respective cell lines. For hepatitis C virus, a genotype 1b stable replicon assay with a luciferase readout was used
- To evaluate the effect of ABI-4334 on incoming capsids, HepG2-sodium/bile acid cotransporter (NTCP) cells were infected with HBV at multiplicity of infection (MOI) of 2500 viral genome equivalents (vge)/cell for 1 hour, followed by drug treatment. Three hours post-treatment, cells were harvested and lysed with 1% NP-40 lysis buffer; cytoplasmic fractions were electrophoresed on a native agarose gel and then blotted onto a nylon membrane for probing against HBV DNA
- To evaluate the effect of ABI-4334 on cccDNA formation, HepG2-NTCP cells were infected with HBV at MOI of 500 vge/cell for 3 hours, followed by drug treatment. At Day 4, cells were harvested, and total DNA was extracted using a modified HIRT DNA-extraction procedure. Total DNA was treated with T5 exonuclease to digest linear and nicked double-stranded DNA, followed by EcoRI digestion to linearize cccDNA. Extracted cccDNA was analyzed by Southern blotting

## Results

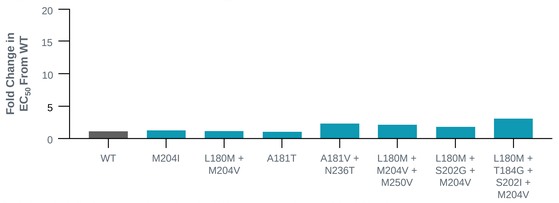
### Figure 2. ABI-4334 activity against core inhibitor binding pocket variants




$EC_{50}$, half-maximal effective concentration; WT, wild-type.

- ABI-4334 showed a potent activity profile against a panel of core inhibitor binding pocket variants (**Figure 2**)[14]
- ABI-4334 retained activity against 14/16 core inhibitor binding pocket variants (<3-fold change from wild-type [WT]) and only exhibited reduced sensitivity to T33N and T33Q (fold changes=15.8 and 5.5, respectively)

### Figure 3. ABI-4334 activity against NrtI-resistant mutants




$EC_{50}$, half-maximal effective concentration; NrtI, nucleos(t)ide reverse transcriptase inhibitor; WT, wild-type.

- ABI-4334 maintained sensitivity (<3-fold change from WT) against all NrtI-resistant mutants tested (**Figure 3**)

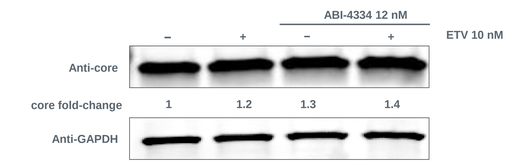
### Table 1. Antiviral activity of ABI-4334 is specific to HBV

| Virus | ABI-4334 $EC_{50}$ (μM) | ABI-4334 $CC_{50}$ (μM) | Positive control | $EC_{50}$ | $CC_{50}$ |
|---|---|---|---|---|---|
| AdV | >10 | >10 | ddC (μM) | 5.3 | >100 |
| HCV | >10 | >10 | GS-7977 (μM) | 0.07 | >5 |
| HSV-1 | >10 | >10 | acyclovir (μM) | 1.0 | >100 |
| IFV | >10 | >10 | baloxavir (μM) | 0.0023 | 29.37 |
| RSV | >10 | >10 | ALS-8112 (μM) | 0.18 | >10 |
| HRV | >10 | >10 | pleconaril (μM) | 0.01 | >5 |
| HIV-1 | >20 | 13.36 | AZT (nM) | 1.08 | >100 |

AdV, adenovirus; AZT, azidothymidine; $CC_{50}$, half-maximal cytotoxic concentration; ddC, 2′, 3′-dideoxycytidine; $EC_{50}$, half-maximal effective concentration; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; HRV, human rhinovirus; HSV-1, herpes simplex virus type 1; IFV, influenza virus; RSV, respiratory syncytial virus.

- The activity of ABI-4334 is specific to HBV, with no antiviral activity observed with any of the other 7 viruses tested (**Table 1**)

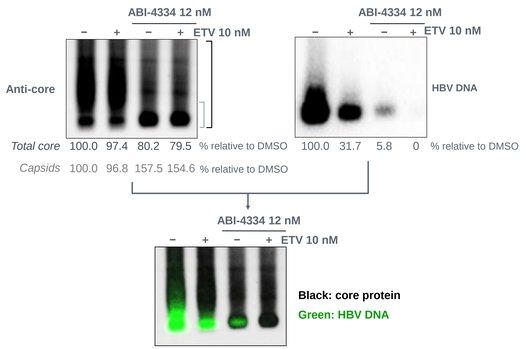
### Figure 4. Impact of ABI-4334 on core protein levels




ETV, entecavir; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

- Treatment with ABI-4334, with or without ETV, has no impact on intracellular core protein, with core protein levels <1.5-fold compared to untreated cells (**Figure 4**)

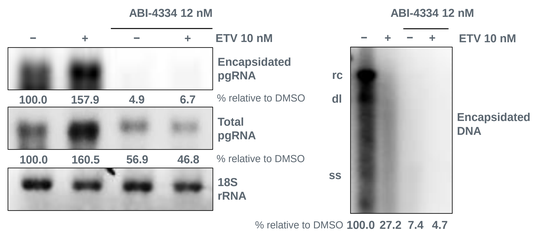
### Figure 5. ABI-4334 results in formation of empty capsids




DMSO, dimethyl sulfoxide; ETV, entecavir; HBV, hepatitis B virus

- A 1.5-fold increase in capsid number relative to untreated cells was observed in ABI-4334–treated cells. The capsids were predominantly empty, with HBV DNA detected at 5.8% compared to dimethyl sulfoxide (DMSO) control (**Figure 5**)

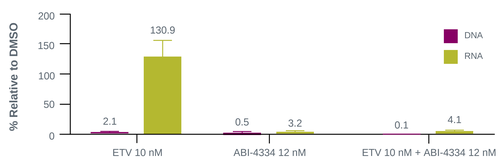
### Figure 6. ABI-4334 inhibits encapsidation of pgRNA and formation of rcDNA




dl, duplex linear; DMSO, dimethyl sulfoxide; ETV, entecavir; pgRNA, pregenomic RNA; rc, relaxed circular; rRNA, ribosomal RNA; ss, single-stranded.

- Treatment with ABI-4334 causes strong inhibition of pgRNA packaging (95% inhibition compared to DMSO control), which results in suppression of intracellular relaxed circular DNA (93% inhibition compared to DMSO control) (**Figure 6**)

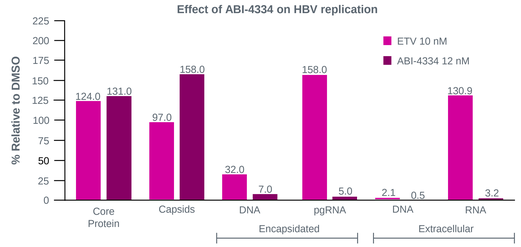
### Figure 7. Extracellular secretion of HBV DNA and RNA is inhibited by ABI-4334




DMSO, dimethyl sulfoxide; ETV, entecavir; HBV, hepatitis B virus.

- Reductions in extracellular HBV RNA and DNA were observed with ABI-4334–treated cells (3.2% and 0.5% relative to DMSO control, respectively) (**Figure 7**)

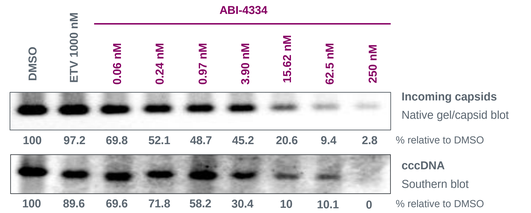
### Figure 8. ABI-4334 activity is consistent with a Class II core inhibitor




DMSO, dimethyl sulfoxide; ETV, entecavir; HBV, hepatitis B virus; pgRNA, pregenomic RNA.

- HBV core inhibitors are broadly classified into Class I and Class II based on their MOA[15]
  - Class I core inhibitors cause capsid mis-assembly, leading to formation of large aberrant core polymers that are prone to degradation or intracellular aggregation
  - Class II core inhibitors accelerate capsid assembly, resulting in the formation of empty capsids that lack HBV pgRNA
- ABI-4334 inhibits HBV pgRNA packaging into nascent capsids, suppressing formation of HBV RNA and HBV DNA-containing particles and their secretion, consistent with the activity of Class II core inhibitors (**Figure 8**)

### Figure 9. ABI-4334 prematurely disrupts incoming capsids, resulting in lower levels of cccDNA formation




cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; ETV, entecavir.

- Treatment with ABI-4334 results in premature disruption of incoming capsids in the cytoplasm ($EC_{50}$ of 0.48 nM), which results in reductions of cccDNA formation ($EC_{50}$ of 0.93 nM) (**Figure 9**)

## Conclusions

- ABI-4334 is a next-generation potent core inhibitor with multiple MOAs that demonstrated broad coverage across core inhibitor binding pocket variants and NrtI-resistant mutants
- The activity of ABI-4334 against pgRNA encapsidation is consistent with Class II core inhibitors
- ABI-4334 prematurely disrupts incoming HBV capsids, which prevents formation of cccDNA
- A Phase 1a first-in-human study with ABI-4334 in healthy participants is planned for the second half of 2022

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