A Novel Class of Orally Available Small Molecules Potently Inhibit Hepatitis B and D Virus Entry

SAT-195

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Background

- Chronic hepatitis B and D virus (HBV and HDV) infection affect an estimated 296 million and 12 million patients worldwide, respectively[1,2]
- HDV is a satellite virus that requires the hepatitis B surface antigen (HBsAg) to infect hepatocytes[3,4]
- Patients with HBV and chronic HDV infection have faster disease progression and a greater risk of developing liver-related outcomes compared to patients without HDV[5-7]
- The daily injectable HBV/HDV entry inhibitor bulevirtide targets the host receptor Na^+ taurocholate co-transporting polypeptide (NTCP), preventing HBV and HDV from entering hepatocytes[8,9]
- Bulevirtide has been shown to lower HDV serum RNA levels and normalize alanine aminotransferase (ALT) levels in co-infected individuals when combined with tenofovir as well as to lower HBsAg levels when combined with pegylated interferon-alpha[10,11]
- There is a need for potent orally-administered entry inhibitors to improve the treatment of patients with chronic HDV

Objective

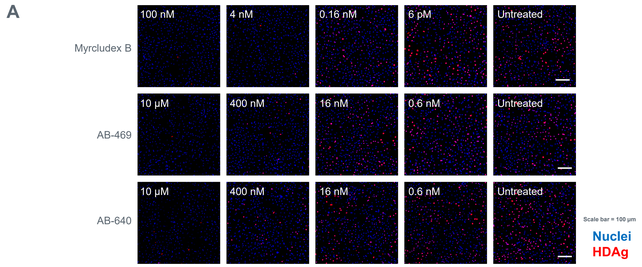
- To characterize the potencies and properties of structurally-differentiated orally-bioavailable small molecule HBV/HDV entry inhibitors

Methods

- **HBV and HDV infection:**
  - **Hepatitis B e antigen (HBeAg):**
    - Primary human hepatocytes (PHHs) were infected with HBV at a multiplicity of infection (MOI) of 300 viral genome equivalents (vge)/cell and co-treated with compounds. 24 hours later, cells were washed and fresh media without inhibitors was added. Cell culture media was harvested at 8 days post-infection (dpi) and secreted HBeAg was measured via an enzyme-linked immunosorbent assay (ELISA)
    - HepG2-NTCP cells were infected with HBV (50 vge/cell) and cotreated with inhibitors. At 5 dpi, viral supernatants were harvested and an HBeAg ELISA was performed
  - **Hepatitis D antigen (HDAg):**
    - PHHs were infected with HDV at an MOI of 20 vge/cell. 24 hours later, cells were washed and fresh media without inhibitors was added. Immunofluorescence analysis was performed on Day 5 for HDAg and analyzed via high-content imaging
    - HepG2-NTCP cells were infected with HDV (20 vge/cell) and co-treated with inhibitors. At 5 dpi, in-cell ELISA was performed for HDAg
  - **HDAg immunofluorescence analysis (IFA):**
    - PHHs were pretreated with compounds 2 hours prior to infection with HDV at an MOI of 20 vge/cell. The next day, cells were washed and fresh media without inhibitors was added. At 5 dpi, cells were processed for immunofluorescence staining of HDAg using a mouse monoclonal anti-HDAg antibody
- **Serum shift assay:**
  - HepG2-NTCP cells were infected with HBV or HDV in the presence of fetal bovine serum (FBS) and physiologically relevant levels of human serum albumin (HSA; 45 mg/mL) and alpha1-acid glycoprotein (AAG; 0.7 mg/mL) and then were compared to a standard infection carried out in media with FBS alone. To compensate for HBV particles that bound to HSA and AAG, the MOI was increased to 500 vge/cell in comparison to the standard infection at 50 vge/cell. Half-maximal effective concentration (EC_{50}) values were generated by quantifying the secretion of HBeAg into culture supernatants by ELISA at 5 dpi
- **NTCP inhibition:**
  - HEK293T cells expressing human NTCP were pre-incubated for 30 minutes with entry inhibitors followed by a 2-minute incubation with a fluorescent bile acid salt derivative. Fluorescent bile acid uptake was measured by flow cytometry
- **PreS1 binding competition:**
  - HEK293T cells stably expressing human NTCP were co-incubated with myristoylated preS1-Alexa-594 peptide and inhibitors for 10 minutes. Binding of fluorescent peptide was measured by flow cytometry
- **Metabolic stability:**
  - Metabolic stability was determined at 1 µM of testing concentration with cynomolgus monkey and human liver microsomes (LMs) using liquid chromatography–tandem mass spectrometry (LC-MS) detection
- **CYP and hERG inhibition:**
  - CYP inhibition was determined at 10 µM of testing concentration with human LMs using diclofenac (2C9), bufuralol (2D6), testosterone (3A4) and midazolam (3A4) as probe substrates and detected with LC-MS. hERG inhibition was determined at 10 µM concentration with Chinese hamster ovary cell lines expressing hERG channels of P29
- **Pharmacokinetic (PK) studies:**
  - Selected compounds (1 mg/kg intravenous and 5 mg/kg oral) were analyzed in cynomolgus monkeys

Results

Figure 1. Entry Inhibitors Efficiently Inhibit HDV and HBV Infection in PHHs



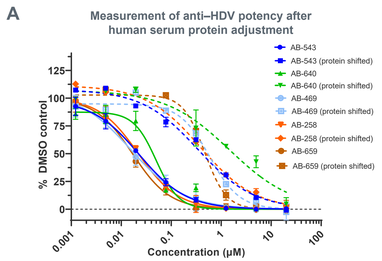
C

Compound	HDV EC_{50} (nM)	HBV EC_{50} (nM)
MyrB	2.8	1.4
AB-543	NA	324.1
AB-640	98.7	236.4
AB-469	92.3	114.2
AB-258	NA	627.9
AB-659	NA	187.3

EC_{50}, half-maximal effective concentration; HBV, hepatitis B virus; HDAg, hepatitis D antigen; HDV, hepatitis D virus; MyrB, myrcludex B; PHH, primary human hepatocyte; NA, not available.

- Novel entry inhibitors efficiently inhibited HDV and HBV entry in PHHs (**Figure 1**)
- Entry inhibitors efficiently inhibited HDV entry in PHHs as demonstrated by IFA (**Fig. 1A**). Dose-response curves of selected inhibitors quantified by image analysis (**Fig. 1B**). Summary of HDV and HBV EC_{50} values (**Fig. 1C**).

Figure 2. Novel Entry Inhibitors Efficiently Inhibit HBV and HDV Infection in NTCP-Expressing Hepatoma Cells



B

Compound	HDV EC_{50} at 5% FBS (nM)	EC_{50} at 5% FBS + 45 mg/mL HSA and 0.7 mg/mL AAG (nM)	Fold shift
AB-543	22.8	503.2	22
AB-640	47.3	1457.8	31
AB-469	15.0	536.9	36
AB-258	21.9	429.5	20
AB-659	13.4	508.4	38

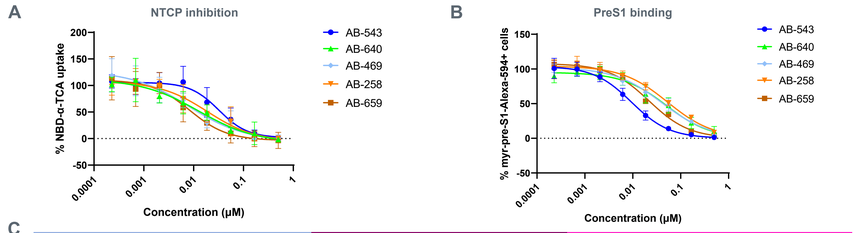
C

Compound	HBV EC_{50} at 5% FBS (nM)	EC_{50} at 5% FBS + 45 mg/mL HSA and 0.7 mg/mL AAG (nM)	Fold shift
AB-543	3.0	63.8	21
AB-640	10.9	376.0	35
AB-469	7.8	286.6	37
AB-258	13.6	242.9	18
AB-659	4.8	120.7	25

AAG, alpha1-acid glycoprotein; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; FBS, fetal bovine serum; HBV, hepatitis B virus; HDV, hepatitis D virus; HSA, human serum albumin; NTCP, Na^+ taurocholate co-transporting polypeptide.

- Novel entry inhibitors efficiently inhibited HBV and HDV entry in HepG2-NTCP cells (**Figure 2**)
- Dose-response curve analysis with human serum factors affected HDV potencies of entry inhibitors (**Fig. 2A**). Summary of HDV and HBV EC_{50} values **(Fig. 2B and C)**. Human serum factors affected HBV and HDV potencies of entry inhibitors by 18- to 37-fold and 20- to 38-fold changes in EC_{50} values, respectively

Figure 3. NTCP Inhibition and PreS1 Binding in HEK293 Cells



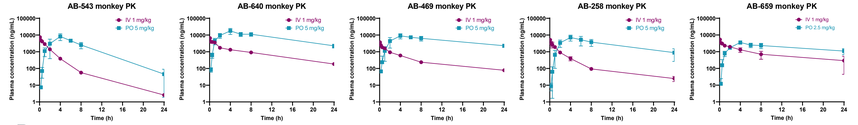
C

Compound	NTCP IC_{50} (nM)	PreS1 IC_{50} (nM)
AB-543	40.0	18.0
AB-640	1.2	1.9
AB-469	0.6	3.0
AB-258	2.2	6.3
AB-659	2.8	4.7

IC_{50}, half-maximal inhibitory concentration; NTCP, Na^+ taurocholate co-transporting polypeptide.

- Novel entry inhibitors interfere with NTCP-dependent bile acid uptake and HBV preS1 binding (**Figure 3**)
- The compounds inhibited NTCP-dependent bile acid uptake (half-maximal inhibitory concentration [IC_{50}]=0.6-40 nM; **Fig. 3A**) and preS1 binding IC_{50}=1.9-18 nM (**Fig. 3B**)
- Summary of NTCP- and preS1 binding inhibition IC_{50} values (**Fig. 3C**)

Figure 4. PK and ADME Profile of AB-543, AB-640, AB-469, AB-258, and AB-659



B

ADME properties	AB-543	AB-640	AB-469	AB-258	AB-659
LM %remaining @45 min (human/cyno)	100/99	95/100	100/89	100/100	98/98
hERG % inhibition at 10 µM	2	4	8	10	1
Kinetic solubility (µM)	77	46	44	76	45

N=2 or 3.
ADME, absorption, distribution, metabolism, and excretion; cyno, cynomolgus; IV, intravenous; LM, liver microsome; min, minute; PK, pharmacokinetics; PO, oral.

- Cynomolgus monkey is predicted to be the most relevant species for *in vivo* evaluation and human PK prediction
- The novel potent entry inhibitors AB-543, AB-640, AB-469, AB-258, and AB-659 possess desired PK profiles (**Fig. 4A**), showing 100% bioavailability in monkey and high oral exposure with terminal half-lives ranging from 3-7 hours
- Compounds AB-543, AB-640, AB-469, AB-258, and AB-659 demonstrated acceptable ADME properties (**Fig. 4B**):
  - Good metabolic stability with more than 85% remaining after 45-minute incubation in monkey and human, respectively
  - Good apparent permeability in Caco-2 cells with no efflux
  - No major CYP inhibition and hERG liabilities
  - Moderate aqueous kinetic solubility
- AB-659 has the potential to achieve desired minimum concentration coverage with once-daily 100-mg dosing based on human PK projection using allometric scaling

Conclusions

- This study identified a novel class of highly-potent, orally-bioavailable HBV/HDV entry inhibitors with good drug-like properties
- The identified inhibitors interfere with preS1 binding and NTCP-mediated bile acid uptake, supporting that the molecular target is NTCP
- Lead optimization of this series of entry inhibitors is in progress, with nomination of a development candidate anticipated in 2023

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ACKNOWLEDGMENTS

- Writing and editorial support were provided by Duncan McCloskey, PhD, of AlphaBioCom, a Red Nucleus company, and were funded by Assembly Biosciences, Inc.
- This study was sponsored by Assembly Biosciences, Inc.

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Presented at the European Association for the Study of the Liver (EASL) – The International Liver Congress 2023, June 21–24, 2023