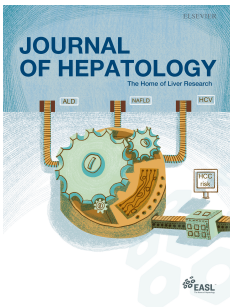


# Evaluation of RNAi Therapeutics VIR-2218 and ALN-HBV for Chronic Hepatitis B: Results From Randomized Clinical Trials

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## BACKGROUND

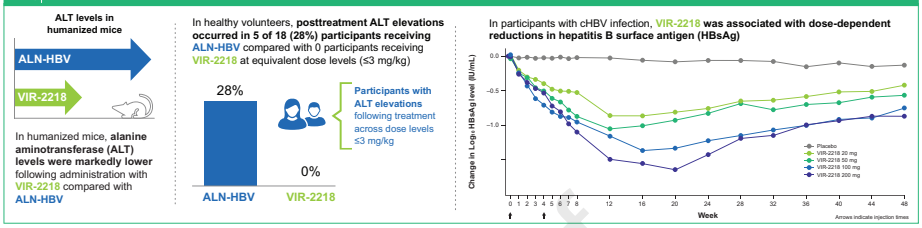
Current management of chronic hepatitis B virus (cHBV) infection requires lifelong treatment with oral antivirals. **ALN-HBV** and **VIR-2218** are investigational RNAi therapeutics that target all major HBV transcripts. **The objectives of these studies were to evaluate the safety and tolerability of a single dose of ALN-HBV and VIR-2218 in healthy adult volunteers and to evaluate the antiviral activity of VIR-2218 in adult participants with cHBV infection.**

## SUMMARY

**VIR-2218 demonstrated an encouraging hepatic safety profile in preclinical and clinical studies as well as dose-dependent HBsAg reductions in participants with cHBV infection.**

## METHODOLOGY


## RESULTS



# Evaluation of RNAi Therapeutics VIR-2218 and ALN-HBV for Chronic Hepatitis B: Results From Randomized Clinical Trials

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**Keywords**: siRNA, HBV, virology, HBV surface antigen

**Conflicts of Interest**

EG served as speaker/advisor/consultant for AbbVie, Abbot Diagnostics, Assembly Biosciences, Gilead Sciences, GSK, and Vir Biotechnology. Y-SL served as speaker/advisor/consultant for AbbVie, Arbutus Biopharma, Assembly Biosciences, Brii Biosciences, Bayer Healthcare, GSK, Gilead Sciences, Janssen, Spring Bank Pharmaceuticals, Roche, Vaccitech, and Vir Biotechnology; and received grant/research support from Bayer Healthcare and Gilead Sciences. JBK was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the study and is a current employee of Design Therapeutics, Carlsbad, California, USA. VJ is employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA. LS was employed at Vir Biotechnology at the time of the study, reports stock ownership in Vir Biotechnology and is a current employee of CareDx Inc. AIB was employed at Vir Biotechnology at the time of the study, is listed as an inventor on several patents and applications held by Vir relating to VIR-2218 and is a current employee of Denali Therapeutics, San Francisco, California, USA. SAH was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the

study and is a current employee of Beam Therapeutics, Cambridge, Massachusetts, USA. ALC is an employee of Vir Biotechnology and reports stock ownership in Vir Biotechnology. FAL is an employee of Vir Biotechnology and reports stock ownership in Vir Biotechnology. MMJ is employed by Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA and reports stock ownership in Alnylam Pharmaceuticals. DJC is an employee of Vir Biotechnology and reports stock ownership in Vir Biotechnology. CK was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the study, is a former employee of Sigilon Therapeutics and is a current employee of Takeda Pharmaceutical Company, Cambridge, Massachusetts, USA. LS-L was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the study and is a current employee of Intellia Therapeutics, Cambridge, Massachusetts, USA, reports stock ownership in Intellia Therapeutics and Taysha Gene Therapies, and serves on the Board of Directors for Alliance for Regenerative Medicine, Oligonucleotide Therapeutics Society, and Taysha Gene Therapies. GH is a paid consultant of 54Gene, Inc. JT is employed by Richmond Pharmacology, was Principal Investigator for the ALN-HBV study in healthy volunteers, and is an Honorary Senior Research Fellow at St George's University of London. He has no other interests to declare in relation to this work. PH was employed at Alnylam during the time the studies were designed and performed. SM was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the study. YIA-R was employed at Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA at the time of the study and is a current employee of Chroma Medicine. CMH is an employee of Vir Biotechnology and reports stock ownership in Vir Biotechnology. PSP is an employee of Vir Biotechnology and reports stock ownership in Vir Biotechnology. M-FY served as speaker/advisor/consultant for AbbVie, Aligos Therapeutics, Antios Therapeutics, Arbutus

Biopharma, Arrowhead Pharmaceuticals, Assembly Biosciences, Bristol-Myers Squibb, Dicerna Pharmaceuticals, Finch Therapeutics, Fujirebio Incorporation, GSK, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, Clear B Therapeutics, Springbank Pharmaceuticals, Silverback Therapeutics, Roche, Sysmex Corporation, and Vir Biotechnology.

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**Author Contributions:** EG, JBK, VJ, AIB, SAH, ALC, CK, LS-L, GH, JT, PH, YIA-R, CMH, and PSP participated in study design; EG, Y-SL, SAH, CK, JT, YIA-R, and M-FY collected data; Y-SL, JBK, VJ, LS, AIB, SAH, ALC, FAL, MMJ, DJC, CK, SM, YIA-R, CMH, PSP, and M-FY analyzed and interpreted the data; and EG, Y-SL, JBK, LS, SAH, MMJ, DJC, LS-L, JT, PSP, and M-FY drafted and revised the manuscript. All authors approved the final version of the manuscript for submission.

**Trial registration:** ClinicalTrials.gov Identifier: NCT02826018 and NCT03672188

**ABSTRACT**

**Background & Aims**: Current treatment for chronic hepatitis B virus (cHBV) infection requires lifelong treatment. New therapy aimed towards HBV functional cure would represent a clinically meaningful treatment advancement. ALN-HBV and VIR-2218 (modified from ALN-HBV by Enhanced Stabilization Chemistry Plus technology reducing off-target, seed-mediated binding while maintaining on-target antiviral activity) are investigational RNAi therapeutics that target all major HBV transcripts.

**Methods**: We report the safety of single doses of VIR-2218 and ALN-HBV in humanized mice, a cross-study comparison of single doses of VIR-2218 and ALN-HBV safety in human heathy volunteers (n=24 and n=49, respectively), and the antiviral activity of two monthly doses of 20, 50, 100, 200 mg of VIR-2218 (total n=24) vs. placebo (n=8) in participants with cHBV infection.

**Results:** In humanized mice, alanine aminotransferase (ALT) levels were markedly lower following administration with VIR-2218 compared with ALN-HBV. In healthy volunteers, posttreatment ALT elevations occurred in 28% of participants receiving ALN-HBV compared with none in those receiving VIR-2218. In participants with cHBV infection, VIR-2218 was associated with dose-dependent reductions in hepatitis B surface antigen (HBsAg). The greatest mean reduction of HBsAg at Week 20 in participants receiving 200 mg was 1.65 log IU/mL. The HBsAg reduction was maintained at 0.87 log IU/mL at Week 48. No participants had serum HBsAg loss or hepatitis B surface antibody seroconversion.

**Conclusions**: VIR-2218 demonstrated an encouraging hepatic safety profile in preclinical and clinical studies as well as dose-dependent HBsAg reductions in patients with cHBV infection. These data support future studies with VIR-2218 as part of combination regimens with a goal of HBV functional cure.

## IMPACT AND IMPLICATIONS

A significant unmet need exists for therapies for chronic HBV (cHBV) infection that achieve functional cure. We report clinical and nonclinical data on two investigational siRNAs that target HBx, ALN-HBV and VIR-2218, demonstrating that incorporation of enhanced stabilization chemistry plus (ESC+) technology in VIR-2218 reduces the propensity to cause ALT elevations as compared to the parent compound, ALN-HBV. We also show that VIR-2218 reduces hepatitis B surface antigen levels in a dose-dependent manner in participants with cHBV infection. These studies support the continued development of VIR-2218 as part of therapeutic regimen for cHBV infection with the goal of a functional cure and are important for HBV researchers and physicians.

## INTRODUCTION

Approximately 290 million people are living with chronic hepatitis B virus (cHBV) infection worldwide.[1] If left untreated, cHBV infection could result in active chronic liver disease and often progresses to cirrhosis, liver failure, hepatocellular carcinoma (HCC), and death.[2]

Currently approved treatments for cHBV infection include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and pegylated-interferon (PEG-IFN).[3] Long-term NRTI therapy reduces but does not eliminate the risk of HCC and is expected to be administered lifelong for most patients.[3] Hepatitis B surface antigen (HBsAg) loss rates remain low with NRTIs (0%-3% of patients) and PEG-IFN (3%-7%).[3] These limitations underscore the need for new, finite therapies that further reduce the risk of HCC. This goal is known as an HBV functional cure.[4]

HBV infection is associated with the expression of HBV proteins, notably HBsAg. It is hypothesized that the presence of large quantities of HBsAg contributes to T- and B-cell dysfunction.[5-13] This immune exhaustion impairs the host's ability to eradicate or control the HBV infection.[5-7] In animal models, knockdown of HBV antigens has been shown to enhance immune control.[14]

One method to reduce HBsAg is RNA interference (RNAi) using small interfering RNA (siRNA).[15-18] Delivery of siRNA to the liver is achieved by conjugation to a triantennary N-acetyl galactosamine (GalNAc) ligand that binds to hepatocytes through the asialoglycoprotein receptor.[19-21] Furthermore, chemical modifications of siRNA leads to exceptional metabolic

stability, and accumulation in acidic intracellular compartments resulting in a prolonged pharmacodynamic effect.[22]

Overlapping templates within the X region of the HBV genome [23] allow for a single siRNA to selectively and effectively target all HBV transcripts.[19] Knockdown of the HBV X protein has the potential benefit of indirectly inhibiting X-mediated upregulation of covalently closed circular DNA (cccDNA) transcription.[24] By targeting the X region near Direct Repeat 2 in a genomic region that remains intact in most integration events, knockdown of both HBV cccDNA and HBV integrated DNA transcripts ensures equipotent HBsAg knockdown in both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients. For these reasons, RNAi therapeutics offer an attractive approach for HBV functional cure.

ALN-HBV and VIR-2218 are GalNAc-conjugated siRNAs, discovered by Alnylam Pharmaceuticals, that target a site complementary to a 19-nucleotide sequence matching to position 1577 to 1596 of the HBV genome that is encoded in all major HBV messenger RNA transcripts. ALN-HBV is modified using Enhanced Stabilization Chemistry (ESC) consisting of 2′-deoxy-2′-fluoro, 2′-*O*-methyl ribose sugar modifications and phosphorothioate (PS) backbone modifications.

As described here, ALN-HBV was associated with alanine aminotransferase (ALT) level elevations in a clinical study of healthy volunteers. Evidence points to RNAi-mediated off-target effects (i.e. interference of non-HBV transcripts) as a possible mechanism.[21,25] This is due to binding of siRNA to off-target transcripts, mediated through the seed region of the siRNA guide

strand and the complementary binding site of the mRNA, referred to herein as seed-mediated binding. To mitigate these effects, the ESC modification of ALN-HBV was further modified using a process described as ESC Plus (ESC+) in a novel siRNA named VIR-2218 (ALN-HBV02; **Figure 1**). VIR-2218 has an identical sequence as ALN-HBV, except for the single substitution of a glycol nucleic acid (GNA) modification within the seed region (**Figure S1**).[20] We hypothesized this ESC+ design would reduce off-target binding while maintaining on-target activity against HBV transcripts.[26] VIR-2218 and ALN-HBV maintain similar activity in an HBV–adeno-associated virus mouse model.[25] RNA sequencing (RNA-seq) analysis demonstrated fewer differentially expressed genes with VIR-2218 compared with ALN-HBV, consistent with reduced off-target effects of the ESC+ design. Further details on the chemistry of ALN-HBV and VIR-2218 are described separately by Schlegel *et al*.[25]

Here, we report on: (i) the safety of VIR-2218 compared with ALN-HBV in humanized mice, (ii) a cross-study comparison of VIR-2218 and ALN-HBV in human heathy volunteers, and (iii) the antiviral activity of VIR-2218 in participants with cHBV infection.



## METHODS

### Preclinical Evaluation of ALN-HBV and VIR-2218 in a Chimeric Mouse Model

The hepatic safety of VIR-2218 and ALN-HBV were evaluated in the liver-chimeric PXB-Mouse® (PhoenixBio, New York, NY, USA), in which at least 70% of the animal's liver is repopulated with normal human hepatocytes.[27] The PXB-Mouse® was used as a preclinical model to predict the safety of VIR-2218 and ALN-HBV in humans. The number of animals used for the study was the minimum necessary for obtaining scientifically valid results. All the

experimental procedures used to treat live animals in this study were approved by the Animal Ethics Committee of PhoenixBio (Resolution No.: 2004).

Male PXB mice at 12 to 18 weeks of age were administered subcutaneous injections of ALN-HBV or VIR-2218 at dose levels of 12 mg/kg, 36 mg/kg, or 100 mg/kg (n = 4 mice per dosing group) on Days 0, 21, 28, 35, and 42. All doses were calculated based on the individual body weights of the mice measured prior to administration on the days of dosing. Blood was collected for analysis twice weekly for 7 weeks. The hALT1 levels were determined using Drichem NX500sV (Fujifilm, Tokyo, Japan).

**Clinical Evaluation of ALN-HBV and VIR-2218 in Healthy Volunteers and Participants With cHBV Infection**

ALN-HBV-001 and VIR-2218-1001 were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Guidelines for Good Clinical Practice, and applicable regulatory and ethical committee requirements. Written informed consent was obtained before each participant entered the respective study and before initiation of protocol-specified procedures. Each study was reviewed and approved by applicable regulatory bodies and ethics committees (see Supplemental Methods).

**(A) ALN-HBV-001 and VIR-2218-1001 Investigational Clinical Studies in Healthy Volunteers**

ALN-HBV-001 was a phase 1, participant-blinded, randomized, placebo-controlled, single-ascending-dose study whereby healthy participants received a single subcutaneous injection of

ALN-HBV at 0.1, 0.3, 1, or 3 mg/kg or placebo on study Day 1. The study was conducted at a single center in the United Kingdom. At each dose level, 4 or 8 participants were randomly assigned 3:1 to receive active study drug or placebo. Participants were 18 to 65 years of age with no uncontrolled medical conditions and normal ALT and direct bilirubin levels. Follow-up occurred for 4 weeks post dose. The primary endpoints were incidence of AEs and clinical laboratory test results. A change was made to the planned analysis to present AE grading as investigator reported (mild, moderate, or severe) instead of according to the Common Terminology Criteria for Adverse Events version 4.0 grading system as originally planned. ALN-HBV-001 was terminated early to focus on advancement of VIR-2218, and not due to safety concerns.

VIR-2218-1001 was a phase 1/2, randomized, double-blind, placebo-controlled study of subcutaneously administered VIR-2218 in healthy adult volunteers and noncirrhotic adult participants with cHBV infection who were on NRTI therapy. The primary objectives of the first part of the study were to evaluate the safety and tolerability of a single dose of VIR-2218 in healthy volunteers.

The first part of this study was conducted at a single site in New Zealand. Healthy volunteers received a single subcutaneous injection of VIR-2218 of 50, 100, 200, 400, 600, or 900 mg on study Day 1. Syringe masking was used to maintain blinding. At the start of each cohort, 2 sentinel participants were randomly assigned 1:1 to VIR-2218 or placebo, dosed concurrently, and monitored for 24 hours. The remaining participants were randomly assigned 5:1 to VIR-

2218 or placebo. Dosing was suspended or stopped if pre-specified criteria were met (see Supplemental Methods).

Participants were 18 to 55 years of age, without clinically significant medical conditions and a creatinine clearance ≥90 mL/min (per Cockcroft-Gault formula), with ALT, AST, and bilirubin level at or below the ULN. Follow-up occurred for 12 weeks post dose. The primary endpoints were incidence of AEs and clinical assessments, including laboratory test results.

**(B) VIR-2218-1001 Clinical Study in Participants With cHBV Infection**

The second part of VIR-2218-1001 evaluated multiple ascending doses of VIR-2218 in noncirrhotic participants with HBeAg-negative or HBeAg-positive cHBV infection who were on NRTI therapy for ≥6 months with HBV DNA <90 IU/mL. The study was conducted at 14 centers in Australia, Hong Kong, Republic of Korea, New Zealand, and Thailand. Participants received 2 subcutaneous VIR-2218 injections, 4 weeks apart, of 20, 50, 100, or 200 mg. At each dose level, 4 or 8 participants were randomly assigned 3:1 to VIR-2218 or placebo. Four HBeAg-negative participants received the 20-mg dose, 8 HBeAg-negative and 4 HBeAg-positive participants received the 50-mg dose, 8 HBeAg-negative participants received the 100-mg dose, and 4 HBeAg-negative and 4 HBeAg-positive participants received the 200-mg dose.

Participants were 18 to 65 years of age, with positive serum HBsAg for ≥6 months, HBsAg level >150 IU/mL and no clinically significant medical conditions other than cHBV infection and ALT or AST levels ≤2 × ULN. Participants were excluded if they had evidence of cirrhosis; a history of chronic liver disease from any cause other than cHBV infection; bilirubin,

prothrombin time, or international normalized ratio values greater than ULN; or a previous history of hepatic decompensation.

Participants were followed for 12 weeks post–second dose. Extended follow-up was required for participants with a >10% decrease in HBsAg at Week 16 compared to Day 1 pre-dose. Visits occurred every 4 weeks from Weeks 20 to 48 or until the HBsAg returned to >90% of the Day 1 pre-dose level. Dosing was stopped if pre-specified criteria were met (see Supplemental Methods).

The following viral parameters were measured to evaluate the antiviral activity of VIR-2218: HBsAg, hepatitis B core related antigen (HBcrAg), and HBV RNA. The primary endpoints were incidence of AEs and clinical assessments including but not limited to laboratory test results. Secondary antiviral endpoints included maximum reduction of serum HBsAg level from Day 1 until Week 16, serum HBsAg loss, sustained HBsAg loss for ≥6 months, anti-HBs seroconversion, and HBeAg loss and/or anti-HBe seroconversion (HBeAg-positive participants only).

**Statistical Analysis**

No formal sample size calculations were conducted. For ALN-HBV-001, up to 142 participants were initially planned to be enrolled in the study across various dose levels. For study VIR-2218-1001, up to 209 participants (56 healthy volunteers and up to 153 participants with cHBV infection) were initially planned to complete the study. For all parts of both studies, descriptive

statistics were used for continuous variables, and frequencies and percentages were used for categorical and ordinal variables.

## RESULTS

### (i) Preclinical Evaluation of ALN-HBV and VIR-2218 in a Chimeric Mouse Model

To compare the in vivo hepatic safety of ALN-HBV versus VIR-2218, human ALT 1 (hALT1) levels were evaluated in a humanized liver mouse model.[27] As shown in **Figure 2a**, hALT1 levels were markedly lower following administration of VIR-2218 compared with ALN-HBV at equivalent dose levels up to 100 mg/kg. With ALN-HBV, a clear dose-dependent increase in hALT1 levels was observed, whereas no relationship between hALT1 and dose level was observed with VIR-2218.

### (ii) Clinical Evaluation of ALN-HBV and VIR-2218 in Healthy Volunteers

In the ALN-HBV-001 clinical trial, 24 healthy volunteers were randomly assigned to receive a single dose of ALN-HBV (0.1, 0.3, 1, or 3 mg/kg) or placebo. In the VIR-2218-1001 study, 50 healthy volunteers were randomly assigned, of whom 49 volunteers received a single fixed dose of VIR-2218 (50, 100, 200, 400, 600, or 900 mg) or placebo (**Figure S2**). One participant assigned to the 400-mg VIR-2218 group withdrew consent before receiving study treatment and was excluded from the analysis. The majority of participants in both studies completed all scheduled safety follow-ups.

Within the ALN-HBV-001 and VIR-2218-1001 studies, demographic characteristics were generally well balanced across treatment groups (**Table S1**). Additionally, across studies,

demographic characteristics were generally well balanced except for a trend toward lower body mass index in ALN-HBV-001 compared with VIR-2218-1001 (mean: 22.0 vs 24.5 $kg/m^2$, respectively).

In ALN-HBV-001, the only AEs reported in more than 1 ALN-HBV–treated participant were nasopharyngitis and headache. These were mild events that occurred in 2 ALN-HBV–treated participants each and resolved without intervention. One injection-site reaction was reported, a mild event in the ALN-HBV 3.0-mg/kg group that resolved the following day without treatment. With the exception of ALT level elevation, as further described next, no clinically significant changes in clinical laboratory parameters, vital signs, or electrocardiograms (ECGs) were observed across treatment groups.

In VIR-2218-1001, a similar rate of AEs was observed in active treatment groups compared with placebo treatment groups (**Table S2**). The most common AE was headache, which occurred in 9 of 37 (24%) and 2 of 12 (17%) VIR-2218– and placebo-treated participants, respectively. A total of 7 of 37 (19%) VIR-2218–treated participants versus 0 placebo-treated participants experienced injection-site reactions, all of which were grade 1 in severity, resolved without intervention, and only 1 was considered related to study drug by the investigator. A non-related grade 3 AE of respiratory tract infection was observed in the 600-mg cohort. No dose-related trends in types or incidence of AEs were observed. No clinically significant changes in laboratory parameters, vital signs, or ECGs were observed across treatment groups.

A summary of the highest postbaseline ALT value relative to the upper limit of normal (ULN) for each participant in ALN-HBV-001 and VIR-2218-1001 is presented in **Figure 2 (b-c)**. In VIR-2218-1001 (**Figure 2c**), the mean mg/kg dose levels for the 50-, 100-, and 200-mg dose cohorts were 0.8, 1.5, and 2.7 mg/kg, respectively, which are similar to the dose levels received in ALN-HBV-001 (**Figure 2b**). In ALN-HBV-001, postbaseline ALT values above the ULN were observed in 5 of 18 (28%) participants across dose levels (**Figure 2b**). In VIR-2218-1001, postbaseline ALT values greater than ULN were observed in 0 participants at comparable dose levels (0.8-2.7 mg/kg; **Figure 2c**). Furthermore, VIR-2218-1001 evaluated dose levels approximately 5-fold higher than the highest dose evaluated in ALN-HBV-001. A low propensity to cause ALT level elevations was maintained at higher doses of VIR-2218 equating up to 13 mg/kg.

No ALT elevations in either study were associated with increases in bilirubin above ULN. No changes in functional status of the liver (e.g., albumin or coagulation parameters) or clinical signs/symptoms of hepatic dysfunction were observed.

**(iii) Clinical Evaluation of VIR-2218 in Participants With cHBV Infection**

Thirty two participants with cHBV infection were randomly assigned to receive 2 monthly doses of VIR-2218 (20, 50, 100, or 200 mg) or placebo (**Figure S3**). All participants were dosed with the allocated treatment and included in the analysis. Only 2 of the VIR-2218 participants failed to complete the full 48 weeks of study visits.

Demographics and baseline characteristics were generally well balanced across treatment groups (**Table 1**). Overall, participants were predominantly male and Asian. Baseline HBsAg levels were similar across treatment groups, with expectedly younger age and higher baseline HBsAg levels observed in HBeAg-positive cohorts.

A summary of AEs is presented in **Table 2**. Overall, AEs were reported in 13 of 24 (54%) participants in the VIR-2218 treatment groups and 2 of 8 (25%) participants in the placebo treatment groups. The most common AE was headache, which occurred in 6 of 24 (25%) and 0 of 8 (0%) VIR-2218– and placebo-treated participants, respectively. A single injection-site reaction of Grade 1 injection-site pain occurred (100-mg cohort). One VIR-2218-related serious AE of grade 2 headache was reported, which resolved with intravenous hydration and non-narcotic pain medication.

Grade 1 ALT level elevations were observed in 5 of 24 (21%) VIR-2218–treated participants (50-mg cohort [n = 2]; 100-mg cohort [n = 1]; and 200-mg cohort [n = 2]) and 1 of 8 (13%) placebo-treated participants. Four of 5 active participants with ALT level elevation who received VIR-2218 and the single placebo-treated participant with ALT level elevation were HBeAg positive. No dose-response relationship was observed. Additionally, all ALT level elevations were asymptomatic and not associated with changes in other functional parameters. All ALT level elevations resolved during the study.

The mean dose levels for each dose of 20 mg, 50 mg, 100 mg, and 200 mg were 0.3 mg/kg, 0.7 mg/kg, 1.5 mg/kg, and 3.3 mg/kg, respectively (**Figure 2d**). The maximum ALT levels were

higher among HBeAg-positive participants as compared with healthy volunteers (**Figure 2c**) or HBeAg-negative participants, but were generally <2.3 × ULN for those dosed up to 200 mg (**Figure 2d; Figure S4**). No trends or clinically significant changes in other laboratory parameters, vital signs, or ECGs were observed.

The mean log reduction in HBsAg level in each dose cohort plotted against time is depicted in **Figure 3**. Reductions in HBsAg were observed across all VIR-2218 groups relative to placebo. Higher doses of VIR-2218 were associated with greater HBsAg reduction and more delayed HBsAg rebound. In participants receiving 200 mg VIR-2218, the greatest mean reduction of HBsAg occurred at Week 20 (1.65 log IU/mL). HBeAg status did not impact maximum HBsAg response achieved. In the 50-mg and 200-mg cohorts, the mean (SD) maximum HBsAg decline was 1.37 (0.61) $\log_{10}$ IU/mL in HBeAg-negative participants and 1.36 (0.51) $\log_{10}$ IU/mL in HBeAg-positive participants (**Figure 4**).

A total of 12 (50%) participants (1 of 3 in 20-mg, 4 of 9 in 50-mg, 4 of 6 in 100-mg, and 3 of 6 in 200-mg VIR-2218 cohorts) experienced an HBsAg reduction to an absolute level <100 IU/mL, all of whom achieved nadir by Week 16. In the 7 participants receiving 100 mg and 200 mg of VIR-2218 who achieved HBsAg level reduction to <100 IU/mL, 5 maintained HBsAg levels <100 IU/mL at Week 48. HBsAg level reductions were more sustained in higher dose cohorts, with mean (SD) reductions of 0.87 (0.55) $\log_{10}$ IU/mL in the 200 mg cohort and 0.75 (0.51) $\log_{10}$ IU/mL in the 100 mg cohort at Week 48 (**Figure 3**).

No participants had serum HBsAg loss or hepatitis B surface antibody (anti-HBs) seroconversion. One HBeAg-positive participant had HBeAg loss at Week 24 (less than the lower limit of quantitation of 0.11 IU/mL) and hepatitis B e antibody (anti-HBe) seroconversion at Week 16, of which both were sustained through last follow-up at Week 48. No apparent difference was observed in maximum HBsAg level decline or rebound rates between patients with or without ALT level elevations.

The majority of HBeAg-negative study participants did not have measurable HBV RNA at baseline, which precluded the ability to analyze post-treatment effects in most participants. However, post-treatment reductions in HBcrAg, HBeAg and HBV RNA in HBeAg-positive patients are described in **Figure S5**.

## DISCUSSION

Although HBV vaccination should eventually halt HBV transmission, global eradication through vaccination is estimated to take another 90 years and will not benefit the almost 300 million adults living with cHBV infection.[17,28] The availability of a safe and effective finite treatment would significantly improve treatment adherence and uptake, thereby reducing HBV-related morbidity and mortality.[17]

By silencing viral protein synthesis, translation inhibitors should directly inhibit hepatitis B virion and subviral particle production. They may also indirectly boost host innate and adaptive immune responses. Translation inhibitors are the backbone of novel HBV cure regimens currently being evaluated in clinical studies, in combination with other new molecular entities.[17]

ALN-HBV is the parent molecule siRNA targeting the X gene of HBV. The development was discontinued following the observation of dose-dependent, asymptomatic, and transient ALT level elevations in phase 1 studies in healthy volunteers to further the development of VIR-2218. VIR-2218 is an ESC+ version of ALN-HBV designed to reduce binding of off-target host RNAs,[25] thereby potentially improving the hepatic safety relative to ALN-HBV.

The potential to improve hepatic safety was first demonstrated preclinically. In an in vitro study in HepG2.2.15 cells, RNA-seq analysis demonstrated fewer differentially expressed genes and a lower magnitude of gene dysregulation in cells treated with VIR-2218 compared with those treated with ALN-HBV, supporting reduced off-target effects with ESC+ siRNA.[25] More notably, in chimeric mice with humanized liver, VIR-2218 showed a markedly reduced propensity to cause hALT1 level elevations relative to ALN-HBV at equivalent dose levels.

These findings are supported by clinical studies in healthy volunteers and participants with cHBV infection. Single doses of ALN-HBV in healthy volunteers were associated with dose-dependent ALT level elevations, including ALT elevations up to 8.6 × ULN with the highest dose of 3 mg/kg. At similar doses, VIR-2218 was not associated with any ALT level elevations. Additionally, VIR-2218 was evaluated up to a dose of 900 mg in healthy volunteers, or approximately 5-fold the highest dose evaluated with ALN-HBV. At 900 mg, mild ALT elevations were observed but did not exceed 3.0 × ULN. All ALT level elevations were asymptomatic with no other evidence of impaired hepatic function. Lastly, in participants with cHBV infection, ALT level elevations were infrequent, did not exceed 2.3 × ULN, and did not

correlate with dose level. Collectively, the preclinical to clinical translation supports the potential of ESC+ technology to improve the hepatic safety profile of siRNAs.

The antiviral results in the phase 2 study in participants with cHBV infection demonstrate that a single siRNA target within the X coding region is associated with similar HBsAg reductions in both HBeAg-negative and HBeAg-positive participant populations, suggesting the target sequence is preserved in both HBV cccDNA and integrated DNA transcripts. Following 2 monthly doses of VIR-2218, dose-dependent HBsAg reductions were observed through the final follow-up visit at Week 48, with the maximum HBsAg reductions generally observed by Week 20. In addition, 50% of participants across all dose levels of VIR-2218 achieved HBsAg levels <100 IU/mL. At 100 and 200 mg doses, the HBsAg reduction was more sustained during follow-up. It is noteworthy that HBsAg level <100 IU/mL has been associated with a significantly higher chance of subsequent HBsAg loss.[29] The durability of HBsAg response associated with only 2 monthly doses of VIR-2218 is indicative of a prolonged pharmacodynamic effect. The prolonged pharmacodynamic effect of VIR-2218 is consistent with the effects of GalNAc-conjugated siRNA therapeutics used for other indications and possibly attributed, in part, to the acid stability of siRNA leading to accumulation within endosomes in the cytoplasm and a pharmacokinetic depot effect.[22]

There are some limitations to this analysis. First, the limited sample size within each cohort is not sufficient to observe rare AEs as well as HBsAg seroclearance. However, VIR-2218 was generally well tolerated based on the types and severity of AEs reported and a clear dose-related HBsAg reductive effect was observed. Also, due to the limited sample size, we were unable to

compare the magnitude of HBsAg level reduction in participants with different baseline HBsAg levels. Second, genotype could not be determined in most participants due to baseline viral DNA suppression. However, nearly all participants were Asian and enrolled in the Asia and the Pacific regions, which may limit the HBV genotypes diversity in this study. It is yet to be determined whether these results are generalizable to other populations, such as Caucasian patients more commonly infected with HBV genotype A or D as opposed to genotypes B or C in the Asian population.[30] Third, participants with severe fibrosis/cirrhosis were not included in this study and the safety of VIR-2218 will need to be assessed separately in these populations. Lastly, cross-trial comparisons between ALN-HBV-001 and VIR-2218-1001 should be interpreted with caution due to differences in the study designs and patient populations.

In conclusion, the preclinical and clinical results of VIR-2218 support continued development for a functional cure of cHBV infection. Although no instances of HBsAg loss were observed in this study, it is hypothesized that when combined with a targeted immune stimulatory agent, VIR-2218 could play a key role in achieving a functional cure in patients with cHBV infection. Importantly, any functional cure treatment will have to be safe and well tolerated. The hepatic safety profile demonstrated to date by VIR-2218 is therefore encouraging.

**List of Abbreviations**: HBV, hepatitis B virus; ESC+, Enhanced Stabilization Chemistry Plus; cHBV, chronic hepatitis B virus; HCC, hepatocellular carcinoma; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PEG-IFN, pegylated-interferon; HBsAg, hepatitis B surface antigen; siRNA, short interfering RNA; GalNAc, N-acetyl galactosamine; HBeAg, hepatitis B e antigen; PS, phosphorothioate; ALT, alanine aminotransferase; GNA, glycol nucleic acid; RNA-

seq, RNA sequencing; hALT1, human ALT 1; AE, adverse event; AST, aspartate aminotransferase; ECG, electrocardiogram; ULN, upper limit of normal; SD, standard deviation; anti-HBe, hepatitis B e antibody.

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**Data Sharing Statement**

All data requests will be reviewed by the study sponsor (Vir Biotechnology, Inc.) and an agreement may be required. Requests for data may be made to Daniel Cloutier (dcloutier@vir.bio).

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Author names in bold designate shared co-first authorship.

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**Table 1. Summary of Demographics and Baseline Characteristics in a Phase 2 Study in Participants With cHBV Infection (VIR-2218-1001)**

| | HBeAg-negative participants | | | | | HBeAg-positive participants | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| | | VIR-2218 | | | | | VIR-2218 | | |
| **Participants, n (%)** | **Placebo (n = 6)** | **20 mg (n = 3)** | **50 mg (n = 6)** | **100 mg (n = 6)** | **200 mg (n = 3)** | **Placebo (n = 2)** | **50 mg (n = 3)** | **200 mg (n = 3)** | **VIR-2218 (n = 24)** |
| Mean age, y (SD) | 44 (7) | 40 (9) | 43 (11) | 45 (6) | 55 (4) | 59 (8) | 35 (10) | 34 (13) | 42 (10) |
| Male, n (%) | 3 (50) | 2 (67) | 5 (83) | 5 (83) | 0 (0) | 1 (50) | 1 (33) | 2 (67) | 15 (63) |
| Race, n (%) | | | | | | | | | |
| Asian | 6 (100) | 3 (100) | 5 (83) | 5 (83) | 3 (100) | 2 (100) | 3 (100) | 3 (100) | 22 (92) |
| White | 0 (0) | 0 (0) | 0 (0) | 1 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (4) |
| Other | 0 (0) | 0 (0) | 1 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (4) |
| Region/country, n (%) | | | | | | | | | |
| Australia | 0 (0) | 0 (0) | 0 (0) | 2 (33) | 0 (0) | 0 (0) | 0 (0) | 2 (67) | 4 (17) |
| Hong Kong, China | 2 (33) | 1 (33) | 3 (50) | 0 (0) | 0 (0) | 0 (0) | 1 (33) | 1 (33) | 6 (25) |
| New Zealand | 0 (0) | 0 (0) | 2 (33) | 1 (17) | 0 (0) | 1 (50) | 1 (33) | 0 (0) | 4 (17) |
| Republic of Korea | 1 (17) | 1 (33) | 1 (17) | 2 (33) | 2 (67) | 0 (0) | 1 (33) | 0 (0) | 7 (29) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Thailand | 3 (50) | 1 (33) | 0 (0) | 1 (17) | 1 (33) | 1 (50) | 0 (0) | 0 (0) | 3 (13) |
| Mean BMI, $kg/m^2$ (SD) | 21 (3) | 22 (4) | 25 (4) | 23 (3) | 23 (2) | 24 (2) | 27 (4) | 25 (4) | 24 (3) |
| Mean $log_{10}$ HBsAg level, IU/mL, (SD) | 3.5 (0.4) | 3.3 (0.3) | 3.3 (0.5) | 3.4 (0.5) | 3.3 (0.4) | 3.2 (0.3) | 3.5 (0.3) | 3.9 (0.6) | 3.4 (0.5) |
| Mean baseline ALT level, U/L, (SD)* | 21.8 (17.6) | 15.3 (4.6) | 23.5 (14.9) | 14.3 (5.0) | 10.0 (4.0) | 26.5 (10.6) | 27.7 (18.6) | 26.0 (17.7) | 19.3 (12.4) |
| Mean fibrosis score (SD) | 4.7 (0.9) | 5.8 (0.8) | 5.2 (1.5) | 5.1 (1.8) | 5.9 (1.0) | 7.1 (0.4) | 4.9 (1.7) | 5.4 (0.7) | 5.3 (1.3) |
| Cirrhosis, n (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Baseline is defined as the last valid non-missing assessment prior to first study drug administration.

cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; SD, standard deviation; BMI, body mass index; HBsAg, hepatitis B virus surface antigen; ALT, alanine aminotransferase.

**Table 2. AEs Summary in Participants With cHBV Infection.**

| | HBeAg-negative participants | | | | | HBeAg-positive participants | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | VIR-2218 | | | | | VIR-2218 | | |
| **Participants, n (%)** | **Placebo (n = 6)** | **20 mg (n = 3)** | **50 mg (n = 6)** | **100 mg (n = 6)** | **200 mg (n = 3)** | **Placebo (n = 2)** | **50 mg (n = 3)** | **200 mg (n = 3)** | **Overall VIR-2218 (n = 24)** |
| Any TEAE | 1 (17) | 0 (0) | 2 (33) | 5 (83) | 2 (67) | 1 (50) | 2 (67) | 2 (67) | 13 (54) |
| Treatment-related TEAE | 0 (0) | 0 (0) | 1 (17) | 2 (33) | 1 (33) | 0 (0) | 1 (33) | 0 (0) | 5 (21) |
| Grade ≥3 TEAE* | 0 (0) | 0 (0) | 0 (0) | 1 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (4) |
| Serious TEAE[†] | 0 (0) | 0 (0) | 0 (0) | 1 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (4) |
| Treatment-related serious TEAE[†] | 0 (0) | 0 (0) | 0 (0) | 1 (17) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (4) |
| TEAE leading to discontinuation of study drug | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| TEAE resulting in death | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

*Grade 3 nonserious TEAE of hypophosphatemia (considered not related to the study drug), which is a known adverse reaction of a concomitant medication tenofovir disoproxil fumarate.

[†]Grade 2 serious TEAE of headache considered to be related to study drug by the investigator, but the Sponsor determined that the constellation of concurrent symptoms (fever, headache, nausea, vomiting, and dehydration) were more consistent with a viral syndrome than a drug reaction and assessed the event as not related to study drug.

AE, adverse event; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; TEAE, treatment-emergent adverse event.

## Figure Legends

### Figure 1. Enhanced stabilization chemistry plus (ESC+).

Enhanced Stabilization Chemistry Plus (ESC+) was designed to decrease seed-mediated off-target binding while maintaining on-target activity and has demonstrated an improved hepatic safety profile and therapeutic index in rodents. For further details on this strategy, please see our companion manuscript.[25]

miRNA, microRNA; mRNA, messenger RNA; UTR, untranslated region; GNA, glycol nucleic acid.

### Figure 2. Comparison of post-treatment ALT levels between ALN-HBV and VIR-2218 in (A) nonclinical and (B-D) clinical studies.

(A) Post-treatment hALT levels were measured over time using a chimeric mouse model with ALN-HBV and VIR-2218 with doses administered on Days 0, 21, 28, 35, and 42. For individual-level data, see **Figure S6**. (B) Maximum ALT levels were measured following a single dose of ALN-HBV in healthy volunteers. (C) Maximum ALT levels were measured following a single dose of VIR-2218 in healthy volunteers. In Figure 2c, 2 healthy volunteers had baseline ALT values greater than ULN. (D) Maximum ALT levels were measured following 2 doses of VIR-2218 in participants with cHBV infection. In Figure 2d, 3 participants with cHBV infection had baseline ALT values greater than ULN. In Figure 2c and 2d, the color scheme represents the dose levels; the weight-based dose levels in mg/kg correspond to the fixed-dose administration of VIR-2218 that was based on the participant's actual body weight.

ALT, alanine aminotransferase; hALT, human alanine aminotransferase; SD, standard deviation; PBS, phosphate-buffered saline; ULN, upper limit of normal; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen.

**Figure 3. Post-treatment mean HBsAg reduction over time in participants with cHBV infection (study VIR-2218-1001).**

Arrows indicate injection times (Weeks 0 and 4).

The mean HBsAg level reduction was calculated based on available HBsAg levels at each timepoint: the majority of participants had available data at all timepoints; the limited missing data is described in the supplement. For participant-level data, see **Figure S7**.

HBsAg, hepatitis B virus surface antigen; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen.

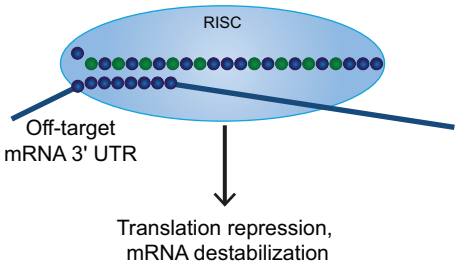
**Figure 4. Individual maximum post-treatment HBsAg level reductions in participants with cHBV infection (study VIR-2218-1001).**

For each box, the center line represents the median, the box limits represent the upper and lower quartiles, and the whiskers extend to the minimum and maximum.

HBsAg, hepatitis B virus surface antigen; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen.

**No ESC+ conjugate**

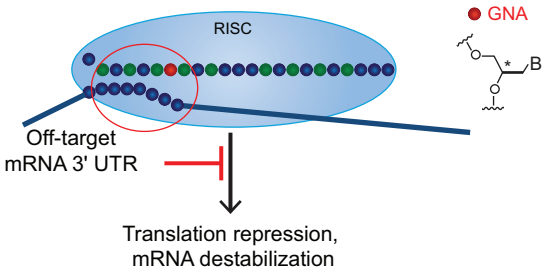
**miRNA-like seed-mediated binding to off-targets**



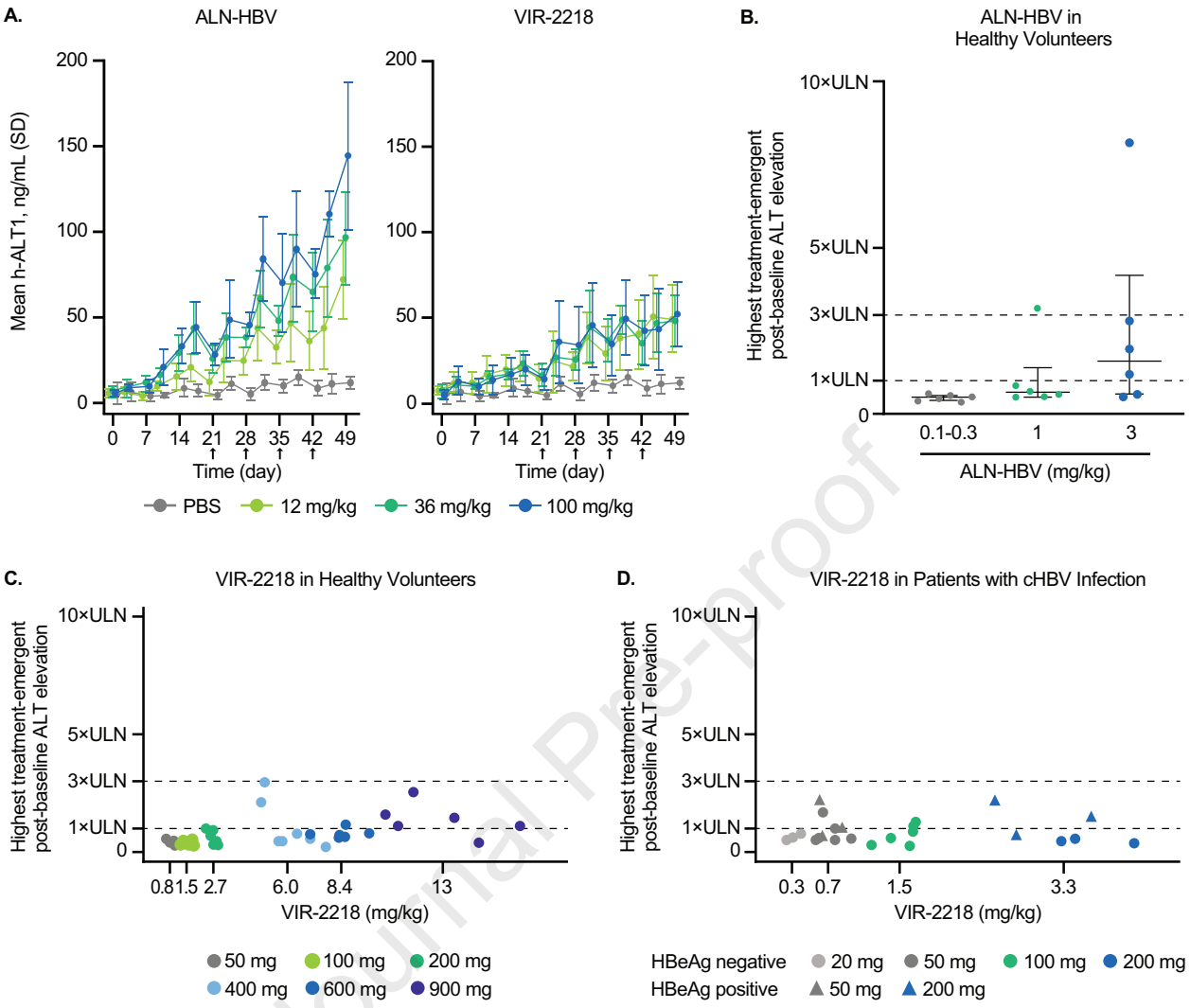

**Off-target effects**

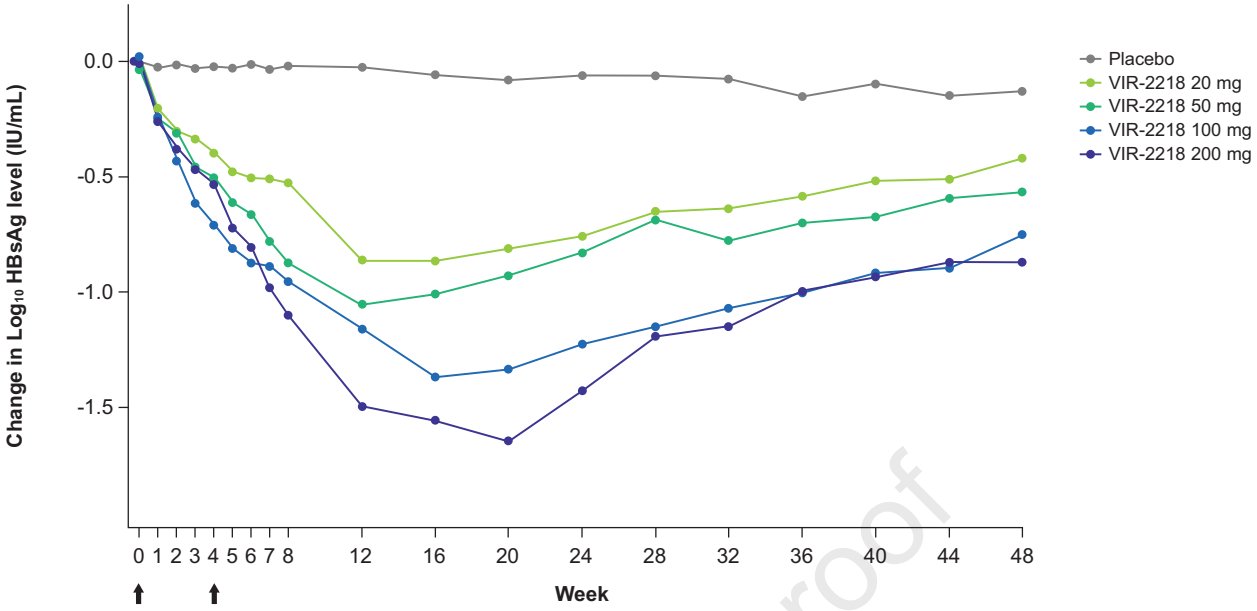
**ESC+ conjugate**

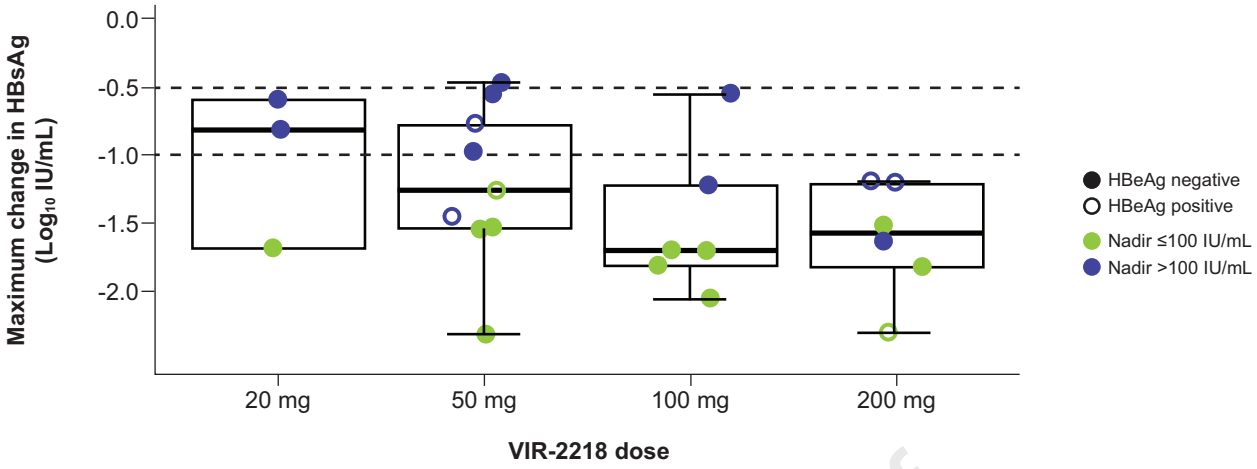
**Destabilize seed-mediated binding to off-targets; retain on-target activity**




**Off-target effects minimized**







## HIGHLIGHTS

- VIR-2218 and ALN-HBV are siRNAs targeting all HBV mRNA transcripts
- VIR-2218 is a glycol nucleic acid-modified version of ALN-HBV
- ALT elevations were more pronounced with ALN-HBV than VIR-2218 in mice and humans
- VIR-2218 reduced HBsAg levels in participants with chronic HBV infection
- VIR-2218 had a positive hepatic safety profile in preclinical and clinical studies