Research Article
Viral Hepatitis

JOURNAL
OF HEPATOLOGY

Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: Results from randomized clinical trials

Ed Gane[1,*], Young-Suk Lim[2], Jae B. Kim[3,a,b], Vasant Jadhav[3], Ling Shen[4], Anna I. Bakardjiev[4,a,c], Stephen A. Huang[3,a,d], Andrea L. Cathcart[4], Florian A. Lempp[4], Maja M. Janas[3], Daniel J. Cloutier[4], Charalambos Kaittanis[3,a,e], Laura Sepp-Lorenzino[3,a,f], Gregory Hinkle[3], Jorg Taubel[5], Patrick Haslett[3,a], Stuart Milstein[3], Yesseinia I. Anglero-Rodriguez[3], Christy M. Hebner[4], Phillip S. Pang[4], Man-Fung Yuen[6]

Journal of Hepatology **2023**. vol. 79 | 924–932

Background & Aims: Current therapy for chronic hepatitis B virus (cHBV) infection involves lifelong treatment. New treatments that enable HBV functional cure would represent a clinically meaningful advance. ALN-HBV and VIR-2218 are investigational RNA interference therapeutics that target all major HBV transcripts.
Methods: We report on: i) the safety of single doses of VIR-2218 (modified from ALN-HBV by enhanced stabilization chemistry plus technology to reduce off-target, seed-mediated binding while maintaining on-target antiviral activity) and ALN-HBV in humanized mice; ii) a cross-study comparison of the safety of single doses of VIR-2218 and ALN-HBV in healthy human volunteers (n = 24 and n = 49, respectively); and iii) the antiviral activity of two doses of 20, 50, 100, 200 mg of VIR-2218 (total n = 24) *vs.* placebo (n = 8), given 4 weeks apart, in participants with cHBV infection.
Results: In humanized mice, alanine aminotransferase (ALT) levels were markedly lower following administration of VIR-2218 compared with ALN-HBV. In healthy volunteers, post-treatment 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.
Trial registration: ClinicalTrials.gov identifiers: NCT02826018 and NCT03672188.

Introduction

Approximately 290 million people are living with chronic hepatitis B virus (cHBV) infection worldwide.[1] If left untreated, cHBV infection can 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 can induce HBsAg loss (functional cure) and further reduce the risk of HCC.[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]

Keywords: siRNA; HBV; virology; HBV surface antigen.
Received 6 October 2022; received in revised form 27 March 2023; accepted 15 May 2023; available online 7 June 2023
* Corresponding author. Address: NZCR House, 3 Ferncroft Street, Grafton, Auckland, 1010, New Zealand; Tel.: +64 6421 548371, fax: +64 0800 61 62 64.
E-mail address: EdGane@adhb.govt.nz (E. Gane).
[a] At the time of the study.
[b] Current affiliation: Design Therapeutics, Carlsbad, California, USA.
[c] Current affiliation: Denali Therapeutics, San Francisco, California, USA.
[d] Current affiliation: Beam Therapeutics, Cambridge, Massachusetts, USA.
[e] Current affiliation: Takeda Pharmaceutical Company, Cambridge, Massachusetts, USA.
[f] Current affiliation: Intellia Therapeutics, Cambridge, Massachusetts, USA.
https://doi.org/10.1016/j.jhep.2023.05.023

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 lead 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 to potentially achieving 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 1,577 to 1,596 of the HBV genome, which is encoded in all major HBV mRNA transcripts. ALN-HBV is modified using enhanced stabilization chemistry (ESC) consisting of 2′-deoxy-2′-fluoro, 2′-*O*-methyl ribose sugar modifications and phosphorothioate backbone modifications.

As described here, ALN-HBV was associated with elevations in alanine aminotransferase (ALT) levels 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+) into a novel siRNA named VIR-2218 (ALN-HBV02; Fig. 1). VIR-2218 has an identical sequence as ALN-HBV, except for the single substitution of a glycol nucleic acid modification within the seed region (Fig. 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 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]

Herein, 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 healthy volunteers, and (iii) the antiviral activity of VIR-2218 in participants with cHBV infection.

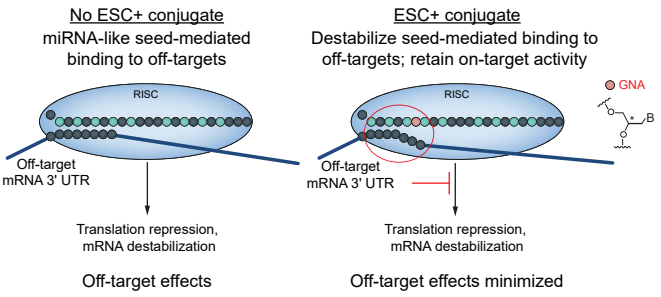


Fig. 1. Enhanced stabilization chemistry plus (ESC+). ESC+ was designed to decrease seed-mediated off-target binding while maintaining on-target activity and has demonstrated an improved hepatic safety profile and therapeutic index in rodents. For further details on this strategy, please see our companion manuscript.[25] ESC+, enhanced stabilization chemistry plus; GNA, glycol nucleic acid; miRNA, microRNA; UTR, untranslated region.

Materials and 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 human ALT 1 (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 supplementary methods).

ALN-HBV-001 and VIR-2218-1001 investigational clinical studies in healthy volunteers

ALN-HBV-001 was a phase I, 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 UK. At each dose level, four or eight 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 was carried out for 4 weeks post dose. The primary endpoints were incidence of adverse events (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 advancing the development of VIR-2218, and not due to safety concerns.

VIR-2218-1001 was a phase I/II, randomized, double-blind, placebo-controlled study of subcutaneously administered VIR-2218 in healthy adult volunteers and non-cirrhotic 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, two sentinel participants were randomly assigned 1:1 to VIR-2218 or placebo, dosed concurrently, and monitored for 24 h. 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 supplementary 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, aspartate aminotransferase, and bilirubin level at or below the upper limit of normal (ULN). Follow-up was carried out for 12 weeks post dose. The primary endpoints were incidence of AEs and clinical assessments, including laboratory test results.

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 non-cirrhotic 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 two subcutaneous VIR-2218 injections, 4 weeks apart, of 20, 50, 100, or 200 mg. At each dose level, four or eight participants were randomly assigned 3:1 to VIR-2218 or placebo. Four HBeAg-negative participants received the 20 mg dose, eight HBeAg-negative and four HBeAg-positive participants received the 50 mg dose, eight HBeAg-negative participants received the 100 mg dose, and four HBeAg-negative and four 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 aspartate aminotransferase levels ≤2x 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 supplementary methods).

The following viral parameters were measured to evaluate the antiviral activity of VIR-2218: HBsAg, hepatitis B core-related antigen, 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, hepatitis B surface antibody (anti-HBs) seroconversion, and HBeAg loss and/or hepatitis B e antibody (anti-HBe) seroconversion (HBeAg-positive participants only).

Statistical analysis

No formal sample size calculations were conducted. For ALN-HBV-001, the initial plan was to enroll up to 142 participants in the study across various dose levels. For study VIR-2218-1001, the initial plan was to enroll up to 209 participants (56 healthy volunteers and up to 153 participants with cHBV infection) 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

Preclinical evaluation of ALN-HBV and VIR-2218 in a chimeric mouse model

To compare the *in vivo* hepatic safety of ALN-HBV *vs.* VIR-2218, hALT1 levels were evaluated in a humanized liver mouse model.[27] As shown in Fig. 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.

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 (Fig. 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 BMI in ALN-HBV-001 compared to VIR-2218-1001 (mean: 22.0 *vs.* 24.5 kg/m^2, respectively).

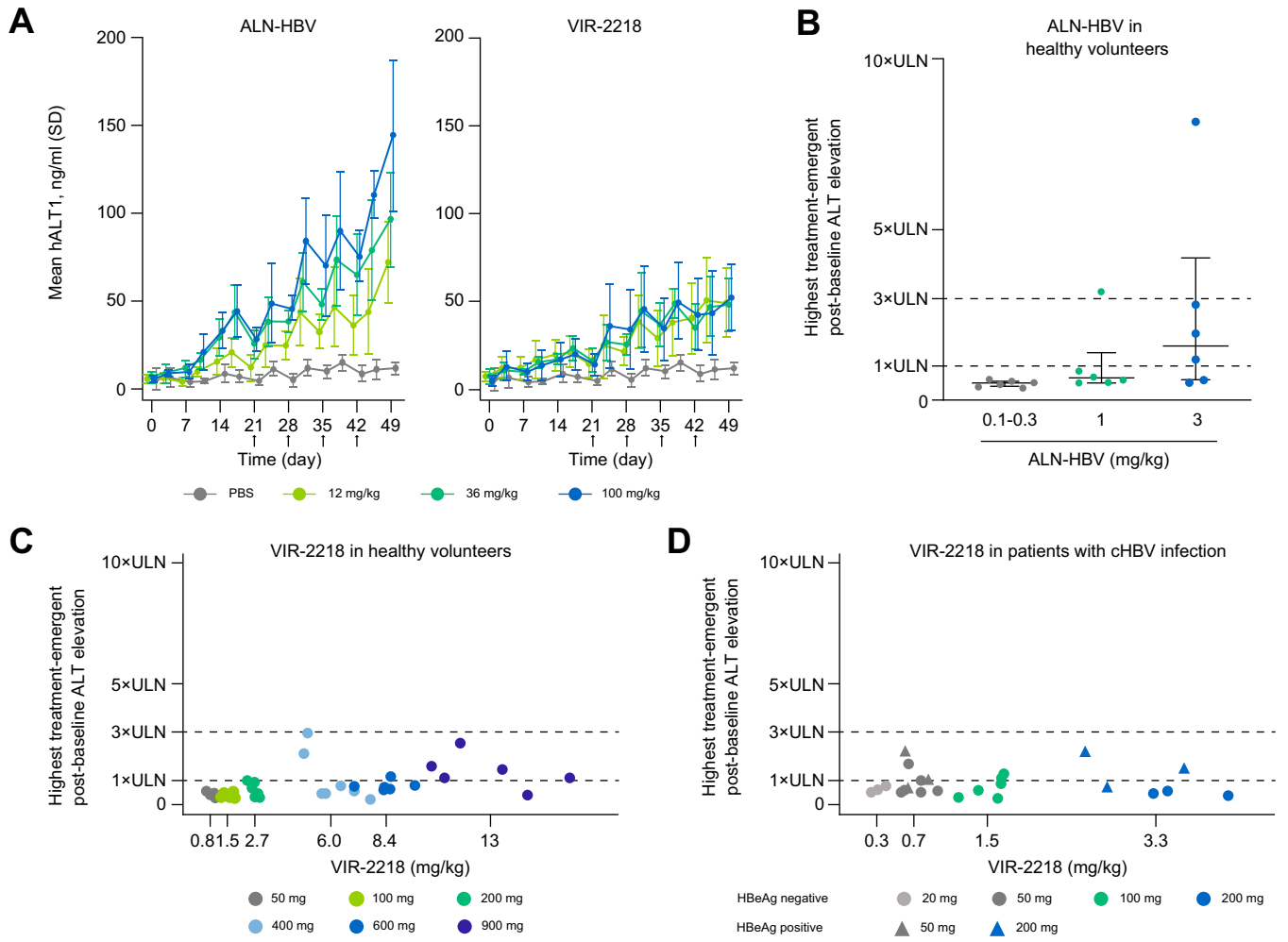


Fig. 2. Comparison of post-treatment ALT levels between ALN-HBV and VIR-2218 in non-clinical and clinical studies. (A) Post-treatment hALT levels were measured over time using a chimeric mouse model with ALN-HBV and VIR-2218 with doses administered on Days 0, 21, 28, 35, and 42. For individual-level data, see Fig. 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. Two healthy volunteers had baseline ALT values greater than ULN. (D) Maximum ALT levels were measured following two doses of VIR-2218 in participants with cHBV infection. Three participants with cHBV infection had baseline ALT values greater than ULN. (C,D) 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; cHBV, chronic hepatitis B virus; hALT, human alanine aminotransferase; HBeAg, hepatitis B e antigen; ULN, upper limit of normal.

In ALN-HBV-001, the only AEs reported in more than one ALN-HBV–treated participant were nasopharyngitis and headache. These were mild events that occurred in two 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 elevations, 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 *vs.* 0 placebo-treated participants experienced injection-site reactions, all of which were grade 1 in severity, resolved without intervention, and only one of which was considered by the investigator to be related to the study drug. 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 post-baseline ALT value relative to the ULN for each participant in ALN-HBV-001 and VIR-2218-1001 is presented in Fig. 2B and C. In VIR-2218-1001 (Fig. 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 (Fig. 2B). In ALN-HBV-001, post-baseline ALT values above the ULN were observed in 5 of 18 (28%) participants across dose levels (Fig. 2B). In VIR-2218-1001, post-baseline ALT values greater than ULN were observed in 0 participants at comparable dose levels (0.8-2.7 mg/kg; Fig. 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 elevations was maintained at higher doses of VIR-2218, equating to 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.

Clinical evaluation of VIR-2218 in participants with cHBV infection

Thirty-two participants with cHBV infection were randomly assigned to receive two doses of VIR-2218 (20, 50, 100, or 200 mg) or placebo, given 4 weeks apart (Fig. S3). All participants were dosed with the allocated treatment and included in the analysis. Only two of the VIR-2218 participants failed to complete the full 48 weeks of study visits.

Demographic 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 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 five active participants with ALT elevation who received VIR-2218 and the single placebo-treated participant with ALT elevation were HBeAg positive. No dose-response relationship was observed. Additionally, all ALT elevations were asymptomatic and not associated with changes in other functional parameters. All ALT 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 (Fig. 2D). The maximum ALT levels were higher among HBeAg-positive participants than healthy volunteers (Fig. 2C) or HBeAg-negative participants, but were generally <2.3x ULN for those dosed up to 200 mg (Fig. 2D; Fig. 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 Fig. 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 (Fig. 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 seven participants receiving 100 mg and 200 mg of VIR-2218 who achieved an HBsAg reduction to <100 IU/ml, five maintained HBsAg levels <100 IU/ml at Week 48. HBsAg level reductions were more sustained in higher dose cohorts, with

Table 1. Summary of demographic and baseline characteristics in a phase II study in participants with cHBV infection (VIR-2218-1001).

	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)
Mean age, yr (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

ALT, alanine aminotransferase; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.
*Baseline is defined as the last valid non-missing assessment prior to first study drug administration.

Table 2. Summary of AEs 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)

AE, adverse event; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; TEAE, treatment-emergent adverse event.
*Grade 3 non-serious 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 by the investigator to be related to the study drug, but the Sponsor determined that the constellation of concurrent symptoms (fever, headache, nausea, vomiting, and dehydration) was more consistent with a viral syndrome than a drug reaction and assessed the event as not related to study drug.

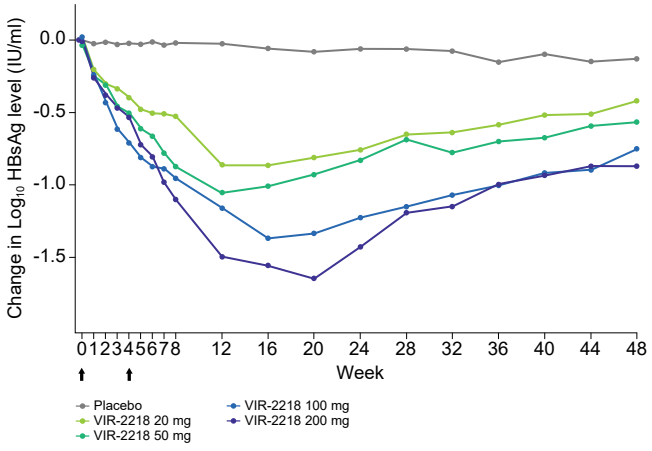


Fig. 3. Post-treatment mean HBsAg reduction over time in participants with cHBV infection (study VIR-2218-1001). Arrows indicate injection times (Weeks 0 and 4). The mean HBsAg level reduction was calculated based on available HBsAg levels at each time point: the majority of participants had available data at all time points; the limited missing data are described in the supplement. For participant-level data, see Fig. S7. cHBV, chronic hepatitis B virus; HBsAg, hepatitis B surface antigen.

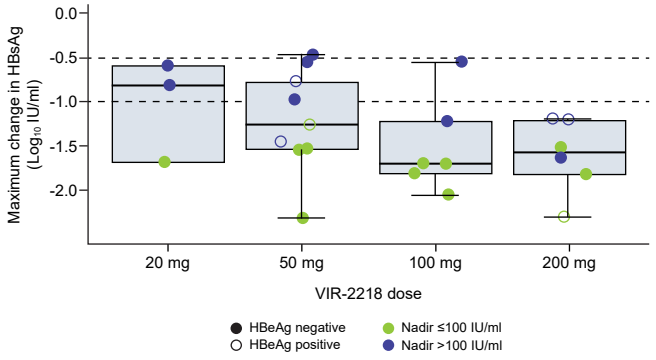


Fig. 4. Individual maximum post-treatment HBsAg level reductions in participants with cHBV infection (study VIR-2218-1001). For each box, the center line represents the median, the box limits represent the upper and lower quartiles, and the whiskers extend to the minimum and maximum. cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

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 (Fig. 3).

No participants had serum HBsAg loss or 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 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 analysis of post-treatment effects in most participants. However, post-treatment reductions in hepatitis B core-related antigen, HBeAg, and HBV RNA in HBeAg-positive patients are described in Fig. 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 elevations in phase I 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 sequencing 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 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 elevations, including ALT elevations up to 8.6x ULN with the highest dose of 3 mg/kg. At similar doses, VIR-2218 was not associated with any ALT elevations. Additionally, VIR-2218 was evaluated at 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 3.0x ULN. All ALT 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 2.3x 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 II 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 participants, suggesting the target sequence is preserved in both HBV cccDNA and integrated DNA transcripts. Following two doses of VIR-2218, given 4 weeks apart, 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 two doses of VIR-2218, given 4 weeks apart, 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-Pacific region, which may limit HBV genotype diversity in this study. It is yet to be determined whether these results are generalizable to other populations, such as White 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.

Affiliations

[1]University of Auckland and New Zealand Clinical Research, Auckland, New Zealand; [2]Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea; [3]Alnylam Pharmaceuticals, Cambridge, Massachusetts, USA; [4]Vir Biotechnology, Inc., San Francisco, California, USA; [5]Richmond Pharmacology, Ltd.; St George's University of London, London, UK; [6]Department of Medicine and State Key Laboratory of Liver Research, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; anti-HBe, hepatitis B e antibody; anti-HBs, hepatitis B surface antibody; cccDNA, covalently closed circular DNA; cHBV, chronic hepatitis B virus; ECG, electrocardiogram; ESC(+), enhanced stabilization chemistry (plus); GalNAc, N-acetylgalactosamine; hALT1, human ALT 1; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PEG-IFN, pegylated-interferon; RNAi, RNA interference; siRNA, short interfering RNA; ULN, upper limit of normal.

Financial support

The ALN-HBV-001 study was funded by Alnylam Pharmaceuticals and the VIR-2218-1001 study was funded by Vir Biotechnology, Inc. Medical editorial and writing support were provided by Kristin Runkle, PhD, and Courtney St. Amour, PhD, of Lumanity Scientific Inc., and were funded by Vir Biotechnology, Inc.

Conflicts of Interest

EG served as speaker/advisor/consultant for AbbVie, Abbott 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 & Dohme, Clear B Therapeutics, Springbank Pharmaceuticals, Silverback Therapeutics, Roche, Sysmex Corporation, and Vir Biotechnology.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' 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.

Acknowledgments

The authors thank all the investigators, study staff, and volunteers involved in the studies. The authors thank Martin Maier, PhD, and Mark Schlegel, PhD, of Alnylam Pharmaceuticals for their review of and contributions to the manuscript. Medical editorial and writing support were provided by Kristin Runkle, PhD, and Courtney St. Amour, PhD, of Lumanity Scientific Inc., and were funded by Vir Biotechnology, Inc.

Data availability 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).

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.05.023.

References

Author names in bold designate shared co-first authorship

[1] Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3:383–403.
[2] Seto WK, Lo YR, Pawlotsky JM, Yuen MF. Chronic hepatitis B virus infection. Lancet 2018;392:2313–2324.
[3] European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–398.
[4] Anderson RT, Lim SG, Mishra P, Josephson F, Donaldson E, Given B, et al. Challenges, considerations, and principles to guide trials of combination therapies for chronic hepatitis B virus. Gastroenterology 2019;156:529–533 e524.
[5] Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol 2016;64:S71–S83.
[6] Maini MK, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. J Hepatol 2016;64:S60–S70.
[7] Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. J Clin Invest 2018;128:4588–4603.
[8] Isogawa M, Chung J, Murata Y, Kakimi K, Chisari FV. CD40 activation rescues antiviral CD8(+) T cells from PD-1-mediated exhaustion. PLoS Pathog 2013;9:e1003490.
[9] Mueller SN, Ahmed R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. Proc Natl Acad Sci U S A 2009;106:8623–8628.
[10] Richter K, Brocker T, Oxenius A. Antigen amount dictates CD8+ T-cell exhaustion during chronic viral infection irrespective of the type of antigen presenting cell. Eur J Immunol 2012;42:2290–2304.
[11] Schietinger A, Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. Trends Immunol 2014;35:51–60.
[12] Tay SS, Wong YC, McDonald DM, Wood NA, Roediger B, Sierro F, et al. Antigen expression level threshold tunes the fate of CD8 T cells during primary hepatic immune responses. Proc Natl Acad Sci U S A 2014;111:E2540–E2549.
[13] Wherry EJ. T cell exhaustion. Nat Immunol 2011;12:492–499.
[14] **Michler T**, **Kosinska AD**, Festag J, Bunse T, Su J, Ringelhan M, et al. Knockdown of virus antigen expression increases therapeutic vaccine efficacy in high-titer hepatitis B virus carrier mice. Gastroenterology 2020;158:1762–1775 e1769.
[15] Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-targeted therapeutics. Cell Metab 2018;27:714–739.
[16] **Bajan S**, **Hutvagner G**. RNA-based therapeutics: from antisense oligonucleotides to miRNAs. Cells 2020;9:137.
[17] Gane E. The roadmap towards cure of chronic hepatitis B virus infection. J R Soc N Z 2020:1–20.
[18] Wooddell CI, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 2017;9:eaan0241.
[19] Flisiak R, Jaroszewicz J, Lucejko M. siRNA drug development against hepatitis B virus infection. Expert Opin Biol Ther 2018;18:609–617.
[20] **Janas MM**, **Schlegel MK**, Harbison CE, Yilmaz VO, Jiang Y, Parmar R, et al. Selection of GalNAc-conjugated siRNAs with limited off-target-driven rat hepatotoxicity. Nat Commun 2018;9:723.
[21] Nair JK, Willoughby JL, Chan A, Charisse K, Alam MR, Wang Q, et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. J Am Chem Soc 2014;136:16958–16961.
[22] Brown CR, Gupta S, Qin J, Racie T, He G, Lentini S, et al. Investigating the pharmacodynamic durability of GalNAc-siRNA conjugates. Nucleic Acids Res 2020;48:11827–11844.
[23] Datta S, Chatterjee S, Veer V, Chakravarty R. Molecular biology of the hepatitis B virus for clinicians. J Clin Exp Hepatol 2012;2:353–365.
[24] **Decorsiere A**, **Mueller H**, **van Breugel PC**, **Abdul F**, Gerossier L, Beran RK, et al. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature 2016;531:386–389.
[25] **Schlegel MK**, **Janas MM**, Jiang Y, Barry JD, Davis W, Agarwal S, et al. From bench to bedside: improving the clinical safety of GalNAc-siRNA conjugates using seed-pairing destabilization. Nucleic Acids Res 2022;50:6656–6670.
[26] Gane E, Schwabe C, Taubel J, Bakardjiev AI, Huang SA, Janas MM, et al. Impact of ESC+ technology on the hepatic safety profile of GalNAc-delivered, HBV-targeted RNAi therapeutics. Poster presented at: The Digital International Liver Congress; August 27–29, 2020.
[27] Tateno C, Kawase Y, Tobita Y, Hamamura S, Ohshita H, Yokomichi H, et al. Generation of novel chimeric mice with humanized livers by using hemizygous cDNA-uPA/SCID mice. PLoS One 2015;10:e0142145.

[28] Nayagam S, Thursz M, Sicuri E, Conteh L, Wiktor S, Low-Beer D, et al. Requirements for global elimination of hepatitis B: a modelling study. Lancet Infect Dis 2016;16:1399–1408.
[29] Tseng TC, Liu CJ, Su TH, Wang CC, Chen CL, Chen PJ, et al. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology 2011;141:517–525. 525 e511-512.
[30] Chu CJ, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterology 2003;125:444–451.

Journal of Hepatology, Volume *79*

Supplemental information

Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: Results from randomized clinical trials

Ed Gane, Young-Suk Lim, Jae B. Kim, Vasant Jadhav, Ling Shen, Anna I. Bakardjiev, Stephen A. Huang, Andrea L. Cathcart, Florian A. Lempp, Maja M. Janas, Daniel J. Cloutier, Charalambos Kaittanis, Laura Sepp-Lorenzino, Gregory Hinkle, Jorg Taubel, Patrick Haslett, Stuart Milstein, Yesseinia I. Anglero-Rodriguez, Christy M. Hebner, Phillip S. Pang, and Man-Fung Yuen

Evaluation of RNAi therapeutics VIR-2218 and ALN-HBV for chronic hepatitis B: Results from randomized clinical trials

Ed Gane, Young-Suk Lim, Jae B. Kim, Vasant Jadhav, Ling Shen, Anna I. Bakardjiev, Stephen A. Huang, Andrea L. Cathcart, Florian A. Lempp, Maja M. Janas, Daniel J. Cloutier, Charalambos Kaittanis, Laura Sepp-Lorenzino, Gregory Hinkle, Jorg Taubel, Patrick Haslett, Stuart Milstein, Yesseinia I. Anglero-Rodriguez, Christy M. Hebner, Phillip S. Pang, Man-Fung Yuen

Table of contents

Supplementary methods

Ethics Committees for Study ALN-HBV-001 and VIR-2218-1001

Each study was reviewed and approved by applicable regulatory bodies and ethics committees. For study ALN-HBV-001, this was London Bridge Research Ethics Committee (UK Health Research Authority). For study VIR-2218-1001, the institutional review boards/independent ethics committees were as follows: Health and Disability Ethics Committees (HDEC); St. Vincent's Human Research Ethics Committee (HREC); Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB); Asan Medical Center Institutional Review Board; Seoul National University Hospital Institutional Review Board; Pusan National University Hospital Institutional Review Board; Ethics Committee of the Faculty of Tropical Medicine, Mahidol University; Siriraj Institutional Review Board Human Research Protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University; Institutional Review Board, Faculty of Medicine, Chulalongkorn University; Khon Kaen University Ethics Committee in Human Research; and Human Research Ethics Committee, Faculty of Medicine, Prince of Songkla University. For both studies, participants were randomly assigned via an interactive response system.

Criteria for Suspending or Stopping Dosing in Healthy Volunteers

Cohort dosing was suspended or stopped if a sentinel participant experienced a grade ≥3 treatment-related AE, if ≥1 participant experienced a grade 3 study drug–related rash, if ≥2 participants experienced the same grade ≥3 study drug–related AE, if ≥1 participant had a study drug–related serious AE, or if ≥1 participant experienced a grade 4 rash.

Criteria for Suspending or Stopping Dosing in Participants With cHBV Infection

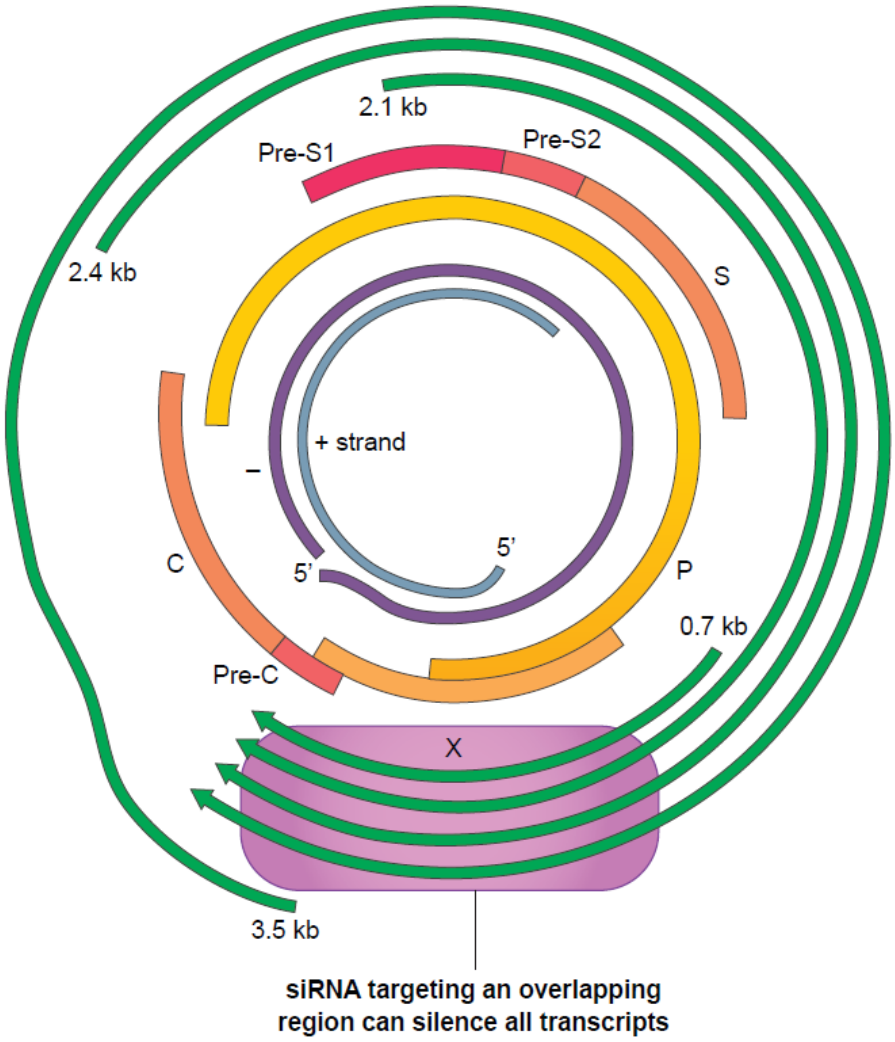
Participants who received 1 dose of study drug continued treatment as scheduled unless serum ALT level >10 × ULN, serum ALT level >5 × ULN with no change in HBsAg (defined as a <50% decrease from the baseline predose value), serum ALT or AST level >3 × ULN with a concomitant total bilirubin level >2 × ULN, or any clinical manifestations of hepatic decompensation.

Supplemental Results

Missing Data in the Analysis of Post-treatment HBsAg Reduction Over Time in Participants With cHBV Infection (Study VIR-2218-1001)

All HBeAg-negative participants completed all follow-up visits except for 1 in the 50-mg dose cohort who missed the Week 28 and 32 visits and 5 participants in the placebo group with last visits at Week 16, 24, 28, and 32, respectively. All HBeAg-positive participants completed all follow-up visits except for 1 participant in the 50-mg dose cohort who had their last follow-up visit at Week 28, 1 participant in the 200-mg dose cohort who missed the Week 28 visit and had last follow-up visit at Week 36, and 2 participants in the placebo group with last visits at Week 16 and 24, respectively.

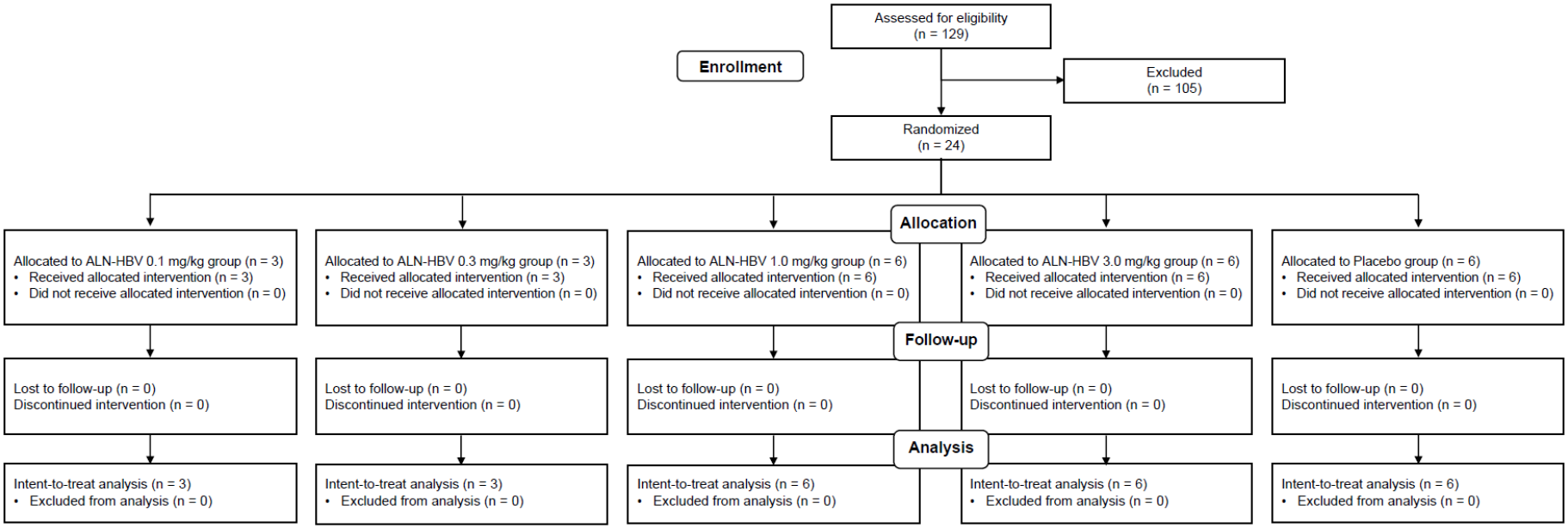
Fig. S1. VIR-2218 target within the HBV genome.



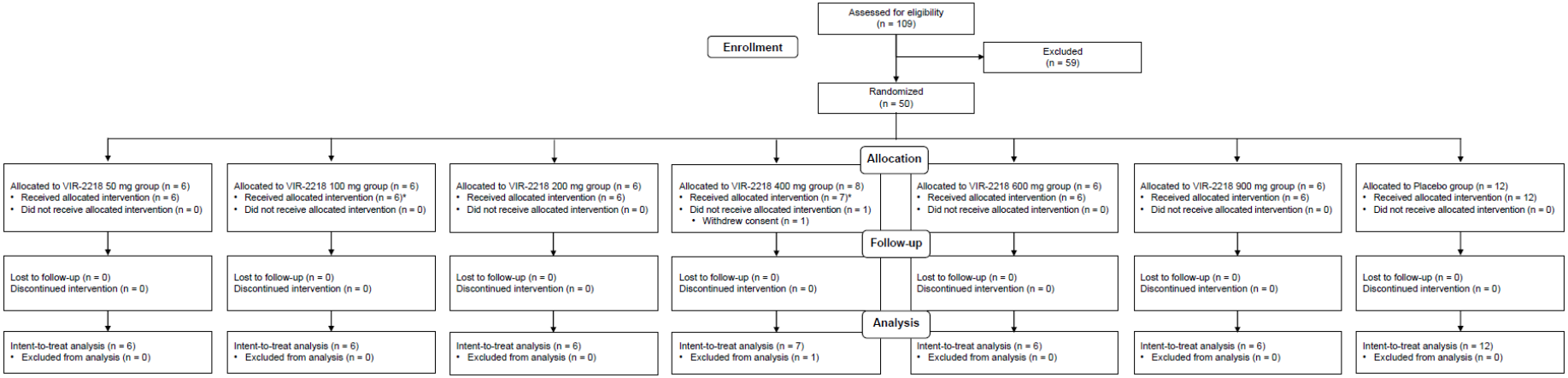
HBV, hepatitis B virus; siRNA, short interfering RNA.

Fig. S2. CONSORT flow diagram for healthy volunteers in the (a) ALN-HBV-001 and (b) VIR-2218-1001 studies.

A.



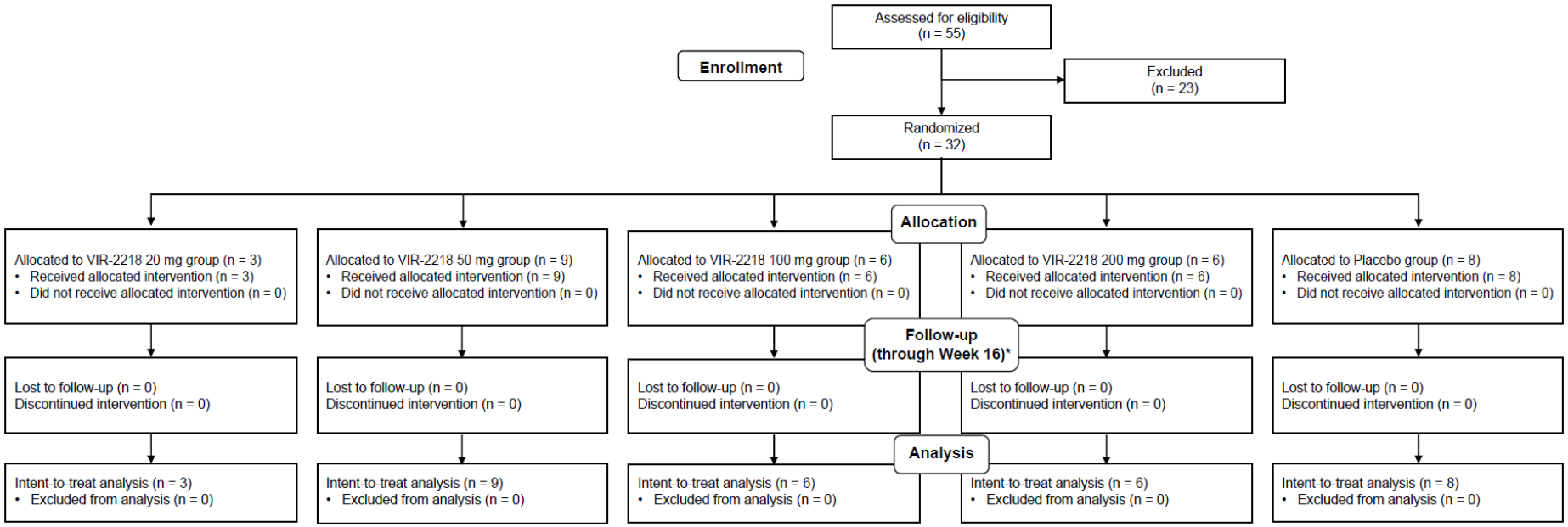
B.



*A total of 2 participants received a partial dose of VIR-2218; 1 participant in the 100-mg cohort received 0.4 mL of the planned 0.5-mL volume and 1 participant in the 400-mg cohort received 1.5 mL or the planned 2.0-mL volume.

CONSORT, Consolidated Standards of Reporting Trials.

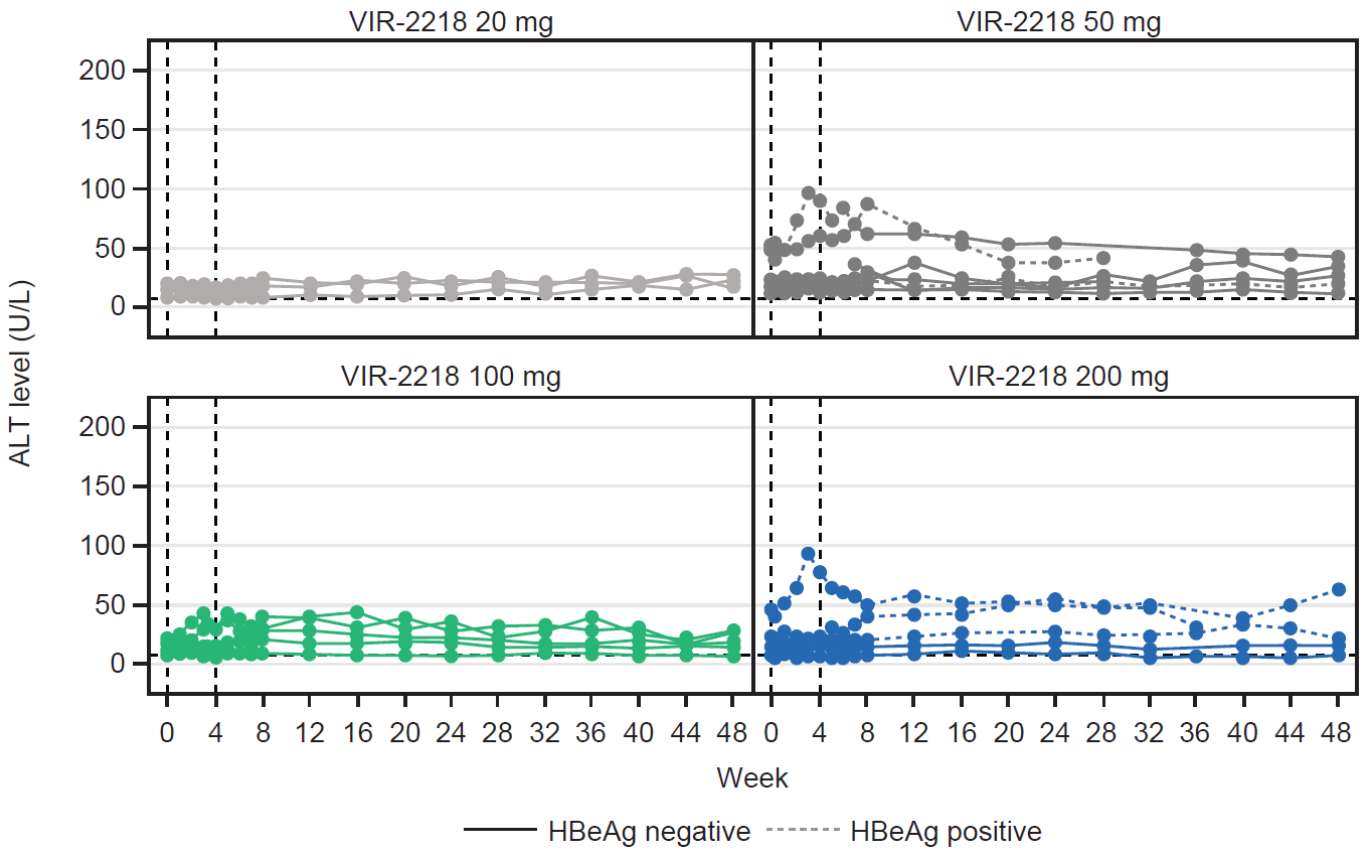
Fig. S3. CONSORT flow diagram for participants with cHBV infection in the VIR-2218-1001 study.



*All participants completed regular follow-up of 16 weeks. Participants with >10% HBsAg level reduction at Week 16 underwent extended follow-up, which was not completed by 2 participants (both of whom were HBeAg positive); 1 participant in the 50-mg cohort had their last follow-up visit at Week 28, and 1 participant in 200-mg cohort missed the Week 28 visit and had their last follow-up visit at Week 36.

CONSORT, Consolidated Standards of Reporting Trials; cHBV, chronic hepatitis B virus; HBsAg, hepatitis B virus surface antigen HBeAg, hepatitis B e antigen.

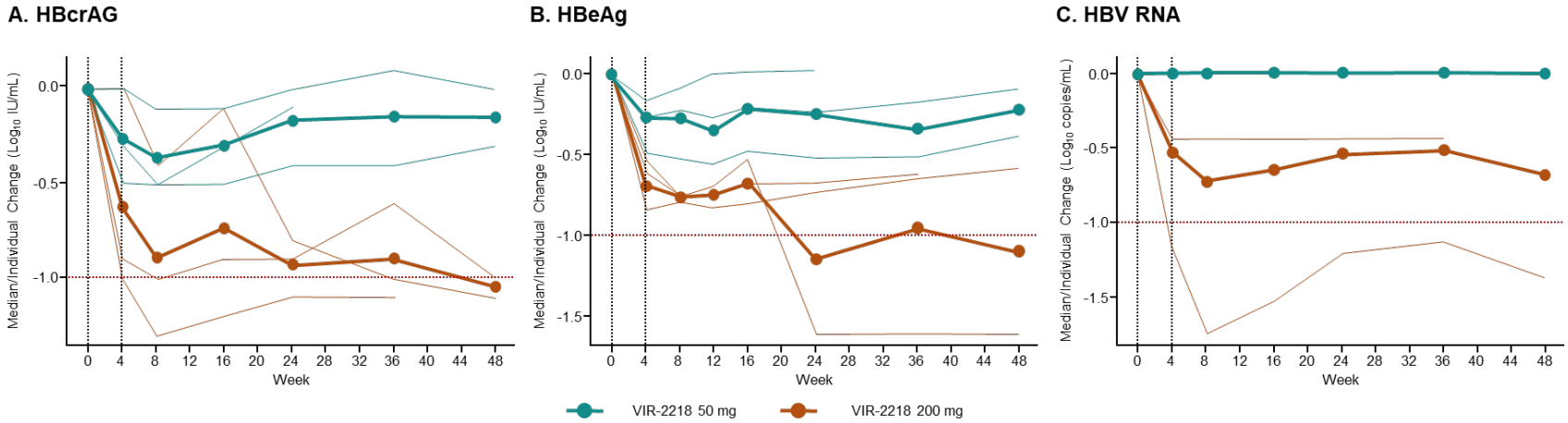
Fig. S4. ALT levels* in all participants with cHBV infection through Week 48 (study VIR-2218-1001).



*The upper limit of normal for male and female participants is 43 U/L and 34 U/L, respectively.

ALT, alanine aminotransferase; cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen.

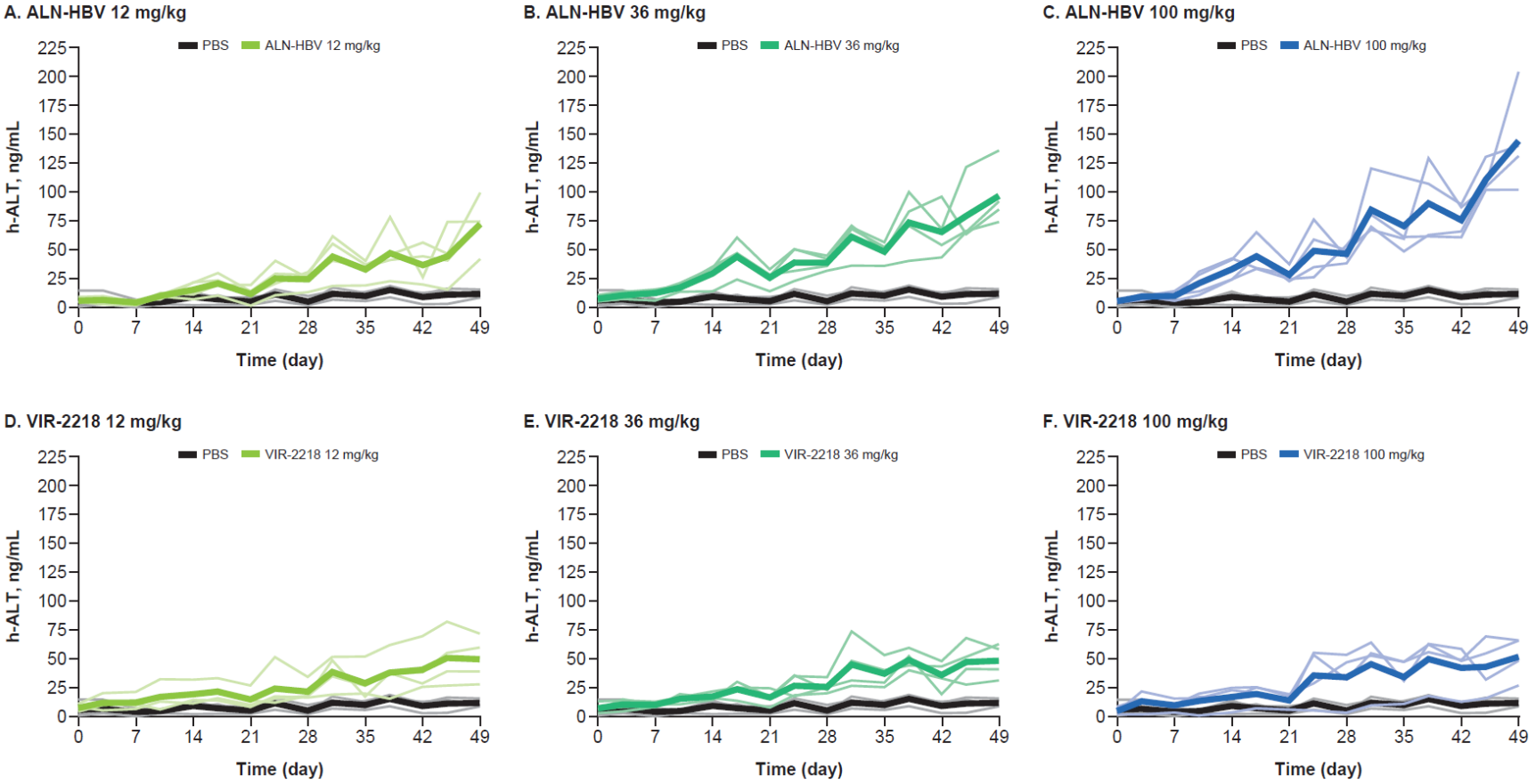
Fig. S5. Dose-dependent reductions in (A) HBcrAg, (B) HBeAg and (C) HBV RNA in HBeAg-positive participants.[*]



[*]Individual-level (solid lines) and median (dotted lines) data are shown. All values < LLOQ were imputed to 1 significant unit below LLOQ; 2 of 3 participants in the 50 mg cohort had < LLOQ HBV RNA at baseline.

HBeAg, hepatitis B e antigen; HBcrAg, hepatitis B core-related antigen; LLOQ, lower limit of quantitation.

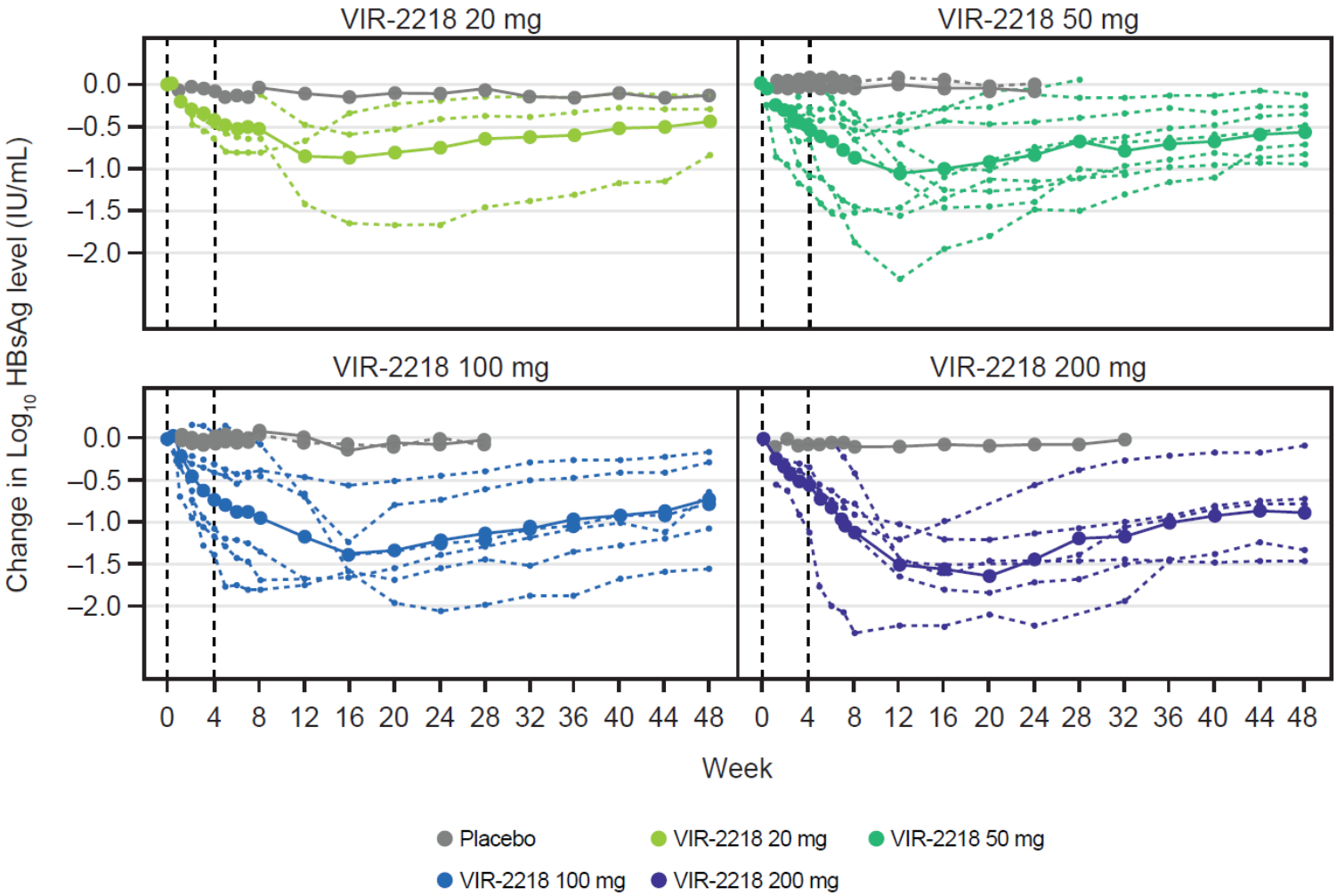
Fig. S6. Individual-level data for comparison of post-treatment ALT levels between ALN-HBV and VIR-2218 in nonclinical studies.*



*Individual-level and mean data are shown; the data correspond to the mean data plotted in Figure 2a.

ALT, alanine aminotransferase; hALT, human alanine aminotransferase.

Fig. S7. Participant-level data for post-treatment mean HBsAg level reduction over time in participants with cHBV infection (study VIR-2218-1001).*



*Individual-level and mean data are shown; the data correspond to the mean data plotted in Figure 3.

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

Table S1. Summary of Demographics of Healthy Volunteers From the ALN-HBV-001 and VIR-2218-1001 Studies

	ALN-HBV					
Participants, n (%)	**Overall (n = 18)**	**0.1 mg/kg (n = 3)**	**0.3 mg/kg (n = 3)**	**1.0 mg/kg (n = 6)**	**3.0 mg/kg (n = 6)**	**Placebo (n = 6)**
Mean age, y (SD)	24.3 (5.9)	30.7 (4.0)	24.3 (6.7)	24.8 (7.2)	20.5 (0.8)	26.8 (5.5)
Male, n (%)	10 (55.6)	2 (66.7)	1 (33.3)	2 (33.3)	5 (83.3)	4 (66.7)
Race, n (%)						
White	10 (55.6)	2 (66.7)	1 (33.3)	4 (66.7)	3 (50.0)	2 (33.3)
Black or African American	4 (22.2)	0 (0.0)	2 (66.7)	1 (16.7)	1 (16.7)	0 (0.0)
Asian	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	1 (16.7)
Other	2 (11.1)	1 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	3 (50.0)
Mean BMI, kg/m^2 (SD)	22.0 (3.0)	23.5 (2.4)	19.4 (1.6)	23.0 (3.1)	21.7 (3.3)	25.3 (2.2)

	VIR-2218							
Participants, n (%)	**Overall (n = 49)**	**50 mg (n = 6)**	**100 mg (n = 6)**	**200 mg (n = 6)**	**400 mg (n = 7)**	**600 mg (n = 6)**	**900 mg (n = 6)**	**Placebo (n = 12)**
Mean age, y (SD)	26.7 (6.1)	25.0 (3.0)	23.3 (4.0)	26.7 (3.8)	24.3 (3.7)	28.8 (6.3)	32.5 (9.5)	26.5 (6.7)
Male, n (%)	18 (36.7)	0 (0.0)	2 (33.3)	3 (50.0)	0 (0.0)	3 (50.0)	3 (50.0)	7 (58.3)
Race, n (%)								

Asian	9 (18.4)	2 (33.3)	3 (50.0)	0 (0.0)	0 (0.0)	2 (33.3)	1 (16.7)	1 (8.3)
White	28 (57.1)	2 (33.3)	2 (33.3)	5 (83.3)	5 (71.4)	3 (50.0)	3 (50.0)	8 (66.7)
Other	12 (24.5)	2 (33.3)	1 (16.7)	1 (16.7)	2 (28.6)	1 (16.7)	2 (33.3)	3 (25.0)
Mean BMI, kg/m^2 (SD)	24.5 (3.1)	23.1 (4.6)	22.9 (2.7)	24.2 (2.0)	25.1 (3.9)	26.0 (1.4)	25.7 (4.0)	24.4 (2.4)

SD, standard deviation; BMI, body mass index.

Table S2. AEs Summary in Healthy Volunteers from the ALN-HBV-001* and VIR-2218-1001* Studies

	ALN-HBV					
Participants, n (%)	**Overall (n = 18)**	**0.1 mg/kg (n = 3)**	**0.3 mg/kg (n = 3)**	**1.0 mg/kg (n = 6)**	**3.0 mg/kg (n = 6)**	**Placebo (n = 6)**
Any AE	12 (66.7)	2 (66.7)	1 (33.3)	3 (50.0)	6 (100.0)	2 (33.3)
Treatment-related AE	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)
Severe AE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serious AE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AE leading to discontinuation of study	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

	VIR-2218							
Participants, n (%)	**Overall (n = 49)**	**50 mg (n = 6)**	**100 mg (n = 6)**	**200 mg (n = 6)**	**400 mg (n = 7)**	**600 mg (n = 6)**	**900 mg (n = 6)**	**Placebo (n = 12)**
Any TEAE	28 (57)	4 (67)	3 (50)	4 (67)	5 (71)	3 (50)	3 (50)	6 (50)
Treatment-related TEAE	3 (6)	0 (0)	1 (17)	0 (0)	1 (14)	1 (17)	0 (0)	0 (0)
Grade ≥3 TEAE[†]	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)
Serious TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Treatment-related serious TEAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TEAE leading to discontinuation of study	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Safety population.

[†]One participant in the 600-mg cohort experienced a grade 3 nonserious AE of respiratory tract infection (considered not related to study drug).

AE, adverse event; treatment-emergent adverse event.