# Preclinical characterization of a novel liver-focused small molecule efficiently inhibiting hepatitis B virus by activating type I interferon signaling

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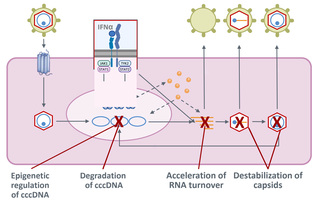
## BACKGROUND

- Chronic hepatitis B virus (cHBV) infection is a significant global health problem
  - Worldwide, an estimated 296 million people have cHBV infection, resulting in about 820,000 deaths each year, mostly due to cirrhosis and hepatocellular carcinoma[1–4]
- Interferon-alpha (IFNα) interferes with multiple steps of the viral life cycle (**Figure 1**)
- Pegylated-IFNα has immunomodulatory and antiviral activities, leading to hepatitis B surface antigen (HBsAg) clearance (functional cure) in some patients[5,6]
  - Poor tolerability of IFNα limits its use in the clinic[1]
- Orally-bioavailable IFNα-like therapeutics with better tolerability are needed and would expand the population of patients that could benefit from the curative mechanisms of IFNα

**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; TYK2, tyrosine kinase.

## OBJECTIVE

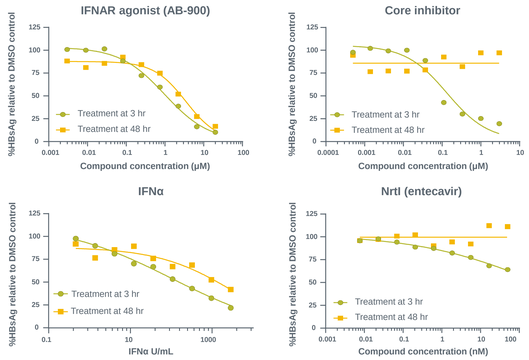
- Development of an orally-bioavailable small molecule liver-focused drug inducing IFNα signaling with the goal of curing patients with cHBV

## METHODS

- HBV infection of primary human hepatocytes (PHHs):
  - PHHs were infected with HBV at 300 viral genome equivalents (vge)/cell and treated with compounds at 3 hours postinfection. The next day, cells were washed and fresh media with compound or IFNα was added. Cell culture media was harvested at 8 days postinfection (dpi) and secreted hepatitis B e antigen (HBeAg) or HBsAg were measured via an enzyme-linked immunosorbent assay (ELISA)
- Hepatitis C virus (HCV) and Zika virus (ZIKV) replicon cells:
  - NanoLuc luciferase reporter assay:
    - Huh-7 and baby hamster kidney cells stably replicating HCV or ZIKV were treated with agonists for 2 days post-plating. Luciferase activities were analyzed using a Nano-Glo Luciferase Assay System
- Encephalomyocarditis virus (EMCV):
  - Cell protection assay:
    - Human A549 cells were infected with EMCV and subsequently treated with agonists for 2 days. Cell viability was assessed using CellTiter-Glo
- Interferon-stimulated gene (ISG) induction:
  - In vivo: Real-time quantitative polymerase chain reaction (RT-qPCR): RNA was extracted from mouse liver and peripheral blood mononuclear cells (PBMCs) were treated with agonists or murine IFNα. RT-qPCR analysis was conducted using customized microarray plates of 22 mouse ISG probes
  - In vitro: PHHs were treated with dimethyl sulfoxide (DMSO), agonist, or IFNα for 16 hours. Cells were lysed, and RNA was collected for hybridization and prepped with nCounter MAX Prep Station and Digital Analyzer with Host Response v1.1 Panel
- Determination of signal transducer and activator of transcription 1 (STAT1) and Janus kinase 1 (JAK1) phosphorylation by western blot:
  - HeLa cells were treated with DMSO, interferon-alpha receptor (IFNAR) agonist, or human IFNα for 20 minutes with or without JAK-1 inhibitors. STAT1 phosphorylation was assessed by western blot using phosphorylated (p)STAT1 and pSTAT3 antibodies
  - Mouse L929 cells were treated with DMSO, agonist, mouse IFNα, or human IFNα for 20 minutes. Western blot was conducted with pSTAT1, pSTAT3, pJAK1, and glyceraldehyde 3-phosphate dehydrogenase antibodies
- Pharmacokinetic (PK) studies:
  - Select compounds had PK parameters assessed in Sprague Dawley rats at 1 mg/kg for intravenous administration and 5 mg/kg for oral administration

## RESULTS

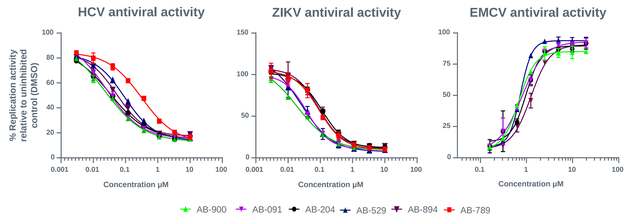
**Figure 2.** IFNAR Agonists Inhibit HBV Infection in PHHs




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

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

**Figure 3.** IFNAR Agonists Have Broad Antiviral Activity

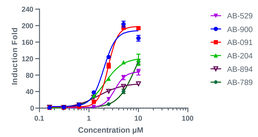



| $EC_{50}$ values (μM) | | | | | | |
|---|---|---|---|---|---|---|
| Compound | AB-900 | AB-091 | AB-204 | AB-529 | AB-894 | AB-789 |
| **HCV** | 0.02 | 0.03 | 0.03 | 0.09 | 0.05 | 0.3 |
| **ZIKV** | 0.02 | 0.04 | 0.1 | 0.03 | 0.09 | 0.09 |
| **EMCV** | 0.7 | 0.8 | 1.0 | 0.8 | 1.3 | 0.3 |

$EC_{50}$, half-maximal effective concentration; DMSO, dimethyl sulfoxide; EMCV, encephalomyocarditis virus; HCV, hepatitis C virus; IFNAR, interferon-alpha receptor; ZIKV, Zika virus.

- Novel IFNAR agonists efficiently inhibited hepatitis C, encephalomyocarditis and Zika virus as expected when the IFNα pathway is activated (**Figure 3**)

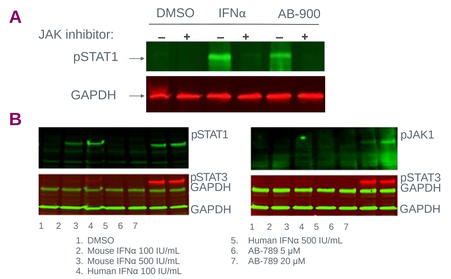
**Figure 4.** IFNAR Agonists Induce ISRE Reporter Activity in HEK Cells




HEK, human embryonic kidney; IFNAR, interferon-alpha receptor; ISRE, interferon-stimulated response element.

- IFNAR agonists induced interferon-stimulated response element (ISRE) reporter activity in human embryonic kidney 293 reporter cells (**Figure 4**)
- $EC_{50}$ values of ISRE induction ranged from 1.6 μM–4.9 μM (**Figure 4**)
  - There were differences in maximum stimulation among the IFNAR agonists

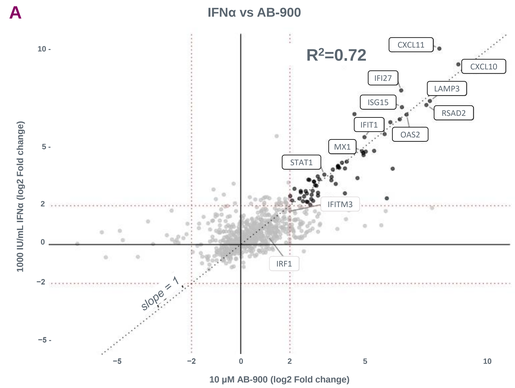
**Figure 5.** AB-900 and AB-789 Activate JAK-STAT Signaling




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 induced STAT1 phosphorylation in human HeLa cells. Induction is sensitive to JAK1 inhibitors (**Figure 5A**)
- AB-789 induced STAT1-, 3- and JAK1 phosphorylation in a dose-dependent manner in murine L929 cells (**Figure 5B**)

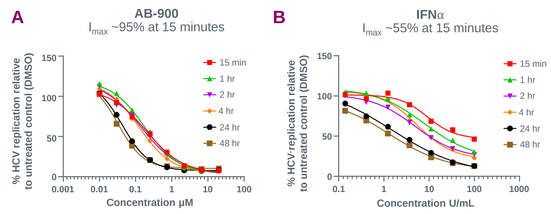
**Figure 6.** IFNAR Agonists Mimic IFNα In Vitro and In Vivo




GBP, guanylate-binding protein; HPRT, hypoxanthine phosphoribosyltransferase; IFI, interferon-induced protein; IFITM, interferon-induced transmembrane; IFNα, interferon-alpha; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; m, micro; OAS, oligoadenylate synthetase; OASL, OAS-like; PBMC, peripheral blood mononuclear cell; RTP, receptor transporter protein; STAT, signal transducer and activator of transcription.

- AB-900 mimics IFNα by inducing ISGs in PHHs comparable to IFNα (**Figure 6A**)
- Similar magnitudes of changes in ISGs were observed between murine IFNα and AB-789 in mice (**Figure 6B**)

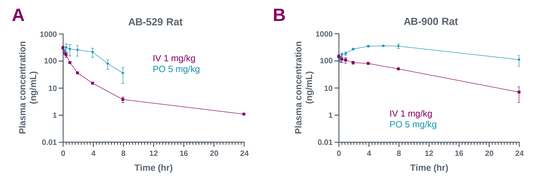
**Figure 7.** AB-900 Rapidly Induces Long-Lasting Antiviral Effects




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

- AB-900 quickly induced 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 7A**)
- IFNα induced a long-lasting antiviral state only if treated for at least 24 hours, with an $I_{max}$ of viral replication >95% (**Figure 7B**)
  - In contrast to the IFNAR agonist, 15 minutes of IFNα exposure to target cells resulted only in ~55% viral replication inhibition (**Figure 7B**)

**Figure 8.** Pharmacokinetics of AB-529 and AB-900




**C**

| Rat PO pharmacokinetics | AB-529 | AB-900 |
|---|---|---|
| $t_{1/2}$ (hr) | 1.6 | 8.5 |
| %F | 84 | 100 |

F, bioavailability; hr, hour; IV, intravenous; PO, by mouth; $t_{1/2}$, terminal half-life.

- Novel IFNAR agonists demonstrated diverse PK profiles:
  - AB-529 is a representative high clearance agonist with limited systemic exposure and a terminal half-life ($t_{1/2}$) of 1.6 hours and oral bioavailability of 84% (**Figure 8A and 8C**)
  - AB-900 has a long $t_{1/2}$ (8.5 hours), with moderate liver exposure and oral bioavailability of 100% (**Figure 8B and 8C**)

## CONCLUSIONS

- Novel IFNAR agonists inhibit HBV and other viruses in vitro
- The agonists tested in this study closely mimic IFNα by activating IFN signaling via the JAK–STAT pathway, leading to ISG induction in human and mouse cells, in vitro and in vivo
- PK data demonstrate that the agonists have desirable liver exposure and oral absorption
- Lead optimization of multiple agonists are in progress

**REFERENCES**

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**ACKNOWLEDGMENTS**

- Writing and editorial support was provided by Gregory Suess, PhD, of AlphaBioCom, LLC, and funded by Assembly Biosciences, Inc.
- This study was sponsored by Assembly Biosciences, Inc.

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**Presented at The Liver Meeting (AASLD), November 4–8, 2022**