

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

WED-377

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Background

- Chronic hepatitis D virus (HDV) infection (cHDV), the most severe form of viral hepatitis, is currently an incurable disease affecting an estimated 12 to 72 million patients worldwide^{1,2}
- Patients suffering from cHDV coinfecting 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 monoinfection⁶
- 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 liver⁷⁻¹⁰
- 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 that may improve the long-term clinical outcomes of patients suffering from cHDV

Objective

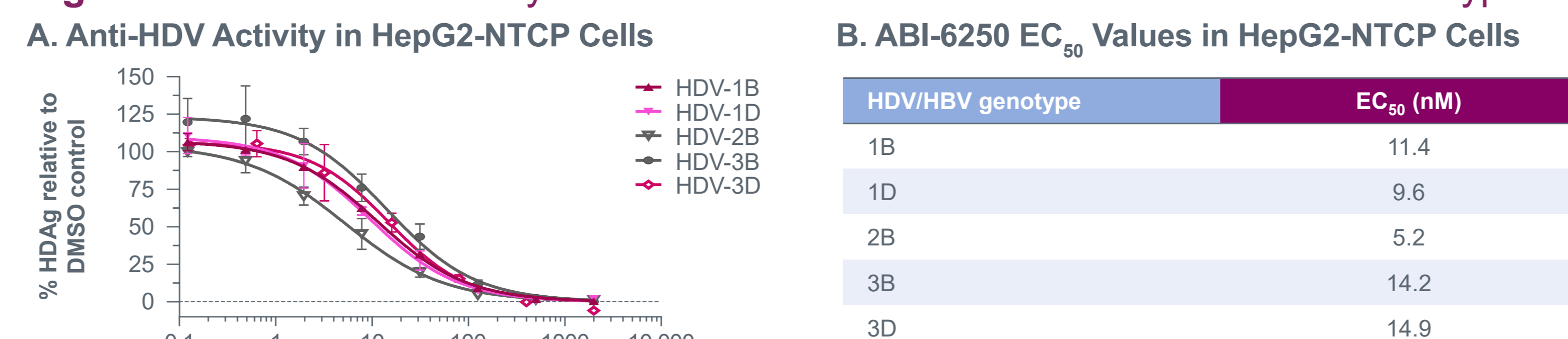
- To describe the preclinical profile of ABI-6250, an orally bioavailable small-molecule entry inhibitor that is a clinical drug candidate for the treatment of cHDV

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 (EC₅₀) 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. EC₅₀ 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 coinubated 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 μ M 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

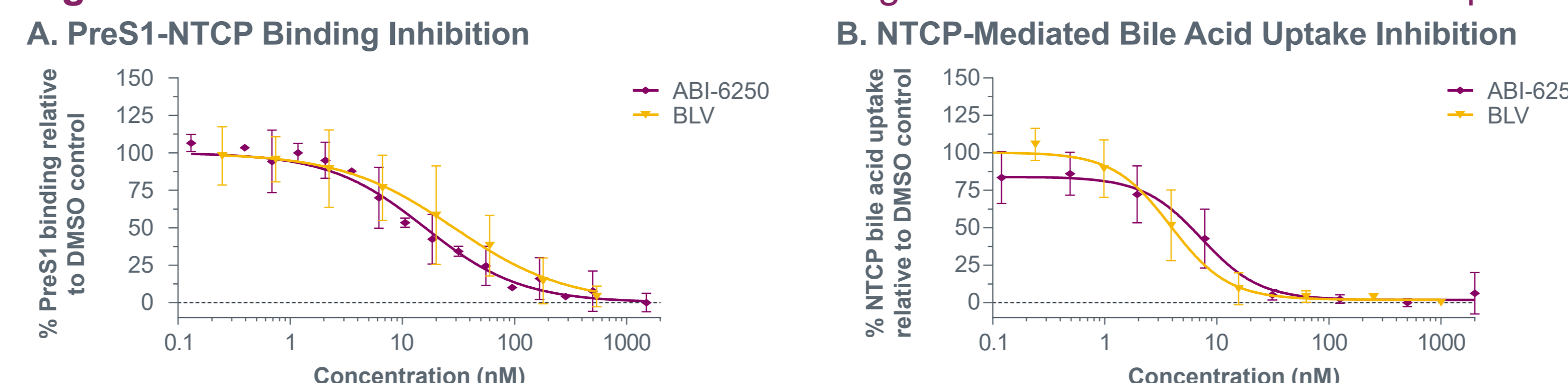
Figure 1. ABI-6250 Efficiently Inhibited HDV Infection of the Most Prevalent Genotypes



DMSO, dimethyl sulfoxide; EC₅₀, 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 EC₅₀ 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* EC₅₀ value 35-fold (data not shown)

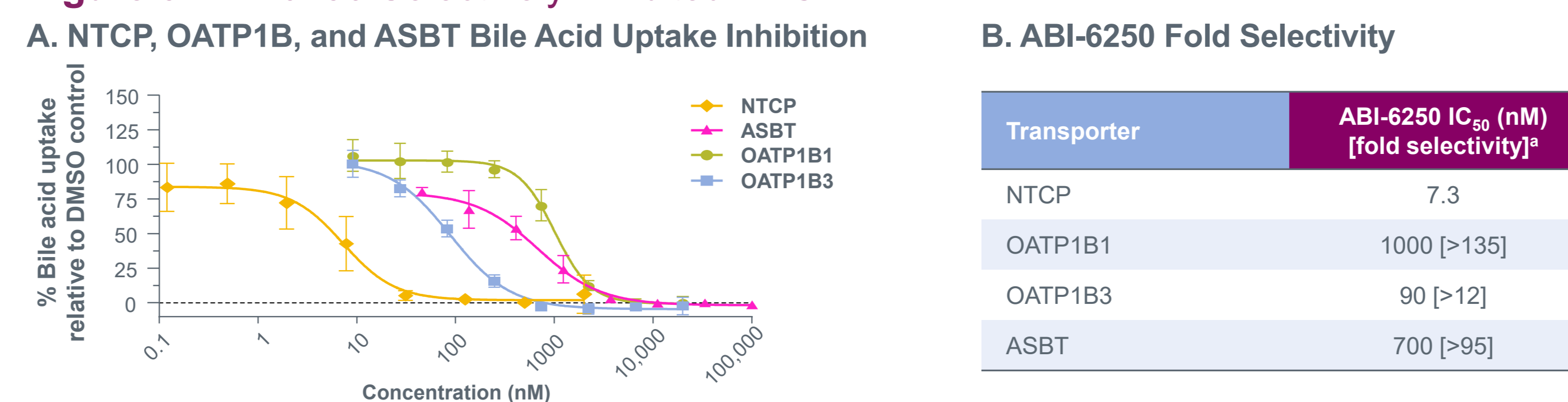
Figure 2. ABI-6250 Inhibited PreS1-NTCP Binding and NTCP-Mediated Bile Acid Uptake



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 (IC₅₀) 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 IC₅₀ values of 7.3 and 3.9 nM, respectively (Figure 2B)

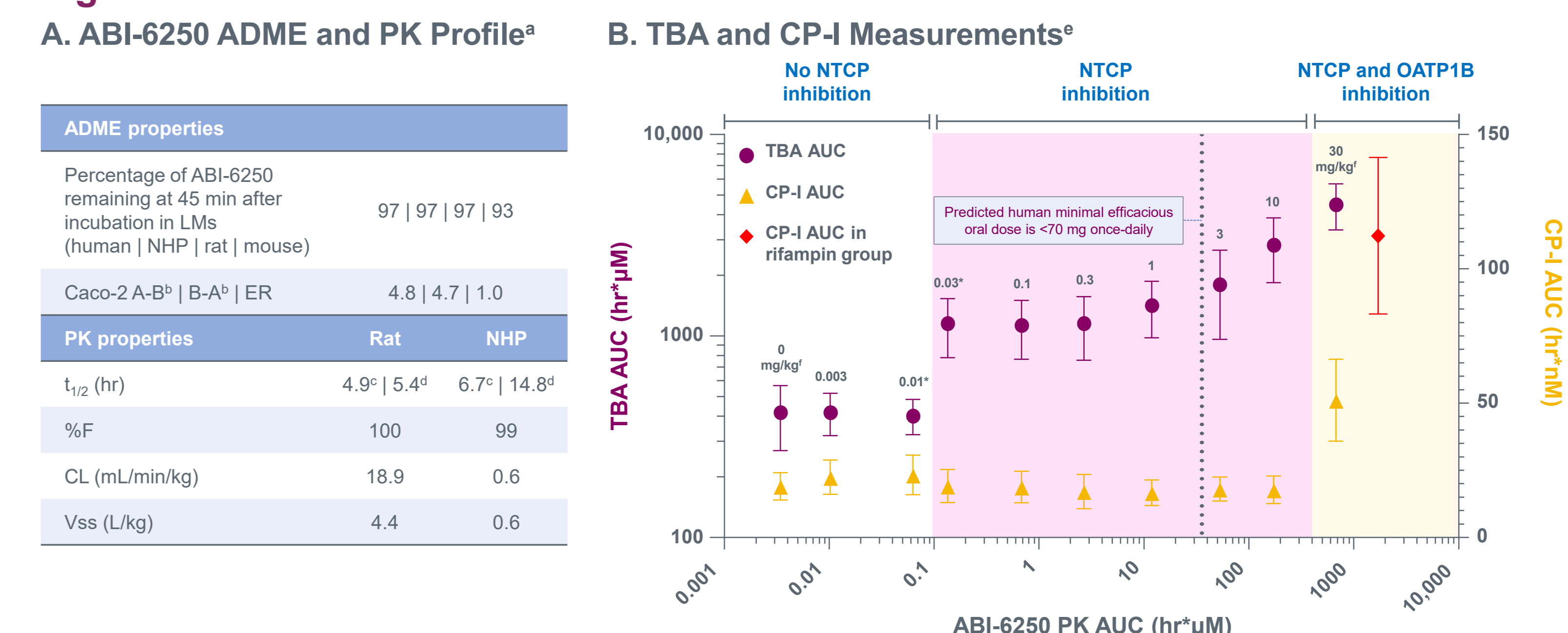
Figure 3. ABI-6250 Selectively Inhibited NTCP



^aNumbers in brackets indicate fold selectivity calculated as the ratio of IC₅₀ bile acid transporter to IC₅₀ NTCP. ASBT, apical sodium-dependent bile acid transporter; DMSO, dimethyl sulfoxide; IC₅₀, half-maximal inhibitory concentration; OATP, organic anion-transporting protein; NTCP, sodium taurocholate cotransporting polypeptide.

- 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 IC₅₀ 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 IC₅₀ bile acid transporter to IC₅₀ NTCP; Figure 3B)

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



^an = 2 or 3. ^b10⁻⁶ cm/s. ^cIf ABI-6250 is administered intravenously. ^dIf ABI-6250 is administered orally. ^eThe x-axis values for vehicle, 0.003 mg/kg and rifampin groups are arbitrarily set. ^fDoses of ABI-6250 in NHPs; n = 6 per dose. ^gFor 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 microsomes; NHP, non-human primate; PD, pharmacodynamics; PK, pharmacokinetics; t_{1/2}, terminal half-life; TBA, total bile acid; V_{ss}, 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 rat 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 4.6 to 15.7 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 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 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