Research article

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Long-term open-label vebicorvir for chronic HBV infection: Safety and off-treatment responses

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Background & Aims: The investigational first-generation core inhibitor vebicorvir (VBR) demonstrated safety and antiviral activity over 24 weeks in two phase IIa studies in patients with chronic HBV infection. In this long-term extension study, patients received open-label VBR with nucleos(t)ide reverse transcriptase inhibitors (NrtIs).

Methods: Patients in this study (NCT03780543) previously received VBR + NrtI or placebo + NrtI in parent studies 201 (NCT03576066) or 202 (NCT03577171). After receiving VBR + NrtI for ≥52 weeks, stopping criteria (based on the treatment history and hepatitis B e antigen status in the parent studies) were applied, and patients either discontinued both VBR + NrtI, discontinued VBR only, or continued both VBR + NrtI. The primary efficacy endpoint was the proportion of patients with HBV DNA <20 IU/ml at 24 weeks off treatment.

Results: Ninety-two patients entered the extension study and received VBR + NrtI. Long-term VBR + NrtI treatment led to continued suppression of HBV nucleic acids and, to a lesser extent, HBV antigens. Forty-three patients met criteria to discontinue VBR + NrtI, with no patients achieving the primary endpoint; the majority of virologic rebound occurred ≥4 weeks off treatment. Treatment was generally well tolerated, with few discontinuations due to adverse events (AEs). There were no deaths. Most AEs and laboratory abnormalities were related to elevations in alanine aminotransferase and occurred during the off-treatment or NrtI-restart phases. No drug–drug interactions between VBR + NrtI and no cases of treatment-emergent resistance among patients who adhered to treatment were observed.

Conclusions: Long-term VBR + NrtI was safe and resulted in continued reductions in HBV nucleic acids following completion of the 24-week parent studies. Following treatment discontinuation, virologic relapse was observed in all patients. This first-generation core inhibitor administered with NrtI for at least 52 weeks was not sufficient for HBV cure.

Clinical trial number: NCT03780543.

Impact and implications: Approved treatments for chronic hepatitis B virus infection (cHBV) suppress viral replication, but viral rebound is almost always observed after treatment discontinuation, highlighting an unmet need for improved therapies with finite treatment duration producing greater therapeutic responses that can be sustained off treatment. First-generation core inhibitors, such as vebicorvir, have mechanisms of action orthogonal to standard-of-care therapies that deeply suppress HBV viral replication during treatment; however, to date, durable virologic responses have not been observed after treatment discontinuation. The results reported here will help researchers with the design and interpretation of future studies investigating core inhibitors as

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possible components of finite treatment regimens for patients with cHBV. It is possible that next-generation core inhibitors with enhanced potency may produce deeper and more durable antiviral activity than first-generation agents, including vebicorvir.

Introduction

Chronic HBV infection (cHBV) represents a significant public health burden. Worldwide, ~296 million people have cHBV, and ~900,000 die annually from HBV-related causes, primarily from complications of cirrhosis and/or hepatocellular carcinoma.[1–3] Effective cHBV treatment is essential to reduce these risks. Current treatment includes finite injectable IFNα and chronic oral nucleos(t)ide reverse transcriptase inhibitors (NrtIs). IFNα[4,5] and NrtIs[6,7] both demonstrate on-treatment antiviral activity, but durable off-treatment virologic responses are rare. For therapeutic regimens to achieve 'functional cure' (defined as sustained suppression of HBV DNA <lower limit of quantification [LLOQ] for ≥6 months post-treatment and undetectable HBsAg with/without HBsAg seroconversion),[8] novel combination approaches incorporating mechanisms complementary to existing treatments are needed.

Vebicorvir (VBR) is an investigational, novel, pangenotypic, first-generation core inhibitor that inhibits HBV replication via mechanisms distinct from NrtIs: inhibition of pregenomic RNA (pgRNA) encapsidation, which prevents assembly and release of viral particles, and disruption of viral capsids, which prevents the formation of covalently closed circular (ccc)DNA. In phase IIa studies, when combined with NrtIs, VBR led to deeper reductions in HBV DNA and pgRNA *vs.* NrtI monotherapy over 24 weeks of treatment in both virologically-suppressed (VS; study 201)[9] and treatment-naive (TN; study 202)[10] patients with cHBV. However, mean reductions in HBsAg in patients who received VBR + NrtI in studies 201 and 202 were not significantly different from patients who received placebo (PBO) + NrtI. This report presents the results of an open-label, long-term extension in which patients who previously participated in study 201 or 202 received VBR + NrtI for up to 148 weeks.

Patients and methods

Study population and design

Study 211 was a phase II, open-label, multicentre extension study (NCT03780543) evaluating the safety and efficacy of VBR + NrtI in patients with cHBV who had previously completed 24 weeks of treatment in either study 201 (NCT03576066)[9] or 202 (NCT03577171).[10] Patients were enrolled from 24 sites in the USA, Canada, Hong Kong, New Zealand, and the UK. Complete inclusion and exclusion criteria and details on treatment compliance are provided in the Supplementary materials.

All patients in study 211 received open-label 300 mg VBR (Assembly Biosciences, Inc., South San Francisco, CA, USA), administered as three 100-mg tablets once daily along with standard-of-care NrtI per the manufacturer's instructions. The VBR dose regimen was the same as that used in the parent studies, which was determined from the phase Ib study.[11] Most patients from study 201 took tenofovir disoproxil fumarate or tenofovir alafenamide as their NrtI at baseline, whereas all patients from study 202 received entecavir (ETV) along with VBR or PBO.

Patients could be treated for up to 148 weeks. The actual duration of treatment for each patient in study 211 was based on their respective HBV treatment history (*i.e.* VS or TN) and HBeAg status (positive or negative) at baseline in their parent study, along with their individual virologic response in study 211 at Week (W) 52. Based on these factors, each patient was assigned to one of three protocol-specified treatment actions (TAs): discontinue both VBR + NrtI, discontinue VBR only and continue NrtI alone, or continue both VBR + NrtI (Table S1).

Individual safety and virologic responses in study 211 were influenced by patient characteristics at baseline in the parent studies and the respective treatments received—that is, VBR + NrtI or PBO + NrtI. Therefore, data from study 211 are reported according to HBeAg status and treatment assignment in the parent study. Additionally, study 211 data are reported from three treatment phases: 'on-treatment', during VBR + NrtI therapy; 'off-treatment', for patients who discontinued VBR + NrtI; and 'after NrtI restart', for patients requiring reintroduction of antivirals after stopping both VBR + NrtI (Table S2). The study design is shown in Fig. S1.

This study was conducted in accordance with the principles of the Declaration of Helsinki, Council for International Organizations of Medical Sciences International Ethical Guidelines, and applicable Good Clinical Practice guidelines. Investigative sites obtained written informed consent before patients were enrolled.

Endpoints

The primary endpoint was the proportion of patients with HBV DNA <20 IU/ml (LLOQ) at 24 weeks off treatment. Secondary endpoints included incidence of adverse events (AEs), premature discontinuations as a result of AEs, abnormal safety laboratory results, proportion of patients with abnormal alanine aminotransferase (ALT) at study 211 baseline who achieved normal ALT at end of treatment (EOT) and end of study (EOS), and incidence of viral rebound off treatment. A complete list of exploratory endpoints is included in the Supplementary material.

Safety assessments

Primary safety assessments included the number of AEs, defined as any untoward medical occurrence in a treated patient regardless of the causal relationship with treatment. The severity of each AE and laboratory abnormality was assessed by the investigator according to the Division of AIDS Toxicity Grading of Laboratory Abnormalities and Clinical AEs. Additional definitions of AEs are described in the Supplementary material. Full schedules of efficacy, safety, and pharmacokinetic (PK) assessments are described in Tables S3–S6 and the Supplementary material.

Assays

Methodological details for measuring HBV DNA, HBV pgRNA, total nucleic acids (TNA; HBV DNA + pgRNA), and antigens are described in the Supplementary material.

To assess for potential HBV sequence changes associated with viral resistance or blunted treatment response, serum samples

from patients with on-treatment viral rebound (≥1 $\log_{10}$ HBV DNA increase from the on-treatment nadir) were selected for sequencing of HBV core and polymerase/reverse transcriptase genes. Plasma samples quantifying VBR and NrtI concentrations were collected predose at W48 and analysed at the bioanalytical laboratory (Agilex, Thebarton, Australia) using validated methodologies.

Statistical analysis

Baseline characteristics and demographics were summarised using the all-enrolled analysis set, which included all patients enrolled in study 211. Efficacy-related endpoints were assessed using the full analysis set, which included all patients who received any dose of study drug and had at least one postdose assessment for the endpoint of interest.

The safety population included all patients who received at least one dose of study drug. PK evaluation included all patients in the safety population who had available VBR or NrtI PK data. Sample size was not based on statistical considerations and consequently descriptive statistics are used throughout. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Patient disposition

Overall, 92/98 (94%) patients (69/73 [95%] from study 201; 23/25 [92%] from study 202) were enrolled between December 2018 and June 2019. Patient disposition is shown in Fig. 1. All enrolled patients received VBR + NrtI during the on-treatment phase. Forty-three of 69 (62%) patients from study 201 discontinued VBR + NrtI during study 211, with 18/69 (26%) discontinuing VBR and continuing NrtI. Of the 43 patients from study 201 who discontinued VBR + NrtI, 30/43 (70%) restarted NrtI after discontinuation, with nine of 43 (21%) remaining off treatment at EOS. No patients from study 202 discontinued both VBR + ETV, with six of 23 (26%) discontinuing VBR and continuing ETV. The duration of time patients spent in the on- and off-treatment phase is described in the Supplementary material.

Baseline demographics and disease characteristics

At study 211 baseline, overall mean age was 44 years, with 62/92 (67%) patients aged <50 years. Most patients were male (52/92; 57%) and Asian (80/92; 87%). Some baseline demographics varied according to the respective parent study, with greater proportions of patients originating from study 201 being older (mean age 46 *vs.* 36 years) and male (64% *vs.* 35%) *vs.* those from study 202 (Table S7). Additional baseline disease characteristics for patients in study 211 are shown in Table S8 and described in further detail in the Supplementary material.

Primary efficacy endpoint

Forty-three patients (20 HBeAg-positive, 23 HBeAg-negative) from study 201 met the protocol-specified TA criteria to discontinue VBR + NrtI. Following discontinuation, all patients experienced virologic relapse with no patients achieving HBV DNA <LLOQ at 24 weeks off treatment (Fig. 2). Of the patients who were HBeAg-negative, 16/23 (70%) relapsed by the 4W follow-up visit, whereas seven of 23 (30%) relapsed by the 12W or 16W follow-up visits. Of the patients who were HBeAg-positive, 17/18 (94%) relapsed by the 4W follow-up visit (one patient had relapsed by the 12W follow-up visit). No patients from study 202 met the TA criteria to discontinue VBR + ETV.

Changes in HBV nucleic acids and antigens during the on-treatment phase

Among patients who were HBeAg-negative who received PBO + NrtI and VBR + NrtI in study 201, most had HBV DNA target not detected (TND) at on-treatment baseline and at EOT using the COBAS TaqMan assay (Table 1). With the Assembly assay, a greater percentage of patients with available samples had HBV DNA TND at on-treatment baseline, with all patients who were HBeAg-negative having HBV DNA TND at EOT. Approximately half of patients who were HBeAg-positive from study 201 who received PBO + NrtI and VBR + NrtI had HBV DNA TND at on-treatment baseline and 30–50% had HBV DNA TND at EOT by the COBAS TaqMan assay. When assessed by the Assembly assay, five of 16 (31%) and 18/27 (67%) patients who were HBeAg-positive who received PBO + NrtI and VBR + NrtI, respectively, during study 201 had HBV DNA TND at on-treatment baseline, with numerically more patients having HBV DNA TND at EOT in study 211. When assessed by the Assembly assay, no patients from study 202 had HBV DNA TND at on-treatment baseline, with none of five and one of eight (13%) patients who received PBO + NrtI and VBR + NrtI, respectively, during study 202 having HBV DNA TND at W2 in study 211. When assessed using the COBAS TaqMan assay, no patients from study 202 had HBV DNA TND at on-treatment baseline and at W2 in study 211. Per the COBAS TaqMan assay, six of 23 (26%) patients from study 202 had HBV DNA TND at EOT (the percentage of patients was greater when assessed by the Assembly assay [eight of 13 (62%)]; Table 1). Because mean baseline levels of HBV DNA were greater in patients who received PBO + NrtI from study 202 *vs.* patients who received VBR + NrtI (Table S8), there were greater mean reductions in HBV DNA at EOT among patients who received PBO + ETV in study 202 *vs.* patients who received VBR + ETV (Fig. 3A).

Changes in HBV pgRNA and TNA during the on-treatment phase are shown in Table 1. Because mean baseline levels of pgRNA were greater in patients who received PBO + NrtI from study 201 *vs.* patients who received VBR + NrtI, there were greater mean reductions in pgRNA levels among patients who received PBO + NrtI in study 201 at EOT *vs.* patients who received VBR + NrtI in study 201. These findings were also observed for TNA, as only patients who were HBeAg-positive showed a mean change from on-treatment baseline in TNA at EOT. Greater reductions in HBV pgRNA in patients who received VBR + ETV *vs.* patients who received PBO + ETV from study 202 led to different levels of HBV pgRNA between treatment groups at on-treatment baseline. Given this, greater mean reductions from on-treatment baseline in mean HBV pgRNA were observed among study 202 patients who had received PBO + ETV *vs.* VBR + ETV at EOT (Fig. 3B).

Minimal mean changes from on-treatment baseline at EOT in HBV antigens were observed (Table S9). At EOT, one patient (PBO + ETV) from study 202 achieved HBeAg seroconversion (defined as antigen loss with the appearance of antibodies), which was maintained through EOS. At EOS, two additional patients achieved HBeAg seroconversion (both received VBR + NrtI in study 201). No patients achieved HBsAg seroconversion.

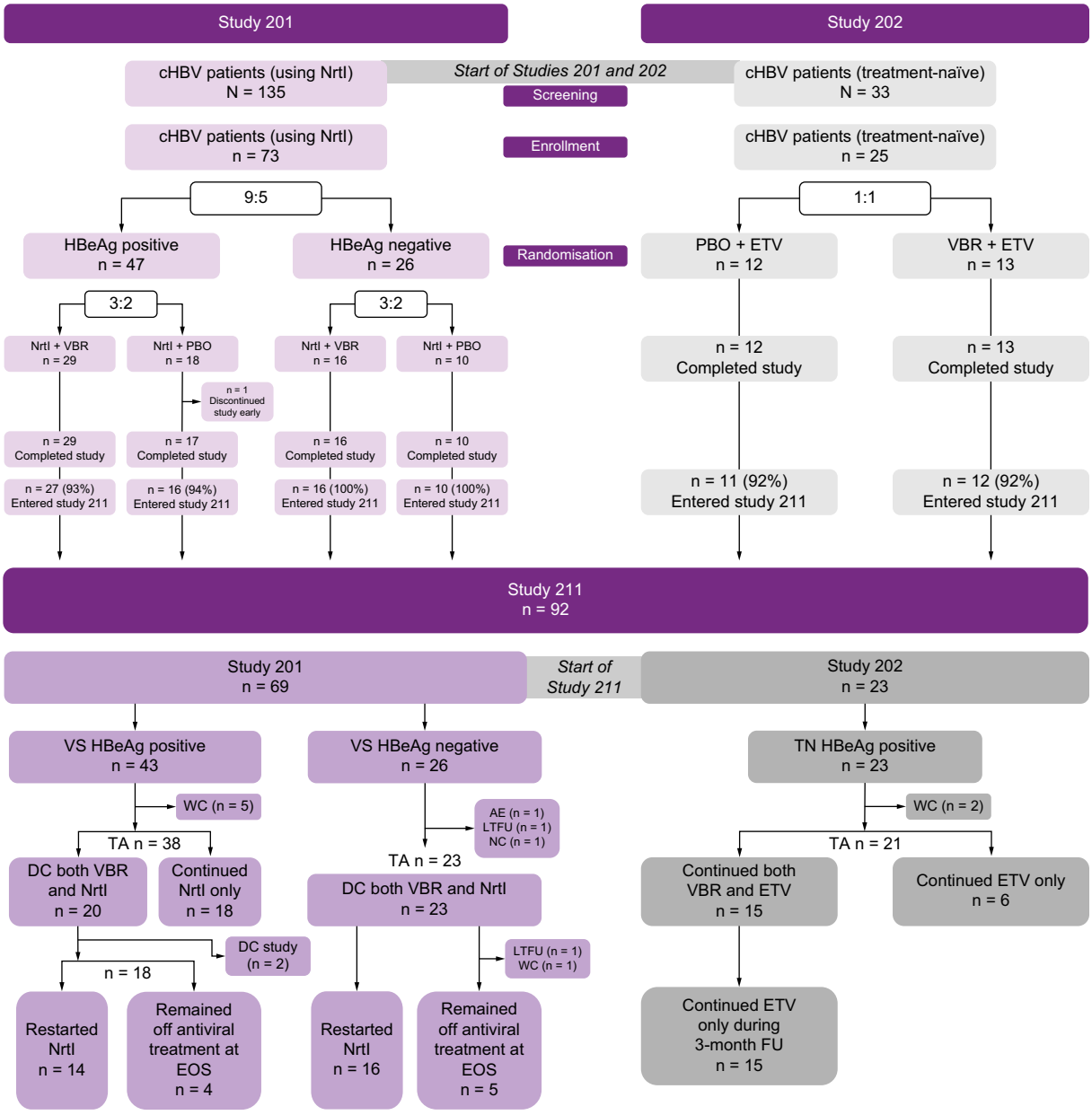

Fig. 1. Study disposition in patients from studies 201 and 202 taking VBR in study 211. Study 211 outcomes are based on the treatment history of the patients in parent studies 201 and 202. AE, adverse event; cHBV, chronic hepatitis B virus infection; DC, discontinued; EOS, end of study; ETV, entecavir; FU, follow-up; LTFU, lost to follow-up; NC, non-compliance; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; TA, treatment action; TN, treatment-naive; VBR, vebicorvir; VS, virologically-suppressed; WC, withdrew consent.

Changes in HBV nucleic acids and antigens in patients who discontinued both VBR + NrtI during the off-treatment phase

Mean HBV DNA and TNA increases from off-treatment baseline at the end of the off-treatment period were greater among patients who were HBeAg-positive *vs.* HBeAg-negative from study 201 (Table 2). The one patient who was HBeAg-positive from study 201 with pgRNA results available, who discontinued both VBR + NrtI, had a change from baseline to end of off-treatment phase of 4.7 $\log_{10}$ U/ml. Individual patient HBV DNA levels by HBeAg status in the parent studies throughout the off-treatment phase are shown in Fig. 2. During the off-treatment phase, 17/20 (85%) patients who were HBeAg-positive and 16/23 (70%) patients who were HBeAg-negative relapsed with HBV DNA levels >2,000 IU/ml.

Mean antigen levels tended to be slightly greater at the end of the off-treatment period *vs.* off-treatment baseline in patients who discontinued both VBR + NrtI during the off-treatment phase (Table S10). Mean HBV DNA declined ~4 $\log_{10}$ from baseline among patients who discontinued VBR + NrtI when they restarted NrtI (Table S11). Mean decreases in HBV antigens among patients who restarted NrtI after discontinuing VBR + NrtI were ≤1 $\log_{10}$ for all antigens (Table S12). Among patients who discontinued VBR and continued NrtI/ETV during the off-treatment phase, the mean increase from baseline in HBV DNA

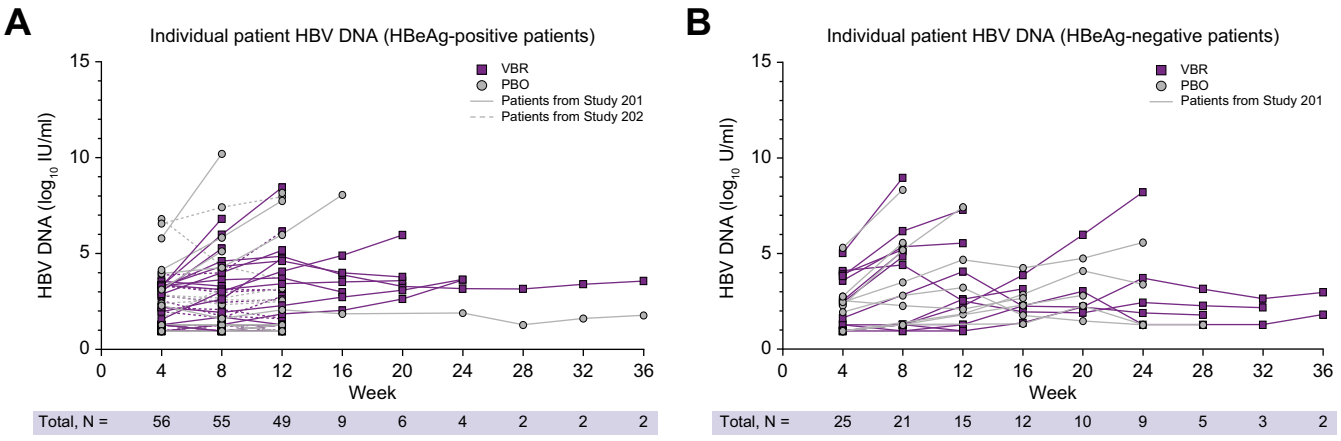

Fig. 2. Individual patient HBV DNA during the off-treatment phase in patients from study 211. HBV DNA levels in (A) patients who were HBeAg-positive and (B) patients who were HBeAg-negative during the off-treatment phase in study 211. Parent study designation as well as treatment received during the parent studies are shown. No patients remained <LLOQ of 20 IU/ml (1.3 log_{10} IU/ml) off treatment. LLOQ, lower limit of quantification; PBO, placebo, VBR, vebicorvir.

Table 1. Observed changes in HBV DNA and pgRNA during the on-treatment phase in study 211 (FAS).

	Patients originating from study 201 (on-treatment phase)					
	VS HBeAg (-)			VS HBeAg (+)		
	PBO + NrtI n = 10	VBR + NrtI n = 16	Total n = 26	PBO + NrtI n = 16	VBR + NrtI n = 27	Total n = 43
HBV DNA TND						
Baseline*	6/10 (60)	13/16 (81)	19/26 (73)	8/15 (53)	16/27 (59)	24/43 (57)
HBV DNA TND						
EOT*	8/10 (80)	13/16 (81)	21/26 (81)	8/16 (50)	8/27 (30)	16/43 (37)
HBV DNA TND						
Baseline†	9/10 (90)	12/16 (75)	21/26 (81)	5/16 (31)	18/27 (67)	23/43 (53)
HBV DNA TND						
EOT†	10/10 (100)	14/14 (100)	24/24 (100)	13/15 (87)	23/27 (85)	36/42 (86)
HBV DNA change from baseline, log_{10} IU/ml, mean (SD)						
EOT	-0.1 (0.14)	0.2 (0.81)	0.1 (0.64)	0.0 (0.23)	0.2 (0.61)	0.1 (0.51)
HBV pgRNA change from baseline, log_{10} U/ml, mean (SD)						
EOT	0 (0.00)	0.1 (0.38)	0.1 (0.29)	-1.4 (1.02)	0 (0.47)	-0.5 (0.98)
HBV TNA change from baseline, log_{10} U/ml, mean (SD)‡						
EOT	0 (0.00)	0 (0.00)	0 (0.00)	-1.2 (1.10)	0.0 (0.14)	-0.5 (0.91)

	Patients originating from study 202 (TN HBeAg +; on-treatment phase)		
	PBO + ETV n = 11	VBR + ETV n = 12	Total N = 23
HBV DNA TND			
Baseline*	0	0	0
Week 2*	0	0	0
HBV DNA TND			
EOT*	3/11 (27)	3/12 (25)	6/23 (26)
HBV DNA TND			
Baseline†	ND§	ND§	ND§
Week 2†	0¶	1/8 (13)	1/13 (8)
HBV DNA TND			
EOT†	5/5 (100)	3/8 (38)	8/13 (62)
HBV DNA change from baseline, log_{10} IU/ml, mean (SD)			
EOT	-2.1 (1.08)	-0.8 (0.70)	-1.4 (1.09)
HBV pgRNA change from baseline, log_{10} U/ml, mean (SD)			
EOT	-3.0 (1.38)	-0.6 (0.75)	-1.7 (1.60)

Data shown are n/N (%) unless otherwise stated.
EOT, end of treatment; ETV, entecavir; FAS, full analysis set; LOD, limit of detection; ND, not determined; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; pgRNA, pregenomic RNA; TN, treatment-naive; TNA, total nucleic acids; TND, target not detected; VBR, vebicorvir; VS, virologically-suppressed.
* Assessed by COBAS TaqMan; LOD = 10 IU/ml.
† Assessed by Assembly Biosciences, Inc. HBV DNA assay; LOD = 5 IU/ml.
‡ TNA = HBV DNA + HBV pgRNA.
§ Patient HBV DNA TND levels were ND given that values were well above the LOD for the less sensitive COBAS assay.
¶ The denominator for patients originating from study 202 is 5 for PBO + ETV.

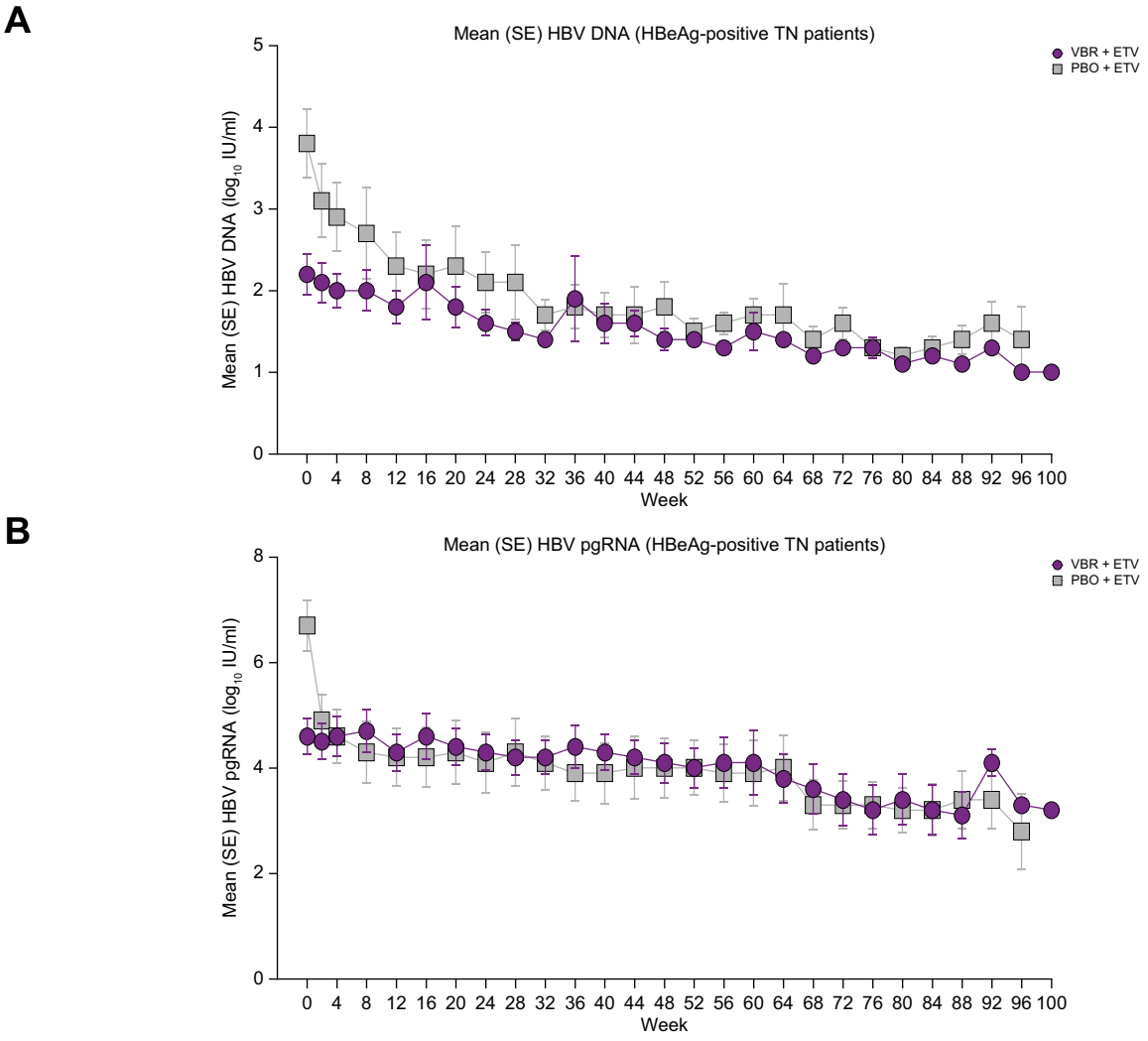

Fig. 3. HBV DNA and pgRNA change from baseline in patients from study 202 (FAS; on-treatment phase). Changes from baseline in (A) HBV DNA and (B) HBV pgRNA in study 211 from patients previously enrolled in study 202. ETV, entecavir; FAS, full analysis set; PBO, placebo; pgRNA, pregenomic RNA; TN, treatment-naive; VBR, vebicorvir.

was ~1 log_{10} IU/ml for patients from study 202, with no notable changes observed among patients from study 201. Mean increases from baseline in HBV pgRNA and TNA were numerically greater for patients from study 201 *vs.* 202. Mean HBV pgRNA and TNA increased from baseline during the off-treatment phase among patients who continued NrtI/ETV (Table S13; Fig. S2). When VBR was discontinued but NrtI/ETV was continued, there was a rebound in mean pgRNA of ~2 log_{10} U/ml, as early as the 4W follow-up visit, that persisted throughout EOS. Mean HBV DNA rebounded ~1 log_{10} IU/ml in patients from study 202 but did not rebound in patients from study 201. No notable changes were observed in mean antigen levels from off-treatment baseline to last visit among patients who discontinued VBR and continued NrtI/ETV only (Table S14; Fig. S3).

Table 2. Observed changes in HBV DNA and pgRNA during the off-treatment phase in patients from study 211 (FAS).

	Patients originating from study 201 (off-treatment phase)	
	VS HBeAg (-) discontinue both n = 23	**VS HBeAg (+) discontinue both n = 18**
HBV DNA baseline, log_{10} IU/ml	1.0 (0.11)	1.2 (0.16)
Change from baseline at end of off-treatment	3.6 (2.41)	4.8 (2.55)
HBV pgRNA baseline, log_{10} U/ml	NA	1.5 (ND)*
Change from baseline at end of off-treatment	NA	4.7 (ND)*
HBV TNA baseline, log_{10} U/ml	1.3 (0.00)	1.3 (0.06)
Change from baseline at end of off-treatment	2.6 (2.29)	4.0 (2.51)

Data shown are mean (SD).
FAS, full analysis set; NA, not applicable; ND, not determined; pgRNA, pregenomic RNA; TNA, total nucleic acids; VS, virologically-suppressed.
* n = 1 with available pgRNA results.

Table 3. Summary of safety by treatment phase in patients from study 211 (SAS).

On-treatment phase					
	Patients originating from study 201			Patients originating from study 202	
Patients reporting:	**VS HBeAg (-) n = 26**	**VS HBeAg (+) n = 43**	**Total n = 69**	**TN HBeAg (+) n = 23**	**Overall total n = 92**
TEAE	16 (62)	26 (60)	42 (61)	12 (52)	54 (59)
Grade 1	9 (35)	11 (26)	20 (29)	6 (26)	26 (28)
Grade 2	7 (27)	14 (33)	21 (30)	3 (13)	24 (26)
Grade 3	0	1 (2)	1 (1)	3 (13)	4 (4)
TEAE related to study drug	3 (12)	5 (12)	8 (12)	2 (9)	10 (11)
TE SAE	0	0	0	1 (4)	1 (1)
TEAE leading to study drug discontinuation	0	0	0	2 (9)	2 (2)
TEAEs found in ≥5% of the total patient population					
Upper respiratory tract infection	3 (12)	6 (14)	9 (13)	1 (4)	10 (11)
Nasopharyngitis	1 (4)	3 (7)	4 (6)	2 (9)	6 (7)
Fatigue	1 (4)	3 (7)	4 (6)	1 (4)	5 (5)

Discontinued both VBR + NrtI (off-treatment phase, patients originating from study 201)

Patients reporting:	**VS HBeAg (-) n = 23**	**VS HBeAg (+) n = 18**	**Total N = 41**
AE	10 (43)	7 (39)	17 (41)
Grade 1	2 (9)	3 (17)	5 (12)
Grade 2	5 (22)	3 (17)	8 (20)
Grade 3	3 (13)	1 (6)	4 (10)
AE related to study drug	0	0	0
SAE	2 (9)	0	2 (5)
AEs found in ≥5% of the total patient population			
ALT increased	6 (26)	5 (28)	11 (27)
AST increased	2 (9)	0	2 (5)
Headache	1 (4)	1 (6)	2 (5)
Nausea	2 (9)	0	2 (5)
Back pain	2 (9)	0	2 (5)

Discontinued both VBR + NrtI, then restarted NrtI (NrtI-restart phase, patients originating from study 201)

Patients reporting:	**VS HBeAg (-) n = 16**	**VS HBeAg (+) n = 14**	**Total N = 30**
AE	3 (19)	6 (43)	9 (30)
Grade 1	1 (6)	2 (14)	3 (10)
Grade 2	0	3 (21)	3 (10)
Grade 4	2 (13)	1 (7)	3 (10)
AE related to study drug	0	0	0
SAE	0	0	0
AEs found in ≥5% of the total patient population			
ALT increased	2 (13)	3 (21)	5 (17)

Continued NrtI/ETV (off-treatment phase)

	Patients originating from study 201	Patients originating from study 202
Patients reporting:	**VS HBeAg (+) continue NrtI only n = 18**	**TN HBeAg (-) continue ETV only n = 6**
AE	0	1 (17)
Grade 1	0	1 (17)

Data shown are n (%).
AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ETV, entecavir; NrtI, nucleos(t)ide reverse transcriptase inhibitor; SAE, serious adverse event; SAS, safety analysis set; TE, treatment-emergent; TEAE, treatment-emergent adverse event; TN, treatment-naive; VBR, vebicorvir; VS, virologically-suppressed.

Safety

Mean (SD) treatment duration during study 211 was 63.5 (17.5) weeks, with most patients (66/92; 72%) receiving study drugs between 48 and 72 weeks. Overall exposure ranged from 1.1 to 103.9 weeks.

A summary of treatment-emergent adverse events (TEAEs) and AEs by treatment phase is reported in Table 3. During the on-treatment phase, 54/92 (59%) patients reported one or more TEAEs. One patient who was HBeAg-positive who received PBO + ETV in study 202 developed on-treatment serious AEs (SAEs) not related to study drug, and another patient (HBeAg-positive who received VBR + NrtI in study 202) developed TEAEs possibly related to study drug—see Supplementary material for more information. No grade 4 TEAEs or deaths occurred during the on-treatment phase. The most common TEAEs were upper respiratory tract infection (10/92; 11%), nasopharyngitis (six of 92; 7%), and fatigue (five of 92; 5%), most of which were grade 1. No trends in the incidence of TEAEs related to treatment received or HBeAg status in the parent studies were noted. On-treatment rash occurred in nine of 69 (13%) patients from study 201 (all grade 1 with variable onset) and none in study 202. All but one case resolved before EOS, and none led to study drug discontinuation. No patients met the ALT flare criteria. Treatment-emergent laboratory abnormalities are summarised in Table S15.

During the off-treatment phase, there were no notable differences in safety between patients who were HBeAg-positive

or -negative from study 201 who discontinued both VBR + NrtI; 17/41 (41%) patients reported an AE (see Supplementary material for information surrounding two patients [both HBeAg-negative] who reported SAEs). No grade 4 AEs or deaths occurred in patients who discontinued both VBR + NrtI. The most common AEs were increases in ALT, increases in aspartate aminotransferase (AST), back pain, headache, and nausea. Two patients had a grade 3 laboratory abnormality (ALT and AST increase), and one had a grade 4 laboratory abnormality (ALT increase). During the NrtI-restart phase, most AEs and laboratory abnormalities were related to ALT and AST increases with no SAEs or deaths. Following NrtI restart, ALT returned to prediscontinuation levels with no events of hepatic decompensation. After discontinuing both VBR + NrtI, 22 patients had increases in ALT: one grade 4, two grade 3, and 12 grade 2. Further detail on ALT elevations and normalisations is provided in the Supplementary material.

Emergence of resistance-associated variants

Nine patients experienced on-treatment viral rebound, and Sanger sequencing showed no core inhibitor binding pocket substitutions or NrtI resistance mutations for eight out of nine patients tested at all time points. One patient had a T109I resistance-associated substitution in the core gene. This patient (who received VBR + NrtI in study 201) had persistently low HBV DNA through on-treatment W32 and had the T109I mutation detected as a mixture as early as W4 during study 201. Five of the nine patients had virologic rebound in a setting of noncompliance with study drug, including the patient with T109I detected in the core gene.

Pharmacokinetics

Summary statistics for predose concentrations of VBR, ETV, and tenofovir are described in Table S16. In general, W48 predose VBR and NrtI concentrations were comparable with those observed in the parent studies, supporting the lack of a drug–drug interaction.[12]

Discussion

This study was designed to assess the safety and antiviral activity of long-term, open-label VBR + NrtI in patients with cHBV. In patients originating from studies 201 and 202, long-term VBR + NrtI led to further reductions in HBV DNA and pgRNA at EOT in study 211 and was generally well tolerated, with few discontinuations and no deaths. No resistance-associated substitutions were observed in patients who were adherent to study drug. PK data from this study are consistent with the parent studies and support a lack of drug–drug interactions between VBR and NrtIs following longer-term administration.

Mean HBV DNA and pgRNA declined during the on-treatment period among patients receiving open-label VBR. Compared with study 211 baseline, there was an increase in the percentage of patients achieving HBV DNA TND at EOT. The observed on-treatment change in viral parameters varied and was influenced by the previous status of patients in the parent studies. Administration of VBR + NrtI resulted in slight numeric, but not clinically significant, decreases in HBV antigens. Additionally, neither HBsAg loss nor HBsAg seroconversion was observed in any patient, which likely correlates with intrahepatic viral persistence.[13] Therefore, we conclude that long-term treatment with VBR + NrtI does not result in functional cure.

Long-term administration of VBR + NrtI was well tolerated, with most TEAEs being grade 1/2 and no grade 4 TEAEs or deaths reported. The nature and frequency of the observed TEAEs were similar between patients who were HBeAg-positive and -negative and those who were TN or VS at the start of the parent studies. Most grade 3/4 AEs and laboratory abnormalities occurred in the off-treatment or NrtI-restart phases and were related to elevations in ALT following cessation of antiviral treatment. Among patients meeting predefined criteria for stopping antiviral therapy, discontinuation of VBR + NrtI was well tolerated, with no hepatic decompensation events and limited AEs and ALT elevations.

Although long-term treatment with VBR + NrtI provided deep reductions in HBV DNA and pgRNA at EOT, failure to maintain HBV DNA <LLOQ after cessation of all antiviral therapy points to the need for more potent and/or additional therapies and novel combinations to work toward finite treatment and functional cure. The failure thus far to achieve functional cure off treatment is likely because of the inability of current treatment regimens to interfere with cccDNA formation and maintenance. Although combination therapy with clinically approved NrtIs and IFNα results in greater HBsAg loss than either monotherapy, the fact that neither of these agents eliminates cccDNA means that off-treatment viral rebound is likely.[14] Therefore, it will be of great importance for future agents to have greater efficacy against the cccDNA reservoir. Although first-generation core inhibitors, such as VBR, demonstrate trough plasma concentrations above the protein-adjusted EC_{50} ($paEC_{50}$) values for HBV DNA and cccDNA formation, next-generation core inhibitors may have enhanced potency with $paEC_{50}$s multiple-fold (up to 900-fold higher *vs.* VBR) above that required for inhibition of capsid disassembly and prevention of cccDNA formation, often called 'secondary mechanisms' of core inhibitors (beyond their effects on capsid assembly).[15–17] Future studies incorporating next-generation core inhibitors may show higher levels of antiviral activity than what was observed in this study.

An important caveat in this study is the treatment assignment and HBeAg status of patients in the parent studies. In general, patients who were HBeAg-negative and received VBR + NrtI in study 201 entered study 211 with low levels of viral parameters, which made assessing changes in these parameters in study 211 difficult. Conversely, patients who were HBeAg-positive from study 202 and received PBO + NrtI had high levels of viral parameters at study 211 baseline, resulting in viral changes that were more apparent with long-term VBR + NrtI. Furthermore, this study followed patients who discontinued all antiviral treatment (*i.e.* VBR + NrtI) for approximately 4–36 weeks. Given that levels of certain viral parameters, such as HBsAg, may take years to diminish under antiviral treatment,[13] it is likely that this short period of follow-up did not capture potential longer-term declines in HBsAg in these patients. As no patients met the primary study endpoint, NrtIs were generally restarted, and consequently the period of follow-up when patients were not receiving any antiviral treatment was insufficient to determine if there were any continued declines in viral parameters. In summary, open-label, long-term, once-daily VBR + NrtI was safe and well tolerated, with few discontinuations and no deaths. Although deeper levels of viral suppression were observed with

the VBR + NrtI combination, durable virologic outcomes were not observed in patients who met the criteria to discontinue antiviral treatment. VBR has been investigated further in two triple-combination, open-label, phase II studies – with both NrtI and IFNα, and with NrtI and the investigational RNA inhibitor AB-729. VBR did not show superior efficacy in key viral parameters in these triple-combination studies *vs.* dual combinations without VBR – hence clinical development of VBR was discontinued.

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cccDNA, covalently closed circular DNA; cHBV, chronic hepatitis B virus infection; EOS, end of study; EOT, end of treatment; ETV, entecavir; IFNα, interferon alpha; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; $paEC_{50}$, protein-adjusted half-maximal effective concentration; PBO, placebo; pgRNA, pregenomic RNA; PK, pharmacokinetics; SAE, serious adverse event; TA, treatment action; TEAE, treatment-emergent adverse event; TN, treatment-naive; TNA, total nucleic acids; TND, target not detected; VBR, vebicorvir; VS, virologically-suppressed; W, week.

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Conflicts of interest

M-FY reports being an advisor/consultant for AbbVie, AiCuris, Aligos Therapeutics, Antios Therapeutics, Arbutus Biopharma, Arrowhead Pharmaceuticals, Assembly Biosciences, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, Fujirebio Incorporation, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Roche, Sysmex Corporation, Tune Therapeutics, Vir Biotechnology, and Visirna Therapeutics and receiving grant/research support from AbbVie, Arrowhead Pharmaceuticals, Assembly Biosciences, Fujirebio Incorporation, Gilead Sciences, Immunocore, Roche, and Sysmex Corporation. SF reports receiving fees for speaking and teaching and/or serving on advisory committees for AbbVie, Assembly Biosciences, Gilead Sciences, Janssen, Lupin, Novo Nordisk, Pfizer, and Springbank Pharma. XM reports being a consultant and being on the speakers' bureau for Gilead Sciences. TTN reports receiving research grant support from Assembly Biosciences and Gilead Sciences. TH reports being on the advisory committee, review panel, or consulting for AbbVie, Bristol-Myers Squibb, Gilead Sciences, Mallinckrodt Pharmaceuticals, Merck, and Organovo and receiving research support from AbbVie, Allergan, Assembly Biosciences, Astra Zeneca, Boehringer Ingelheim, Bristol-Myers Squibb, CARA, Cytodyn, DURECT Corporation, Enanta Pharmaceuticals, Galectin Therapeutics, Gilead Sciences, Grifols, Intercept Pharmaceuticals, Janssen, Merck, Mirum, Novartis, Novo Nordisk, Nucorion Pharmaceuticals, Pfizer, Salix Pharmaceuticals, Sonic Incytes, Terns Pharmaceuticals, and Valeant. H-WH reports serving on the National Advisory Board and receives research grant support from Gilead Sciences. ME reports receiving grants from AbbVie, Bristol-Myers Squibb, Eisai, Gilead Sciences, and Roche and serving on advisory boards for AbbVie, Bristol-Myers Squibb, Gilead Sciences, and Merck. RGN reports having served on advisory boards and as a speaker for Gilead Sciences, Janssen, and Merck and having conducted research for AbbVie, Gilead Sciences, Janssen, and Merck. JSP reports receiving research grants from Assembly Biosciences and GlaxoSmithKline and consulting fees from Gilead Sciences. IMJ reports being a consultant or on advisory boards for AbbVie, Aligos Therapeutics, Arbutus Biopharma, Gilead Sciences, Intercept Pharmaceuticals, Janssen, and Roche; having conducted research (all payments to institution) for Assembly Biosciences, Bristol-Myers Squibb, Cymabay, Eli Lilly, Enanta Pharmaceuticals, Genfit, Gilead Sciences, Intercept, Janssen, Merck, and Novo Nordisk; receiving payment from the Chronic Liver Disease Foundation for manuscript preparation; and participating on a Data Monitoring Committee for Altimmune, Arrowhead Pharmaceuticals, Galmed, GlaxoSmithKline, and Takeda. WSA reports being a member of the speaking bureau for Gilead Sciences and Intercept Pharmaceuticals and has received research grants from Genfit, GlaxoSmithKline, Intercept Pharmaceuticals, Ipsen, Madrigal, Mirum, Pfizer, and Zydus. S-HH reports being a consultant and being on the speakers' bureau for Gilead Sciences. EJG reports serving on advisory boards for AbbVie, Aligos Therapeutics, Assembly Biosciences, Gilead Sciences, GlaxoSmithKline, Intellia, Janssen, Roche, Vir Biotechnology, and Virion and having served as a speaker for Abbott Diagnostics, AbbVie, and Gilead Sciences. KZ, RY, and SJK report being employees of and holding stock interest in Assembly Biosciences. JM and LMS report being former employees of and holding stock interest in Assembly Biosciences. MBo reports being a member of the speaking bureau for AbbVie, Gilead Sciences, and Intercept Pharmaceuticals and has received research support from Assembly Biosciences, Boehringer Ingelheim, Gilead Sciences, Intercept Pharmaceuticals, Inventiva, and Viking Therapeutics. FW reports being a study investigator for AbbVie. AR reports receiving grant support, lecture fees, and advisory board fees from AbbVie, Celgene, Gilead Sciences, Intercept Pharmaceuticals, Merck, and Novartis. MBe reports having no conflicts of interest. NR reports advising, being on the speakers' bureau for, and receiving grants from AbbVie and Gilead Sciences; being on the speakers' bureau for Onyx and Salix; and having received grants from Bristol-Myers Squibb and Merck. SC reports receiving clinical trial–related payments from Assembly Biosciences. DTD reports being a consultant for Gilead Sciences and Intercept Pharmaceuticals. 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KA reports being on the advisory board, a consultant, and a speaker for AbbVie, Aligos, Arbutus Biopharma, Assembly Biosciences, Bristol-Myers Squibb, Gilead Sciences, Immunocore, Janssen, Merck, Novartis, Roche, Shinogi, Sobi, and Vir Biotechnology and receiving grants from Bristol-Myers Squibb, Gilead Sciences, and Roche. MSS reports receiving grants from AbbVie, Assembly Biosciences, GlaxoSmithKline, Janssen, the National Institutes of Health, and Vir Biotechnology and receiving personal fees from AbbVie, Antios Therapeutics, Arbutus Biopharma, Atea Pharmaceuticals, Gilead Sciences, GlaxoSmithKline, F2G, Immunocore, Precision Biosciences, and Virion Therapeutics.

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

Authors' contributions

All authors meet authorship criteria set forth by the International Committee for Medical Journal Editors, have significantly contributed to and approved the final submitted version of the manuscript, and take responsibility for the integrity of the work. Study oversight: M-FY, SF, XM, TTN, TH, H-WH, ME, RGN, JSP, IMJ, WSA, S-HH, EJG, SJK, LMS, MBo, FW, AR, MBe, NR, SC, DTD, PYK, ERS, HSB, JL, KA, MSS. Experiments and procedures: M-FY, SF, XM, TTN, TH, H-WH, ME, RGN, JSP, IMJ, WSA, S-HH, EJG, RY, MBo, FW, AR, MBe, NR, SC, DTD, PYK, ERS, HSB, JL, KA, MSS. Data acquisition: M-FY, SF, XM, TTN, TH, H-WH, ME, RGN, JSP, IMJ, WSA, S-HH, EJG, RY, JM, MBo, FW, AR, MBe, NR, SC, DTD, PYK, ERS, HSB, JL, KA, MSS. Data analysis: M-FY, SF, IMJ, JM, SJK, LMS, JL, KA, MSS. Data interpretation: All authors. Critical revision of the manuscript: All authors.

Data availability statement

Data can be made available to researchers upon reasonable request.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100999.

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Author names in bold designate shared co-first authorship.

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Supplemental information

Long-term open-label vebicorvir for chronic HBV infection: Safety and off-treatment responses

Man-Fung Yuen, Scott Fung, Xiaoli Ma, Tuan T. Nguyen, Tarek Hassanein, Hie-Won Hann, Magdy Elkhashab, Ronald G. Nahass, James S. Park, Ira M. Jacobson, Walid S. Ayoub, Steven-Huy Han, Edward J. Gane, Katie Zomorodi, Ran Yan, Julie Ma, Steven J. Knox, Luisa M. Stamm, Maurizio Bonacini, Frank Weilert, Alnoor Ramji, Michael Bennett, Natarajan Ravendhran, Sing Chan, Douglas T. Dieterich, Paul Yien Kwo, Eugene R. Schiff, Ho S. Bae, Jacob Lalezari, Kosh Agarwal, and Mark S. Sulkowski

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Supplementary methods

Inclusion criteria

Patients who met the following inclusion criteria were eligible for enrolment:

1. Willing and able to provide informed consent.

2. Previously enrolled in a study of vebicorvir (VBR) and completed the treatment period, with demonstrated compliance in the opinion of the investigator.

3. For female patients, agreed to use an effective birth control method for the duration of the study and follow-up, were surgically sterile for at least 6 months, or were at least 2 years postmenopausal with serum follicle-stimulating hormone levels consistent with a postmenopausal status. Effective birth control methods included male or female condom (could not be used together due to increased risk of breakage), vasectomy, intrauterine device (IUD), diaphragm, or cervical cap. Female patients of childbearing potential were required to have a negative pregnancy test.

4. All heterosexually active male patients agreed to use an effective birth control method for the duration of the study and follow-up. Effective birth control methods included male or female condom (could not be used together due to increased risk of breakage), vasectomy, hormone-based contraception (only female partner of a male patient), IUD, diaphragm, or cervical cap.

5. Agreed to adhere to lifestyle considerations including abstaining from alcohol abuse (defined as alcohol consumption exceeding 2 standard drinks per day on average [1 standard drink=10 grams of alcohol]); the use of illicit, herbal or other substances; and unnecessary over-the-counter medications throughout study duration.

6. Were in good general health except for chronic HBV infection (cHBV).

7. Had the ability to take oral medication and were willing to adhere to the Study 211 regimen in the opinion of the investigator.

Exclusion criteria

Patients who met any of the following exclusion criteria were not eligible for enrolment:

1. Had evidence of resistance-associated variants or lack of compliance on a previous study of VBR.
2. Had treatment-emergent adverse events (TEAEs) or laboratory abnormalities deemed clinically significant and possibly or probably related to drug while on a previous study of VBR that in the opinion of the investigator or the Sponsor made the patient unsuitable for Study 211.
3. Had current clinically significant cardiac or pulmonary disease; chronic or recurrent renal or urinary tract disease; liver disease other than HBV; endocrine disorder; autoimmune disorder; diabetes mellitus requiring treatment with insulin or hypoglycaemic agents; neuromuscular, musculoskeletal, or mucocutaneous conditions requiring frequent treatment; seizure disorders requiring treatment; or other medical conditions requiring frequent medical management or pharmacologic or surgical treatment that in the opinion of the investigator or the Sponsor made the patient unsuitable for the study.
4. Females who were lactating or pregnant or wished to become pregnant within the duration of Study 211.

Treatment compliance

Patients were asked to return used study drug bottles and any unused study drug at study visits. To monitor compliance, study sites conducted tablet counts on these returned bottles. Patients who forgot to return bottles were asked to return them at the next study visit.

In cases where the patients forgot to take the study drug at the scheduled time on a given day, they were instructed to take the day's dose as long as it was within 8 hours of their scheduled dose of the day. These patients were instructed not to "catch up" and take twice the dose on the following day. If a patient reported having missed 2 or more consecutive doses, or multiple missed single doses, then the medical monitor was contacted before any further action was taken. These missed doses were to be recorded in the source documents. If a patient demonstrated continued noncompliance with study drug dosing, despite educational efforts, the investigator would contact the medical monitor to discuss discontinuation of the patient from the study.

Exploratory endpoints

- Mean change from Baseline in $\log_{10}$ serum HBeAg
- Mean change from Baseline in $\log_{10}$ serum HBsAg
- Incidence of patients with loss or change in $\log_{10}$ HBsAg or $\log_{10}$ HBeAg (<0.5, ≥0.5 to 1.0, or >1.0 in viral antigens) at end of treatment (EOT) and end of follow-up
- Incidence of patients with HBsAg seroconversion (loss of HBsAg and appearance of HBsAg antibody) or HBeAg seroconversion (loss of HBeAg and appearance of HBeAg antibody)

- Incidence of patients with detectable HBV DNA by PCR at Baseline whose HBV DNA becomes target not detected (TND)
- Quantitative changes from Baseline in viral RNA on treatment and through end of follow-up
- Quantitative changes in serum hepatitis B core-related antigen (HBcrAg) levels on treatment and through end of follow-up
- Incidence of HBsAg or HBeAg seroconversion in patients up to 3 years off therapy
- Incidence of patients requiring retreatment following DNA undetected through 3 years off therapy
- Incidence of patients with emergence of HBV resistance–associated variants
- If differences are seen in outcomes/adverse events (AEs) between racial or ethnic groups: pharmacogenomic correlations with clinical outcomes in patients who have provided an optional informed consent and sample in Study 201 or 202
- Quantitative levels of VBR and nucleos(t)ide reverse transcriptase inhibitor (NrtI) in plasma

Definition of AEs

Serious adverse events (SAEs) were defined as any events considered life threatening or that resulted in death, inpatient hospitalisation (or prolongation of existing hospitalisation), or persistent disability/incapacity. Rash and alanine aminotransferase (ALT) flare were AEs of special interest. ALT flares were defined as ALT >2× Baseline and ≥10× the upper limit of normal (ULN; defined by the American Association for the

Study of Liver Diseases [AASLD]) or ALT >2× the on-treatment nadir and ≥10× ULN. TEAEs were any AEs with an onset date on or after the study drug start date and no later than 28 days after permanent discontinuation of study drug. Clinical and laboratory AEs were coded using the Medical Dictionary for Regulatory Activities (version 21.0).

Virologic assay methodology

HBV DNA was measured by COBAS TaqMan Version 2.0 (Roche Diagnostics, Mannheim, Germany; lower limit of quantification [LLOQ]=20 IU/mL; limit of detection [LOD]=10 IU/mL) and by a semi-quantitative gel-based assay developed by Assembly Biosciences, Inc. (South San Francisco, CA, USA; LOD=5 IU/mL). Two quantitative pregenomic RNA (pgRNA) assays developed by Assembly Biosciences, Inc. (South San Francisco, CA, USA) were utilised, one for use in samples with high levels of HBV DNA from Study 202 (LLOQ=135 U/mL) and one for samples with low levels of HBV DNA from Study 201 (LLOQ=35 U/mL). Total nucleic acids (composite HBV DNA+pgRNA) were assessed by a novel assay developed by Assembly Biosciences, Inc. (South San Francisco, CA, USA; LLOQ=20 U/mL). The novel assays developed by Assembly Biosciences, Inc. have been described previously.[1] HBeAg (LLOQ=0.11 IU/mL) and HBsAg (LLOQ=0.05 IU/mL) were quantified using the Architect i2000SR assays (Abbott Diagnostics, Lake Forest, IL, USA). Quantification of HBcrAg (LLOQ=1 kU/mL) was performed using the Lumipulse G assay (Fujirebio, Malvern, PA, USA). All viral parameters were assessed at Covance Central Laboratory Services (now LabCorp, multiple locations) with the exception of HBcrAg levels, which were measured at the University of Hong Kong. ALT was assessed against normal ranges set by Covance

Central Laboratory Services (ULN of 34 U/L for females and 43 U/L for males) and AASLD guidelines (ULN of 25 U/L for females and 33 U/L for males).[2]

Three-year off-treatment follow-up

All patients who discontinued both VBR+NrtI were followed for up to 3 years from the date of treatment discontinuation to assess the durability of virologic response. Patients had an unscheduled visit to notify them of the treatment action (TA) to be implemented, at which point each individual patient's visit schedule was reset; patients then returned to the clinic for follow-up every 4 weeks for visits at 4, 8, 12, 16, 20, and 24 weeks posttreatment discontinuation, then every 8 weeks for visits at 32, 40, and 48 weeks posttreatment discontinuation, and then every 12 weeks until completion of the 3-year follow-up. Additional unscheduled visits were performed at the investigator's discretion. Following completion of the visit 3 years after VBR+NrtI discontinuation, patients exited the study and were placed under the routine care of their respective physicians.

Twelve-week follow-up on NrtI alone

All patients who discontinued VBR only and continued NrtI alone were followed for 12 weeks from the date of VBR discontinuation. Patients then had an unscheduled visit to notify them of the TA to be implemented, at which point each individual patient's visit schedule was reset; patients then returned to the clinic for follow-up visits at 4, 8, and 12 weeks after discontinuation of VBR. Additional unscheduled visits were performed at the investigator's discretion. Following completion of the follow-up visit 12

weeks after discontinuation of VBR, patients exited the study and were placed under the routine care of their respective physicians.

Continuation of treatment with VBR+NrtI

All patients who continued VBR+NrtI beyond Week 52 returned to the clinic for visits every 4 weeks until Week 148. Patients were then notified of their TA by phone and would continue their planned study visit schedule. At Week 148, patients were evaluated for virologic response and either discontinued both VBR+NrtI and were followed for up to 3 years, or discontinued VBR only, continued NrtI alone, and were followed for 12 weeks.

Criteria to restart NrtI following discontinuation of both VBR+NrtI

Patients who discontinued both VBR+NrtI were followed to assess the durability of virologic response. The investigator used clinical judgement to determine when to restart NrtI. However, NrtI therapy was reintroduced if any of the following criteria listed below were met:

- ALT >10× ULN
- Direct bilirubin >2.0× ULN
- International Normalised Ratio (INR) >1.5
- ALT >3× ULN and HBV DNA >100,000 IU/mL
- ALT >ULN and HBV DNA >2000 IU/mL on 3 consecutive visits at least 1 month apart
- Any clinical decompensation, regardless of HBV DNA level

- Physician or patient's decision

If any of these criteria were met, then patients could have an unscheduled visit to notify them to restart NrtI. Patients' visit schedules were then reset upon restarting NrtI; patients returned for follow-up visits at 4, 8, and 12 weeks after restarting NrtI and would then complete participation in the study.

Supplementary results

Duration of time patients had on and off treatment

The mean (SD) duration of the on-treatment phase for patients from Study 201 (57.6 [12.9] weeks) was lower than that of patients from Study 202 (81.0 [18.2] weeks). In patients from Study 201 who discontinued VBR+NrtI, the mean (SD) duration of the off-treatment phase was similar between HBeAg-positive (20.9 [8.82] weeks) and -negative patients (20.6 [11.0] weeks) and was of similar duration for the NrtI-restart phase (13.4 [2.46] and 14.2 [4.20] weeks, respectively). Patients who discontinued VBR and continued on NrtI/entecavir (ETV) also had a similar duration of off-treatment phase.

Description of Baseline disease characteristics for Study 211

Mean duration of HBV infection and number of years on current NrtI treatment were longer among patients from Study 201 than among Study 202 patients (treatment duration for Study 202 patients at Study 211 Baseline was 24 weeks). As expected, mean Baseline HBV DNA measured by COBAS TaqMan among patients from Study 201 were mostly TND. A higher percentage of Study 201 HBeAg-negative patients had

HBV DNA TND at Study 211 Baseline than did HBeAg-positive patients. Mean Baseline HBV DNA was not different between treatment groups among patients from Study 201. Study 211 Baseline characteristics from Study 202 showed mean HBV DNA was greater among placebo (PBO)+ETV than among VBR+ETV patients. No Study 202 patients had HBV DNA TND at Study 211 Baseline (**Table S7**).

Mean HBV pgRNA at Study 211 Baseline was higher among Study 201 HBeAg-positive patients than among negative patients. HBeAg-positive patients who received PBO+NrtI in Study 201 had greater mean pgRNA at Baseline versus patients who received VBR+NrtI. Mean HBV pgRNA at Study 211 Baseline among HBeAg-positive patients from Study 202 was numerically greater in patients who received PBO+ETV versus VBR+ETV. No HBeAg-positive patients from Study 202 had HBV pgRNA <LLOQ at Study 211 Baseline (**Table S7**).

Mean levels of HBV antigens were lower among patients from Study 201 versus patients from Study 202 at Study 211 Baseline. Mean levels of HBV antigens at Study 211 Baseline were similar between patients who had received VBR+NrtI/ETV and PBO+NrtI/ETV in the parent studies. Mean ALT levels were lower among patients from Study 201 versus Study 202 at Study 211 Baseline, with ALT levels being slightly higher among HBeAg-positive versus -negative patients from Study 201. Patients who received PBO+ETV in Study 202 had higher mean ALT levels at Study 211 Baseline versus patients who received VBR+ETV (**Table S7**). Of the 4 patients from Study 201 with abnormal ALT at Baseline, 2 had ALT ≤ULN at EOT, and 1 had ALT ≤ULN at end of study (EOS). Of the 7 patients from Study 202 with abnormal ALT at Baseline, 5 had ALT ≤ULN at EOT and at EOS (data not shown).

Narratives for SAEs and AEs leading to discontinuation of treatment

On-treatment phase

A 33-year-old Asian male patient who was treatment-naïve with HBeAg-positive cHBV infection was initially enrolled in Parent Study 202 and randomised to receive placebo PBO+ETV. The patient completed 24 weeks of blinded treatment in Study 202 and was enrolled in Study 211 to receive open-label VBR+ETV. The patient's medical history included abdominal tenderness, and the patient reported a Grade 1 AE of stress in Study 202, which was ongoing at Baseline of Study 211. No concomitant medications were reported. During Study 211, the patient reported AEs of Grade 1 conjunctivitis (Study Days 52–60) and Grade 1 insomnia (Study Days 162–215). On Study Day 253, the patient presented to the emergency room agitated, distressed over his social situation, and speaking about wanting to kill himself, though later he reported that he was misunderstood, as English is not his native language. A Grade 2 AE of anxiety, a Grade 1 AE of palpitations, and a Grade 3 SAE of suicidal ideation were reported. The patient was hospitalised and discontinued VBR on Study Day 255. The patient's mood improved, and he denied further suicidal ideation. He was discharged on Study Day 258, and the SAE of suicidal ideation was resolved. The investigator considered all AEs not related to study drug.

Off-treatment phase

Patient 1

A 46-year-old White male who was virologically-suppressed (VS) with HBeAg-negative cHBV infection was initially enrolled in Parent Study 201 and was randomised to receive VBR and continue NrtI (ETV). The patient completed 24 weeks of blinded treatment in Study 201 and was enrolled in Study 211 to receive open-label VBR+NrtI. The patient's medical history included Barrett oesophagus, hiatal hernia, oesophagitis, and oesophageal reflux. No concomitant medications were reported. The patient met the TA criteria to discontinue treatment, and both VBR and ETV were discontinued on Study Day 426. On Study Day 563 (137 days after discontinuation of study drug), the patient was brought to the emergency room by friends who stated that the patient was confused. The evaluating physician believed the patient may have had a seizure and was in a post-ictal state. The patient was admitted to the hospital for further evaluation, during which he was treated with lorazepam and levetiracetam for the suspicion of seizure. The results from a magnetic resonance imaging scan and electroencephalogram were normal. He was found to be in atrial fibrillation, for which he received aspirin. The patient reported back pain. Chest X-ray, electrocardiogram, and echocardiogram were normal. The patient was discharged from the hospital in stable condition. A Grade 3 SAE of seizure, a Grade 2 AE of atrial fibrillation, and a Grade 2 AE of back pain were resolved. The investigator considered all AEs not related to study drug.

Patient 2

A 55-year-old Black or African American female who was VS with HBeAg-negative cHBV infection initially enrolled in Study 201 and was randomised to receive PBO and continue NrtI (tenofovir alafenamide fumarate [TAF]). The patient completed

24 weeks of blinded treatment in Study 201 and was enrolled in Study 211 to receive open-label VBR+NrtI. The patient's medical history included obesity, hypertension, coronary artery disease with prior stenting, insomnia, and depression. No concomitant medications were reported.

On Study Day 255, the patient switched NrtI from TAF to ETV due to insurance issues. On Study Day 280, the patient experienced Grade 1 AEs of toothache and tooth infection, for which she received amoxicillin and ibuprofen. On Study Day 311, the patient experienced Grade 1 papular rash, possibly related to study drug, for which she received hydrocortisone cream. The patient met the TA criteria to discontinue treatment, and both VBR+NrtI were discontinued on Study Day 427.

On Study Day 512 (85 days after discontinuation of study drug), the patient experienced a Grade 3 SAE of procedural haemorrhage. The day prior, the patient had undergone plastic surgery, including abdominoplasty, liposuction, and breast augmentation. The patient was being discharged from the centre, and upon standing to get into the car, the patient felt dizzy and passed out. The patient was brought back upstairs to the clinic, where she passed out again; she was subsequently kept at the medical centre overnight for observation. While at the medical centre, the patient complained of persistent dizziness and decided to go to the emergency room. Upon her admission to the emergency room, the patient's haemoglobin level was 5.3 g/dL (reference range: 12.0–16.0 g/dL), her platelet count was 137 k/mcL (reference range: 140–400 k/mcL), and her INR was 1.1 (reference range: 0.8–1.2). The patient was found to have had acute blood loss in the left anterior abdominal wall as a complication of abdominoplasty. The patient was admitted and received 2.5 units of packed red blood

cells via transfusion. A Grade 3 AE of procedural pain was also reported. No additional surgery was performed. On Study Day 516, the patient's haemoglobin level was 7.9 g/dL; the patient was discharged, and the AEs were considered resolved. The investigator considered the AEs of procedural haemorrhage and procedural pain not related to study drug.

Details surrounding on-treatment AEs of ALT elevation

During the on-treatment phase, 3 patients reported Grade 3 AEs of ALT increase and laboratory abnormalities of elevated ALT. In these cases, ALT elevations were associated with aspartate aminotransferase elevations, and there were no graded abnormalities in total bilirubin, alkaline phosphatase, albumin, and INR and no signs of hepatic decompensation. No patients met the criteria for ALT flare. One patient discontinued VBR due to the AE of ALT increase and recovered soon after. The other 2 patients with elevated ALT on treatment are described below.

A 36-year-old Black or African American female patient with HBeAg-positive cHBV treated with PBO+ETV in Parent Study 202 enrolled in Study 211 to receive VBR+ETV. The patient had a Grade 3 AE of ALT increase reported on Study Day 8 and a Grade 3 laboratory abnormality of elevated ALT (199 U/L). Levels of ALT peaked on Study Day 22 (296 U/L) and then improved on treatment, and the AE was resolved on Study Day 43. From Study Day 1 to 57, HBV DNA decreased from 3.72 $\log_{10}$ IU/mL to <LLOQ, and HBsAg decreased from 4.63 $\log_{10}$ IU/mL to 3.06 $\log_{10}$ IU/mL. Per the investigator, the elevated ALT occurred in the setting of increased alcohol use and was not considered related to study drug.

Another patient was a 35-year-old Asian male with HBeAg-positive cHBV treated with PBO+NrtI in Parent Study 201 who enrolled in Study 211 to receive VBR+NrtI. On treatment, the patient had a Grade 2 AE of ALT increase reported on Study Day 393 and a Grade 2 laboratory abnormality of elevated ALT (184 U/L). The patient met the TA criteria to discontinue treatment, and both VBR and NrtI were discontinued on Study Day 420. On Study Day 421, Grade 3 elevated ALT (271 U/L) was observed, and a Grade 3 AE of ALT increase was reported. Off treatment, ALT levels remained stably elevated, HBV DNA increased to 5.95 $\log_{10}$ IU/mL, and the patient met laboratory criteria to restart NrtI on Study Day 491 (71 days after stopping treatment). At the last observation on Study Day 575 (84 after NrtI restart), HBV DNA had decreased to 40 IU/mL (1.6 $\log_{10}$ IU/mL), and the ALT level was 111 U/mL (Grade 2). At the end of the study, the Grade 3 AE of ALT increase was considered resolved and the Grade 2 AE of ALT increase was ongoing; the investigator considered both AEs not related to study drug.

Supplementary figures and tables

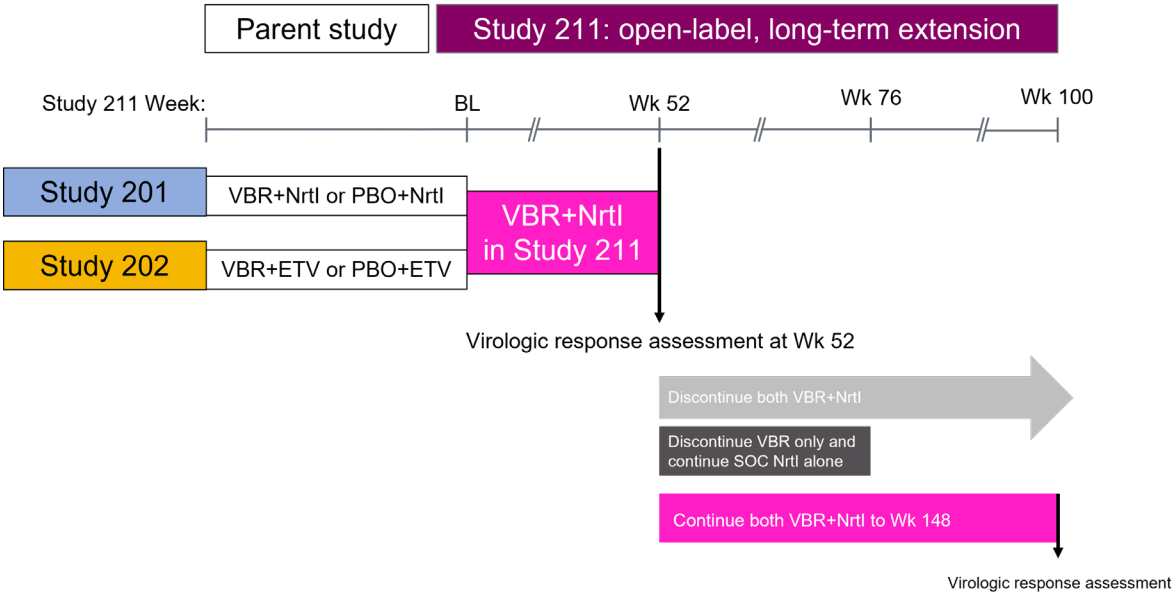

Fig. S1. Study 211 design.

The duration of treatment for each patient was based on the respective HBV treatment history (virologically-suppressed in Study 201 or treatment-naïve in Study 202), hepatitis B e antigen status (positive or negative) at Baseline in the parent study, and their individual virologic response in Study 211. Based on these factors, each patient was evaluated for virologic response and assigned to one of the following treatment actions: discontinue both VBR+NrtI, discontinue VBR only and continue NrtI alone, or continue both VBR+NrtI for up to 148 weeks.

BL, Baseline; ETV, entecavir; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; SOC, standard-of-care; VBR, vebicorvir; Wk, week.

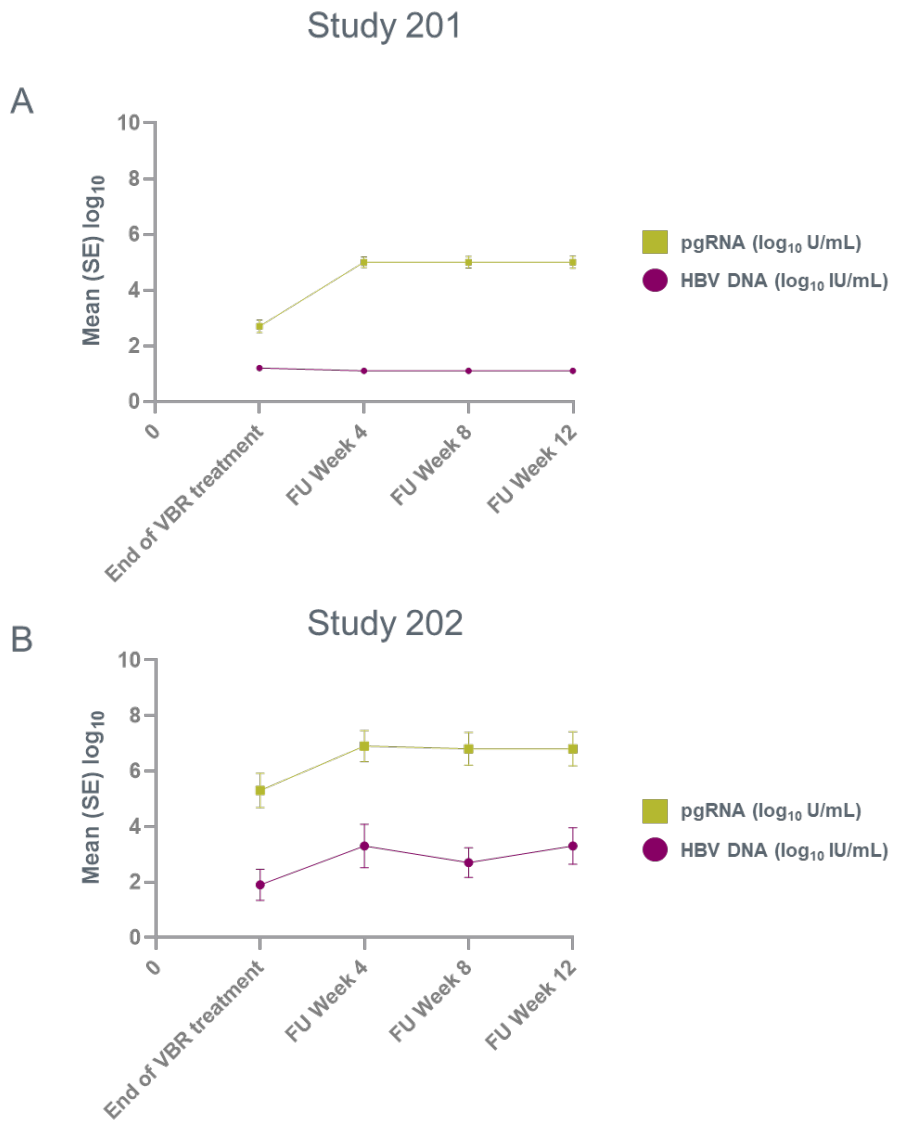

Fig. S2. HBV DNA and HBV pgRNA in patients from Study 211 who discontinued VBR and remained on NrtI.

Mean log_{10} levels of HBV DNA and HBV pgRNA in **(A)** patients from Study 201 and **(B)** patients from Study 202 who discontinued VBR and remained on NrtI.

FU, follow-up; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; SE, standard error; VBR, vebicorvir.

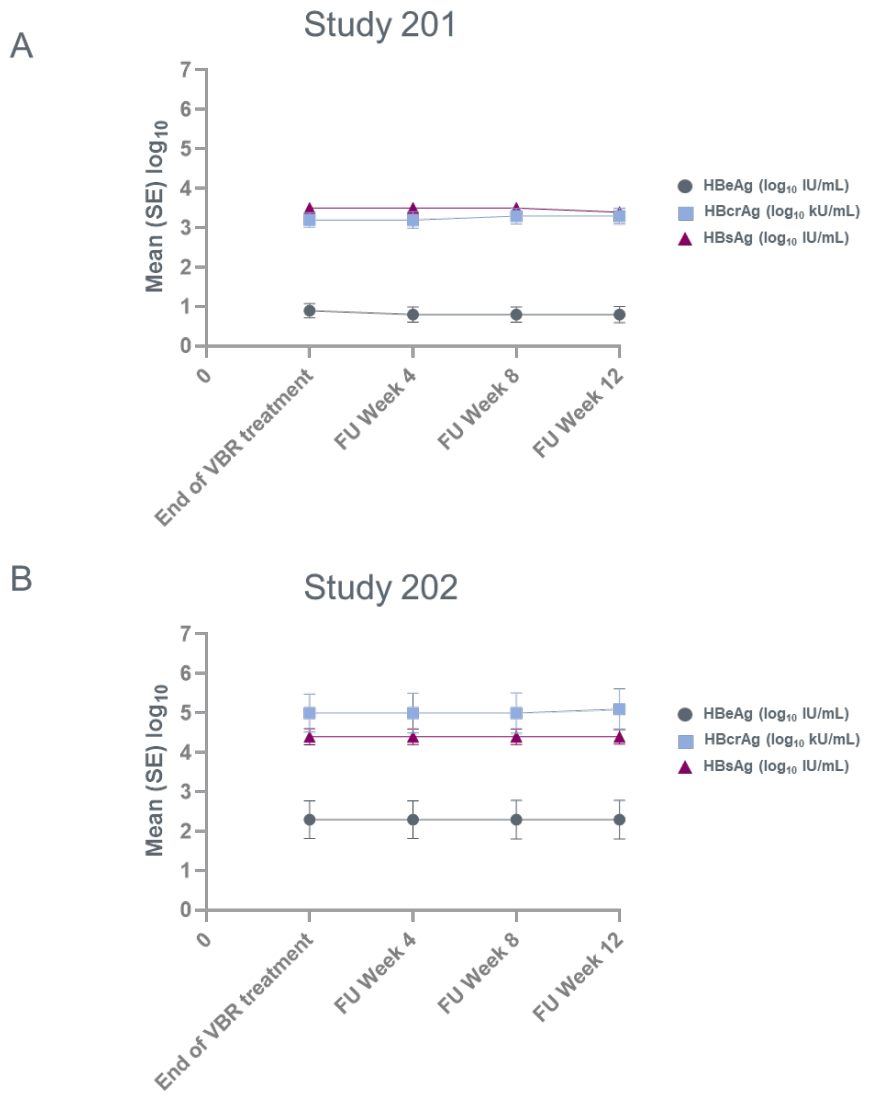

Fig. S3. HBV viral antigens in patients from Study 211 who discontinued VBR and remained on NrtI.

Mean log_{10} levels of HBV viral antigens in **(A)** patients from Study 201 and **(B)** patients from Study 202 who discontinued VBR and remained on NrtI.

FU, follow-up; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; SE, standard error; VBR, vebicorvir.

Table S1. Decision criteria and treatment actions for Study 211 patients previously enrolled in Studies 201 and 202.

Parent study	Treatment history[a]	HBeAg status[a]	Study 211 visit (week)	Decision criteria	Treatment actions
201	VS	–	52[b]	Both VBR+NrtI stopped in all patients	Discontinue both VBR+NrtI and enter long-term, off-treatment follow-up for up to 3 years
201	VS	+	52[b]	If HBV TNA was <20 IU/mL **and** HBeAg ≤5 IU/mL for ≥7 consecutive visits[c]	Discontinue both VBR+NrtI and enter long-term, off-treatment follow-up for up to 3 years
				If HBV TNA was not <20 IU/mL **and** HBeAg ≤5 IU/mL for ≥7 consecutive visits[c]	Discontinue VBR only and continue NrtI alone; enter follow-up on NrtI alone for 12 weeks
202	TN	+	52[b]	If ≥2.5 $\log_{10}$ reduction in HBV pgRNA from Baseline in the	Continue both VBR+ETV for additional 96 weeks (ie, to Week 148)

				parent study or achieved HBV pgRNA <LLOQ	
				If <2.5 log_{10} reduction in pgRNA from Baseline in the parent study or did not achieve HBV pgRNA <LLOQ	Discontinue VBR only and continue ETV alone; enter follow-up on ETV alone for up to 12 weeks
			148	If HBV TNA <20 IU/mL **and** HBeAg ≤5 IU/mL for ≥7 consecutive visits[c]	Discontinue both VBR+ETV and enter long-term, off-treatment follow-up for up to 3 years
				If HBV TNA was not <20 IU/mL **and** HBeAg ≤5 IU/mL for ≥7 consecutive visits[c]	Discontinue VBR only and continue ETV alone; enter follow-up on ETV alone for up to 12 weeks

[a]Treatment history and HBeAg status at Baseline in the parent studies (Study 201 or 202). [b]Patients without virologic assessment at Week 52 were evaluated at the next study visit. [c]Consecutive visits were determined from the last time point at which values were available for all parameters.

ETV, entecavir; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LLOQ, lower limit of quantification; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; TN, treatment-naïve; TNA, total nucleic acids; VBR, vebicorvir; VS, virologically-suppressed.

Table S2. Treatment groups from parent studies reported in Study 211.

Parent study	**Study 201 (NCT03576066)**											
Parent study population	Virologically suppressed											
Parent study HBeAg status	Positive						Negative					
Parent study treatment	VBR+NrtI			PBO+NrtI			VBR+NrtI			PBO+NrtI		
Data reporting period	On-Rx[a]	Off-Rx[b]	NrtI-restart[c]	On-Rx[a]	Off-Rx[b]	NrtI-restart[c]	On-Rx[a]	Off-Rx[b]	NrtI-restart[c]	On-Rx[a]	Off-Rx[b]	NrtI-restart[c]
Parent study	**Study 202 (NCT03577171)**											
Parent study population	Treatment-naïve											
Parent study HBeAg status	Positive											
Parent study treatment	VBR+ETV						PBO+ETV					
Data reporting period	On-Rx[a]		Off-Rx[b]			NrtI-restart[c]	On-Rx[a]		Off-Rx[b]			NrtI-restart[c]

[a]Data reported from the first dose of study drug to the last dose of study drug in Study 211. [b]Data reported after the last dose of study drug in Study 211. [c]Data reported from the time NrtI was restarted after VBR+NrtI were discontinued.

ETV, entecavir; HBeAg, hepatitis B e antigen; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; Rx, treatment; VBR, vebicorvir.

Table S3. Schedule of efficacy, safety, and pharmacokinetic assessments for on-treatment patients through Week 100.

Assessment	0	2	4	8	12	16	20	24	28	32	36	40	44	48	52	56[a]	60	64	68	72	76	80	84	88	92	96	100
Full physical examinations	X							X							X						X						X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications[b] and AE review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dermatologic assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBV DNA and pgRNA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

HBsAg, HBeAg, and HBcrAg[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistry, haematology, and coagulation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PK sample														X													

[a]When the primary endpoint was assessed. [b]All concomitant medications were required to be recorded in the designated electronic case report form from the date informed consent was obtained to 30 days following the last dose of all study drug(s), with the exception of concomitant medications used for treatment of HBV in patients who restarted HBV therapy, which were collected through the end of follow-up. [c]HBcrAg was included as a biomarker and tested at every visit. HBeAg and HBsAg were tested at every visit except for Week 2.

AE, adverse event; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; PK, pharmacokinetic.

Table S4. Schedule of efficacy and safety assessments for on-treatment patients from Weeks 104 to 148.

	Study week											
Assessment	104	108	112	116	120	124	128	132	136	140	144	148
Full physical examinations						X						X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications[a] and AE review	X	X	X	X	X	X	X	X	X	X	X	X
Dermatologic assessment	X	X	X	X	X	X	X	X	X	X	X	X
HBV DNA and pgRNA	X	X	X	X	X	X	X	X	X	X	X	X
HBsAg, HBeAg, and HBcrAg	X	X	X	X	X	X	X	X	X	X	X	X

Chemistry, haematology, and coagulation	X	X	X	X	X	X	X	X	X	X	X	X

[a]All concomitant medications were required to be recorded in the designated electronic case report form from the date informed consent was obtained to 30 days following the last dose of all study drug(s), with the exception of concomitant medications used for treatment of HBV in patients who restarted HBV therapy, which were collected through the end of follow-up.

AE, adverse event; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA.

Table S5. Schedule of efficacy and safety assessments for long-term, off-treatment patients who discontinue both VBR+Nrtl.

				Posttreatment follow-up[a]: 3-year follow-up					
Assessment	4 Wks	8 Wks	12 Wks	20 Wks	24 Wks	32 Wks	40 Wks	48 Wks	Q3 months (Months 15–36)
Full physical examinations	X								
Vital signs	X								
Concomitant medications[b] and AE review	X	X	X	X	X	X	X	X	X
Symptom-derived physical exam		X	X	X	X	X	X	X	X

HBV DNA and pgRNA	X	X	X	X	X	X	X	X	X
HBsAg, HBeAg, and HBcrAg	X	X	X	X	X	X	X	X	X
Chemistry, haematology, and coagulation	X								
Liver panel		X	X	X	X	X	X	X	X

[a]Patients with a posttreatment ALT elevation >2× ULN or HBV DNA >2000 IU/mL were asked to return to the clinic every 2 weeks for an unscheduled visit to monitor liver function and viral load until the patient's lab values resolved or the patient was required to restart NrtI therapy. [b]Any concomitant medications were recorded in the designated electronic case report form from the date informed consent was obtained to 30 days following the last dose of all study drug(s), with the exception of concomitant medications used for treatment of HBV in patients who restarted HBV therapy, which were collected through end of follow-up.

AE, adverse event; ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; Q3 months, every 3 months; ULN, upper limit of normal; VBR, vebicorvir; Wk, week.

Table S6. Schedule of efficacy and safety assessments for patients who either prematurely discontinued the study, stopped VBR only and continued Nrtl alone, or restarted Nrtl after discontinuation of both VBR+Nrtl.

	Posttreatment follow-up: 12-week follow-up		
Assessment	Follow-up 1[a]	Follow-up 2[a]	Follow-up 3[a]/EOS
Full physical examinations	X		
Vital signs	X		
Concomitant medications[b] and AE review	X	X	X
Symptom-derived physical exam		X	X
HBV DNA and pgRNA	X	X	X

HBsAg, HBeAg, and HBcrAg	X	X	X
Chemistry, haematology, and coagulation	X		
Liver panel		X	X

[a]The follow-up 1, 2, and 3 visits occurred 4, 8, and 12 weeks, respectively, after the patient discontinued VBR and continued NrtI, restarted NrtI, or was prematurely terminated from the study. Additional (unscheduled) follow-up visits occurred as clinically indicated. [b]Any concomitant medications were recorded in the designated electronic case report form from the date informed consent was obtained to 30 days following the last dose of all study drug(s), with the exception of concomitant medications used for treatment of HBV in patients who restarted HBV therapy, which were collected through end of follow-up.

AE, adverse event; EOS, end of study; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; VBR, vebicorvir.

Table S7. Baseline demographics for patients in Study 211, all-enrolled analysis set.

	Patients originating from Study 201			Patients originating from Study 202	
Characteristic	**VS HBeAg (–) n=26**	**VS HBeAg (+) n=43**	**Total n=69**	**TN HBeAg (+) n=23**	**Overall total n=92**
Age, years	49 (35, 65)	45 (21, 67)	46 (21, 67)	36 (21, 67)	44 (21, 67)
<50 years, n (%)	14 (54)	29 (67)	43 (62)	19 (83)	62 (67)
Sex, male, n (%)	16 (62)	28 (65)	44 (64)	8 (35)	52 (57)
Race, n (%)					
Asian	20 (77)	38 (88)	58 (84)	22 (96)	80 (87)
Black	3 (12)	1 (2)	4 (6)	1 (4)	5 (5)

Native Hawaiian or other Pacific Islander	0	1 (2)	1 (1)	0	1 (1)
White	2 (8)	2 (5)	4 (6)	0	4 (4)
Other	1 (4)	1 (2)	2 (3)	0	2 (2)
BMI, kg/m^2	24.4 (18.7, 29.9)	24.0 (18.5, 33.8)	24.1 (18.5, 33.8)	23.5 (17.3, 32.7)	24.0 (17.3, 33.8)

Data shown are mean (minimum, maximum) unless indicated otherwise.

BMI, body mass index; HBeAg, hepatitis B e antigen; TN, treatment-naïve; VS, virologically-suppressed.

Table S8. Baseline disease characteristics for patients in Study 211, all-enrolled analysis set.

	Patients originating from Study 201						Patients originating from Study 202		
	VS HBeAg (–) n=26			VS HBeAg (+) n=43			TN HBeAg (+) n=23		
Characteristic	**PBO+Nrtl n=10**	**VBR+Nrtl n=16**	**Total n=26**	**PBO+Nrtl n=16**	**VBR+Nrtl n=27**	**Total n=43**	**PBO+ETV n=11**	**VBR+ETV n=12**	**Total n=23**
Years positive for HBV	20.9 (8.1)	14.8 (12.1)	17.1 (11.0)	11.1 (6.1)	12.2 (8.7)	11.8 (7.8)	11.5 (9.7)	10.2 (8.2)	10.8 (8.8)
Nrtl, n (%)									
ETV	2 (20)	3 (19)	5 (19)	1 (6)	3 (11)	4 (9)	11 (100)	12 (100)	23 (100)
ETV/TDF	0	0	0	0	1 (4)	1 (2)	0	0	0
TAF	5 (50)	6 (38)	11 (42)	5 (31)	8 (30)	13 (30)	0	0	0

TDF	3 (30)	7 (44)	10 (38)	10 (63)	15 (56)	25 (58)	0	0	0
Years on current Nrtl treatment	6.8 (6.0)	3.3 (3.6)	4.7 (4.9)	3.8 (3.0)	5.2 (3.8)	4.7 (3.5)	0.5 (0.01)	0.5 (0.00)	0.5 (0.01)
HBV DNA, log_{10} IU/mL[a]	1.1 (0.17)	1.0 (0.19)	1.1 (0.18)	1.1 (0.23)	1.1 (0.16)	1.1 (0.19)	3.8 (1.38)	2.2 (0.86)	2.9 (1.37)
TND, n (%)	6 (60)	13 (81)	19 (73)	8 (50)	16 (59)	24 (56)	0	0	0
TND at Week 2, n (%)	—	—	—	—	—	—	0	0	0
HBV DNA									
TND, n (%)[b]	9 (90)	12 (75)	21 (81)	5 (31)	18 (67)	23 (53)	ND[c]	ND[c]	ND[c]
TND at Week 2, n (%)[d]	—	—	—	—	—	—	0	1 (13)	1 (8)

HBV pgRNA, log_{10} U/mL[e]	1.5 (0.00)	1.5 (0.03)	1.5 (0.02)	3.3 (1.53)	1.9 (0.63)	2.4 (1.24)	6.7 (1.59)	4.6 (1.19)	5.6 (1.76)
<LLOQ, n (%)	10 (100)	15 (94)	25 (96)	3 (19)	16 (59)	19 (44)	0	0	0
HBV TNA, log_{10} U/mL[f]	1.3 (0.00)	1.3 (0.00)	1.3 (0.00)	2.7 (1.42)	1.4 (0.36)	1.9 (1.12)	ND[g]	ND[g]	ND[g]
<LLOQ, n (%)	10 (100)	12 (75)	22 (85)	4 (25)	17 (63)	21 (49)	ND[g]	ND[g]	ND[g]
HBeAg, log_{10} IU/mL[h]	–1.0 (0.04)	–1.00 (0.00)	–1.0 (0.02)	0.5 (1.00)	0.4 (0.91)	0.4 (0.93)	2.0 (1.44)	2.1 (1.09)	2.1 (1.24)
<LLOQ, n (%)	9 (90)	16 (100)	25 (96)	0	1 (4)	1 (2)	1 (9)	0	1 (4)
HBcrAg, log_{10} kU/mL[i]	0.6 (0.56)	0.4 (0.60)	0.5 (0.58)	2.9 (0.94)	2.8 (0.87)	2.8 (0.89)	5.0 (1.18)	5.0 (1.02)	5.0 (1.07)
<LLOQ, n (%)	3 (30)	7 (44)	10 (38)	0	0	0	0	0	0

HBsAg, log_{10} IU/mL[j]	3.3 (0.64)	3.1 (0.55)	3.2 (0.59)	3.6 (0.54)	3.5 (0.37)	3.6 (0.43)	4.4 (0.50)	4.3 (0.49)	4.4 (0.49)
<LLOQ, n (%)	0	0	0	0	0	0	0	0	0
HBeAb, negative, n (%)	1 (10)	1 (6)	2 (8)	13 (81)	24 (89)	37 (86)	10 (91)	12 (100)	22 (96)
HBsAb, negative, n (%)	10 (100)	16 (100)	26 (100)	16 (100)	27 (100)	43 (100)	9 (82)	8 (67)	17 (74)
ALT, U/L[k]	21 (12.2)	21 (7.0)	21 (9.2)	24 (14.8)	27 (29.4)	26 (24.8)	48 (30.9)	19 (7.1)	33 (26.0)
<ULN, n (%)[k]	1 (10)	1 (6)	2 (8)	1 (6)	1 (4)	2 (5)	6 (55)	1 (8)	7 (30)

Data shown are mean (SD) unless indicated otherwise.

[a]Measured by COBAS TaqMan/central lab (LLOQ=20 IU/mL and LOD=10 IU/mL). [b]Assembly Biosciences, Inc. HBV DNA assay (LOD=5 IU/mL). [c]Patient HBV DNA TND levels were ND given that values were well above the LOD for the less sensitive COBAS assay. [d]Denominators for patients originating from Study 202 are 5, 8, and 13 for PBO+ETV, VBR+ETV, and Total, respectively. [e]Assembly Biosciences, Inc. HBV pgRNA assay (LLOQ=35 U/mL for VS patients and 135 U/mL

for TN patients). [f]HBV TNA composite LLOQ=20 U/mL. [g]ND since all patients were HBV pgRNA positive. [h]Measured by COBAS TaqMan/central lab (LLOQ=0.11 IU/mL). [i]Measured by Fujirebio Lumipulse G (at the University Hong Kong lab; LLOQ=1 kU/mL). [j]Measured by COBAS TaqMan/central lab (LLOQ=0.05 IU/mL. [k]ALT ULN is 34 U/L for females and 43 U/L for males [Covance]).

ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAb, HBeAg antibody; HBeAg, hepatitis B e antigen; HBsAb, HBsAg antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LLOQ, lower limit of quantification; LOD, limit of detection; ND, not determined; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; pgRNA, pregenomic RNA; SD, standard deviation; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TN, treatment-naïve; TNA, total nucleic acids; TND, target not detected; ULN, upper limit of normal; VBR, vebicorvir; VS, virologically-suppressed.

Table S9. Observed changes in HBV antigens during the on-treatment phase in patients from Study 211 (FAS).

Patients originating from Study 201 (on-treatment phase)						
	VS HBeAg (–)			VS HBeAg (+)		
	PBO+NrtI n=10	VBR+NrtI n=16	Total n=26	PBO+NrtI n=16	VBR+NrtI n=27	Total n=43
HBeAg change from Baseline, log_{10} IU/mL						
EOT	ND[a]	ND[a]	ND[a]	–0.1 (0.43)	–0.1 (0.29)	–0.1 (0.35)
Change >1 log_{10} IU/mL, n (%)	ND[a]	ND[a]	ND[a]	0	0	0
Seroconversion, n (%)[b]	ND[a]	ND[a]	ND[a]	0	0	0
HBsAg change from Baseline, log_{10} IU/mL						

EOT	0 (0.16)	–0.1 (0.15)	–0.1 (0.16)	0 (0.05)	–0.1 (0.07)	–0.1 (0.07)
Change >1 log_{10} IU/mL, n (%)	0	0	0	0	0	0
Seroconversion, n (%)[c]	0	0	0	0	0	0
HBcrAg change from Baseline, log_{10} kU/mL						
EOT	–0.1 (0.14)	0.0 (0.41)	0.0 (0.32)	–0.2 (0.21)	–0.1 (0.24)	–0.2 (0.23)
Patients originating from Study 202 (TN HBeAg +; on-treatment phase)						
	PBO+ETV n=11			**VBR+ETV n=12**		**Total n=23**
HBeAg change from Baseline, log_{10} IU/mL						
EOT	–0.7 (0.96)			–0.4 (0.64)		–0.5 (0.81)

Change >1 log_{10} IU/mL, n (%)	1 (9)	1 (8)	2 (9)
Seroconversion, n (%)[b]	1 (9)	0	1 (4)
HBsAg change from Baseline, log_{10} IU/mL			
EOT	–0.6 (1.01)	–0.2 (0.24)	–0.4 (0.73)
Change >1 log_{10} IU/mL, n (%)	1 (9)	0	1 (4)
Seroconversion, n (%)[c]	0	0	0
HBcrAg change from Baseline, log_{10} kU/mL			
EOT	–0.9 (1.12)	–0.5 (0.67)	–0.7 (0.91)

Data shown are mean (SD) unless otherwise stated. [a]ND as all patients were HBeAg-negative. [b]HBeAg seroconversion was defined as loss of HBeAg and appearance of HBeAb. [c]HBsAg seroconversion was defined as loss of HBsAg and appearance of HBsAb.

EOT, end of treatment; ETV, entecavir; FAS, full analysis set; HBcrAg, hepatitis B core-related antigen; HBeAb, antibody to HBeAg; HBeAg, hepatitis B e antigen; HBsAb; antibody to HBsAg; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ND, not determinable; NrtI, nucleos(t)ide reverse transcriptase inhibitor; PBO, placebo; SD, standard deviation; TN, treatment-naïve; VBR, vebicorvir; VS, virologically-suppressed.

Table S10. Observed changes in HBV antigens during the off-treatment phase in patients from Study 211 (FAS).

Patients originating from Study 201 (off-treatment phase)		
	VS HBeAg (–) discontinue both n=23	**VS HBeAg (+) discontinue both n=18**
HBeAg Baseline, log_{10} IU/mL	–1.0 (0.04)	–0.2 (0.51)
Change from Baseline at end of off-treatment	0.3 (0.96)	1.5 (1.88)
HBsAg Baseline, log_{10} IU/mL	3.1 (0.61)	3.5 (0.49)
Change from Baseline at end of off-treatment	0.2 (0.71)	0.3 (0.66)
HBcrAg Baseline, log_{10} kU/mL	0.4 (0.58)	2.2 (0.47)

Change from Baseline at end of off-treatment	1.1 (1.59)	1.7 (1.96)

Data shown are mean (SD).

FAS, full analysis set; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; SD, standard deviation; VS, virologically-suppressed.

Table S11. Observed changes in HBV DNA during the Nrtl-restart phase in patients from Study 211 (FAS).

	Patients originating from Study 201 (Nrtl restart)	
	VS HBeAg (–), discontinued both and restarted Nrtl n=16	**VS HBeAg (+), discontinued both and restarted Nrtl n=14**
HBV DNA		
Baseline, log_{10} IU/mL	5.7 (2.13)	6.8 (2.32)
Change from Baseline at end of Nrtl-restart phase	–4.3 (1.83)	–3.9 (1.10)

Data shown are mean (SD).

FAS, full analysis set; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; Nrtl, nucleos(t)ide reverse transcriptase inhibitor; SD, standard deviation; VS, virologically-suppressed.

Table S12. Observed changes in HBV antigens during the NrtI-restart phase in patients from Study 211 (FAS).

Patients originating from Study 201 (NrtI restart)		
	VS HBeAg (–), discontinued both and restarted NrtI n=16	**VS HBeAg (+), discontinued both and restarted NrtI n=14**
HBeAg Baseline, log_{10} IU/mL	–0.6 (1.17)	1.8 (1.60)
Change from Baseline at end of NrtI-restart phase	–0.3 (0.81)	–0.8 (1.19)
HBsAg Baseline, log_{10} IU/mL	3.5 (0.58)	4.0 (0.72)
Change from Baseline at end	–0.5 (0.78)	0 (0.56)

of NrtI-restart phase		
HBcrAg Baseline, log_{10} kU/mL	2.4 (1.83)	4.6 (1.85)
Change from Baseline at end of NrtI-restart phase	–1.0 (0.78)	–0.8 (1.16)

Data shown are mean (SD).

FAS, full analysis set; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; SD, standard deviation; VS, virologically-suppressed.

Table S13. Observed changes in HBV nucleic acids in patients who continued Nrtl/ETV during the off-treatment phase in patients from Study 211 (FAS).

	Continued Nrtl/ETV (off-treatment phase)	
	Patients originating from Study 201	**Patients originating from Study 202**
	VS HBeAg (+), continued Nrtl only	**TN HBeAg (+), continued ETV only**
	n=18	**n=6**
HBV DNA		
Baseline, $\log_{10}$ IU/mL	1.2 (0.16)	1.9 (1.37)
Change from Baseline at end of off-treatment phase	–0.1 (0.17)	1.3 (1.86)
HBV pgRNA		
Baseline, $\log_{10}$ U/mL	2.7 (0.71)	5.3 (1.53)

Change from Baseline at end of off-treatment phase	2.3 (0.78)	1.5 (1.11)
HBV TNA		
Baseline, log_{10} U/mL[a]	1.7 (0.55)	3.3 (1.02)
Change from Baseline at end of off-treatment phase	2.0 (0.98)	1.4 (1.33)

Data shown are mean (SD). [a]TNA=HBV DNA+HBV pgRNA.

ETV, entecavir; FAS, full analysis set; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; pgRNA, pregenomic RNA; SD, standard deviation; TN, treatment-naïve; TNA, total nucleic acids; VS, virologically-suppressed.

Table S14. Observed changes in HBV antigens in patients who continued Nrtl/ETV during the off-treatment phase in patients from Study 211 (FAS).

	Continued Nrtl/ETV (off-treatment phase)	
	Patients originating from Study 201	**Patients originating from Study 202**
	VS HBeAg (+), continued Nrtl only **n=18**	**TN HBeAg (+), continued ETV only** **n=6**
HBeAg Baseline, log_{10} IU/mL	0.9 (0.77)	2.3 (1.18)
Change from Baseline at end of off-treatment phase	–0.1 (0.30)	0.0 (0.11)
HBsAg Baseline, log_{10} U/mL	3.5 (0.44)	4.4 (0.50)
Change from Baseline at end	0 (0.09)	0 (0.06)

of off-treatment phase		
HBcrAg Baseline, log_{10} U/mL	3.2 (0.78)	5.0 (1.18)
Change from Baseline at end of off-treatment phase	0 (0.22)	0.1 (0.27)

Data shown are mean (SD).

ETV, entecavir; FAS, full analysis set; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NrtI, nucleos(t)ide reverse transcriptase inhibitor; SD, standard deviation; TN, treatment-naïve; VS, virologically-suppressed.

Table S15. Summary of laboratory abnormalities dependent on TA for patients in Study 211 (SAS).

On-treatment phase					
	Patients originating from Study 201			Patients originating from Study 202	
Patients reporting:	**VS HBeAg (–) n=26**	**VS HBeAg (+) n=43**	**Total n=69**	**TN HBeAg (+) n=23**	**Overall total N=92**
Any grade	20 (77)	32 (74)	52 (75)	20 (87)	72 (78)
Grade 1	14 (54)	22 (51)	36 (52)	12 (52)	48 (52)
Grade 2	5 (19)	6 (14)	11 (16)	6 (26)	17 (18)
Grade 3	1 (4)	4 (9)	5 (7)	2 (9)	7 (8)
Glucose increased					
Grade 1	6 (23)	13 (30)	19 (28)	7 (30)	26 (28)
Grade 2	4 (15)	1 (2)	5 (7)	1 (4)	6 (7)

Amylase increased					
Grade 1	2 (8)	10 (23)	12 (17)	5 (22)	17 (18)
Grade 2	1 (4)	0	1 (1)	3 (13)	4 (4)
Grade 3	0	1 (2)	1 (1)	0	1 (1)
AST increased					
Grade 1	1 (4)	6 (14)	7 (10)	0	7 (8)
Grade 2	0	2 (5)	2 (3)	2 (9)	4 (4)
Grade 3	1 (4)	0	1 (1)	1 (4)	2 (2)
ALT increased					
Grade 1	3 (12)	4 (9)	7 (10)	1 (4)	8 (9)
Grade 2	1 (4)	0	1 (1)	0	1 (1)
Grade 3	0	1 (2)	1 (1)	2 (9)	3 (3)
Creatinine increased					
Grade 1	6 (23)	3 (7)	9 (13)	1 (4)	10 (11)

Grade 2	0	1 (2)	1 (1)	0	1 (1)
Glucose decreased					
Grade 1	4 (15)	0	4 (6)	4 (17)	8 (9)
Grade 2	0	2 (5)	2 (3)	0	2 (2)
Urate increased					
Grade 1	5 (19)	3 (7)	8 (12)	2 (9)	10 (11)
Total bilirubin increased					
Grade 1	2 (8)	0	2 (3)	2 (9)	4 (4)
Grade 2	0	1 (2)	1 (1)	0	1 (1)
Lipase increased					
Grade 1	0	3 (7)	3 (4)	0	3 (3)
Grade 2	0	1 (2)	1 (1)	0	1 (1)

Sodium increased					
Grade 1	0	3 (7)	3 (4)	1 (4)	4 (4)
Bicarbonate decreased					
Grade 1	0	0	0	1 (4)	1 (1)
Grade 2	0	1 (2)	1 (1)	1 (4)	2 (2)
Sodium decreased					
Grade 1	0	2 (5)	2 (3)	1 (4)	3 (3)
Haemoglobin decreased					
Grade 1	0	2 (5)	2 (3)	2 (9)	4 (4)
Platelets decreased					
Grade 1	0	2 (5)	2 (3)	0	2 (2)

Grade 2	0	0	0	1 (4)	1 (1)
Prothrombin intl normalised ratio increased					
Grade 1	1 (4)	1 (2)	2 (3)	0	2 (2)
Grade 2	0	1 (2)	1 (1)	0	1 (1)
Grade 3	0	1 (2)	1 (1)	0	1 (1)

Discontinued both VBR+Nrtl (off-treatment phase, patients originally from Study 201)			
Patients reporting:	**VS HBeAg (–) n=23**	**VS HBeAg (+) n=18**	**Total N=40**
Any grade	14 (64)	14 (78)	28 (70)
Grade 1	6 (27)	3 (17)	9 (23)
Grade 2	7 (32)	9 (50)	16 (40)
Grade 3	0	2 (11)	2 (5)
Grade 4	1 (5)	0	1 (3)
ALT increased			

Grade 1	4 (18)	3 (17)	7 (18)
Grade 2	6 (27)	6 (33)	12 (30)
Grade 3	0	2 (11)	2 (5)
Grade 4	1 (5)	0	1 (3)
AST increased			
Grade 1	6 (27)	4 (22)	10 (25)
Grade 2	2 (9)	6 (33)	8 (20)
Grade 3	1 (5)	0	1 (3)
Glucose increased			
Grade 1	3 (15)	4 (25)	7 (19)
Grade 2	1 (5)	0	1 (3)
Amylase increased			
Grade 1	1 (6)	3 (20)	4 (13)
Urate increased			

Grade 1	3 (15)	0	3 (8)

Discontinued both VBR+Nrtl, then restarted Nrtl (Nrtl-restart phase, patients originally from Study 201)			
Patients reporting:	**VS HBeAg (–) n=16**	**VS HBeAg (+) n=14**	**Total N=30**
Any grade	14 (88)	13 (93)	27 (90)
Grade 1	6 (38)	4 (29)	10 (33)
Grade 2	4 (25)	5 (36)	9 (30)
Grade 3	2 (13)	2 (14)	4 (13)
Grade 4	2 (13)	2 (14)	4 (13)
ALT increased			
Grade 1	3 (19)	4 (29)	7 (23)
Grade 2	4 (25)	4 (29)	8 (27)
Grade 3	1 (6)	2 (14)	3 (10)
Grade 4	2 (13)	2 (14)	4 (13)
AST increased			
Grade 1	3 (19)	5 (36)	8 (27)

Grade 2	1 (6)	3 (21)	4 (13)
Grade 3	3 (19)	1 (7)	4 (13)
Grade 4	0	1 (7)	1 (3)
Glucose increased			
Grade 1	2 (13)	3 (23)	5 (18)
Grade 3	1 (7)	0	1 (4)
Amylase increased			
Grade 1	3 (21)	1 (8)	4 (15)
Bilirubin increased			
Grade 1	1 (6)	2 (14)	3 (10)
Urate increased			
Grade 1	1 (7)	2 (15)	3 (11)

	Continued Nrtl/ETV (off-treatment phase)	
	Patients originating from Study 201	**Patients originating from Study 202**
Patients reporting:	**VS HBeAg (+) continued Nrtl only n=18**	**TN HBeAg (–) continued ETV only n=6**
Any grade	2 (11)	1 (17)
Grade 1	2 (11)	1 (17)
Urate increased		
Grade 1	0	1 (25)[a]
Total bilirubin increased		
Grade 1	1 (6)	0
Lipase increased		
Grade 1	1 (8)[b]	0

Data shown are n (%). [a]n=4. [b]n=13.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ETV, entecavir; HBeAg, hepatitis B e antigen; intl, international; NrtI, nucleos(t)ide reverse transcriptase inhibitor; SAS, safety analysis set; TA, treatment action; TN, treatment-naïve; VBR, vebicorvir; VS, virologically-suppressed.

Table S16. Summary statistics of plasma concentration (ng/mL) for VBR, ETV, and TFV (PK analysis set) in patients from Study 211.

	VBR Wk 48 predose	ETV Wk 48 predose	TFV (TAF) Wk 48 predose	TFV (TDF) Wk 48 predose
N	64	27	15	21
Mean	1560	0.962	20.8	113
SD	679	2.01	15.1	106
% CV	43.7	209.3	72.5	93.1
Median	1640	0.494	15.7	81.7
Minimum	0	0.128	10.9	16.0
Maximum	3160	10.8	71.1	424

CV, coefficient of variation; ETV, entecavir; PK, pharmacokinetic; SD, standard deviation; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; VBR, vebicorvir; Wk, week

References

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