TOP-109

VIR-2218 and VIR-3434 Therapy Is Efficacious in Preclinical Models of Hepatitis Delta Virus Infection

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Introduction

- Chronic hepatitis delta virus (HDV) infection represents the most severe form of viral hepatitis with limited treatment options. HDV is a satellite virus of hepatitis B virus (HBV) that depends on HBV-derived hepatitis B surface antigen (HBsAg) for envelopment and viral dissemination in the liver[1]
- VIR-2218 is an investigational interfering RNA (RNAi) therapeutic that targets a highly conserved region within the HBV X open reading frame and demonstrates potent *in vitro* and *in vivo* antiviral activity. VIR-2218 is conjugated to an N-acetylgalactosamine ligand to enable targeted delivery to the liver[2]
- VIR-3434 is an investigational monoclonal antibody (mAb) targeting the antigenic loop of HBsAg with pan-genotypic neutralizing activity *in vitro*. Treatment with VIR-3434 inhibits viral spread and leads to elimination of circulating HBsAg *in vivo* (**Figure 1**). The mAb carries an engineered Fc that extends serum half-life (LS mutation) and increases binding to activating Fc gamma receptors (FcγRs) FcγRIIa and IIIa but decreases binding to inhibitory FcγRIIb (XX2/GAALIE mutation)[3]
- VIR-2218 and VIR-3434 are currently in clinical trials as mono- and combination therapy in HBV-monoinfected and HBV/HDV-coinfected individuals

Conclusions

- VIR-3434 targets the conserved antigenic loop within HBsAg present on both HBV and HDV virions and mediates pan-genotypic neutralization of HDV *in vitro* with >10,000-fold higher potency than HBV-specific immunoglobulins
- *In vivo*, the parental molecule of VIR-3434 reduced the levels of HBsAg, HDV, and HBV viremia in HBV/HDV-coinfected liver-chimeric mice
- Single and combination treatments with VIR-2218 and VIR-3434 of HBV/HDV-coinfected primary human hepatocytes *in vitro* reduced HBV antigens as well as secreted infectious HDV virions. *In vivo* combination evaluations are currently ongoing
- These data support the development of VIR-2218 and VIR-3434 for treatment of patients with chronic HBV/HDV coinfection

References: 1. Urban S, et al. *Gut*. 2021;70(9):1782-1794. **2.** Lim Y-S, et al. *J Hepatol*. 2022;77(suppl 1):S69-S70. **3.** Lempp FA, et al. Poster presented at: American Association for the Study of Liver Diseases (AASLD); 12-15 November 2021; Virtual. **4.** Küttner G, et al. *Mol Immunol*. 1999;36(10): 669-683. **5.** Giersch K, et al. *JHEP Rep*. 2023;5(4):100673.

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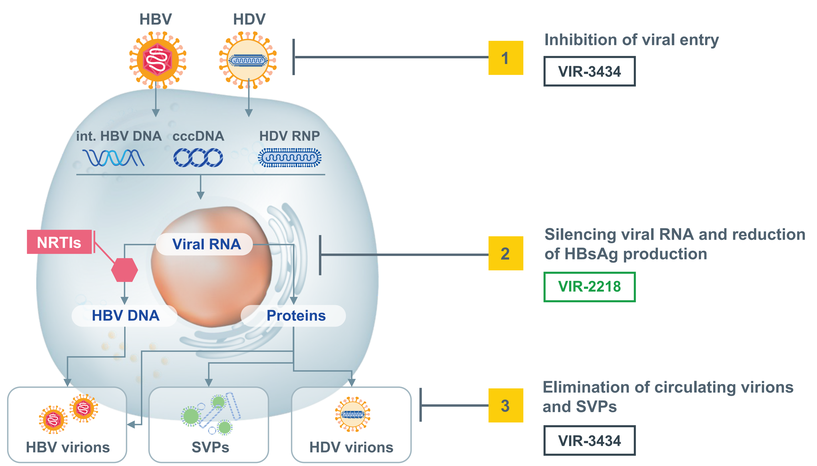
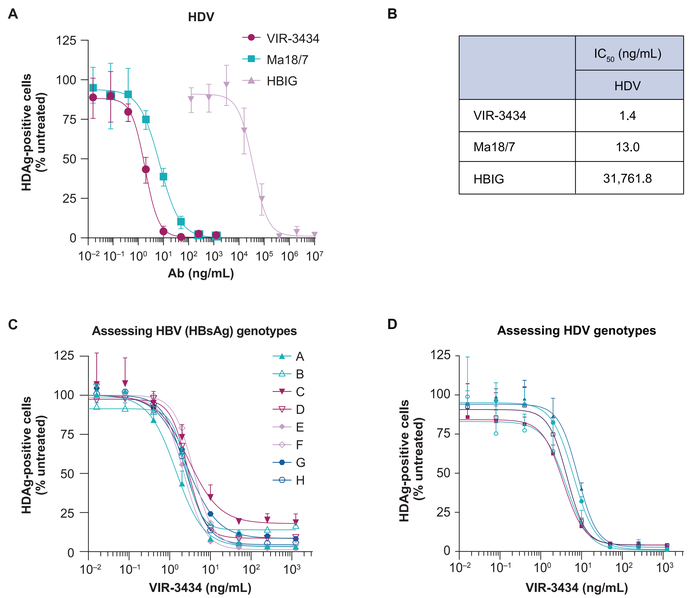
Figure 1. VIR-2218 and VIR-3434 Target Different Steps in the HBV and HDV Replication Cycles



cccDNA, covalently closed circular DNA; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; int., integrated; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; RNP, ribonucleoprotein; SVP, subviral particle.

Results

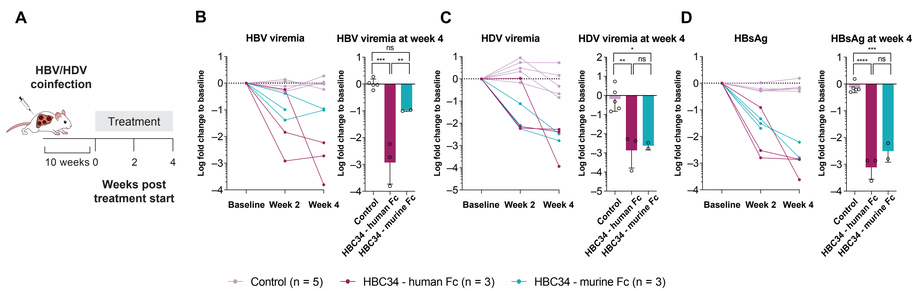
Figure 2. VIR-3434 Neutralizes HDV Infection With Pan-genotypic Activity



	IC_{50} (ng/mL)
	HDV
VIR-3434	1.4
Ma18/7	13.0
HBIG	31,761.8

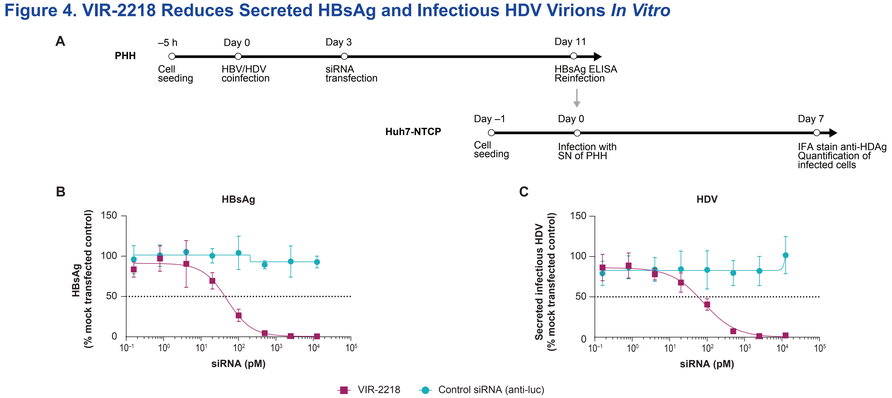
***In vitro* neutralization of HDV with VIR-3434. (A)** Huh7-NTCP cells were infected with HDV (genotype 1, enveloped with HBsAg of HBV genotype A) in the presence of VIR-3434, Ma18/7 (anti-preS1),[4] or HBIG. Cells were immunostained for HDAg and quantified 7 days post infection. **(B)** Summary of IC_{50} values. The geometric mean of 2 independent experiments is shown. **(C)** HDV (genotype 1) was enveloped with HBsAg of 8 different HBV genotypes (A-H), and neutralization by VIR-3434 was assessed in Huh7-NTCP cells. **(D)** Different HDV genotypes (1 and 5-8) were enveloped with HBsAg of HBV genotype A, and neutralization by VIR-3434 was assessed in Huh7-NTCP cells. Note that neutralization could not be assessed for HDV genotypes 2 to 4 due to insufficient viral titers.
Ab, antibody; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDAg, hepatitis delta antigen; HDV, hepatitis D virus; IC_{50}, half maximal inhibitory concentration.

Figure 3. VIR-3434 Reduces HBV and HDV Viremia in Chronically Infected Liver-chimeric Mice



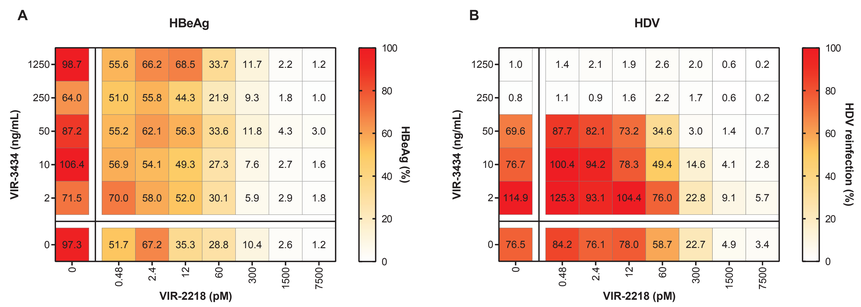
HBC34/VIR-3434 reduces HBV and HDV viremia as well as circulating HBsAg *in vivo* in chronically infected liver-chimeric mice. (A) Human liver-chimeric uPA/SCID beige mice were coinfected with HBV (genotype D) and HDV (genotype 1)[5] for 10 weeks until stable coinfection was achieved. Mice were treated for 4 weeks with HBC34 (the parental molecule of VIR-3434) either carrying the native human or an engineered murine Fc portion. **(B/C)** HBV/HDV viremia was assessed in serum by qPCR. **(D)** HBsAg quantification from mouse sera was performed using the Architect HBsAg assay (Abbott Ireland Diagnostics, Sligo, Ireland). One animal in the murine Fc group was sacrificed at week 2. Each circle represents 1 animal. The mean ± SD is shown in the bar graphs. Statistical differences were analyzed by 1-way ANOVA. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, ns p > 0.05.
ANOVA, analysis of variance; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; ns, nonsignificant; qPCR, quantitative polymerase chain reaction; SD, standard deviation; uPA/SCID, urokinase-type plasminogen activator/severe combined immunodeficiency.

Figure 4. VIR-2218 Reduces Secreted HBsAg and Infectious HDV Virions *In Vitro*



VIR-2218 reduces HBsAg and secreted HDV virions. (A) *In vitro* antiviral efficacy of VIR-2218 was determined in an HBV/HDV coinfection model of PHH. After treatment with siRNA, secreted HBsAg was quantified by ELISA **(B)** and secreted infectious HDV virions were quantified by reinfection of Huh7-NTCP cells **(C)**.
ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HDAg, hepatitis delta antigen; HDV, hepatitis D virus; HBsAg, hepatitis B surface antigen; Huh7-NTCP, sodium taurocholate co-transporting polypeptide stable expressing Huh7 cells; IFA, immunofluorescence assay; luc, luciferase; PHH, primary human hepatocytes; siRNA, small interfering RNA; SN, supernatant.

Figure 5. *In Vitro* Drug Combination Study of VIR-2218 and VIR-3434



***In vitro* antiviral efficacy of VIR-2218/VIR-3434 combination treatment was determined in an HBV/HDV coinfection model of PHH.** PHH were coinfected with HBV/HDV and treated with combinations of VIR-2218/VIR-3434 on day 3 post infection. At day 12 post infection, HBeAg **(A)** was quantified by ELISA, and secreted infectious HDV virions were quantified by reinfection of Huh7-NTCP cells **(B)**.
Note that there is no antiviral effect of VIR-3434 on HBeAg, as mAb treatment was started after initial infection. No signs of antagonism were observed.
ELISA, enzyme-linked immunosorbent assay; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; mAb, monoclonal antibody; PHH, primary human hepatocytes.

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