Preclinical characterization of ABI-4334, a novel, highly potent core inhibitor for the treatment of chronic hepatitis B virus infection

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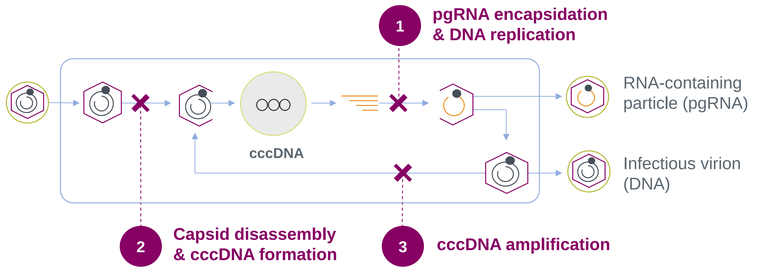
BACKGROUND

- Core inhibitors are a novel class of hepatitis B virus (HBV) direct-acting antivirals, with the potential to increase on-treatment responses and increase cure rates after finite treatment
- Core inhibitors have multiple mechanisms of action (MOAs) (**Figure 1**):
  - Inhibition of pregenomic (pg) RNA encapsidation, which blocks the assembly and release of new viral particles containing pgRNA or HBV DNA
  - Disruption of incoming capsids, which block the establishment of de novo covalently closed circular (ccc) DNA during infection
- Core inhibitors exert their greatest antiviral activity against viral replication; however, potent antiviral activity via both MOAs may be important for optimal patient responses[1]
- Vebicorvir[2], ABI-H3733[3,4], and ABI-4334 represent structurally distinct core inhibitors with increasing potency against both HBV DNA and cccDNA formation

Figure 1. Core inhibitor mechanisms of action



cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA.

OBJECTIVE

- To characterize the preclinical properties of ABI-4334, a novel core inhibitor with high potency against pgRNA encapsidation and cccDNA formation

METHODS

- The antiviral activity of ABI-4334 was measured in AD38 and HepG2-NTCP cell lines, as well as in primary human hepatocytes (PHH). AD38 cells were induced by removal of tetracycline and treated with ABI-4334 for 4 days. HepG2-NTCP and PHH were infected with HBV and treated with ABI-4334 on Day 1. 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)
  - Extracellular hepatitis B surface antigen was measured by ELISA (cccDNA prevention)
  - Intracellular pgRNA was measured by branched DNA assay using a pgRNA-specific probe (cccDNA prevention)
- Protein-adjusted half-maximal effective concentrations (paEC$_{50}$) were determined in AD38 cells cultured with tetracycline-free media supplemented with 2% fetal bovine serum, 45 mg/mL human serum albumin (HSA), and 0.7 mg/mL alpha acidic glycoprotein (AAG). Four days following compound treatment, intracellular HBV DNA was measured by quantitative polymerase chain reaction (qPCR)
- A panel of HBV genotypes (A-J) and core inhibitor binding pocket variants were assessed for ABI-4334 sensitivity in HepG2 cells. Constructs were transiently transfected on Day 1, and then 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 qPCR
- Cytotoxicity (up to 20 µM) was measured in 6 cell lines and peripheral blood mononuclear cells (PBMCs) using CellTiterGlo 2.0 (Promega)
- Single-dose pharmacokinetic (PK) studies of ABI-4334 were conducted in rat, mouse, dog, and monkey at 1 mg/kg delivered intravenously. Noncompartmental analysis was done to determine PK parameters from preclinical species, and then allometric scaling was performed to derive human PK parameters for ABI-4334 (drug clearance [CL] and steady state volume of distribution [V_{ss}]) for a 70-kg person
- Simulation of human concentration-time PK profile was conducted for an 11-day, 300-mg, once daily (QD) dosing of ABI-4334, using the human-scaled PK parameters with an assumption of 50% bioavailability

RESULTS

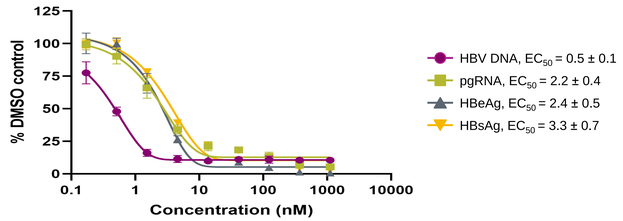
- ABI-4334 potently inhibits pgRNA encapsidation and cccDNA formation in PHH (0.5–3.3 nM) as well as in AD38 (1.2 nM) and HepG2-NTCP (0.4–2.9 nM) cell lines (**Table 1** and **Figure 2**)

Table 1. Potency of ABI-4334 in AD38, HepG2-NTCP, and PHH

Cells	Parameter	ABI-4334 EC$_{50}$ ± SD (nM)[a]
AD38	HBV DNA	1.2 ± 0.3
HepG2-NTCP	HBV DNA	0.4 ± 0.1
	HBV RNA	1.6 ± 0.8
	HBeAg	1.8 ± 0.4
	HBsAg	2.9 ± 0.3
PHH	HBV DNA	0.5 ± 0.1
	HBV RNA	2.2 ± 0.4
	HBeAg	2.4 ± 0.5
	HBsAg	3.3 ± 0.7

[a]EC$_{50}$ values represent mean ± SD of at least 3 independent experiments.
EC$_{50}$, half-maximal effective concentration; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PHH, primary human hepatocytes; SD, standard deviation.

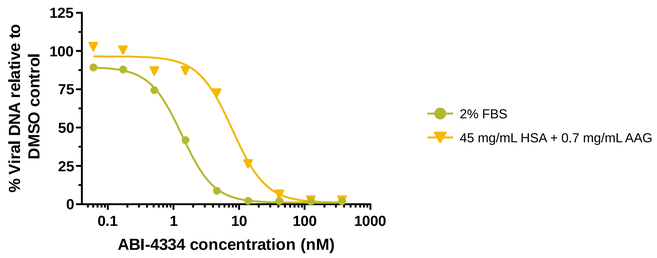
Figure 2. ABI-4334 potently inhibits pgRNA encapsidation and cccDNA formation in PHH



cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; EC$_{50}$, half-maximal effective concentration; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; PHH, primary human hepatocytes.

- In cell-based antiviral assays incorporating HSA and AAG, ABI-4334 has a serum shift of 5.5, which resulted in paEC$_{50}$ measurements of 2.8 nM for pgRNA encapsidation and 13.2 nM for cccDNA formation (**Figure 3**)

Figure 3. Serum protein shift value for ABI-4334 AD38 cells



Parameter	ABI-4334 (n = 3)
Serum protein shift (fold)	5.5 ± 0.3
HBV DNA paEC$_{50}$ (nM)	2.8
cccDNA paEC$_{50}$ (nM)	13.2

AAG, alpha acidic glycoprotein; cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; EC$_{50}$, half-maximal effective concentration; FBS, fetal bovine serum; HBV, hepatitis B virus; HSA, human serum albumin; pa, protein-adjusted.

- ABI-4334 exhibits pan-genotypic activity, with single-digit nM potency for genotypes A–J (**Table 2**)

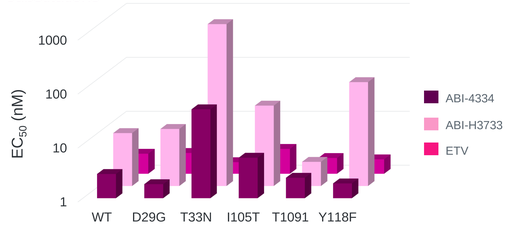
Table 2. ABI-4334 exhibits pan-genotypic HBV activity

Genotype	ABI-4334 EC$_{50}$ (nM)	ETV EC$_{50}$ (nM)
A	0.9	0.7
B	0.8	0.9
C	0.7	0.9
D	1.3	1.1
E	4.5	1.6
F	0.4	1.5
G	3.6	1.9
H	1.8	0.9
I	0.6	1.1
J	1.0	1.7

EC$_{50}$, half-maximal effective concentration; ETV, entecavir; HBV, hepatitis B virus.

- The activity profile of ABI-4334 against a panel of core inhibitor binding pocket variants was improved relative to ABI-H3733, which has an improved resistance profile relative to other core inhibitors.[5] ABI-4334 retained activity against 4 out of 5 variants (<2-fold change from wild type) and only exhibited reduced sensitivity to T33N (fold change = 15.8) (**Figure 4**)

Figure 4. ABI-4334 is potent against core inhibitor binding pocket substitutions



Core protein substitution	Fold change in EC$_{50}$ vs WT: ABI-4334	ABI-H3733	ETV
D29G	0.7	1.2	1.0
T33N	15.8	>104.7	0.7
I105T	2.0	3.2	1.2
T109I	0.9	0.3	0.8
Y118F	0.7	8.8	0.8

EC$_{50}$, half-maximal effective concentration; ETV, entecavir; WT, wild-type.

- No cytotoxicity was observed across 6 cell lines and PBMCs (half-maximal cytotoxic concentration [CC$_{50}$] >20 µM) (**Table 3**)

Table 3. Lack of ABI-4334 cytotoxicity in human cells

Cells/cell lines	ABI-4334 CC$_{50}$ (µM)[a]
Huh7	>20
HepG2	>20
HEK-293	>20
HeLa-H1A	>20
NCI-H226	>20
MOLT-4	>20
PBMC	>20

[a]20 µM was the highest concentration tested.
CC$_{50}$, half-maximal cytotoxic concentration.

- Noncompartmental analysis found that ABI-4334 had moderate clearance and high volume of distribution (**Table 4**)

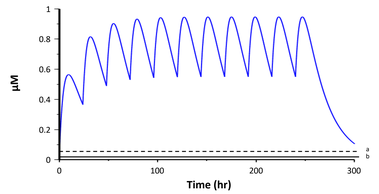
Table 4. PK parameters from preclinical species

Species	Rat	Mouse	Dog	Monkey
IV dose (mg/kg)	1	1	1	1
Body weight (kg)	0.3	0.02	10	5
CL (mL/min/kg)	15.2	13.4	3.6	7.3
V_{ss} (L/kg)	2.6	0.9	3.2	2.4
$T_{1/2}$ (hr)	2.7	1.2	16.8	5.7
F%	46%	72%	93%	24%

CL, drug clearance; F, bioavailability; IV, intravenous; PK, pharmacokinetic; $T_{1/2}$, half-life; V_{ss}, steady state volume of distribution.

- Allometric scaling resulted in CL and V_{ss} estimates of 3.45 mL/min/kg and 4.48 L/kg, respectively, in a 70-kg human. Human PK modeling predicts that a 300 mg QD dose of ABI-4334 will achieve a trough concentration (C_{min}) value of 600 nM, which is 196-fold greater than the pgRNA encapsidation paEC$_{50}$ and 42-fold greater than the cccDNA formation paEC$_{50}$ (**Figure 5**)

Figure 5. Human PK prediction for ABI-4334 (300 mg QD)



[a]cccDNA paEC$_{50}$ = 0.0132 µM. [b]HBV DNA paEC$_{50}$ = 0.0028 µM.
cccDNA; covalently closed circular DNA; EC$_{50}$, half-maximal effective concentration; HBV, hepatitis B virus; pa, protein-adjusted; QD, once daily.

CONCLUSIONS

- ABI-4334 is a novel, orally bioavailable core inhibitor with single-digit nM potency against both pgRNA encapsidation and cccDNA formation
- Human PK modeling predicts that a 300 mg QD dose of ABI-4334 will achieve C_{min} values 196- and 42-fold over pgRNA encapsidation and cccDNA formation paEC$_{50}$ measurements, respectively
- Phase 1 studies with ABI-4334 are planned for 2022

REFERENCES

1) Cai D, et al. Presented at: EASL 2021; June 23–26, 2021. **2)** Huang Q, et al. *Antimicrob Agents Chemother*. 2020;AAC.01463–20. **3)** Huang Q, et al. Presented at: EASL 2019; April 10–15, 2019. **4)** Gane E, et al. Presented at: AASLD 2021; November 12–15, 2021. **5)** Cai D, et al. Presented at: AASLD 2020; November 13–16, 2020.

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