Pre-Clinical Characterization of Novel Liver-Focused Small Molecules Efficiently Inhibiting Hepatitis B Virus by Activating Type I Interferon Signaling

HBV INTERNATIONAL MEETING

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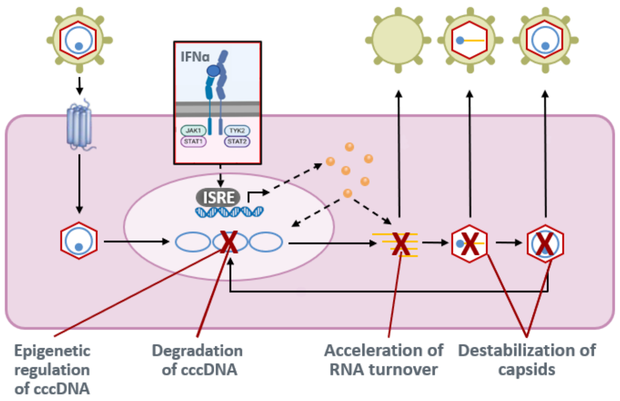
BACKGROUND

- Chronic hepatitis B virus infection (cHBV) is a significant global health problem
  - Worldwide, an estimated 296 million people have cHBV, resulting in about 820,000 deaths each year, mostly due to cirrhosis and hepatocellular carcinoma[1]
- Nucleos(t)ide reverse transcriptase inhibitors (NrtIs) reduce HBV DNA, but treatment is indefinite and demonstrates a low rate of functional cure, necessitating lifelong administration[2,3]
- Interferon-alpha (IFNα) interferes with multiple steps of the viral life cycle (**Figure 1**)
- Pegylated (PEG)-IFNα has immunomodulatory and antiviral activities, leading to hepatitis B surface antigen (HBsAg) clearance (functional cure) in some patients[4,5] and at a higher rate than for NrtIs[6,7]
  - Poor tolerability of IFNα limits its use in the clinic[8]
- Orally-bioavailable, liver-targeted IFNα-like therapeutics with improved tolerability have the potential to increase the proportion of patients achieving functional cure through this mechanism

Figure 1. HBV Replication Cycle and IFNα



cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; IFNα, interferon-alpha; ISRE, interferon-stimulated response element; JAK, Janus kinase; STAT, signal transducer and activator of transcription; TYK, tyrosine kinase.

OBJECTIVE

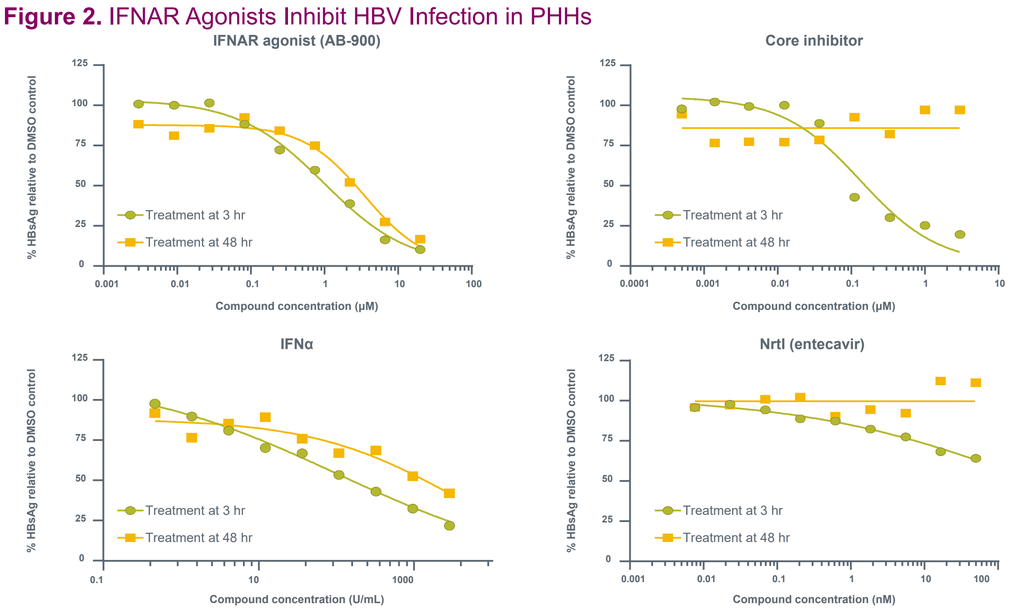
- To characterize a novel class of orally-bioavailable small molecules that inhibit HBV through activation of IFN signaling

METHODS

- HBV infection of primary human hepatocytes (PHHs):
  - PHHs were infected with HBV at 300 viral genome equivalents/cell and treated with IFNα receptor (IFNAR) agonists at 3 hours post-infection. The next day, cells were washed, and fresh medium with agonist or IFNα was added. Cell culture medium was harvested at 8 days post-infection, and secreted hepatitis B e antigen (HBeAg) or HBsAg was measured via an enzyme-linked immunosorbent assay
- Hepatitis C virus (HCV) replicon cells (NanoLuc luciferase reporter assay):
  - Huh-7 cells stably replicating HCV were treated with agonists for 2 days post-plating. Luciferase activities were analyzed using a Nano-Glo luciferase assay
  - Demonstration of long-lasting antiviral effects: Incubation of replicon cells with agonist or IFNα for indicated time points was followed by the removal of agonist or IFNα. Cells were then left untreated until 48 hours post-initial treatment and then analyzed as described above
- Interferon-stimulated gene (ISG) induction:
  - *In vivo* (mice): RNA was extracted from mouse liver, and peripheral blood mononuclear cells (PBMCs) treated with IFNAR agonists or murine IFNα. Real-time quantitative polymerase chain reaction analysis was conducted using customized plates of 22 mouse ISG probes
  - *In vitro*: PHHs were treated with dimethyl sulfoxide (DMSO), IFNAR agonist, or IFNα, for 16 hours. Cells were lysed, RNA isolated for hybridization to an nCounter Host Response version 1.1 Panel, and analyzed using the nanoString nCounter Analysis System
- Determination of signal transducer and activator of transcription (STAT) 1 and Janus kinase (JAK) 1 phosphorylation by Western blot:
  - HeLa cells were treated with DMSO, IFNAR agonist, or human IFNα for 20 minutes with or without JAK1 inhibitors. STAT1 phosphorylation was assessed by Western blot using phosphorylated (p)STAT1 and pSTAT3 antibodies
- Pharmacokinetic (PK) and pharmacodynamic (PD) studies in nonhuman primates (NHP):
  - PK parameters were assessed in NHPs given AB-091 50 mg/kg orally and PD parameters were assessed in NHPs given AB-091 50 mg/kg orally and 0.04 mg/kg PEG-IFNα
  - PBMCs were isolated at 2-, 6-, and 24-hours post-dose and liver biopsies were taken at 6 hours post-dose. Total RNA and ISG induction were monitored by genome-wide RNA sequencing

RESULTS

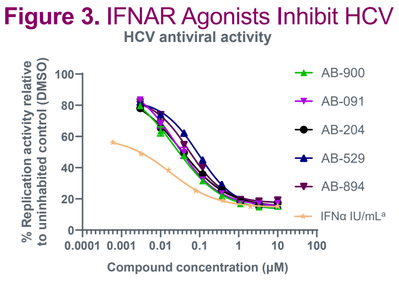
Figure 2. IFNAR Agonists Inhibit HBV Infection in PHHs



DMSO, dimethyl sulfoxide; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFNα, interferon-alpha; IFNAR, interferon-alpha receptor; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PHH, primary human hepatocyte.

- A novel IFNAR agonist (AB-900) inhibits HBV at both time points post-infection (**Figure 2**)
  - AB-900 and IFNα inhibit HBsAg secretion whether given before or after covalently closed circular (ccc)DNA establishment (48 hours post-infection)
  - In contrast, the core inhibitor only inhibits HBsAg secretion at 3 hours post-infection (prior to cccDNA establishment)
  - Entecavir poorly inhibits HBsAg at 3 hours post-infection (half-maximal effective concentration [EC_{50}] >100 nM) and is inactive at 48 hours post-infection
- HBsAg and HBeAg EC_{50} values for IFNAR agonists in HBV-infected PHHs are 0.9 to 6.2 μM and 1.0 to 3.5 μM, respectively

Figure 3. IFNAR Agonists Inhibit HCV RNA Replication in Hepatoma Cells

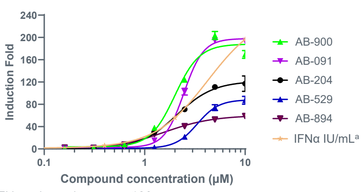


	EC_{50} Values (μM)					
	AB-900	AB-091	AB-204	AB-529	AB-894	IFNα IU/mL
HCV	0.02	0.03	0.03	0.09	0.05	1.8

[a]IFNα values shown are 100x.
DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; HCV, hepatitis C virus; IFNα, interferon-alpha; IFNAR, interferon-alpha receptor.

- As expected, IFNAR agonists efficiently inhibit HCV replication by activating IFNα (**Figure 3**)

Figure 4. IFNAR Agonists Induce ISRE Reporter Activity in HEK293 Cells

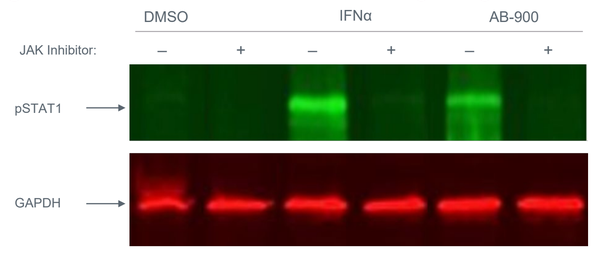


	EC_{50} Values (μM)					
	AB-900	AB-091	AB-204	AB-529	AB-894	IFNα IU/mL
ISRE	2.00	2.46	2.09	3.34	1.56	425.7

[a]IFNα values shown are 100x.
HEK, human embryonic kidney; IFNα, interferon-alpha; IFNAR, interferon-alpha receptor; ISRE, interferon-stimulated response element.

- IFNAR agonists induce interferon-sensitive response element (ISRE) reporter activity in human embryonic kidney (HEK) 293 reporter cells (**Figure 4**)
- Half-maximal inhibitory concentration values of ISRE reporter activity range from 0.1 to 9.0 μM in HEK293 cells (**Figure 4**)
  - The IFNAR agonists show differences in maximum stimulation

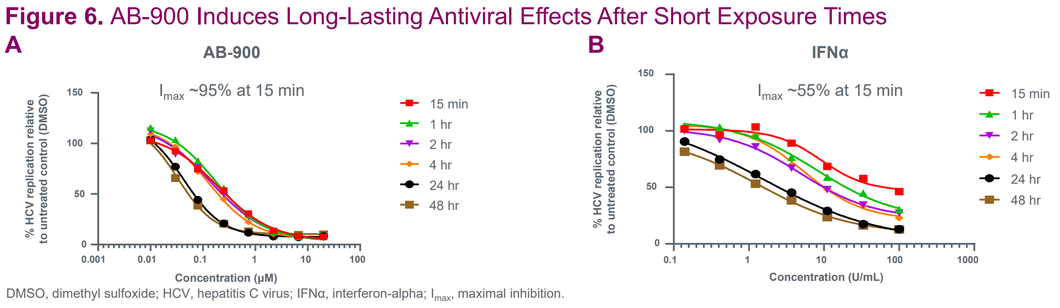
Figure 5. AB-900 Activates JAK-STAT Signaling in HeLa cells



DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFNα, interferon-alpha; JAK, Janus kinase; p, phosphorylated; STAT, signal transducer and activator of transcription.

- AB-900 induces STAT1 phosphorylation in HeLa cells; induction is sensitive to JAK1 inhibition (**Figure 5**)

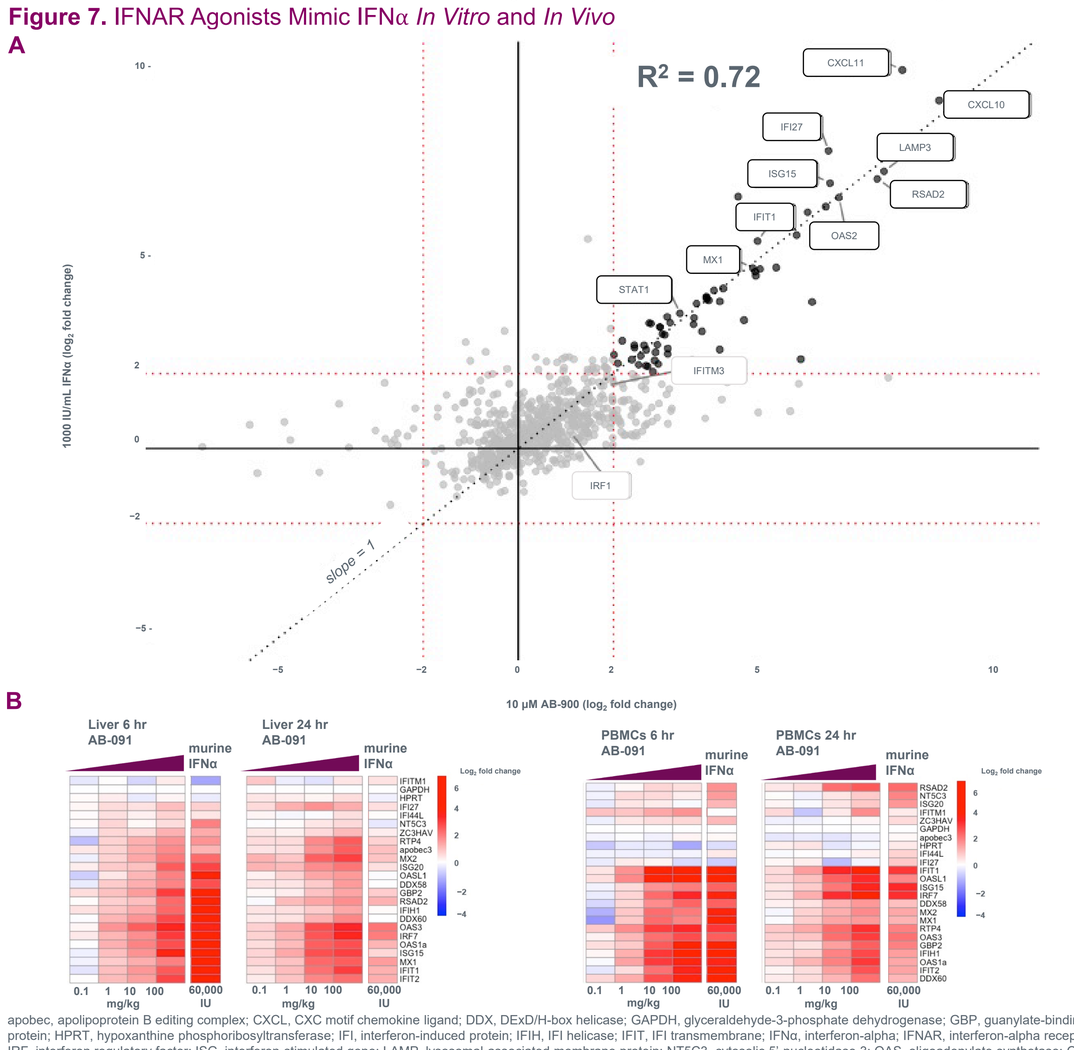
Figure 6. AB-900 Induces Long-Lasting Antiviral Effects After Short Exposure Times



DMSO, dimethyl sulfoxide; HCV, hepatitis C virus; IFNα, interferon-alpha; I_{max}, maximal inhibition.

- AB-900 induces a long-lasting antiviral state after only 15 minutes of exposure in HCV replicon cells, with a maximal inhibition (I_{max}) of viral replication >95% (**Figure 6A**)
- IFNα induces a long-lasting antiviral state only if treated for at least 24 hours, with an I_{max} of viral replication >95% (**Figure 6B**)
- In contrast to AB-900, 15 minutes of IFNα exposure to infected target cells results only in ~55% viral replication inhibition (**Figure 6B**)

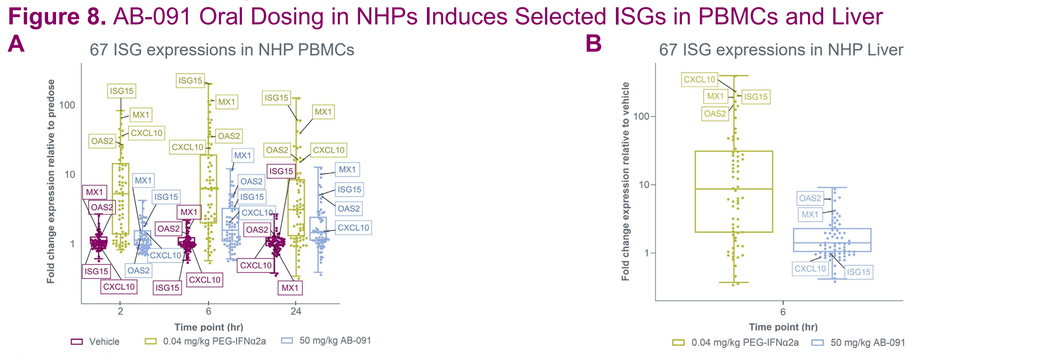
Figure 7. IFNAR Agonists Mimic IFNα *In Vitro* and *In Vivo*



apobec, apolipoprotein B editing complex; CXCL, CXC motif chemokine ligand; DDX, DExD/H-box helicase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GBP, guanylate-binding protein; HPRT, hypoxanthine phosphoribosyltransferase; IFI, interferon-induced protein; IFIH, IFI helicase; IFIT, IFI transmembrane; IFNα, interferon-alpha; IFNAR, interferon-alpha receptor; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; LAMP, lysosomal-associated membrane protein; NT5C3, cytosolic 5′-nucleotidase 3; OAS, oligoadenylate synthetase; OASL, OAS-like; PBMC, peripheral blood mononuclear cell; RSAD, radical S-adenosyl methionine domain; RTP, receptor transporter protein; STAT, signal transducer and activator of transcription; ZC3HAV, zinc finger CCCH-type antiviral protein.

- AB-900 mimics IFNα by inducing ISGs comparably to IFNα in PHHs (**Figure 7A**)
- In mice, similar magnitudes of change in ISGs are observed between murine IFNα and AB-091 (**Figure 7B**)
- PK information is shown in **Figure 9A**

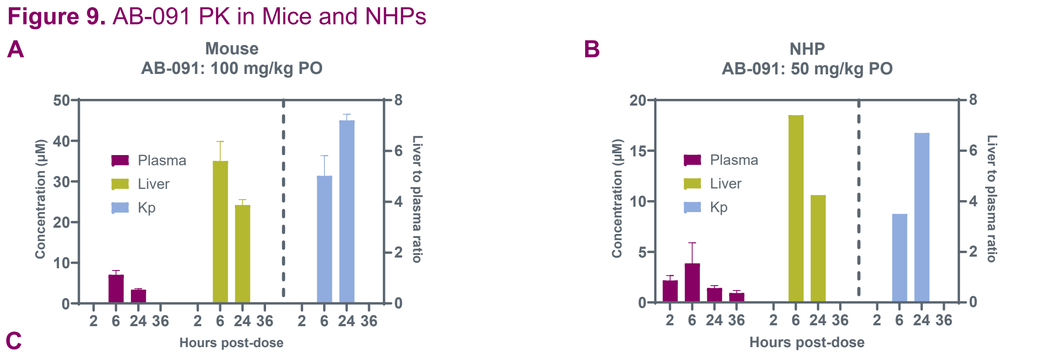
Figure 8. AB-091 Oral Dosing in NHPs Induces Selected ISGs in PBMCs and Liver



CXCL, CXC motif chemokine ligand; ISG, interferon-stimulated gene; NHP, nonhuman primate; OAS, oligoadenylate synthetase; PBMC, peripheral blood mononuclear cell; PEG-IFN2α, pegylated interferon-alpha 2.

- AB-091 (50 mg/kg) administered to NHPs induces ISG expression at 6- and 24-hours post-dose in PBMCs **(Figure 8A)** and at 6 hours post-dose in liver **(Figure 8B)**
- PK information is shown in **Figure 9B**
- The fold induction of MX1 by AB-091 is comparable to MX1 induction in PBMCs and livers of patients chronically infected with HCV who are treated with IFNα[9]
- In contrast, 0.04 mg/kg of PEG-IFNα (positive control) induces ISGs at all given time points to a significantly greater extent than AB-091. This is likely due to the concentration of PEG-IFNα used in this study, which is 4-fold greater than what is used in human patients

Figure 9. AB-091 PK in Mice and NHPs



C

Mouse PK Parameters	AB-091
$t_{1/2}$ (hours)	14
Kp in Liver	5.0[a]
%F	75

[a]Measured at 6 hours post ABI-091 administration.
F, bioavailability; Kp, partition coefficient; NHP, nonhuman primate; PK, pharmacokinetics; PO, by mouth; $t_{1/2}$, terminal half-life.

- The PK profile of AB-091 in mice (**Figure 9A**) and NHPs (**Figure 9B**) is diverse and demonstrates
  - At 6- and 24- hours post-dose, AB-091 liver-to-plasma ratios between 5- to 7-fold in mice and 4- to 6-fold in NHPs
  - That AB-091 has a terminal half-life of 14 hours, a partition coefficient of 5.0 in the liver, and oral bioavailability of 75% in mice (**Figure 9C**)

CONCLUSIONS

- Novel small molecule IFNAR agonists inhibit HBV and other viruses *in vitro*
- The IFNAR agonists tested in this study closely mimic IFNα by activating ISRE signaling and inducing the JAK-STAT pathway, leading to ISG induction in human liver cells as well as in the liver and PBMCs of NHPs and mice
- PK data demonstrate that a selected agonist has desirable liver exposure and oral absorption
- Lead optimization of multiple IFNAR agonists is in progress

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