SAT426

In Vitro and *In Vivo* Characterization of VIR-2218, an Investigational RNAi Therapeutic Targeting Hepatitis B Virus

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Introduction

- Chronic hepatitis B virus (HBV) infection remains an important global public health problem with significant morbidity and mortality[1,2]
- Continued HBV DNA suppression with nucleoside reverse transcriptase inhibitors (NRTIs) requires lifelong treatment and rarely results in hepatitis B surface antigen (HBsAg) loss
- An RNA interference (RNAi) therapeutic targeting HBV RNAs has the potential to contribute to functional cure with finite treatment by decreasing expression of viral antigens, including tolerogenic HBsAg
- VIR-2218 is an investigational, N-acetylgalactosamine (GalNAc)-conjugated, double-stranded RNAi therapeutic created using Enhanced Stabilization Chemistry Plus (ESC+) that targets within the HBx gene region shared by all HBV transcripts[3,4]

HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; L/M/S-HBsAg, large/medium/small HBsAg; rcDNA, relaxed circular DNA.

Objective

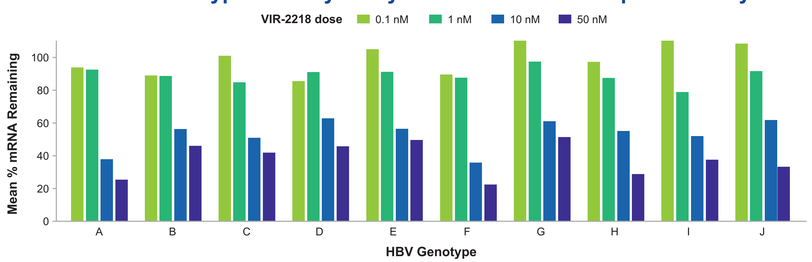
- To determine *in vitro* and *in vivo* antiviral activity of the investigational RNAi therapeutic VIR-2218

Methods

- Pan-genotypic activity was determined using luciferase readout in a psiCHECK™-2 (Promega Corporation, Madison, Wisconsin, USA) plasmid, which incorporates the HBx gene sequence of a specific genotype fused within the 3'-untranslated region of a luciferase gene
- Secreted HBV DNA, HBsAg, and HBeAg levels were measured by quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) in the stably HBV-expressing HepG2.2.15 cell line at Day 6 after reverse transfection of VIR-2218
- The effects of VIR-2218 on HBsAg and HBeAg levels after authentic infection were determined in HBV-infected HepG2 cells expressing Na^+-taurocholate cotransporting polypeptide (NTCP) and primary human hepatocytes (PHH)
- *In vivo,* HBsAg levels were examined following single or multiple subcutaneous (SC) injections of VIR-2218 in mice transduced with adeno-associated virus serotype 8 (AAV8)-HBV encoding HBV genotype D

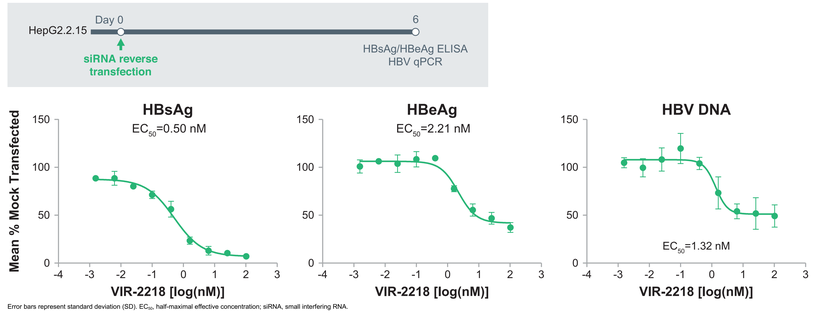
Results

VIR-2218 Pan-Genotypic Activity Analysis in PsiCHECK-2 Reporter Assay



- HBx mRNA knockdown was observed for representative sequences of all 10 HBV genotypes with 22.4–51.4% remaining at the highest dose (50 nM)

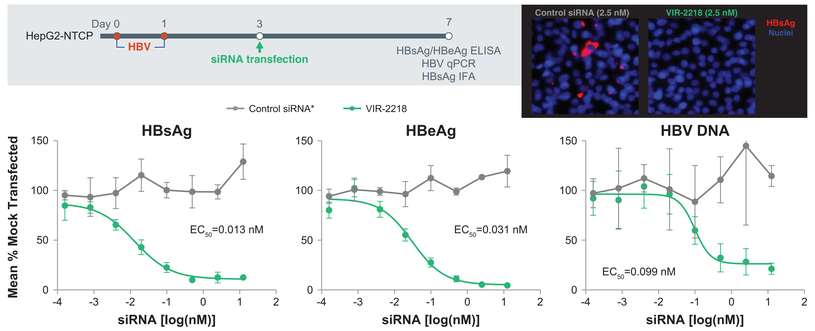
VIR-2218 Activity in an Integrated HBV Cell Line
HepG2.2.15



Error bars represent standard deviation (SD). EC_{50}, half-maximal effective concentration; siRNA, small interfering RNA.

- Dose-dependent reductions of three viral markers (HBsAg, HBeAg, HBV DNA) originating from three different viral mRNAs were observed in cells reverse-transfected with VIR-2218

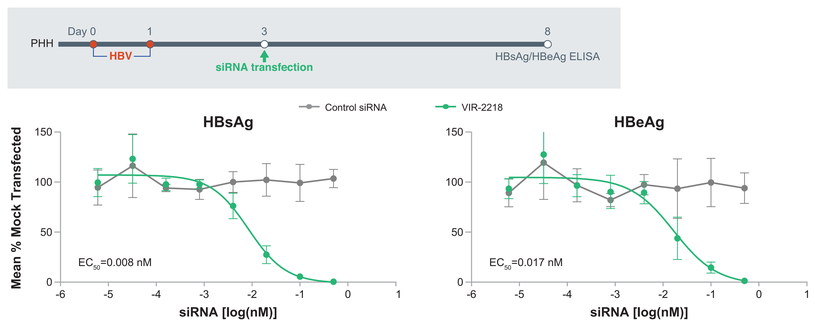
VIR-2218 Activity on *In Vitro* Authentic HBV Infection
HepG2-NTCP Cells



*siRNA targeting the luciferase gene. Error bars represent SD. IFA, immunofluorescence assay.

- VIR-2218 exhibits potent reduction of intracellular and secreted HBsAg, secreted HBeAg and HBV DNA in an authentic infection model

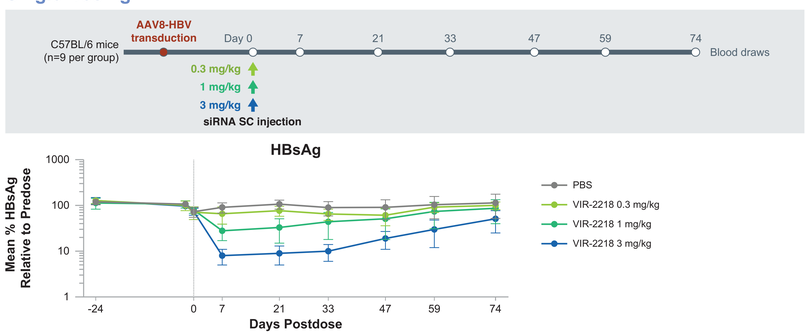
VIR-2218 Activity on *In Vitro* Authentic HBV Infection
Primary Human Hepatocytes



Error bars represent SD.

- VIR-2218 reduced HBsAg and HBeAg with picomolar potency in the PHH HBV infection model

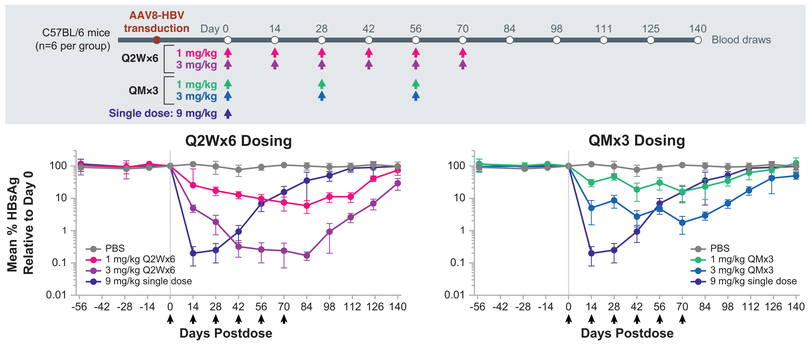
VIR-2218 *In Vivo* Activity in the AAV-HBV Mouse Model
Single Dosing



Error bars represent SD. PBS, phosphate-buffered saline.

- Single SC doses of VIR-2218 at 0.3, 1, and 3 mg/kg resulted in a sustained dose-dependent reduction of serum HBsAg with 34%, 72%, and 92% maximum reduction, respectively

VIR-2218 *In Vivo* Activity in the AAV-HBV Mouse Model
Multiple Dosing



Error bars represent SD.

- Mice receiving 1 or 3 mg/kg doses every other week × 6 or monthly × 3, or a single dose at 9 mg/kg, exhibited dose-dependent serum HBsAg reduction, with a maximum of 2.7- to 2.8-log reduction in the highest dose group

Conclusions

- VIR-2218 targets a highly conserved region of the HBV genome and demonstrates pan-genotypic effects against HBV *in vitro*
- In two authentic *in vitro* infection models, VIR-2218 demonstrated highly effective knockdown of covalently closed circular DNA-derived viral transcript with picomolar potency, leading to the decrease of HBV markers including intracellular and secreted HBsAg
- *In vivo*, VIR-2218 demonstrated potent, sustained antiviral activity in the AAV-HBV mouse model with HBsAg reduction up to 2.8-log
- These data support development of VIR-2218 for treatment of patients with chronic HBV infection

References: 1. EASL 2017 HBV Clinical Practice Guidelines. J Hepatol. 2017;67:370-98; **2.** World Health Organization. Hepatitis B. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Accessed March 28, 2019; **3.** Janas MM, et al. Nat Commun. 2018;9:723; **4.** Schlegel MK, et al. J Am Chem Soc 2017;139:8537-46; **5.** Nassal M. Gut 2015;64:1972-84.

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