Preclinical profiling of ABI-6250, a first-in-class oral therapeutic candidate for chronic hepatitis D

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Introduction

- 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 to improve the long-term clinical outcomes of patients suffering from cHDV

Methods

- **HDV and HBV entry inhibition:**
  - Hepatoma cells or primary human hepatocytes (PHH) were inoculated with HDV (cell culture or patient-derived) or HBV (cell culture-derived) 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
- **HDV and HBV genotyping:**
  - Total RNA extracted from cHDV patient serum/plasma samples were multiplexed during library generation and sequenced on an Illumina NovaSeq X, 2x150 bp paired end reads. RNA-Seq data were analyzed using an in-house sequencing pipeline and aligned to prototypic HDV and HBV genotypes
- **NTCP-mediated bile acid uptake inhibition:**
  - PHHs and Huh7-NTCP cells were preincubated for 60 minutes with ABI-6250, followed by a 50-minute incubation with 3-α-nitrobenzoxadiazole taurocholic acid. After supernatant removal, fluorescence intensity was measured by plate reader and half-maximal inhibitory concentration (IC_{50}) values were calculated using GraphPad Prism 10
- **Cytotoxicity assessment:**
  - Cells were incubated with ABI-6250 for 3-days at 37°C with compounds in suitable cell culture media followed by cytotoxicity determination using CellTiterGlow reagent. Half-maximal cytotoxic concentration (CC_{50}) values were calculated using GraphPad Prism 10
- **Transporter inhibition:**
  - ABI-6250s potential impact on individual transporter was assessed in accordance with established Standard Operating Procedures (SOPs)
- **Pharmacokinetic/Pharmacodynamic (PK/PD) studies:**
  - ABI-6250 PK parameters were obtained following a single oral dosing of cynomolgus monkeys at given concentrations. Plasma samples were used for evaluating PK, coproporphyrin-I (CP-I), a biomarker for organic anion transporting polypeptide 1B (OATP1B) inhibition, and total bile acid (TBA) by LC-MS, LC-MS, and enzymatic cycling, respectively

References

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Disclosures

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

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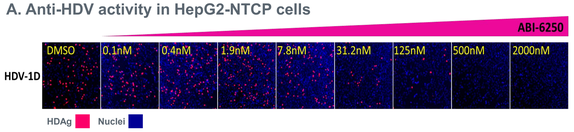
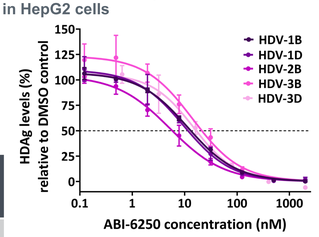
Results

Figure 1. ABI-6250 Efficiently Inhibited HDV & HBV Infection and Bile Acid Uptake

A. Anti-HDV activity in HepG2-NTCP cells



B. ABI-6250 dose-dependent HDV inhibitory effects in HepG2 cells



C. ABI-6250 EC_{50} & IC_{50} values

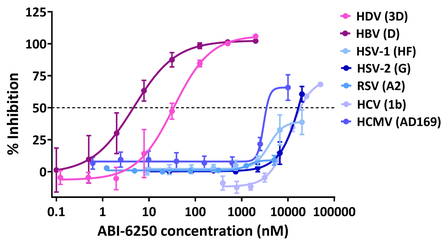
Compound	Cell culture HDV EC_{50} (nM)		Patient HDV EC_{50} (nM)	Cell culture HBV EC_{50} (nM)		Bile Acid IC_{50} (nM)	
	PHH	HepG2-NTCP	HepG2-NTCP	PHH	HepG2-NTCP	PHH	Huh7-NTCP
ABI-6250	11 (GT-3B)	5 – 15 (GT-1,2,3, B,D)	21.1*,#	14‡ (GT-A,C,D)	4.7 (GT-D)	2.9	8.3
Bulevirtide	0.6 (GT-3B)	0.5 (GT-3D)	-	0.2 (GT-D)	0.2 (GT-D)	1.9	4.8

*median value of 18 patient isolates; #HDV GT-1: 17x, GT-8:1x; HBV GT-D: 11x, GT-E: 2x, GT unknown: 5x
‡average value of HBV GT-A, C, & D

- ABI-6250 efficiently inhibited HDV and HBV entry and bile acid uptake *in vitro* (**Figure 1**)
- ABI-6250 inhibited HDV in HepG2-NTCP cells demonstrated by HDAg immunofluorescence analysis (**Fig. 1A**) and in-cell ELISA (**Fig.1B**)
- Summary of antiviral EC_{50} & bile acid uptake inhibition IC_{50} values generated in HDV or HBV infected PHHs & HepG2-NTCP cells and PHHs & Huh7-NTCP cells, respectively (**Fig. 1C**)

Figure 2. ABI-6250 Specifically Inhibited HDV and HBV Infection

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)] (**Fig. 2A**)
- Summary of EC_{50} values and selectivity indices (SI) (ratio EC_{50},virus/EC_{50},HBV/HDV) (**Fig. 2B**)

Figure 3. ABI-6250 Had Minimal Effects on Cell Viability

A. ABI-6250 CC_{50} values

Cell Type	ABI-6250 CC_{50} (μM)		
	ABI-6250	Puromycin	Tamoxifen
Huh-7	24.7	1.2	ND
HEK293	>30	0.7	ND
HepG2	>30	1.4	ND
HeLa-H1A	23.5	0.7	ND
MOLT-4	>15	0.3	ND
NCI-H226	>30	0.4	ND
MT-4	>30	0.2	ND
PBMC	>24	0.4	ND
PHH	29.1	1.1	6.7

ND, not determined

- ABI-6250 had minimal effects on cell viability *in vitro* (**Figure 3**)
- Puromycin and/or Tamoxifen were used as positive controls
- Summary of CC_{50} values in a multitude of different cell types (**Fig. 3**)

Figure 4. ABI-6250 Selectively Inhibited NTCP

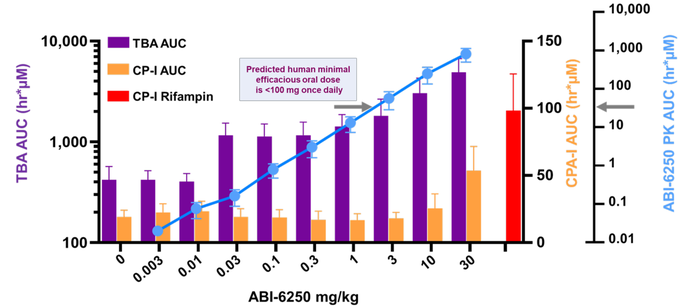
A. Assessment of ABI-6250 transporter inhibition *in vitro*

Transporter	ABI-6250 IC_{50} (μM)	Fold-selectivity
NTCP	0.008	1
BCRP	2.0	250
BSEP	10.2	1275
MRP2	19.4	2425
OAT1	9.7	1213
OAT2	8.4	1050
OATP1B1	0.6	75
OATP1B3	0.1	13
OATP2B1	1.6	200
MDR1	>10 [30%]	>1250
MATE1	>15 [26%]	>1875
MATE2K	>15 [20%]	>1875
OCT1	>15 [30%]	>1875
OCT2	>15 [13%]	>1875
OSTα/β	>15 [0%]	>1875

- ABI-6250 selectively and potently inhibited NTCP (**Figure 4**)
- Individual transporter IC_{50} values were determined in appropriate transporter assay conditions following established SOPs. Reference inhibitors were evaluated side-by-side (data not shown)
- Summary of IC_{50} values & fold-selectivity calculated as the ratio of given transporter IC_{50} to NTCP IC_{50}. Maximum inhibition at given concentration is shown in parentheses (**Fig. 4**)

Figure 5. ABI-6250 Elevates Plasma Total Bile Acids in Cynomolgus Monkeys

A. Total bile acid, coproporphyrin-I, & PK measurements *in vivo*



ABI-6250 is administered orally. The y-axis PK value for the 0.003 mg/kg group is arbitrarily set. Doses of ABI-6250 in monkeys; n = 6 per dose. Arrows indicate human PK projection using allometric scaling; AUC, area under the curve; CPI-I, coproporphyrin-I; TBA, total bile acid;

- Orally administered ABI-6250 elevated TBA levels in cynomolgus monkeys indicating drug-target engagement (**Figure 5**)
- ABI-6250 elevated TBA levels in monkeys 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, elevated CP-I levels (**Fig. 5**)
- ABI-6250 has the potential to achieve the desired minimum efficacious concentration coverage with <100 mg once-daily

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**
- **ABI-6250s PK profile supports low once-daily dosing in patients with chronic HDV**
- **A Phase 1a clinical trial with ABI-6250 is currently ongoing**