Preclinical Profiling of ABI-6250, a Novel, Orally Bioavailable, Small-Molecule Therapeutic Drug Candidate for the Treatment of Chronic Hepatitis D Virus Infection

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Background

- Chronic hepatitis D virus (HDV) infection (cHDV), the most severe form of viral hepatitis, affects an estimated 12 to 72 million patients worldwide[1,2]
- Patients with cHDV coinfected with hepatitis B virus (HBV) vs HBV alone have an increased risk of developing life-threatening liver conditions, such as fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)[3-5]
  - The incidence rates of HCC are more than 2.5-fold higher in patients with HDV/HBV coinfection vs HBV monoinfection[6]
- Both HBV and the satellite virus HDV use sodium taurocholate cotransporting polypeptide (NTCP), a bile acid transporter on hepatocytes, as an entry receptor into the liver[7-10]
- Bulevirtide (BLV), a peptide inhibitor targeting NTCP and the only drug approved by the European Medicines Agency for cHDV, requires daily injections to prevent HDV and HBV entry into hepatocytes[9,11-14]
- There is a medical need for efficacious, orally administered entry inhibitors to improve the long-term clinical outcomes of patients with cHDV

Objective

- To describe the preclinical profile of ABI-6250, an orally bioavailable small-molecule entry inhibitor that is a clinical drug candidate for the treatment of cHDV

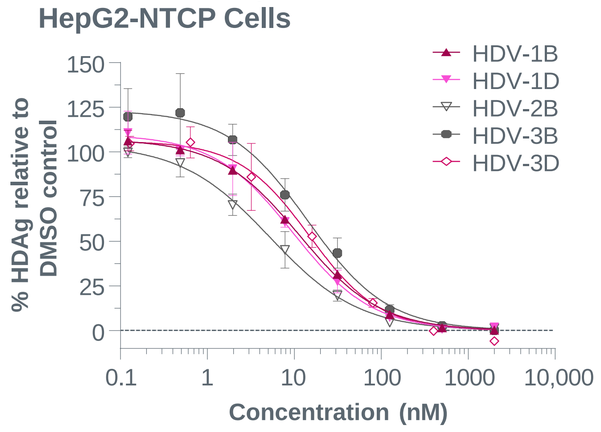
Methods

- **HDV infection: Measurement of hepatitis D antigen (HDAg):**
  - HepG2-NTCP cells were infected with HDV inoculum and cotreated with ABI-6250. After 24 hours, supernatants were removed and fresh media without ABI-6250 were added. At 5 days postinfection (dpi), HDAg was measured by an in-cell enzyme-linked immunosorbent assay (ELISA) to generate half-maximal effective concentration (EC_{50}) values
- **Human serum shift assay:**
  - HDV-infected cells in the presence of fetal bovine serum (FBS) and physiological levels of human serum albumin (45 mg/mL) and alpha-1-acid glycoprotein (0.7 mg/mL) were compared to a standard infection carried out in media with FBS alone. EC_{50} values were generated by quantifying HDAg at 5 dpi by in-cell ELISA
- **NTCP-mediated bile acid uptake inhibition:**
  - HEK293T cells expressing human NTCP were preincubated for 60 minutes with ABI-6250 or BLV, followed by a 50-minute incubation with 3-α-nitrobenzoxadiazole taurocholic acid. After supernatant removal, fluorescence intensity was measured by plate reader
- **Organic anion-transporting protein (OATP) inhibition:**
  - HEK293T cells expressing human OATP1B1 or OATP1B3 were preincubated for 30 minutes with ABI-6250 or BLV followed by a 10-minute incubation with fluorescein-methotrexate. After supernatant removal, fluorescence intensity was measured by flow cytometry
- **PreS1 binding competition:**
  - HEK293T cells stably expressing human NTCP were coincubated with myristoylated preS1-Alexa-594 peptide and ABI-6250 or BLV for 10 minutes. Binding of the fluorescent peptide was measured by flow cytometry
- **Metabolic stability:**
  - Metabolic stability was determined at 1 µM of testing concentration with non-human primate (NHP) and human liver microsomes (LMs) using liquid chromatography–tandem mass spectrometry (LC-MS)
- **Pharmacokinetic (PK) studies:**
  - ABI-6250 PK parameters were obtained following a single intravenous bolus dose at 1 mg/kg and oral dose at 2.5 or 5 mg/kg in Sprague-Dawley rats and NHPs. Samples were analyzed using LC-MS
- **Pharmacodynamic (PD) studies:**
  - Total bile acid (TBA) levels were measured by enzymatic cycling, and coproporphyrin-I (CP-I) was measured using LC-MS

Results

Figure 1. ABI-6250 Efficiently Inhibited HDV Infection of the Most Prevalent Genotypes

A. Anti-HDV Activity in HepG2-NTCP Cells



B. ABI-6250 EC_{50} Values in HepG2-NTCP Cells

HDV/HBV genotype	EC_{50} (nM)
1B	11.4
1D	9.6
2B	5.2
3B	14.2
3D	14.9

DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; HBV, hepatitis B virus; HDAg, hepatitis D antigen; HDV, hepatitis D virus; NTCP, sodium taurocholate cotransporting polypeptide.

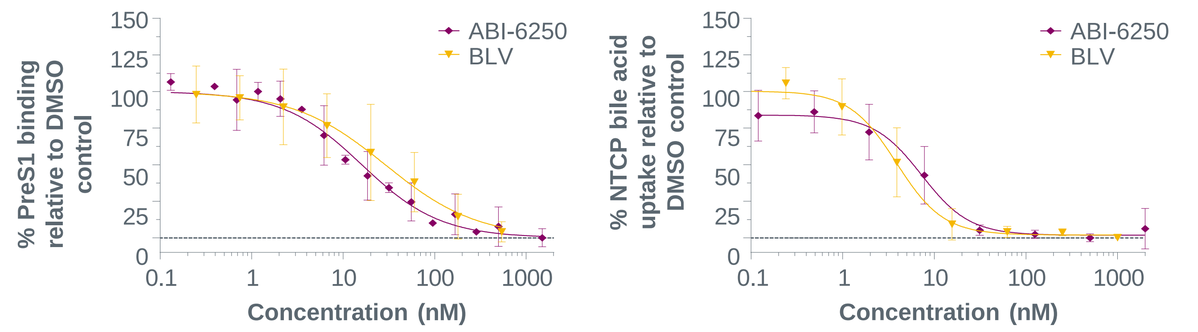
- ABI-6250 efficiently inhibited all tested HDV genotypes (1–3) with HBV genotype B or D envelopes (**Figure 1**)
- ABI-6250 efficiently inhibited HDV entry in HepG2-NTCP cells as demonstrated by HDAg in-cell ELISA (**Figure 1A**), with EC_{50} values ranging from 5.2 to 14.9 nM (**Figure 1B**)
- Human serum factors affected the inhibitory potency of ABI-6250 for HDV genotype 3D by increasing the *in vitro* EC_{50} value 35-fold (data not shown)

Results

Figure 2. ABI-6250 Inhibited PreS1-NTCP Binding and NTCP-Mediated Bile Acid Uptake

A. PreS1-NTCP Binding Inhibition

B. NTCP-Mediated Bile Acid Uptake Inhibition

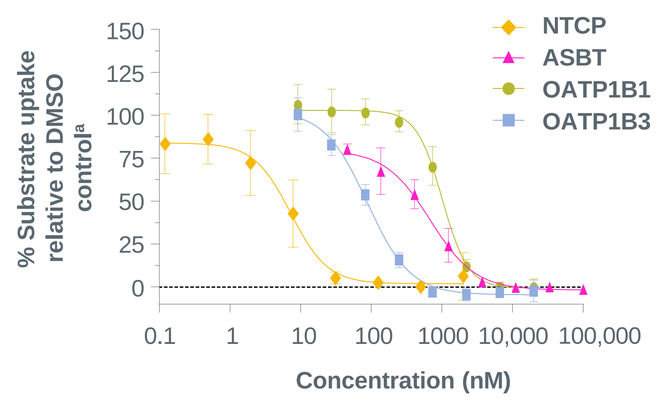


BLV, bulevirtide; DMSO, dimethyl sulfoxide; NTCP, sodium taurocholate cotransporting polypeptide.

- ABI-6250 efficiently inhibited HBV preS1-NTCP binding and NTCP-mediated bile acid uptake in HEK293T NTCP cells (**Figure 2**)
- Dose-response curves showed that ABI-6250 and BLV inhibited preS1-NTCP binding, with half-maximal inhibitory concentration (IC_{50}) values of 16.0 and 28.9 nM, respectively (**Figure 2A**)
- Dose-response curves showed that ABI-6250 and BLV inhibited bile acid uptake, with IC_{50} values of 7.3 and 3.9 nM, respectively (**Figure 2B**)

Figure 3. ABI-6250 Selectively Inhibited NTCP

A. NTCP, OATP1B, and ASBT Uptake Inhibition



B. ABI-6250 Fold Selectivity

Transporter	ABI-6250 IC_{50} (nM) [fold selectivity][b]
NTCP	7.3
OATP1B1	1000 [>135]
OATP1B3	90 [>12]
ASBT	700 [>95]

[a]Bile acid uptake was measured for NTCP and ASBT; methotrexate was measured for OATP1B1/3. [b]Numbers in brackets indicate fold selectivity calculated as the ratio of IC_{50} of the bile acid transporter to IC_{50} of NTCP.
ASBT, apical sodium-dependent bile acid transporter; DMSO, dimethyl sulfoxide; IC_{50}, half-maximal inhibitory concentration; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion-transporting protein.

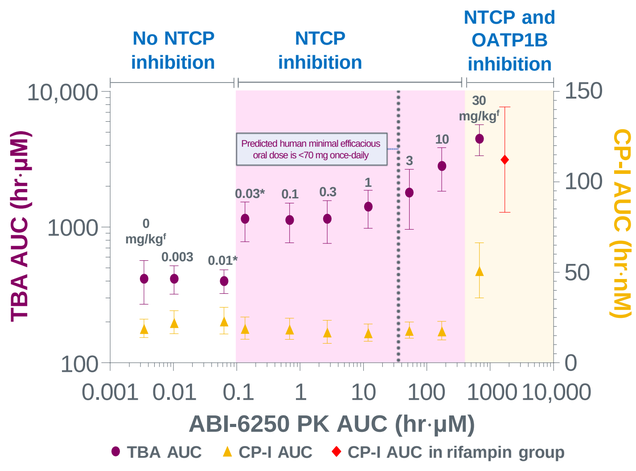
- ABI-6250 had limited inhibitory effects *in vitro* on OATP1B, NTCP, and apical sodium-dependent bile acid transporter (ASBT) activity (**Figure 3**)
- ABI-6250 inhibited OATP1B1-, OATP1B3-, and ASBT-dependent bile acid uptake, with IC_{50} values of 1000, 90, and 700 nM, respectively (**Figure 3A and B**)
- ABI-6250 is a selective NTCP inhibitor, as demonstrated by fold selectivity (calculated as the ratio of IC_{50} of the bile acid transporter to IC_{50} of NTCP; **Figure 3B**)

Figure 4. ABI-6250 Has Favorable ADME and PK/PD Profiles

A. ABI-6250 ADME and PK Profile[a]

ADME properties		
Percentage of ABI-6250 remaining at 45 min after incubation in LMs (human | NHP | rat | mouse)	97 | 97 | 97 | 93	
Caco-2 A-B[b] | B-A[b] | ER	4.8 | 4.7 | 1.0	
PK properties	**Rat**	**NHP**
$t_{1/2}$ (hr)	4.9[c] | 5.4[d]	6.7[c] | 14.8[d]
%F	100	99
CL (mL/min/kg)	18.9	0.6
Vss (L/kg)	4.4	0.6

B. TBA and CP-I Measurements[e]



[a]n = 2 or 3. [b]10^{-6} cm/s. [c]If ABI-6250 is administered intravenously. [d]If ABI-6250 is administered orally. [e]The x-axis values for vehicle, 0.003 mg/kg, and rifampin groups are arbitrarily set. [f]Doses of ABI-6250 in NHPs; n = 6 per dose. *For TBA AUC p<0.01 between 0.01 mg/kg and 0.03 mg/kg. Vertical line indicates human PK projection using allometric scaling.
A-B, A to B permeability; ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; B-A, B to A permeability; CL, clearance; CP-I, coproporphyrin-I; ER, efflux ratio; %F, bioavailability; LM, liver microsome; NHP, non-human primate; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion-transporting protein; PD, pharmacodynamics; PK, pharmacokinetics; $t_{1/2}$, terminal half-life; TBA, total bile acid; Vss, volume of distribution.

- Absorption, distribution, metabolism, and excretion (ADME) and PK/PD studies showed that ABI-6250 has:
  - A favorable ADME and PK/PD profile (**Figure 4**)
  - Good LM stability in human and preclinical species (**Figure 4A**)
  - Good apparent permeability in Caco-2 cells, with no efflux and terminal half-lives ($t_{1/2}$) in rats and NHPs greater than 4.9 hours. The predicted $t_{1/2}$ in humans is over 30 hours when administered orally
  - Approximately 100% bioavailability in NHPs and rats, with $t_{1/2}$ ranging from 4.9 to 14.8 hours
  - Elevated TBA levels at doses up to 30 mg/kg in NHPs, indicating drug-target engagement. CP-I levels, a biomarker for OATP1B inhibition, were not increased at physiologic concentrations of ABI-6250. Rifampin, an OATP1B inhibitor, efficiently elevated CP-I levels (**Figure 4B**)
  - No cytochrome P450 inhibition and human ether-a-go-go-related gene liabilities (data not shown)
  - Demonstrated good metabolic stability, with more than 95% remaining after 45 minutes of incubation in NHP and human LMs (**Figure 4A**)
  - The potential to achieve the desired minimum efficacious concentration coverage with <70 mg once-daily dosing

Conclusions

- ABI-6250 is an NTCP-selective, highly potent, orally bioavailable HDV entry inhibitor
- At clinically relevant concentrations, ABI-6250 elevates total bile acids in non-human primates, indicating target engagement without increasing coproporphyrin-I plasma levels, a biomarker for OATP1B inhibition
- The preclinical PK profile of ABI-6250 supports low once-daily oral dosing in patients with cHDV
- ABI-6250 is expected to enter Phase 1 clinical trials by the end of 2024

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DISCLOSURES

All authors are employees of Assembly Biosciences, Inc., and may own stock