SAT434

VIR-2218 Plus VIR-3434 Combination Therapy Reduces Hepatitis B Virus Surface Antigen Levels *In Vivo*

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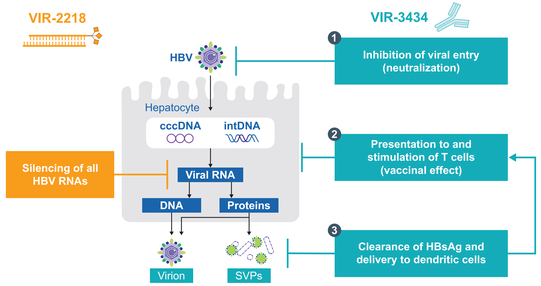
Background and Introduction

- Chronic hepatitis B virus (HBV) infection is a major global public health burden affecting approximately 296 million people worldwide, resulting in an estimated 820,000 deaths annually.[1] A significant unmet medical need remains for a curative, well-tolerated chronic hepatitis B treatment with a finite duration
- Ribonucleic acid (RNA) interference (RNAi) therapeutics targeting HBV RNAs and monoclonal antibodies (mAbs) targeting HBV surface antigen (HBsAg) represent compelling strategies for potentially enabling a functional cure in patients with chronic HBV infection
- VIR-2218 is an investigational small interfering RNA (siRNA) therapeutic that targets the HBx region of the HBV genome and demonstrates potent *in vitro* and *in vivo* antiviral activity.[2] VIR-2218 is conjugated to an N-acetyl galactosamine (GalNAc) ligand to enable targeted delivery to the liver
- VIR-3434 is an investigational neutralizing mAb targeting the antigenic loop of HBsAg with pangenotypic neutralizing activity *in vitro*.[3] Treatment with the parental molecule of VIR-3434 (HBC34) inhibits viral spread and leads to elimination of HBsAg *in vivo*. VIR-3434 carries an engineered fragment crystallizable region (Fc) that extends serum half-life (LS mutation) and increases binding to the activating Fc gamma receptors (FcgRs) FcgRIIa and FcgRIIIa but decreases binding to the inhibitory FcgRIIb (XX2/GAALIE mutation)
- VIR-2218 and VIR-3434 are currently in clinical trials as monotherapy and as a combination
- VIR-2218 and VIR-3434 target different steps in the viral replication cycle (**Figure 1**)

Figure 1. Proposed MOA: VIR-2218 and VIR-3434 Target Different Steps in the HBV Replication Cycle[2,3]



Objective

- The aim of this pre-clinical study is to evaluate the activity of VIR-2218 and VIR-3434 as monotherapy and in combination in two well-established mouse models of HBV infection: C57BL/6 mice transduced with adeno-associated virus 8-hepatitis B virus (AAV8-HBV) (genotype D) and human liver-chimeric PXB® mice infected with HBV (genotype C)

Conclusions

- Both the RNAi therapeutic VIR-2218 and the mAb VIR-3434 demonstrate pangenotypic activity against HBV *in vitro*
- Monotherapy with either VIR-2218 or HBC34-mu (murinized version of VIR-3434) is effective in reducing plasma/serum levels of HBsAg in two HBV mouse models
- Combined treatment improved suppression of plasma/serum HBsAg and HBV DNA over monotherapy and is a promising strategy for a functional HBV cure
- These data support further clinical development of combination therapy with VIR-2218 and VIR-3434 for the potential treatment of patients with chronic HBV infection

References: 1. World Health Organization. Hepatitis B. Published July 27, 2021. Accessed May 5, 2022. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. **2.** Lempp FA et al. Preclinical characterization of VIR-3434, a monoclonal antibody neutralizing hepatitis B virus that facilitates FcγR-mediated elimination of HBsAg. Poster presented at AASLD 2021; November 12-15, 2021; Virtual. **3.** Gane E et al. Poster presented at EASL 2021; June 23-26, 2021; Virtual. **4.** World Health Organization. Hepatitis D. Published July 28, 2021. Accessed May 5, 2022. https://www.who.int/news-room/fact-sheets/detail/hepatitis-d. **5.** Wang W et al. *J Hepatol*. 2021;75:311-323.

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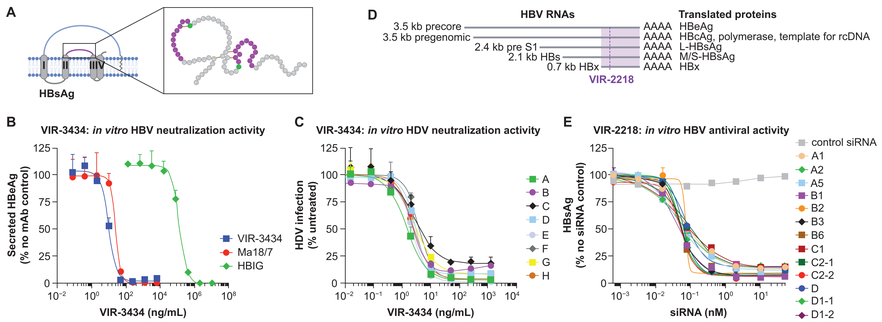
Disclosures: This analysis was funded by Vir Biotechnology, Inc. Third-party writing support was provided by ApotheCom (San Diego, CA) and was funded by Vir Biotechnology, Inc.

https://bit.ly/3xr86Yb

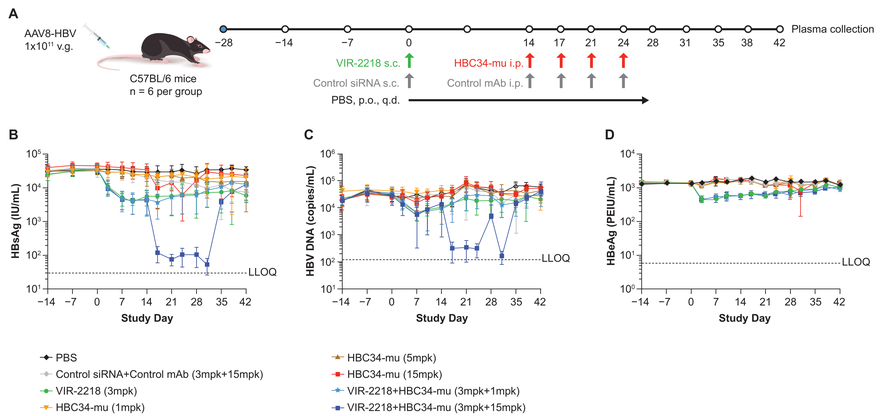
Results

Figure 2. VIR-3434 and VIR-2218 Demonstrate Potent Pangenotypic Activity Against HBV *In Vitro*



(A) VIR-3434 binds to a conserved epitope on the antigenic loop of HBsAg. **(B)** Primary human hepatocytes were infected with HBV (genotype D) in the presence of VIR-3434, preS1-targeting Ma18/7 mAb, or polyclonal hepatitis B immune globulin (HBIG). Secreted HBeAg was quantified as marker for infection 7 days after infection. **(C)** To assess the *in vitro* activity of VIR-3434 against HBV genotypes, we utilized hepatitis D virus (HDV), a satellite virus of HBV found in about 5% of patients with chronic HBV.[4] Because HDV requires the HBV envelope protein for entry into and secretion from hepatocytes, HDV can be used as a tool to study HBsAg from different HBV genotypes.[5] Neutralization by VIR-3434 was assessed in Huh7-NTCP cells that were infected with HDV pseudotyped with HBsAg from eight different HBV genotypes (A-H). **(D)** VIR-2218 targets within the HBx gene region shared by all HBV transcripts. **(E)** To evaluate the *in vitro* antiviral activity of VIR-2218 against representative HBV genotypes, the HBV1.3-overlength genome system, in which all viral RNAs are transcribed under the regulation of authentic HBV promoters, was used. Huh7 cells were transfected with plasmids containing the HBV1.3 genome sequences from 13 isolates of HBV, representing genotypes A-D, followed by transfection of VIR-2218 or control siRNA. HBsAg was used as a readout.

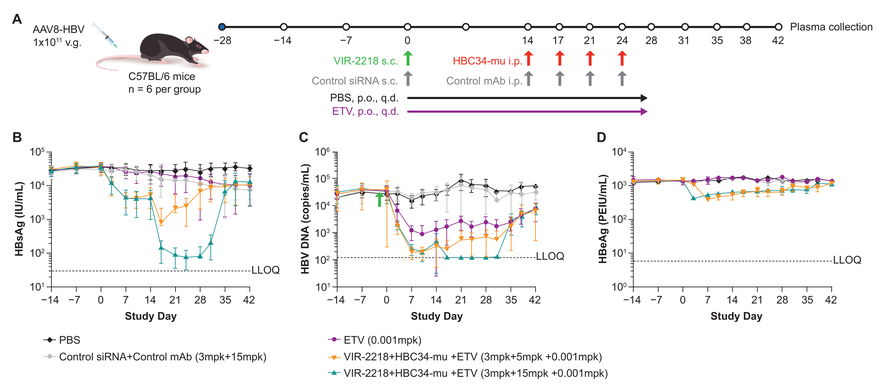
Figure 3. Combination Therapy with VIR-2218 and HBC34-mu[a] Reduced HBsAg and HBV DNA Plasma Levels in AAV8-HBV Mice



(A) Study design. C57BL/6 mice were injected with 1x10^{11} viral genomes of AAV8-HBV (genotype D) in 200 µl of PBS through tail vein. Starting at 28 days after injection (day 0), treatment was initiated as indicated. Blood was drawn for plasma collection as specified. **(B-D)** Plasma samples were analyzed at the indicated time-points for the viral markers HBsAg **(B)**, HBV DNA **(C)**, and HBeAg **(D)**.

- Compared with control, VIR-2218 led to a mild but significant reduction of plasma HBsAg, HBV DNA, and HBeAg levels (maximum mean reductions of 0.89 log, 0.65 log, and 0.51 log, respectively; $P < 0.01$), whereas 15 mpk HBC34-mu monotherapy slightly but significantly reduced plasma HBsAg (maximum mean reduction of 0.79 log, $P < 0.01$)
- The combination of VIR-2218 and 15 mpk HBC34-mu further significantly reduced plasma HBsAg and HBV DNA levels compared with VIR-2218 monotherapy (2.11 log and 2.05 log additional maximum reduction, respectively; $P < 0.01$)

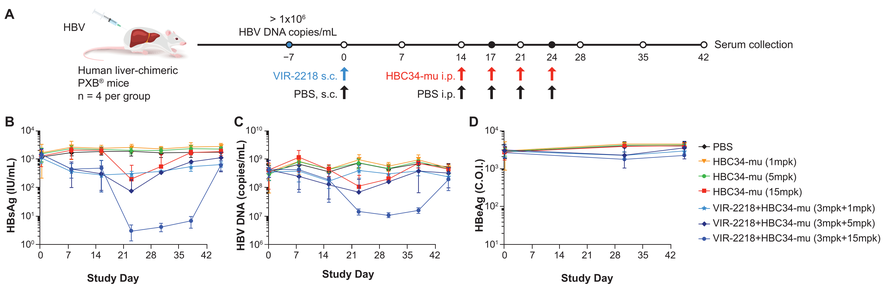
Figure 4. Combination Therapy of VIR-2218 and HBC34-mu[a] with ETV Results in Reduced HBsAg and HBV DNA Plasma Levels in AAV8-HBV Mice



(A) Study design. C57BL/6 mice were injected with 1x10^{11} viral genomes of AAV8-HBV (genotype D) in 200 µl of PBS. Starting at 28 days after injection (day 0), treatment was initiated as indicated. Blood was drawn for plasma collection as specified. **(B-D)** Plasma samples were analyzed at the indicated time-points for the viral markers HBsAg **(B)**, HBV DNA **(C)**, and HBeAg **(D)**.

- As expected, ETV treatment did not significantly lower HBsAg or HBeAg plasma levels but led to a significant reduction in HBV DNA levels compared with the vehicle control (maximum mean reduction of 1.58 log, $P < 0.01$)
- The triple combination of HBC34-mu, VIR-2218, and ETV further reduced HBV DNA levels significantly and in a dose-dependent manner (combination with 5 mpk HBC34-mu: maximum additional reduction of 0.85 log, $P < 0.01$; combination with 15 mpk HBC34-mu: maximum additional reduction of 1.26 log, $P < 0.01$)
- In contrast to ETV monotherapy, the triple combination also led to a significant reduction in HBsAg plasma levels in a dose-dependent manner (combination with 5 mpk HBC34-mu: maximum additional reduction of 1.8 log, $P < 0.01$; combination with 15 mpk HBC34-mu: maximum additional reduction of 2.39 log, $P < 0.01$)
- Compared with the results of ETV monotherapy, plasma HBeAg in mice treated with combination of the 3 articles (HBV02 + HBC34-mu + ETV: 3 + 15 + 0.001 mpk or 3 + 5 + 0.001 mpk) had statistically significant ($P < 0.01$) reductions with maximal mean reductions of 0.52 log or 0.56 log, respectively

Figure 5. VIR-2218 Plus HBC34-mu[a] Combination Therapy Reduces HBsAg and HBV DNA Serum Levels in Human Liver-Chimeric Mice



(A) Study design. Human liver-chimeric mice (PXB® mice) infected with HBV, genotype C, which showed serum HBV DNA levels of 1x10^6 copies/mL or greater on day -7, were included in the study. On day 0, treatment with the different modalities was initiated as indicated. Blood was drawn for serum collection as specified (empty circles). **(B-D)** Serum samples were analyzed at the indicated times for the viral markers HBsAg **(B)**, HBV DNA **(C)**, and HBeAg **(D)**.

- In HBV-infected liver-chimeric mice, 15 mpk HBC34-mu monotherapy resulted in a significant 1.46 log decrease in serum HBsAg ($P < 0.05$). Treatment with the combination VIR-2218 and 15 mpk HBC34-mu showed a significant reduction in serum HBsAg levels (maximum mean reduction of 2.51 log, $P < 0.05$)
- VIR-2218 and 15 mpk HBC34-mu combination also showed a significant reduction in serum HBV DNA levels (1.47 log, $P < 0.05$) compared with levels in the vehicle group, whereas treatment with 15 mpk HBC34-mu alone led to a 0.72 log ($P > 0.05$) decrease

Abbreviations: AAV8-HBV, adeno-associated virus 8-hepatitis B virus; cccDNA, covalently closed circular DNA; ETV, entecavir; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; Huh7, human hepatocarcinoma cells; intDNA, integrated DNA; i.p., intraperitoneal; IU, international unit; LLOQ, lower limit of quantification; L/M/S-HBsAg, large/medium/small HBsAg; mAb, monoclonal antibody; MOA, mechanism of action; mpk, milligrams per kilogram; PBS, phosphate buffered saline; PEIU, Paul Ehrlich Institute units; p.o., oral; q.d., once daily; rcDNA, relaxed circular DNA; RNA, ribonucleic acid; s.c., subcutaneous; siRNA, small interfering RNA; SVP, subviral particle; v.g., vector genome.

[a]HBC34-mu is a murinized version of HBC34, the parental mAb of VIR-3434. VIR-3434 and HBC34-mu showed comparable neutralization potential *in vitro* (data not shown).

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