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

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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 co-infected with hepatitis B virus (HBV) vs HBV alone have an increased risk of developing life-threatening liver conditions, such as fibrosis, cirrhosis, & hepatocellular carcinoma (HCC)[3–5]
- The incidence rates of HCC are more than 2.5-fold higher in patients with HDV/HBV co-infection vs HBV mono-infection[6]
- Both HBV and the satellite virus HDV use sodium taurocholate co-transporting polypeptide (NTCP), a bile acid transporter expressed in hepatocytes, as an entry receptor into the liver[7–10]
- Bulevirtide (BLV), a peptide inhibitor targeting NTCP, is the only drug approved by the European Medicines Agency for cHDV and requires daily injections[9,11-14]
- There is a medical need for an efficacious, orally administered entry inhibitor that may improve the long-term clinical outcomes of patients suffering from cHDV

Objective

- Preclinical profiling of ABI-6250, an orally bioavailable small-molecule entry inhibitor that is a clinical drug candidate for the treatment of cHDV

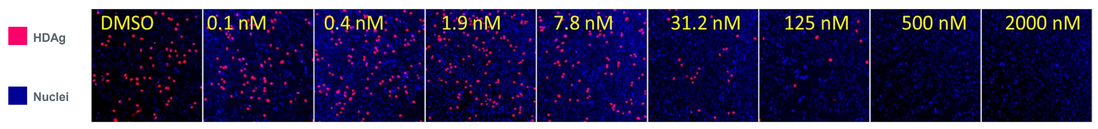
Methods

- **HDV and HBV infection:**
  - Hepatoma cells or primary human hepatocytes (PHH) were inoculated with HDV or HBV and cotreated with ABI-6250. After 24 hours (h), supernatants were removed and fresh media without ABI-6250 were added. At 5 days postinfection (dpi), hepatitis D antigen (HDAg) or hepatitis B e antigen (HBeAg) were measured by an in-cell ELISA or ELISA, respectively, to generate half-maximal effective concentration (EC_{50}) values
- **Time-of-addition study:**
  - Pre-treatment: HepG2-NTCP cells were treated for 24h with ABI-6250 or BLV, washed, and inoculated with HBV. Co-treatment: Cells were treated with ABI-6250 or BLV and inoculated at the same time. Post-treatment: Cells were inoculated with HBV 24h prior treatment with ABI-6250 or BLV
- **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 (NBD-TCA). After supernatant removal, fluorescence intensity was measured by plate reader
- **Bile acid transporter studies:**
  - HEK293T cells expressing human OATP1B1, OATP1B3, or ASBT were preincubated for 30 minutes with ABI-6250 followed by 10 minutes incubation with fluorescein-methotrexate or NBD-TCA. After supernatant removal, fluorescence intensity was measured by flow cytometry
- **PreS1 binding competition:**
  - HEK293T cells stably expressing human NTCP were co-incubated with myristoylated preS1- Alexa-594 peptide and ABI-6250 or BLV for 10 minutes. Binding of the fluorescent peptide was measured by flow cytometry
- **Pharmacokinetic/Pharmacodynamic (PK/PD) studies:**
  - ABI-6250 PK parameters were obtained following a single oral dosing of NHPs at given concentrations. Samples were analyzed using LC-MS. Total bile acid (TBA) levels were measured by enzymatic cycling, and coproporphyrin-I (CP-I) was measured using LC-MS

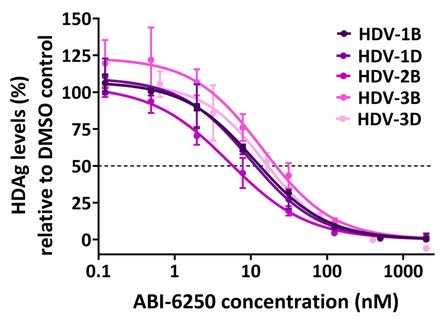
Results

Figure 1. ABI-6250 Potently Inhibits Multiple HDV & HBV Genotypes

A. ABI-6250 Anti-HDV Activity in HepG2-NTCP Cells



B. ABI-6250 Dose-Response Inhibitory Effects in HepG2-NTCP Cells



C. ABI-6250 EC_{50} Values

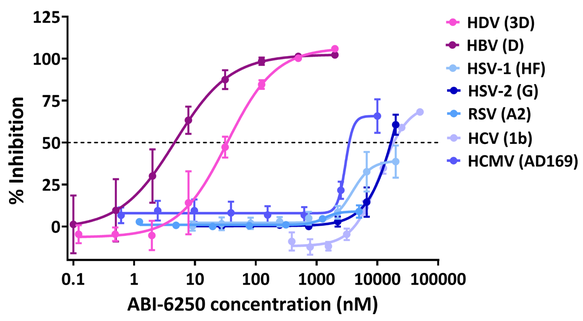
Compound	HDV EC_{50} (nM)		HBV EC_{50} (nM)	
	PHH	HepG2-NTCP	PHH	HepG2-NTCP
ABI-6250	11 (GT-3B)	5 – 15 (GT-1, 2, 3, B, D)	14 (GT-A, C, D)	4.7 (GT-D)
Bulevirtide	0.6 (GT-3B)	0.5 (GT-3D)	0.2 (GT-D)	0.2 (GT-D)

GT, Genotype

- ABI-6250 efficiently inhibited all tested HDV and HBV genotypes (**Figure 1**)
- ABI-6250 efficiently inhibited HDV entry in HepG2-NTCP cells as demonstrated by HDAg immunofluorescence analysis (HDV-1D, EC_{50}= 9.6 nM, **Figure 1A**) and in-cell ELISA (**Figure 1B**).
- Summary of EC_{50} values generated in HDV or HBV infected HepG2-NTCP cells and PHHs (**Figure 1C**)

Figure 2. ABI-6250 Specifically Inhibits HDV & HBV

A. Dose-Response Analysis



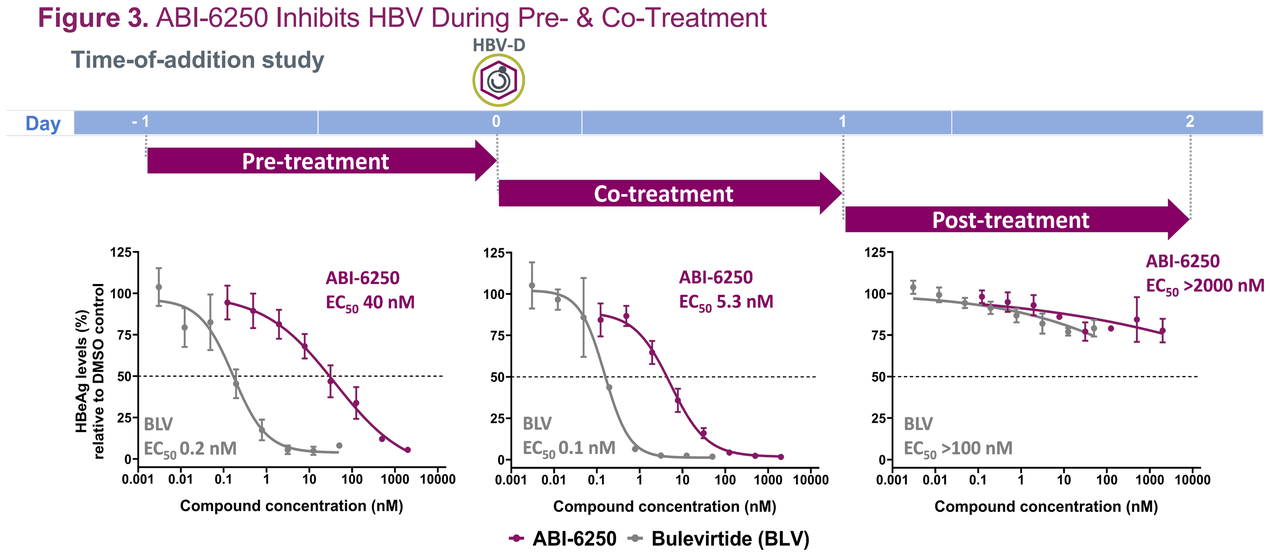
B. ABI-6250 EC_{50} & SI Values

Virus	ABI-6250 EC_{50} & Selectivity Indices (SI)		
	ABI-6250 EC_{50} (nM)	HBV SI	HDV SI
HDV-3D	15	-	-
HBV-D	5	-	-
HSV-1	>20,000	>4,255	>1,342
HSV-2	16,100	3,426	1,080
RSV	>5,000	>1,064	>336
HCV	14,500	3,085	973
HCMV	6,530	1,389	438

- ABI-6250 specifically inhibited HDV and HBV (**Figure 2**)
- Virus specificity of ABI-6250 was evaluated in cell culture by testing against different viruses [Herpes Simplex Virus (HSV), Respiratory Syncytial Virus (RSV), Hepatitis C Virus (HCV), Human Cytomegalovirus (HCMV)] (**Figure 2A**)
- Summary of EC_{50} values and selectivity Indices (SI) (ratio EC_{50},virus/EC_{50},HBV/HDV) (**Figure 2B**)

Results

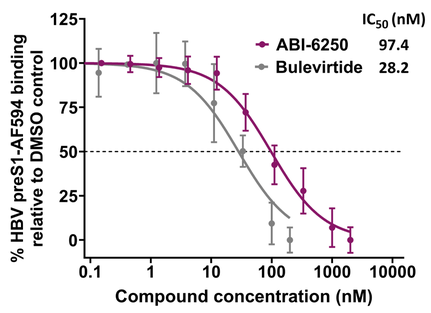
Figure 3. ABI-6250 Inhibits HBV During Pre- & Co-Treatment



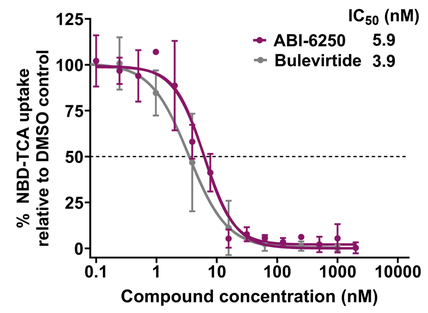
- ABI-6250 inhibited HBV during pre- and co-treatment, not post-treatment (**Figure 3**)
- Time-of-addition study with ABI-6250; HBV inhibition during pre and co-treatment of ABI-6250 or BLV

Figure 4. ABI-6250 Interferes with HBV PreS1 Binding to NTCP & NTCP-Dependent Bile Acid Uptake

A. PreS1-NTCP Binding Inhibition



B. NTCP-Mediated Bile Acid Uptake Inhibition



- ABI-6250 efficiently inhibited HBV preS1-NTCP binding & NTCP-mediated bile acid uptake in HEK293T NTCP cells (**Figure 4**)
- ABI-6250 and BLV inhibited preS1-NTCP binding dose-dependently (**Figure 4A**)
- ABI-6250 and BLV inhibited bile acid uptake dose-dependently (**Figure 4B**)

Figure 5. ABI-6250 Selectively Inhibits NTCP-Dependent Bile Acid Uptake *In Vitro*

A. ABI-6250 BA Uptake Inhibition

Cell type	Bile Acid IC_{50} (nM)	
	ABI-6250	Bulevirtide
PHH	2.9	4.9
Huh7-NTCP	8.3	8.3
HEK293-NTCP	5.9	3.9

B. NTCP, OATP1B, & ASBT BA Uptake Inhibition

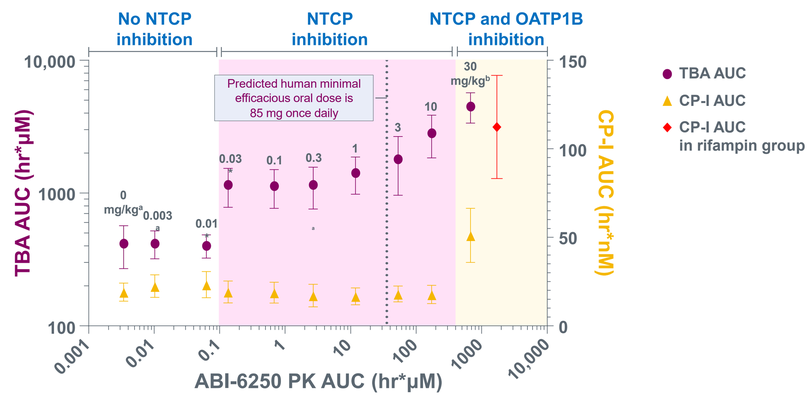
Human transporter	ABI-6250 IC_{50} (nM) [fold selectivity][a]
NTCP	5.9
OATP1B1	1000 [>169]
OATP1B3	90 [>15]
ASBT	700 [>118]

[a]Numbers in parentheses are fold selectivity in relation to NTCP inhibition.

- ABI-6250 inhibited BA uptake in various cells and had limited inhibitory effects on OATP1B & ASBT activity *in vitro* (**Figure 5**)
- ABI-6250 inhibited BA uptake in PHH, & in Huh7- and HEK293-NTCP overexpressing cells (**Figure 5A**)
- ABI-6250 had limited inhibitory effects on OATP1B1-, OATP1B3-, & ASBT-dependent bile acid uptake (**Figure 5B**)
- ABI-6250 is a selective NTCP inhibitor, as demonstrated by fold selectivity (ratio of IC_{50} BA transporter/IC_{50} NTCP)

Figure 6. ABI-6250 Elevates Plasma Bile Acids *In Vivo*

Total Bile Acid & Coproporphyrin-I Measurements in NHPs



[a]The x-axis values for vehicle, 0.003 mg/kg and rifampin groups are arbitrarily set. 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. NHP, non-human primate; NTCP, sodium taurocholate co-transporting polypeptide; PD, pharmacodynamics; PK, pharmacokinetics

- Orally administered ABI-6250 elevated TBA levels in NHPs indicating drug-target engagement (**Figure 6**)
- ABI-6250 elevated TBA levels in NHPs starting at 0.03 mg/kg. 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
- ABI-6250 has the potential to achieve the desired minimum efficacious concentration coverage with 85 mg once-daily dosing

Conclusions

- ABI-6250 is a highly potent, specific, orally bioavailable HDV/HBV entry inhibitor
- At projected clinically relevant concentrations, ABI-6250 elevates total bile acid levels *in vivo* without increasing coproporphyrin-I plasma levels, a biomarker for OATP1B inhibition, indicating specific target engagement
- The PK profile of ABI-6250 supports low once-daily dosing in patients with chronic HDV
- A phase 1a trial with ABI-6250 is currently ongoing

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DISCLOSURES

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

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