Research Article
Viral Hepatitis

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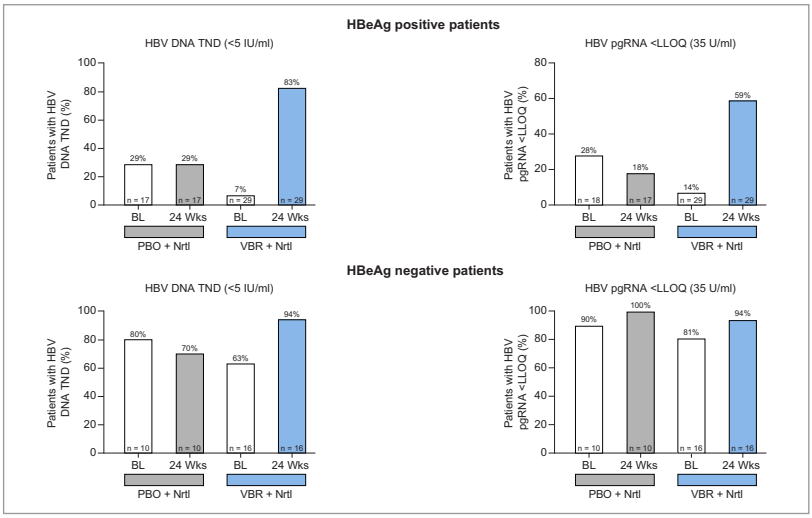
Safety and efficacy of vebicorvir in virologically suppressed patients with chronic hepatitis B virus infection

Graphical abstract



Highlights

- Complete suppression of HBV replication is essential for finite treatment regimens.
- Vebicorvir (VBR) is a novel inhibitor of the HBV core protein.
- VBR interferes with two additional steps in HBV replication than NrtIs.
- Added to NrtI, VBR did not significantly change mean HBV antigens over 24 weeks.
- Added to NrtI, VBR further reduced HBV DNA and pgRNA by high-sensitivity PCR assays.

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Lay summary

Core inhibitors represent a novel approach for the treatment of chronic hepatitis B virus (HBV) infection, with mechanisms of action distinct from existing treatments. In this study, vebicorvir added to existing therapy reduced HBV replication to a greater extent than existing treatment and was generally safe and well tolerated.

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Safety and efficacy of vebicorvir in virologically suppressed patients with chronic hepatitis B virus infection

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Background & Aims: HBV nucleos(t)ide reverse transcriptase inhibitors (NrtIs) do not completely suppress HBV replication. Previous reports indicate persistent viremia during NrtI treatment despite HBV DNA being undetectable. HBV core inhibitors may enhance viral suppression when combined with NrtIs. This phase II trial (NCT03576066) evaluated the efficacy and safety of the investigational core inhibitor, vebicorvir (VBR), in virologically- suppressed patients on NrtIs.

Methods: Non-cirrhotic, NrtI-suppressed patients with chronic HBV were randomised to VBR 300 mg once daily or matching placebo (PBO) for 24 weeks. Treatment was stratified by hepatitis B e antigen (HBeAg) status. The primary endpoint was change from Baseline in serum HBeAg or hepatitis B surface antigen (HBsAg) after 24 weeks.

Results: Of 73 patients enrolled, 47 were HBeAg positive and 26 were HBeAg negative. In HBeAg-positive and -negative patients, there were no differences in the change from Baseline at Week 24 for HBsAg or HBeAg. Using a novel, high-sensitivity assay to detect HBV DNA, a greater proportion of patients with detectable HBV DNA at Baseline achieved undetectable HBV DNA at Week 24 in the VBR+NrtI *vs.* PBO+NrtI group. In HBeAg-positive patients, a greater change from Baseline in HBV pregenomic (pg) RNA was observed at Week 24 with VBR+NrtI *vs.* PBO+NrtI. Treatment-emergent adverse events (TEAEs) in VBR+NrtI patients included upper respiratory tract infection, nausea, and pruritus. No serious adverse events, Grade 4 TEAEs, or deaths were reported.

Conclusions: In this 24-week study, VBR+NrtI demonstrated a favourable safety and tolerability profile. While there were no significant changes in viral antigen levels, enhanced viral suppression was demonstrated by greater changes in DNA and pgRNA with the addition of VBR compared to NrtI alone.

Clinical trials number: NCT03576066.

Lay summary: Core inhibitors represent a novel approach for the treatment of chronic hepatitis B virus (HBV) infection, with mechanisms of action distinct from existing treatments. In this study, vebicorvir added to existing therapy reduced HBV replication to a greater extent than existing treatment and was generally safe and well tolerated.

Keywords: HBV; hepatitis; antiviral; NrtI; cccDNA; core inhibitor; pgRNA.
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Introduction

Chronic hepatitis B virus infection (cHBV) is a global public health problem associated with significant rates of morbidity and mortality.[1,2] Worldwide, it is estimated that >250 million people are living with cHBV, and approximately 1 million die

annually from cHBV-associated liver disease.[1,3,4] The likelihood of developing comorbid liver disease with cHBV is high, with an estimated 5- to 100-fold increase in risk of hepatocellular carcinoma (HCC) among persons with cHBV.[4]

HBV infection is diagnosed through detection of HBV DNA and HBsAg in plasma or serum.[3] Seroclearance of hepatitis B surface antigen (HBsAg), which is regarded as functional cure, is the goal of antiviral therapy.[5] HBV is a DNA virus that relies on reverse transcription of pregenomic (pg)RNA to HBV DNA for replication; hence, nucleos(t)ide reverse transcriptase inhibitors (NrtIs) are standard-of-care for cHBV.[6,7] NrtIs are generally safe, have a high barrier to resistance, and suppress HBV DNA in most patients, but their use rarely leads to loss of viral antigens, thus necessitating lifelong administration. New therapies are needed to provide deeper suppression of HBV replication with the goal of achieving durable, off-treatment virologic response.

To address this need, vebicorvir (VBR), an investigational HBV core inhibitor, was developed to interfere with multiple steps of the HBV viral-replication cycle, reducing HBV DNA, pgRNA, and the transcriptional activity of covalently closed circular (ccc) DNA.[8–10] By interfering with additional steps in HBV replication, the combination treatment of a core inhibitor and NrtI has the potential to provide deeper levels of virologic suppression and to improve treatment outcomes *vs.* NrtI alone. The objective of this phase II study was to evaluate the safety and efficacy of VBR+NrtI in non-cirrhotic, virologically-suppressed patients with cHBV and to test the hypothesis that combination therapy will achieve greater suppression of HBV replication than NrtIs alone.

Patients and methods

Study population and design

This was a phase II, multicentre, double-blind, placebo (PBO)-controlled study evaluating VBR+NrtI in virologically-suppressed patients with cHBV (NCT03576066). The study enrolled patients from 22 sites in 4 countries (United States, Canada, New Zealand, and Hong Kong) between June 2018 and July 2019.

Eligible patients were aged 18–70 years, had cHBV, were non-cirrhotic (F0–F2) with no history of hepatic decompensation, and were in otherwise good health. Patients must have been receiving treatment with an NrtI with HBV DNA ≤lower limit of quantification (LLOQ) by COBAS TaqMan Version 2.0 (Roche Diagnostics, Mannheim, Germany) for ≥6 months, HBsAg >400 IU/ml, and alanine aminotransferase (ALT) ≤5x the upper limit of normal (ULN). Inclusion and exclusion criteria and protocol deviations are provided in the supplementary information.

Vebicorvir 300 mg was self-administered as three 100 mg tablets or matching PBO once daily after a meal, at approximately the same time each day for 24 weeks. Patients were stratified 9:5 (hepatitis B e antigen [HBeAg] positive:HBeAg negative) and 3:2 to receive VBR plus their standard-of-care NrtI (VBR+NrtI) or PBO plus NrtI (PBO+NrtI) by an interactive phone/web response system. Efficacy and safety evaluations were conducted at Day 1 (Baseline) and treatment Weeks 2, 4, 8, 12, 16, 20, and 24.

This study was conducted in compliance with the principles of the Declaration of Helsinki, International Council for Harmonisation guidelines, and all applicable US Code of Federal Regulations. Institutional Review Board/Independent Ethics Committee approval of the study protocol was secured, and patients gave written informed consent before study procedures began.

Assays

HBV genotyping was performed using highly sensitive PCR (DNA) and reverse transcription (RT)-PCR (DNA+pgRNA) assays to detect low copy HBV genomes (Assembly Biosciences, South San Francisco, CA, USA) and by phylogenetic analysis of the HBV reverse transcriptase domain of polymerase (pol/RT), the X region, or the Core region. HBV DNA was measured by COBAS TaqMan Version 2.0 (Roche Diagnostics, Mannheim, Germany) with an LLOQ of 20 IU/ml (1.30 $\log_{10}$ IU/ml)[1,11] and, additionally, by a high-sensitivity assay with a limit of detection (LOD) of 5 IU/ml (0.69 $\log_{10}$ IU/ml; Assembly Biosciences, South San Francisco, CA, USA). Serum HBV pgRNA was measured using a quantitative RT-PCR assay with an LLOQ of 35 U/ml (1.54 $\log_{10}$ U/ml; Assembly Biosciences, South San Francisco, CA, USA).[10,12] Both assays for detection of HBV DNA and HBV pgRNA were developed by Assembly Biosciences and are described in the supplementary information. HBsAg and HBeAg were quantified using the Architect i2000SR assays with LLOQ of 0.05 IU/ml (–1.30 $\log_{10}$ IU/ml) and 0.11 IU/ml (–0.96 $\log_{10}$ IU/ml), respectively (Abbott Diagnostics, Lake Forest, IL, USA). Hepatitis B core-related antigen (HBcrAg) was quantified using the Lumipulse G assay with LLOQ of 1 kU/ml (0 $\log_{10}$ kU/ml; Fujirebio, Malvern, PA, USA). Resistance was monitored by population sequencing of the HBV core protein and pol/RT region (mutant detection limit ≥15%).

Efficacy endpoints

The primary endpoint was change in mean $\log_{10}$ serum HBsAg or HBeAg from Baseline (Day 1) to Week 24. Secondary endpoints included rates of ALT normalisation in patients with abnormal ALT (defined as Division of AIDS grading of ≥1) at Baseline and assessment of safety through adverse events (AEs) and laboratory abnormalities. Exploratory endpoints included change from Baseline in viral parameters, including HBV DNA, HBV pgRNA, and HBcrAg. Seroconversion, defined as loss of HBsAg/HBeAg and the appearance of the respective antibodies, was assessed at Week 24. Patients with persistently detectable HBV DNA on study were subject to resistance sequencing of the reverse transcriptase and core protein regions.

Pharmacokinetic endpoints

The pharmacokinetic (PK) variables were trough levels and (where available) trough-to-peak ratios of VBR on VBR+NrtI therapy compared with PBO+NrtI therapy. PK samples for VBR and NrtI concentrations were collected predose on Day 1 and Weeks 2, 4, 12, and 24 and during follow-up at Week 28; optional post-dose samples were collected 4 (±2) hours after dosing on Day 1 and Weeks 2 and 4. Drug concentrations in plasma were determined using validated methods for VBR and NrtI.

Safety endpoints

Primary safety assessments included the number of patients with treatment-emergent AEs (TEAEs), TEAEs leading to premature discontinuations, and TE laboratory abnormalities. AEs were recorded and monitored by an external data-monitoring committee. A TEAE was defined as any AE that newly appeared or worsened in severity on or after the date of first dose of study drug, but not more than 30 days after a patient's last dose. TE laboratory abnormalities were assessed at each study visit, and observed results were reported as change from Baseline. AEs were assessed at study visits on Day 1, Weeks 2 and 4, and every 4 weeks thereafter until Week 24. Verbatim descriptions of AEs were coded

using Version 21.0 of the Medical Dictionary for Regulatory Affairs. Safety was monitored by a data-monitoring committee, including a safety analysis after all patients had completed Week 12.

Statistical analysis

This study used an intent-to-treat (ITT) population to analyse efficacy-related endpoints between patients in different treatment groups and a safety analysis set to analyse safety-related endpoints between treatment arms. The ITT population consisted of all randomised patients; the safety population included all randomised patients who received at least 1 dose of study drug.

Based on a sample size of 45 HBeAg-positive patients, randomised in a 3:2 ratio, a 2-sample t test with a 2-sided $\alpha = 0.05$ significance level, would have 89.5% power to detect a difference of at least 0.5 $\log_{10}$ (IU/ml) in the mean change from Baseline in serum HBsAg or HBeAg at Week 24. A similar test would have 80% power to detect a treatment difference of at least 0.6 $\log_{10}$ (IU/ml) in the change from Baseline in serum HBsAg at Week 24 in HBeAg-negative patients. An equal SD of 0.5 is assumed for both treatment groups.

Baseline demographics and disease characteristics were reported descriptively. A repeated-measures analysis using observed data from all scheduled visits was performed for analysis of the primary endpoint. This analysis compared groups over time using a linear mixed-effect model repeat measurement that included fixed effects for treatment, visit, treatment-by-visit interaction, and Baseline value. Both HBsAg and HBeAg were reviewed independently and with no control of the α level. For HBV antigens and HBV pgRNA, summary statistics are provided by treatment group for the observed results and change from Baseline $\log_{10}$ at each study visit. For HBV DNA, a repeated-measures analysis using observed data from all scheduled visits for change from Baseline was performed. Where applicable, efficacy endpoints are described with the difference in least-squares mean (LSM) between treatment groups, and 95% CIs are presented.

Safety was summarised using descriptive statistics. Safety data are presented as the total number and percentage of patients experiencing an event. The number and percentage of patients who had normal ALT at Week 24 were compared to the number with abnormal ALT at Baseline. For ALT normalisation, the American Association for the Study of Liver Diseases ULN was used (25 U/L for females and 35 U/L for males). All CIs are 2-sided and use the Clopper-Pearson (exact binomial method) at 95%. Adjustments for multiplicity were not made in any analysis. Statistical analyses were performed using SAS Version 9.4 or higher, unless otherwise specified.

Results

Baseline demographics and disease characteristics

One hundred thirty-five patients were screened, and 73 enrolled. Reasons for exclusion and screening failure are summarised in the supplementary information. Of these 73 patients, 47 were HBeAg positive; 29 were randomised to VBR+NrtI, and 18 were randomised to PBO+NrtI. Of the 26 HBeAg-negative patients, 16 received VBR+NrtI, and 10 received PBO+NrtI. Three HBeAg-positive patients (1 VBR+NrtI and 2 PBO+NrtI) were enrolled in the study with deviations from inclusion criteria. One HBeAg-positive patient randomised to receive PBO+NrtI withdrew from the study after 69 days of treatment due to nonadherence to the study drug; the other 72 (99%) patients completed the study (Fig. 1).

Baseline demographics were similar between populations and treatment arms (Table 1). Most patients were male (64%) and Asian (84%). The mean age of the cohort was 45.3 years (all but 1 patient <65 years-old [range 20–66]). For the total cohort, Baseline disease characteristics were largely similar between treatment arms (Table S1). HBeAg-negative patients tended to be positive for HBV longer and have numerically lower levels of HBV DNA, HBV pgRNA, and HBcrAg compared with HBeAg-positive patients at Baseline (Table 2). Mean (SD) self-reported positivity for HBV infection was 13.2 (9.27) years. At randomisation, 42/73 (58%), 22/73 (30%), and 10/73 (14%) patients were receiving tenofovir disoproxil fumarate (TDF), tenofovir alafenamide fumarate (TAF), and entecavir (ETV), respectively. Mean (SD) duration of current HBV treatment was similar between patients receiving VBR+NrtI, 4.0 (3.72) years, and PBO+NrtI, 4.4 (4.31) years. At Baseline, 45/47 (96%) HBeAg-positive and 26/26 (100%) HBeAg-negative patients had HBV DNA <LLOQ by COBAS TaqMan. When assessed by high-sensitivity methodology, 39/47 (83%) HBeAg-positive and 8/26 (31%) HBeAg-negative patients had detectable HBV DNA >5 IU/ml. At Baseline, 38/47 (81%) HBeAg-positive and 4/26 (15%) HBeAg-negative patients had HBV pgRNA >LLOQ (35 U/ml). Baseline patient sequence information and pre-existing resistance mutations are summarised in Table S2.

Change from Baseline in serum viral antigens

At Week 24, the LSM (95% CI) change from Baseline in $\log_{10}$ serum HBsAg for HBeAg-positive patients receiving VBR+NrtI was 0.028 (−0.009, 0.065) and 0.041 (−0.008, 0.089) for those receiving PBO+NrtI ($p = 0.6855$; Fig. 2A). In HBeAg-negative patients, LSM (95% CI) change from Baseline in HBsAg at Week 24 was 0.087 (0.017, 0.156) and 0.009 (−0.080, 0.097) for VBR+NrtI and PBO+NrtI, respectively ($p = 0.1750$; Fig. 2B). In HBeAg-positive patients, LSM (95% CI) change from Baseline in HBeAg was −0.053 (−0.115, 0.009) and −0.097 (−0.179, −0.015) for VBR+NrtI and PBO+NrtI, respectively ($p = 0.3987$; Fig. 2A).

In HBeAg-positive patients, LSM (95% CI) change from Baseline in HBcrAg was similar between VBR+NrtI (−0.164 [−0.208, −0.120]) and PBO+NrtI (−0.130 [−0.186, −0.073]; $p = 0.3503$; Fig. 2A). Also, in HBeAg-negative patients, there were no differences in LSM (95% CI) change from Baseline in HBcrAg at Week 24 between VBR+NrtI (−0.073 [−0.130, −0.016]) and PBO+NrtI (−0.056 [−0.129, 0.016]; $p = 0.7192$; Fig. 2B). No patients experienced HBsAg seroconversion and 1 HBeAg-positive patient receiving VBR+NrtI experienced HBeAg seroconversion.

Change from Baseline in HBV pgRNA and HBV DNA

In HBeAg-positive patients, a greater mechanism-based change from Baseline at Week 24 in HBV pgRNA was observed with VBR+NrtI (−1.683 [−1.930, −1.436]) *vs.* PBO+NrtI (−0.046 [−0.363, 0.272]; $p<0.0001$; Fig. 3A). Fig. 4A presents the proportions of HBeAg-positive patients with undetectable HBV DNA and HBV pgRNA at Baseline and Week 24 by high-sensitivity analytical methods. Among patients receiving PBO+NrtI, the proportion with HBV DNA <5 IU/ml remained the same at Baseline and Week 24, 5/17 (29%). Among patients receiving VBR+NrtI, the proportion with HBV DNA <5 IU/ml increased from 2/29 (7%) at Baseline to 24/29 (83%) at Week 24. The proportion of PBO+NrtI patients with pgRNA <35 U/ml decreased from 5/18 (28%) at

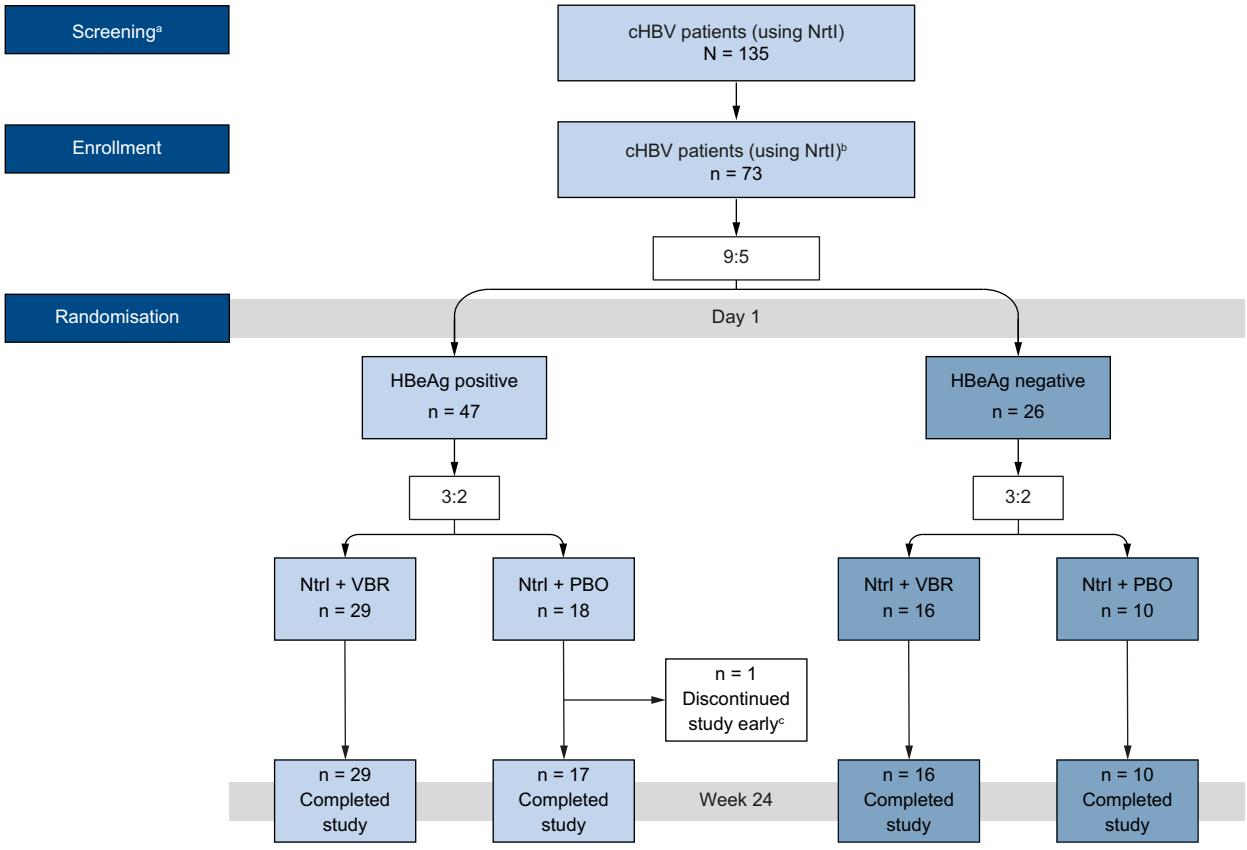

Fig. 1. Patient disposition (ITT population). [a]Virologically suppressed patients with HBeAg-positive or -negative cHBV. [b]Three HBeAg-positive patients (1 VBR+NrtI and 2 PBO+NrtI) were enrolled in the study with deviations from inclusion criteria. These patients were enrolled based on revised enrollment criteria, which were accepted, allowing these patients to continue the study. [c]One PBO+NrtI HBeAg-positive patient discontinued due to noncompliance with the study drug. cHBV, chronic hepatitis B virus; HBeAg, hepatitis B e antigen; ITT, intent-to-treat; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; VBR, vebicorvir.

Baseline to 3/17 (18%) at Week 24. For VBR+NrtI patients, the proportion with HBV pgRNA <35 U/ml increased from 4/29 (14%) at Baseline to 17/29 (59%) at Week 24. Detection of HBV DNA and HBV pgRNA for individual HBeAg-positive patients both at Baseline and Week 24 is shown in Fig. S1. At Week 24, of the 51 patients who had HBV DNA target not detected, 39 (76.5%) also achieved HBV pgRNA <LLOQ.

In HBeAg-negative patients, no differences were observed in mean serum HBV pgRNA between treatments over the 24-week period; HBV pgRNA change from Baseline to Week 24 was VBR+NrtI (−0.083 [−0.089, −0.076]) and PBO+NrtI (−0.088 [−0.096, −0.079]; $p = 0.3502$; Fig. 3B). Fig. 4B presents the proportions of HBeAg-negative patients with undetectable HBV DNA and HBV pgRNA at Baseline and Week 24 by high-sensitivity analytical

Table 1. Baseline demographics (ITT population).

	HBeAg positive (n = 47)		HBeAg negative (n = 26)	
Characteristic	**PBO+NrtI (n = 18)**	**VBR+NrtI (n = 29)**	**PBO+NrtI (n = 10)**	**VBR+NrtI (n = 16)**
Age (years)				
Mean±SD	46.1±12.9	42.1±10.7	46.9±8.3	49.3±7.7
Sex (Female)	8 (44)	8 (28)	5 (50)	5 (31)
Ethnicity				
Hispanic or Latino	1 (6)	1 (3)	0	0
Race[a]				
Asian	15 (83)	26 (90)	9 (90)	11 (69)
Black or African American	2 (11)	0	1 (10)	2 (13)
White	0	2 (7)	0	2 (13)
Other[b]	1 (6)	1 (3)	0	1 (6)
BMI (kg/m^2)				
Mean±SD	23.3±2.84	24.1±3.92	23.9±4.16	24.5±2.79

Data presented as n (%) unless otherwise specified.
ITT, intent-to-treat; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; SD, standard deviation; VBR, vebicorvir.
[a]One patient selected both "Asian" and "Native Hawaiian or Other Pacific Islander" for race and is summarised under "Other" category.
[b]Other includes Native Hawaiian, Pacific Islander, American Indian, and Alaska Native.

Table 2. Baseline disease characteristics (ITT population).

	HBeAg positive (n = 47)		HBeAg negative (n = 26)	
Characteristic	**PBO+NrtI (n = 18)**	**VBR+NrtI (n = 29)**	**PBO+NrtI (n = 10)**	**VBR+NrtI (n = 16)[a]**
Years positive for HBV	10.3±6.10	12.0±8.50	20.4±8.13	14.3±12.11
HBV genotype				
A	1 (6)	2 (7)	2 (20)	4 (25)
B	2 (11)	10 (34)	0	4 (25)
C	11 (61)	8 (28)	1 (10)	1 (6)
D	0	0	0	1 (6)
F	0	1 (3)	0	0
G	1 (6)	0	0	0
Undetermined[b]	3 (17)	8 (28)	7 (70)	2 (13)
NrtI at randomisation				
TDF	13 (72)	18 (62)	4 (40)	7 (44)
TAF	4 (22)	8 (28)	4 (40)	6 (38)
ETV	1 (6)	4 (14)	2 (20)	3 (19)
Duration of current HBV treatment (years)	3.2±2.71	4.6±3.68	6.6±5.81	2.8±3.60
HBV DNA (log_{10} IU/ml; COBAS TaqMan) <LLOQ	18 (100)	27 (93)[c]	10 (100)	16 (100)
HBV DNA (Assembly Biosciences assay) LOD				
TD	12 (67)	27 (93)	2 (20)	6 (38)
TND	5 (28)	2 (7)	8 (80)	10 (63)
All	17 (94)[d]	29 (100)	10 (100)	16 (100)
HBV pgRNA (log_{10} U/ml)	3.16±1.51	3.65±1.46	1.56±0.08	1.66±0.33
<LLOQ	5 (28)	4 (14)	9 (90)	13 (81)
ALT (U/L)	27.2±19.37	26.6±16.30	21.1±9.72	27.0±13.47
HBeAg (log_{10} IU/ml)	0.43±0.96	0.55±0.98	−0.96±0.11	−1.00±0
<LLOQ	0	0	9 (90)[e]	16 (100)
HBsAg (log_{10} IU/ml)	3.57±0.52	3.48±0.40	3.35±0.65	2.99±0.56
<LLOQ	0	0	0	0
HBcrAg (log_{10} kU/ml)	2.95±0.91	3.00±0.95	0.64±0.55	0.49±0.68
<LLOQ	0	0	2 (20)	5 (31)
HBeAb positive	1 (6)	1 (3)	9 (90)	14 (88)
HBsAb positive	0	1 (3)	0	0

Data presented as n (%) or mean±SD. HBV DNA LLOQ (COBAS TaqMan Version 2.0) = 20 IU/ml or 1.30 log_{10} IU/ml. HBV DNA LOD (Assembly Biosciences) = 5 IU/ml or 0.70 log_{10} IU/ml. pgRNA LLOQ = 35 U/ml or 1.54 log_{10} U/ml. HBeAg LLOQ = 0.11 IU/ml or −0.96 log_{10} IU/ml. HBsAg LLOQ = 0.05 IU/ml or −1.30 log_{10} IU/ml. HBcrAg LLOQ = 1 kU/ml or 0 log_{10} kU/ml.

ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAb, hepatitis B e antigen antibody; HBeAg, hepatitis B e antigen; HBsAb, hepatitis B surface antigen antibody; HBsAg, hepatitis B surface antigen; ITT, intent-to-treat; LOD, limit of detection; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; pgRNA, pregenomic RNA; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; TD, target detected; TND, target not detected; VBR, vebicorvir.

[a]4 patients do not have recorded genotype. [b]A combination of not determinable and missing. [c]Randomisation of patients was based on results from screening and not Day 1. Predose Day 1 values are considered as Baseline for comparative purposes. At screening, all patients met protocol eligibility criteria with HBV DNA <20 IU/ml (COBAS TaqMan Version 2.0). Following receipt of the Day 1 laboratory results after initiation of treatment, 2 HBeAg-positive patients were found to have detectable HBV DNA of 25 IU/ml and 443 IU/ml, respectively. [d]1 HBeAg-positive patient discontinued the study early due to noncompliance with study drug. [e]At screening, 1 patient had HBeAg at LOD (0.11 IU/ml). On retest, it was determined to be below the LOD and was considered HBeAg negative for the purpose of randomisation. Day 1 HBeAg was 0.23 IU/ml and all subsequent visits were 0.12 or 0.13 IU/ml.

methods. Importantly, at Baseline, while 26/26 (100%) HBeAg-negative patients had HBV DNA <LLOQ by COBAS TaqMan, only 18/26 (69%) had HBV DNA <5 IU/ml by the high-sensitivity assay (Table 2). At Week 24, for PBO+NrtI, the proportion of patients with HBV DNA <5 IU/ml decreased from 8/10 (80%) at Baseline to 7/10 (70%) at Week 24. However, in VBR+NrtI recipients, the proportion of patients with HBV DNA <5 IU/ml increased from 10/16 (63%) at Baseline to 15/16 (94%) at Week 24 (difference between PBO+NrtI and VBR+NrtI for proportion of patients with HBV DNA <5 IU/ml was p = 0.2642). Overall, 22/26 (85%) virologically- suppressed HBeAg-negative patients had pgRNA <35 U/ml at Baseline (Table 2). At Week 24, the proportion of PBO+NrtI patients with HBV pgRNA <35 U/ml increased from 9/10 (90%) at Baseline to 10/10 (100%). In VBR+NrtI recipients, the proportion of patients with HBV pgRNA <35 U/ml increased from 13/16 (81%) at Baseline to 15/16 (94%) at Week 24. The use of the high-sensitivity HBV DNA and HBV pgRNA assays demonstrates the addition of VBR to NrtI leads to greater viral suppression than can be achieved with NrtI alone. No rebound in HBV DNA (virologic breakthrough) was observed in any patients; therefore, resistance sequencing was not performed.

Pharmacokinetic plasma concentrations

When administered with NrtI, mean trough plasma concentrations of VBR remained consistent throughout the 24-week study (1,280–1,600 ng/ml, 1,310–1,410 ng/ml, and 1,310–1,410 ng/ml for, VBR+ETV, VBR+TAF, and VBR+TDF, respectively; Fig. S2). When examining NrtI plasma concentrations, percent coefficient of variation tended to be numerically lower with ETV (0.35–1.78) than with TAF (9.67–21.30), and both were lower than TDF (72.40–89.10; Table S3). Mean trough plasma concentrations of ETV, TAF, and TDF were compared with the ratios of VBR+NrtI and PBO+NrtI ratio differences <2-fold were not considered clinically significant (Table S3). Overall, PK data from the study indicate no clinically significant drug interactions between VBR and the NrtIs at the dose levels studied.

Safety

At least 1 TEAE was reported by 53% and 29% of patients in the VBR+NrtI and PBO+NrtI groups, respectively; most of these were of Grade 1 and 2 severity, with no patient experiencing a Grade 4 or serious AE (Table 3). One patient in the PBO+NrtI arm

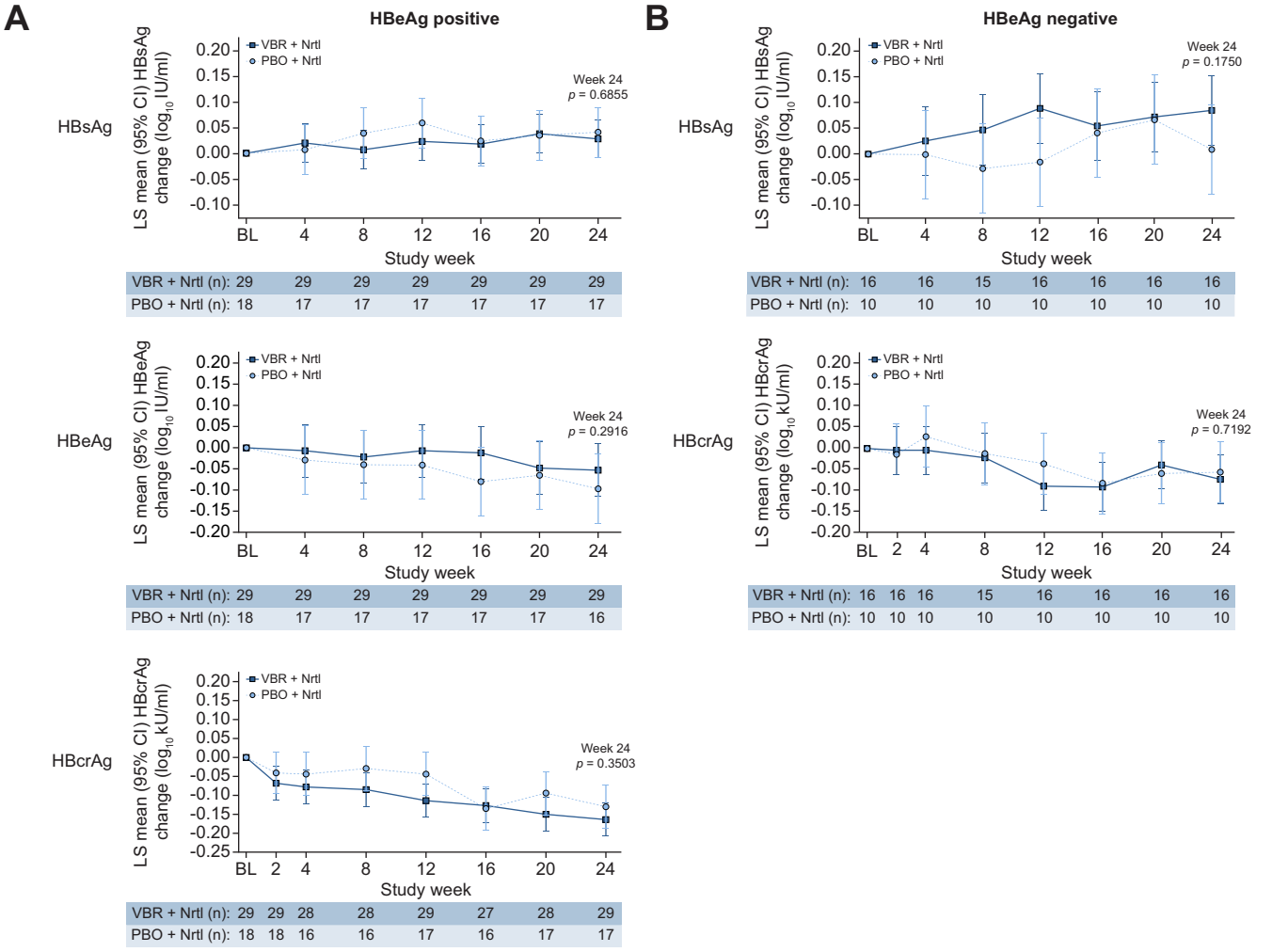

Fig. 2. Mean change from Baseline in viral antigens ITT population. (A) HBeAg-positive and (B) HBeAg-negative patients. None of the viral antigens differed between VBR+NrtI and PBO+NrtI groups at any time points (p>0.05; linear mixed-effects repeated-measure model). The differences in the number of samples tested per time point reflect missing samples and samples not tested due to haemolysis or receipt at the laboratory beyond stability. BL, Baseline; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ITT, intent-to-treat; LS, least squares; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; VBR, vebicorvir.

experienced Grade 3 arthralgia, considered unrelated to study drug and resolved on treatment with celecoxib. No patients receiving VBR+NrtI experienced a Grade 3 AE. No patients had an AE leading to study treatment discontinuation, serious AE, or death. The most common AE in patients in the PBO+NrtI group was arthralgia (n = 2 [7%]), while in the VBR+NrtI group, it was upper respiratory tract infection (n = 5 [11%]). There were 12 events of rash in 7/45 (16%) VBR+NrtI patients, including preferred terms of rash, rash macular, and rash maculo-papular. Most of these cases were macular or maculopapular rash not associated with pruritus, tended to be Grade 1, were self-limiting or resolved following treatment with topical agents, and did not lead to study drug discontinuation. There were no systemic signs, symptoms, or laboratory abnormalities associated with any cases of rash. No ALT flares were observed. A single patient in the VBR+NrtI group had Grade 3 low lymphocytes, which was the only observed laboratory abnormality of Grade 3 or higher (Table 3).

Discussion

Current NrtI therapy for cHBV is generally safe and well tolerated. In 75–80% of patients, HBV DNA can be suppressed to <LLOQ by available assays after 48 weeks of treatment. However, low-level residual viremia remains, which almost universally leads to viral rebound following NrtI cessation, necessitating prolonged, suppressive therapy with NrtIs. Attaining durable, off-treatment virologic responses will likely require combination regimens utilising multiple agents with distinct and complementary mechanisms of action. Profound reduction in HBV replication is expected to be a key component of successful combination therapies that can lead to a functional cure.

In this randomised, phase II clinical study, we tested the hypothesis that blocking additional steps in the HBV replication cycle through the addition of the HBV core inhibitor VBR to existing NrtI therapy would lead to greater levels of viral suppression, with subsequent declines in HBV-related antigens. The

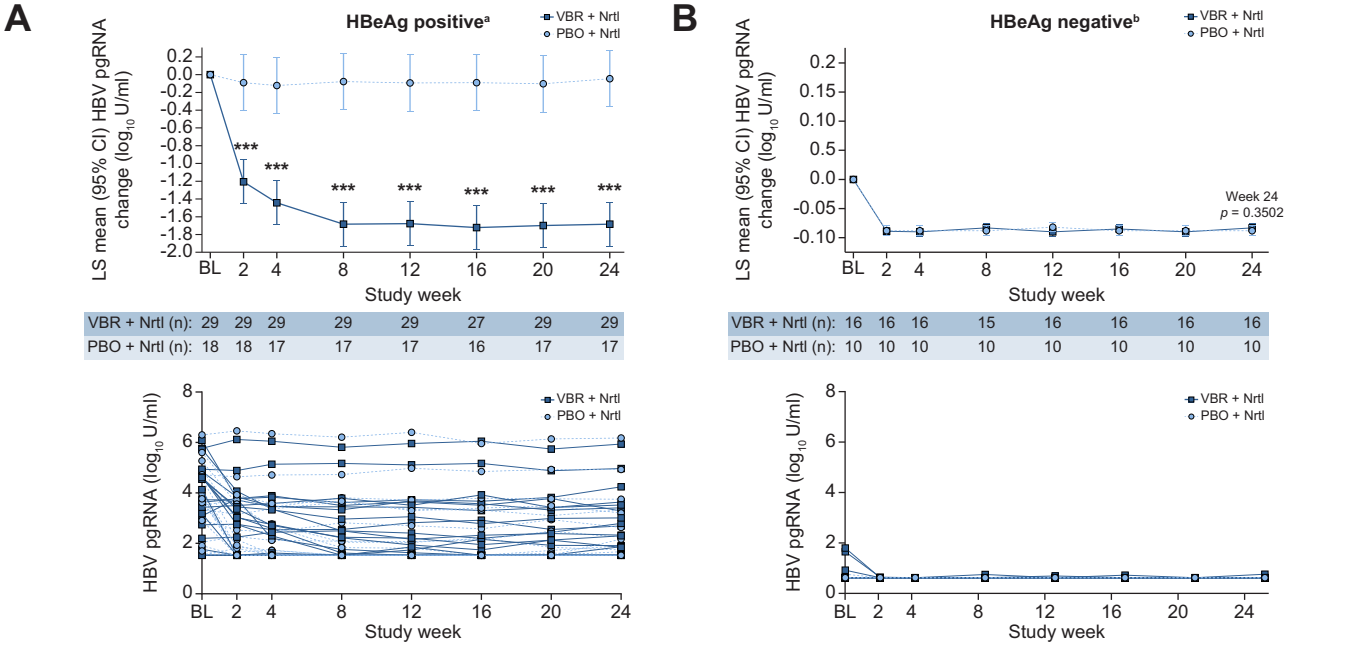

Fig. 3. Mean HBV pgRNA (log_{10} U/ml) change from Baseline over time and individual patient data (ITT population). LS mean change from Baseline (95% CI) for pgRNA (top panels) was different between VBR+NrtI and PBO+NrtI at Week 24 for (A) HBeAg-positive patients (*p <0.0001), but not (B) HBeAg-negative patients (p = 0.3502; linear mixed-effects repeated-measure model). Bottom panels show individual patient HBV pgRNA data. The differences in the number of samples tested per time point reflect missing samples and samples not tested due to haemolysis or receipt at the laboratory beyond stability. [a]9/47 (19%) and 20/46 (43%) patients were <LLOQ at Baseline and at Week 24, respectively. [b]22/26 (85%) and 25/26 (96%) patients were <LLOQ at Baseline and at Week 24, respectively. Only HBeAg-negative patients with detectable values are presented in the individual patient plot. BL, baseline; CI, confidence interval; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ITT, intent-to-treat; LS, least-squares; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; pg, pregenomic; VBR, vebicorvir.

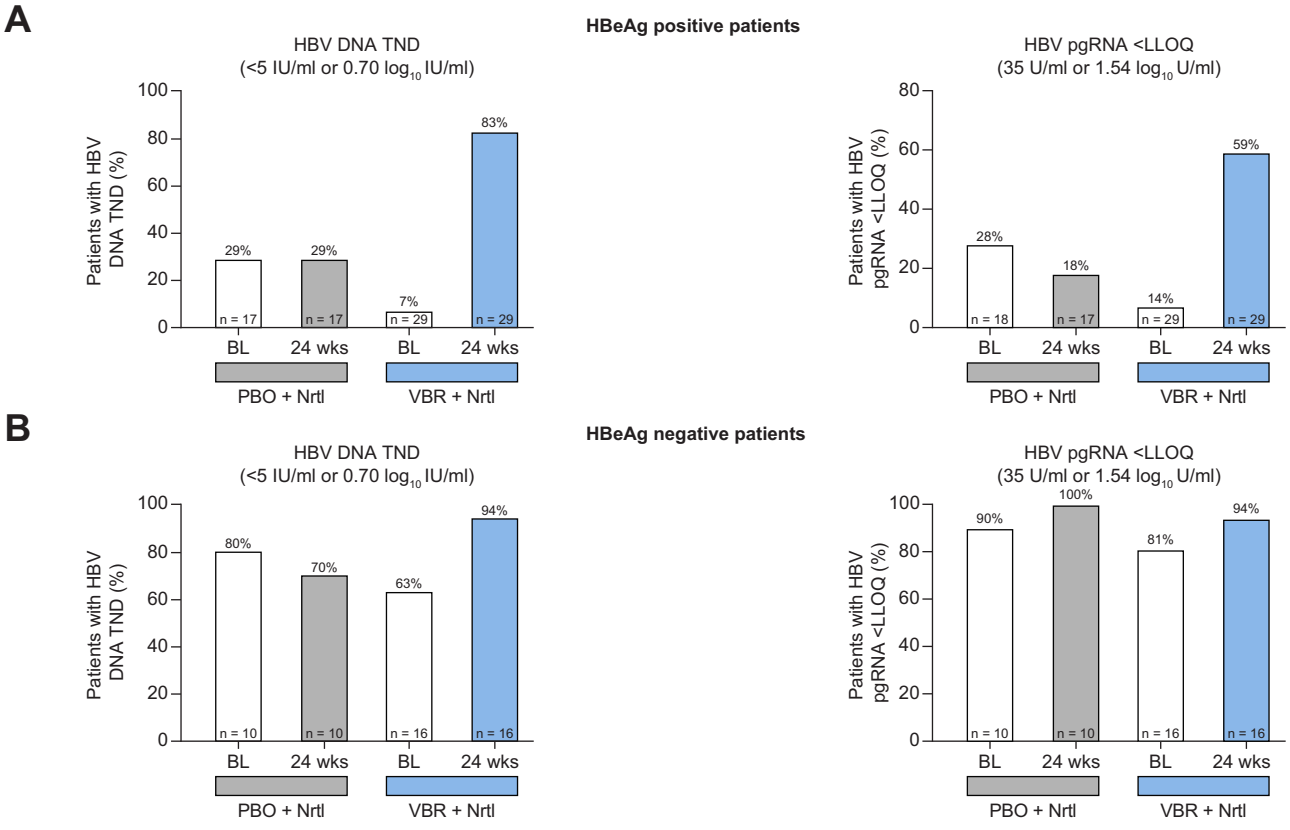

Fig. 4. Proportion of patients with undetectable HBV DNA or HBV pgRNA at Baseline and Week 24. Proportions of (A) HBeAg positive and (B) HBeAg-negative patients with undetectable HBV DNA and HBV pgRNA at Baseline and Week 24 by high-sensitivity analytical methods. BL, Baseline; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; pg, pregenomic; TND, target not detectable; VBR, vebicorvir; Wks, weeks.

Table 3. Summary of adverse events (safety population).

Event	PBO+NrtI (n = 28)	VBR+NrtI (n = 45)
Any TEAE[a]	8 (29)	24 (53)
TEAEs leading to study drug discontinuation	0	0
Deaths	0	0
AEs Grade 3–4	1 (4)	0
Serious AEs	0	0
TEAEs in ≥5% of either population		
Rash	0 (0)	7 (16)
Upper respiratory tract infection	1 (4)	5 (11)
Nausea	0	4 (9)
Pruritus	0	3 (7)
Arthralgia	2 (7)	1 (2)
Grade 3 or 4 laboratory abnormalities in ≥ 1% of patients in any population		
Lymphocytes low	0	1 (2)

Data presented as n (%).
AEs, adverse events; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; TEAE, treatment-emergent adverse event; VBR, vebicorvir.
[a]Preferred terms are coded using the Medical Dictionary for Regulatory Activities Version 21.0. For laboratory abnormalities, treatment-emergent is defined as normal at Baseline then Grade 1–4 at a post-Baseline visit, or increased Grade after Baseline.

study confirmed previous findings of persistent low-level viremia in NrtI-suppressed patients with HBV DNA below the LLOQ of available assays.[13] When assessed by higher-sensitivity assays over the 24-week treatment period, VBR+NrtI resulted in deeper levels of viral suppression in both HBeAg-positive and -negative patients compared to continued NrtI monotherapy. Specifically, with VBR+NrtI, HBV DNA was reduced to a greater extent in both HBeAg-positive and -negative patients compared with PBO+NrtI, suggesting that low-level persistent viremia was not eliminated despite long-term therapy with NrtI alone. The only source of HBV pgRNA is cccDNA, and consequently this marker represents the most direct measure of cccDNA transcriptional activity. Similar findings were observed with HBV pgRNA in HBeAg-positive patients but not HBeAg-negative patients, where HBV pgRNA levels were already very low before the start of VBR treatment. The clinical significance of this greater suppression of HBV DNA and HBV pgRNA with VBR+NrtI needs to be established. HBV integrations, which may result in carcinogenesis,[14] are still detectable after prolonged NrtI therapy.[15,16] According to a recent study, patients virologically-suppressed on NrtI therapy with residual HBV DNA and HBV pgRNA detected by highly sensitive assays had a significantly higher risk of developing HCC.[17] A more profound suppression of HBV nucleic acids by combination antiviral therapy may further decrease the risk of HCC development.[17]

While VBR+NrtI increased HBV DNA and HBV pgRNA suppression, reductions in HBeAg and HBsAg levels from Baseline to Week 24 were similar between both treatments. Since reductions in viral antigens through core inhibition can only occur via reduction of cccDNA transcriptional activity, longer-term treatment may be required to induce downstream HBV antigen reductions. The addition of core inhibitors to novel combination regimens with agents directly interfering with HBV antigen production, such as RNA interference, may improve treatment outcomes through complementary mechanisms. Studies are ongoing with VBR to test this hypothesis.

Current first-generation core inhibitors have a relatively low barrier to resistance when used as a monotherapy.[18] In this study, no patients experienced protocol-specified virologic breakthrough criteria, and therefore, resistance sequencing was not performed.

Standard-of-care NrtIs are generally well tolerated,[5,19] but some core inhibitors in clinical development have been discontinued due to emergent safety concerns.[20–22] Vebicorvir was well tolerated when administered with NrtI for 24 weeks in virologically suppressed, HBeAg-positive and -negative patients. Rashes were observed in several patients taking VBR, which were generally low-grade, self-limiting, or treated with topical agents and did not lead to study drug discontinuation. No VBR and NrtI drug-drug interactions were observed at the dose levels evaluated in this study, which supports continued assessment of VBR in NrtI combination regimens.

This study has several limitations. Treatment stratification was based on HBeAg status (positive *vs.* negative) and assignment to VBR+NrtI or PBO+NrtI. Consequently, there are numerical differences in Baseline disease characteristics between subgroups with interpretation limited by the small sample size. *Ad hoc* analyses show that previous NrtI duration of less than 3 years results in a numerically greater (but not significant) decrease in HBV DNA compared with patients with greater than 3 years of previous NrtI treatment (data not shown). The small number of patients in these subanalyses make interpretation of this finding difficult. These potential differences in Baseline HBV characteristics should be explored in future studies of larger sample size, where additional treatment stratification factors may be incorporated. Although safety, tolerability, and deeper suppression of HBV DNA and HBV pgRNA were demonstrated, the 24-week treatment duration may not be long enough to fully characterise antiviral activity, particularly in regard to HBV antigens, or to reveal potential AEs that may emerge after prolonged treatment. Longer-term studies would also confirm the absence of virologic breakthrough for the VBR+NrtI regimen as demonstrated in this study. Following completion of this study, eligible patients were offered enrollment in a long-term extension study (NCT03780543) in which they received open-label VBR+NrtI for up to 148 weeks. While the study was sufficiently powered to achieve statistical significance across treatment arms, an increase in the number of study patients would help generalise these results to a global population. Patients were not stratified by HBV genotype, and a numerically greater proportion of patients receiving VBR had genotype B, while a numerically greater proportion of patients receiving NrtI monotherapy had genotype C. In a preclinical study, VBR demonstrated broad-spectrum activity against HBV genotypes A, B, C, and D,[23] and it was predicted to have similar activity across HBV genotypes. It is not expected that HBV genotype at Baseline would influence the antiviral potency of VBR in patients with cHBV.

Approximately one-third of virologically-suppressed patients receiving long-term standard-of-care NrtI therapy had persistent low-level viremia when measured by high-sensitivity assays. In HBeAg-positive patients, VBR+NrtI led to greater viral suppression as assessed by HBV DNA and HBV pgRNA compared to continued NrtI monotherapy. Changes in HBV viral antigens from Baseline to Week 24 were similar between both treatment groups. In this study, VBR administered with NrtIs for 24 weeks resulted in no deaths, serious AEs, or AEs leading to discontinuation of VBR, and all TEAEs were Grade 2 or less. No drug-drug interactions with NrtIs and no

treatment-emergent resistance were observed in this study. These data support further clinical investigation of core inhibitors as potential therapeutic agents in patients with cHBV.

Abbreviations

AEs, adverse events; ALT, alanine aminotransferase; cccDNA, covalently closed circular DNA; cHBV, chronic hepatitis B virus infection; ETV, entecavir; HCC, hepatocellular carcinoma; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; ITT, intent-to-treat; LLOQ, lower limit of quantification; LOD, limit of detection; LSM, least-squares mean; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; PK, pharmacokinetic; Pol/RT, polymerase reverse transcriptase; pgRNA, pregenomic RNA; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; TEAE, treatment-emergent adverse event; ULN, upper limit of normal; VBR, vebicorvir.

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Conflict of interest

Man-Fung Yuen reports being an advisor/consultant for and/or having received grant/research support from AbbVie, Aligos Therapeutics, Antios Therapeutics, Arbutus Biopharma, Arrowhead Pharmaceuticals, Assembly Biosciences, Bristol-Myers Squibb, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, Fujirebio Incorporation, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, Roche, Springbank Pharmaceuticals, Silverback Therapeutics, Sysmex Corporation, and Vir Biotechnology. Kosh Agarwal reports being on the advisory board, a consultant, and a speaker for AbbVie, Assembly Biosciences, Aligos, Arbutus, Bristol-Myers Squibb, Gilead Sciences, Immunocore, Janssen, Merck, Novartis, Roche, Sobi, Shinoigi, and Vir; and receiving grants from Bristol-Myers Squibb, Gilead Sciences, and Roche. Xiaoli Ma reports being a consultant and being on the speakers bureau for Gilead Sciences. Tuan T. 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Uri Lopatin reports being a former employee and holding stock interest in Assembly Biosciences. Richard Colonno reports being an employee of and holding stock interest in Assembly Biosciences. Julie Ma reports being an employee of and holding stock interest in Assembly Biosciences. Steven J. Knox reports being an employee of and holding stock interest in Assembly Biosciences. Luisa M. Stamm reports being an employee of and holding stock interest in Assembly Biosciences. Maurizio Bonacini reports being a member of the speaking bureau for Intercept Pharmaceuticals, Gilead Sciences, and AbbVie and has received research support from Assembly Biosciences, Intercept Pharmaceuticals, Viking Therapeutics, and Boehringer Ingelheim. Ira M. 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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors approved the final manuscript prior to submission. Man-Fung Yuen contributed to study oversight, experiments and procedures, data acquisition, data analysis, data interpretation, and critical revision of the manuscript. Kosh Agarwal contributed to study oversight, experiments and procedures, data acquisition, data analysis, data interpretation, and critical revision of the manuscript. Xiaoli Ma contributed to study oversight, experiments and procedures, data acquisition, data interpretation, and critical revision of the manuscript. Tuan T. Nguyen contributed to study oversight, experiments and procedures, data acquisition, data interpretation, and critical revision of the manuscript. Eugene R. Schiff contributed to study oversight, experiments and procedures, data acquisition, data interpretation, and critical revision of the manuscript. Hie-Won L. 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Data availability statement

Data can be made available to researchers upon reasonable request.

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Supplementary data

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