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Original Article

Efficacy, safety, and pharmacokinetics of capsid assembly modulator linvencorvir plus standard of care in chronic hepatitis B patients

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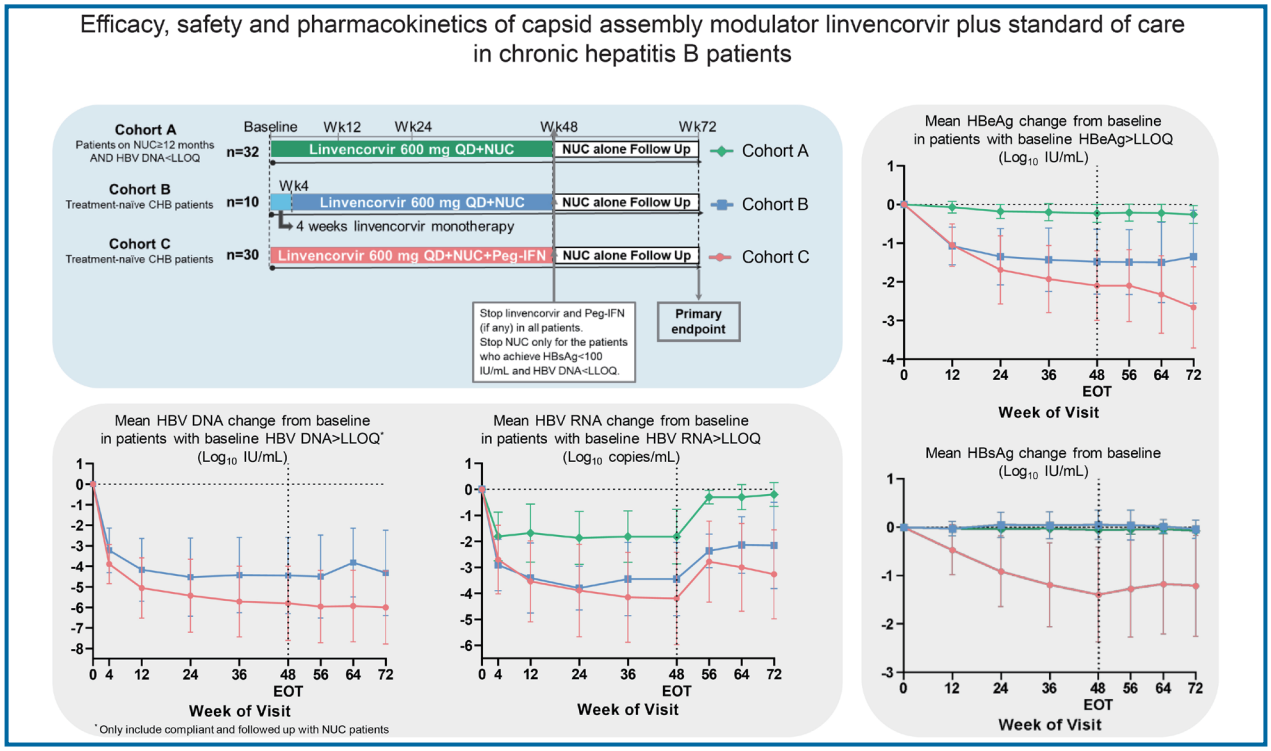
Graphical Abstract



Study Highlights

- Linvencorvir plus SoC was generally safe and well tolerated.
- Linvencorvir on top of SoC demonstrated potent suppression of HBV DNA and RNA including in patients with high viral loads.
- Suppression of HBV RNA was partially sustained during off-linvencorvir period in treatment-naïve patients.
- Linvencorvir plus SoC durably reduced HBeAg and HBcrAg in treatment-naïve patients.
- Linvencorvir on top of Peg-IFN-α and NUC led to an obvious HBsAg decline in treatment-naïve patients including in those with HBV genotype C; however, no HBsAg loss was achieved.

Background/Aims: Four-week treatment of linvencorvir (RO7049389) was generally safe and well tolerated, and showed anti-viral activity in chronic hepatitis B (CHB) patients. This study evaluated the efficacy, safety, and pharmacokinetics of 48-week treatment with linvencorvir plus standard of care (SoC) in CHB patients.

Methods: This was a multicentre, non-randomized, non-controlled, open-label phase 2 study enrolling three cohorts: nucleos(t)ide analogue (NUC)-suppressed patients received linvencorvir plus NUC (Cohort A, n=32); treatment-naïve patients received linvencorvir plus NUC without (Cohort B, n=10) or with (Cohort C, n=30) pegylated interferon-α (Peg-IFN-α). Treatment duration was 48 weeks, followed by NUC alone for 24 weeks.

Results: 68 patients completed the study. No patient achieved functional cure (sustained HBsAg loss and unquantifiable HBV DNA). By Week 48, 89% of treatment-naïve patients (10/10 Cohort B; 24/28 Cohort C) reached unquantifiable HBV DNA. Unquantifiable HBV RNA was achieved in 92% of patients with quantifiable baseline HBV RNA (14/15 Cohort A, 8/8 Cohort B, 22/25 Cohort C) at Week 48 along with partially sustained HBV RNA responses in treatment-naïve patients during follow-up period. Pronounced reductions in HBeAg and HBcrAg were observed in treatment-naïve patients, while HBsAg decline was only observed in Cohort C. Most adverse events were grade 1–2, and no linvencorvir-related serious adverse events were reported.

Conclusions: 48-week linvencorvir plus SoC was generally safe and well tolerated, and resulted in potent HBV DNA and RNA suppression. However, 48-week linvencorvir plus NUC with or without Peg-IFN did not result in the achievement of functional cure in any patient. **(Clin Mol Hepatol 2024;30:191-205)**

Keywords: Linvencorvir; RO7049389; Capsid assembly modulator; Chronic hepatitis B; Phase 2

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Abbreviations:

AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHB, chronic hepatitis B; cccDNA, covalently closed circular DNA; CAM, capsid assembly modulator; CFB, change from baseline; CI, confidence interval; ETV, entecavir; ECG, electrocardiograms; EOT, end of treatment; FU, follow-up; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; LLOQ, lower limit of quantification; NUC, nucleos(t)ide analogue; NA, not applicable; Peg-IFN-α, pegylated interferon-α; pgRNA, pregenomic RNA; PK, pharmacokinetics; SoC, standard of care; SD, standard deviation; SAE, serious adverse event; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; URTI, upper respiratory tract infection

INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global health challenge, and is associated with life-threatening consequences.[1,2] Functional cure, defined as unquantifiable HBV DNA and sustained hepatitis B surface antigen (HBsAg) loss,[3] improves long-term prognosis and is a major therapeutic goal for chronic hepatitis B (CHB) therapy.[4-7] Currently available treatments for CHB, including nucleos(t)ide analogues (NUCs) and pegylated interferon (Peg-IFN), have limitations. NUCs, which inhibit HBV DNA synthesis, are unable to fully suppress viral replication in some patients (or do so very slowly), especially in hepatitis B e antigen (HBeAg) positive patients and those with high viral load.[8,9] NUCs must be taken life-long, have no direct effect on HBV RNA or covalently closed circular DNA (cccDNA), and rarely lead to functional cure.[3,5,7,10,11] Peg-IFN therapy is finite, but results in low rates of functional cure and is associated with side effects.[12] There is therefore a need for novel, well tolerated treatments that can augment viral suppression and help clear HBsAg in combination with current standard of care (SoC).[10]

The HBV capsid is involved in multiple steps of the HBV life cycle and is an important target of antiviral therapies in development.[13,14] Several capsid assembly modulators (CAMs), which inhibit viral replication by inducing the formation of aberrant non-capsid polymers (CAM-A, previously known as Class I) or morphologically normal but nucleic acid-free empty capsids (CAM-E, previously known as Class II),[15] have reached phase 1 and 2 clinical development.[13] Studies to date have shown that 24-week treatment with CAM and NUC leads to suppression of HBV DNA and RNA, but has limited effect on HBV antigens.[14,16,17]

Linvencorvir (RO7049389) is a novel small molecule CAM that induces aberrant capsid assembly, leading to the degradation of viral core protein, thereby inhibiting pregenomic RNA (pgRNA) encapsidation and HBV DNA replication. Further, linvencorvir also induces the disassembly of nucleocapsids, potentially interfering with cccDNA biosynthesis.[18] A first-in-human, three-part phase 1/2 study of linvencorvir has been conducted in healthy volunteers and CHB patients. In Part 1 of the phase 1/2 study, linvencorvir showed favourable safety and pharmacokinetic profiles in healthy volunteers following single ascending doses up to 2,500 mg, and multiple ascending doses up to 1,200 mg/day for 2 weeks.[19] In Part 2, 4-week monotherapy with linvencorvir up to 1,000 mg/day was generally safe and well tolerated, and had potent antiviral activity in viremic CHB patients.[20] Here, we report Part 3 (phase 2 stage) of the phase 1/2 study, in which we evaluated the efficacy, safety and pharmacokinetics of linvencorvir in combination with SoC therapies (NUC with or without Peg-IFN-α) for 48 weeks in virologically-suppressed and treatment-naïve CHB patients.

MATERIALS AND METHODS

Study design and population

This multicenter, non-randomized, non-controlled, open-label phase 2 study (Part 3 of the first-in-human linvencorvir trial) was performed at 16 sites in Taiwan (n=5), Mainland China (n=3), New Zealand (n=2), Thailand (n=2), Australia (n=1), Bulgaria (n=1), Hong Kong (n=1) and Singapore (n=1). This study comprised three treatment cohorts, in which NUC-suppressed or treatment-naïve CHB patients received open-label treatment with linvencorvir plus a first-line NUC (entecavir [ETV], tenofovir alafenamide [TAF], or tenofovir disoproxil fumarate [TDF]) with or without Peg-IFN-α for 48 weeks (Fig. 1). In Cohort A, NUC-suppressed patients received linvencorvir plus NUC therapy for 48 weeks. In Cohort B, treatment-naïve patients received linvencorvir alone for the first 4 weeks of the treatment period, followed by linvencorvir plus NUC therapy for the remaining 44 weeks. Treatment-naïve patients enrolled in Cohort C received linvencorvir plus NUC and Peg-IFN-α therapy throughout the 48-week study treatment period. After the study treatment period, all patients were followed up for 24 weeks with NUC monotherapy, or without NUC if they met protocol-defined NUC stopping criteria (HBsAg below 100 IU/mL and HBV DNA below the lower limit of quantification [LLOQ; 20 IU/mL]) at the end of study treatment (Week 48). During the off-treatment follow-up period, if alanine aminotransferase (ALT) >2 times the upper limit of normal (ULN; 41 U/L for men and 33 U/L for women) was accompanied by confirmed virological relapse, NUC treatment may be restarted at the discretion of the investigator and applicable CHB guidelines.

Eligible patients were aged 18–60 years with CHB (a positive HBsAg or HBV DNA test or HBeAg-positive for more than 6 months before screening), and HBsAg concentration above 250 IU/mL at screening. NUC-suppressed patients were re-

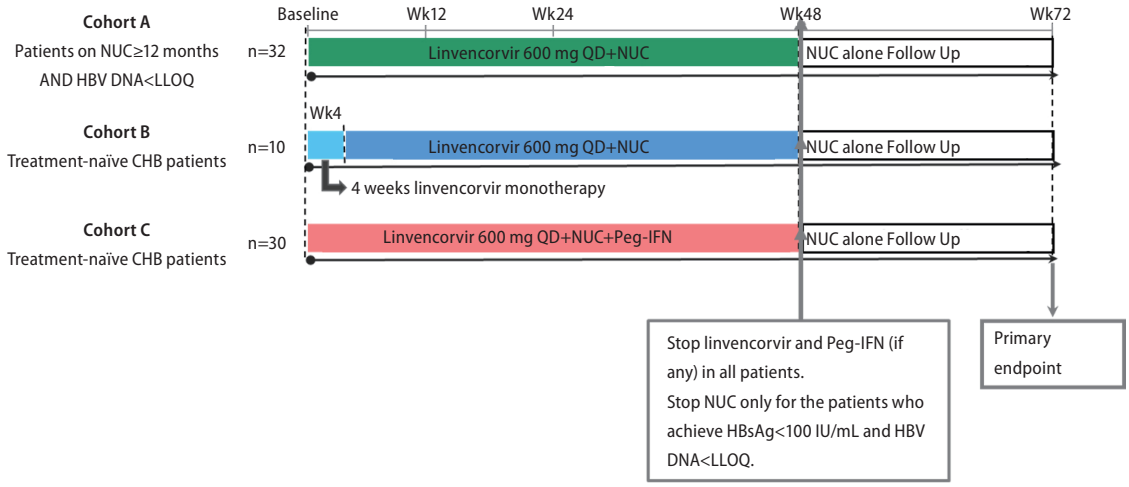

Figure 1. Study design. NUC, nucleos(t)ide analogue; HBV, hepatitis B virus; LLOQ, lower limit of quantification; CHB, chronic hepatitis B; QD, once a day; NUC, nucleos(t)ide analogue; Peg-IFN, pegylated interferon; HBsAg, hepatitis B surface antigen.

quired to have been treated with NUC monotherapy (ETV, TAF, or TDF) for at least 12 months, and must have been on the same NUC therapy for at least 3 months before screening. These patients should have HBV DNA below LLOQ at screening, and ALT ≤2×ULN at screening and Day -1. Treatment-naïve patients were required to have previously received anti-HBV treatments for less than 30 days in total, and to have not received any anti-HBV treatment within 3 months prior to the first study dose. Treatment-naïve patients also required HBV DNA of at least 2×10^4 IU/mL (HBeAg-positive patients) or 2×10^3 IU/mL (HBeAg-negative patients) at screening, and ALT levels between 1–5×ULN at screening and below 5×ULN at Day -1. Full details of the eligibility criteria are provided in the Supplementary Material.

The study was conducted in accordance with Good Clinical Practice standards and the Declaration of Helsinki. The study protocol was approved by the institutional review boards or ethics committees from all participating study centres, and written informed consent was obtained from each participant included in the study.

Procedures

In all three treatment cohorts, linvencorvir 600 mg was administered orally once a day in the fasted state (≥2 hours after a meal and ≥2 hours before the next meal). NUC (ETV, TAF, or TDF) and Peg-IFN-α therapy were administered according to the local label or guidelines. Investigators could refer to Peg-IFN stopping rules recommended in major guidelines.[5,7,21] If Peg-IFN was stopped before the end of the 48-week treatment period, linvencorvir and NUC were to be continued until the end of the treatment period. At the end of the study treatment period, NUC therapy was continued for 24 weeks unless patients met the NUC stopping criteria.

Safety-related clinical and laboratory evaluations, and blood sample collections for the determination of HBV viral dynamic responses were conducted on day-1, during the study treatment period (every 2 weeks for the first 4 weeks and every 4 weeks thereafter), and during the follow-up (every 8 weeks for patients not meeting the NUC stopping criteria; every 2 weeks for the first 12 weeks and every 4 weeks thereafter for patients meeting the NUC stopping criteria). Details of methodologies for determining HBV genotype, and measuring viral dynamic markers are provided in the Supplementary Material. In particular, plasma HBV RNA was quantitatively assessed at Roche Diagnostic International Ltd (for non-Chinese sites) or Q2 Solutions for Chinese sites using a COBAS® HBV RNA test on the Roche COBAS®6800 System.[22,23] Safety assessments included monitoring and recording the occurrence and severity of adverse events (AEs), physical examinations, safety laboratory assessments, vital signs, and 12-lead electrocardiograms (ECGs). AEs and ALT and aspartate aminotransferase (AST) elevations were graded according to the Division of AIDS criteria (Supplementary Table 1).

Plasma samples for pharmacokinetics (PK) analysis were

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collected at the following time points: (1) pre-dose and 1–8 hours post-dose on day 1 and Weeks 4 (Cohort B only) and 24, (2) pre-dose and 1–4 hours post-dose at all other scheduled visits during study treatment; and (3) before and 1–24 hours after the last dose of study treatment.

Endpoints

The primary endpoint in this study was HBV DNA below the LLOQ (20 IU/mL) with HBsAg loss (<0.05 IU/mL) at 24 weeks post-treatment (defined as functional cure in the protocol). Secondary efficacy endpoints included serum HBV DNA and RNA below the LLOQ, HBsAg and HBeAg loss and anti-HBs and anti-HBe seroconversion, quantitative change from baseline for the HBV markers including serum HBV DNA, HBV RNA and HBV antigens. Secondary efficacy endpoints were assessed in each cohort overall and the following patient subgroups: HBeAg-positive and HBeAg-negative, and low and high baseline HBsAg (cutoff: 4 $\log_{10}$ IU/mL). Relationships between secondary efficacy endpoints and HBV genotype and high baseline HBV DNA (>7 $\log_{10}$ IU/mL) were also explored. Other secondary endpoints were the incidence of AEs and most common AEs, and the PK profile of linvencorvir and its metabolites when used in combination with SoC therapies.

Statistical analysis

The sample size for this study was intended to support the assessment of the functional cure rate. Individual cohort sample sizes of at least 10–30 were planned to ensure that the lower 95% confidence interval was above 5–14% if there was an observed functional cure rate of 30%, assuming binomial distribution.

All patients who received at least one dose of linvencorvir were included in the safety and efficacy analysis populations. Efficacy analyses were based on the actual number of patients with valid results at each study visit. For the PK analysis, patients who significantly violated inclusion or exclusion criteria, who deviated significantly from the protocol, or for whom data were unavailable or incomplete which may have influenced the PK analysis were excluded.

For continuous variables, descriptive statistics were calculated. Values below the LLOQ were imputed to numeric values below the LLOQ value to make a conservative calculation of change from baseline values (Supplementary Table 2). For categorical data, the number and proportion of study participants in each category were summarized. Spearman's rank correlation was calculated to determine the relationship between graded treatment-emergent ALT elevations and categorized maximal declines in HBsAg. PK parameters were calculated from a non-compartment analysis using Phoenix software (WinNonlin models version 6.4).

RESULTS

Patient characteristics

Between June 14, 2019 and October 19, 2020, 72 (44%) of 163 screened patients were enrolled in the study: 32 NUC-suppressed patients in Cohort A, 10 and 30 treatment-naïve patients in Cohorts B and C, respectively (Fig. 2). All 72 patients received linvencorvir, and 68 (94.4%) patients completed the 72-week study. Linvencorvir treatment was discontinued early for non-safety reasons in four patients (on days 15 and 62 for two patients in Cohort A, and on days 83 and 237 for two patients in Cohort C).

Baseline demographics and clinical characteristics are shown in Table 1. In Cohorts A and C, patients were predominantly Asian and male, whereas 5 (50%) patients were Asian and 5 (50%) patients were male in Cohort B. HBV DNA levels were below the LLOQ in all Cohort A patients, but 15 (46.9%) patients had quantifiable HBV RNA. Mean baseline HBV DNA levels were 5.73 $\log_{10}$ IU/mL in Cohort B and 6.91 $\log_{10}$ IU/mL in Cohort C. Two (20%) and 18 (60%) patients in Cohorts B and C, respectively, had a high viral load (HBV DNA >7 $\log_{10}$ IU/mL). Eight (80%) and 27 (90%) patients in Cohorts B and C, respectively, had baseline quantifiable HBV RNA. In Cohort A, six (19%) patients had HBV genotype C, as did five patients (50%) in Cohort B, and 11 patients (37%) in Cohort C. NUC-suppressed patients were mainly HBeAg-negative (66% [21/32]), but treatment-naïve patients were mainly HBeAg-positive (63% [25/40]). Mean baseline HBsAg levels across the three cohorts ranged from 3.2 $\log_{10}$ IU/mL in Cohort A to 3.96 $\log_{10}$ IU/mL in Cohort C. More than half of the Cohort C patients had high baseline HBsAg levels (≥ 4 $\log_{10}$ IU/mL).

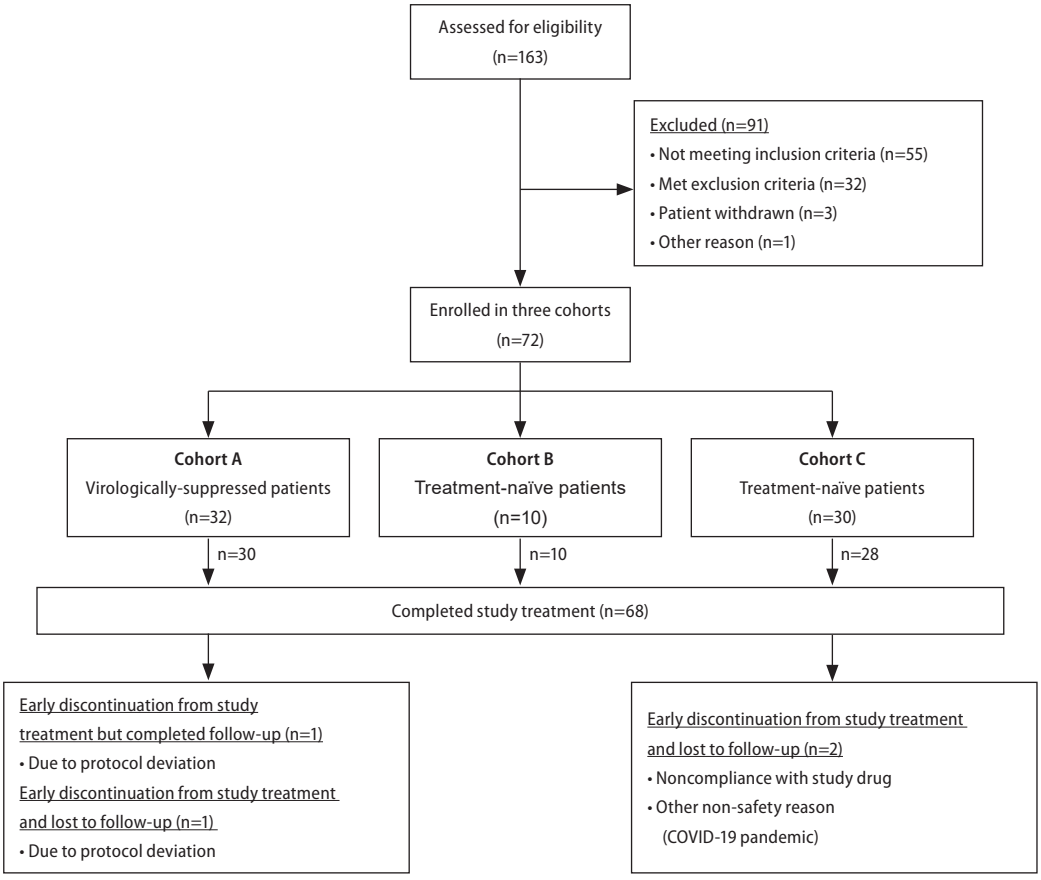

Figure 2. Trial profile.

Primary endpoint

In this study, no patient achieved HBV DNA<LLOQ with HBsAg loss at Week 24 post-study treatment (functional cure).

HBV DNA responses

In NUC-suppressed patients (Cohort A), mean HBV DNA levels remained below the LLOQ throughout the study. In treatment-naïve patients, HBV DNA levels declined by a mean (standard deviation [SD]) of 4.45 (1.86) and 5.80 (1.81) $\log_{10}$ IU/mL at Week 48 in Cohorts B and C, respectively (Fig. 3A). With higher baseline HBV DNA levels, HBeAg-positive patients achieved larger reductions in HBV DNA than HBeAg-negative patients (mean [SD] HBV DNA declines of 5.48 [1.19] vs. 2.90 [1.62] $\log_{10}$ IU/mL, respectively, in Cohort B; 6.97 [0.74] vs. 3.80 [1.20] $\log_{10}$ IU/mL, respectively, in Cohort C) (Fig. 3B). All ten (100%) Cohort B patients achieved HBV DNA below the LLOQ at Week 48, including two HBeAg-positive patients with high viral load. By Week 48, HBV DNA levels reached below the LLOQ in 86% (24/28) of Cohort C patients who completed 48-weeks of study treatment, including in 76% (13/17) of HBeAg-positive patients with high viral load. All the remaining four patients who had not achieved unquantifiable HBV DNA during the study treatment had significantly reduced viral DNA levels (<150 IU/mL) at Week 48. At the end of study treatment, all Cohort B patients entered into the 24-week follow-up with NUC treatment. Five patients in Cohort C met the NUC stopping criteria at Week 48, so they were followed without NUC. During the NUC-alone follow-up period, 96% (26/27) of NUC-compliant Cohorts B and C patients with unquantifiable HBV DNA by Week 48 sustained HBV DNA below the LLOQ; the four patients who had not achieved unquantifiable HBV DNA by Week 48 attained HBV DNA below the LLOQ or maintained low HBV DNA levels. Among the five patients who entered off-treatment follow-up, four patients experienced HBV DNA rebound to quantifiable levels at around Week 56. Three out of them were not retreated at the investi-

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Table 1. Baseline demographics and clinical characteristics

Characteristics	NUC-suppressed Cohort A (n=32)	Treatment-naïve	
		Cohort B (n=10)	Cohort C (n=30)
Age, years	47.2 (8.3)	43.8 (9.8)	32.8 (7.7)
Sex			
Female	13 (41%)	5 (50%)	7 (23%)
Male	19 (59%)	5 (50%)	23 (77%)
Race			
Asian	25 (78%)	5 (50%)	28 (93%)
White	6 (19%)	4 (40%)	1 (3%)
Other	1 (3%)	1 (10%)	1 (3%)
Previous NUC treatment, months	98.9 (53.8)	0 (0)	0.1 (0.4)*
Min–max	16.9–213.9	0–0	0–2.0*
HBV DNA			
log_{10} IU/mL	<LLOQ	5.73 (1.86)	6.91 (1.89)
>7 log_{10} IU/mL	0	2 (20%)	18 (60%)
HBV RNA			
≥LLOQ	15 (47%)	8 (80%)	27 (90%)
log_{10} copies/mL‡	2.58 (1.06)	4.14 (1.41)	5.1 (1.93)
HBsAg,			
log_{10} IU/mL	3.20 (0.52)	3.48 (0.68)	3.96 (0.9)
≥4 log_{10} IU/mL	4 (13%)	1 (10%)	16 (53%)
HBeAg			
Positive†	11 (34%)	6 (60%)	19 (63%)
Negative	21 (66%)	4 (40%)	11 (37%)
log_{10} IU/mL§	0.32 (0.77)	1.06 (0.85)	2.73 (0.60)
HBcrAg			
≥LLOQ	25 (78%)	9 (90%)	27 (90%)
log_{10} U/mL‡	4.68 (1.04)	5.90 (1.67)	7.29 (1.88)
HBV genotype			
A	1 (3%)	0	1 (3%)
B	8 (25%)	0	13 (43%)
C	6 (19%)	5 (50%)	11 (37%)
D	0	5 (50%)	2 (7%)
Unknown	17 (53%)	0	3 (10%)
ALT			
U/L	20.66 (7.30)	59.40 (36.57)	94.10 (42.96)
Normal	32 (100%)	1 (10%)	2 (7%)
>1–2xULN	0	7 (70%)	11 (37%)
>2–5xULN	0	2 (20%)	17 (57%)

Data are presented as mean (standard deviation) or number (%).
ALT, alanine aminotransferase; HBV, hepatitis B virus; HBcrAg, hepatitis core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LLOQ, lower limit of quantification; NUC, nucleos(t)ide analogue; ULN, upper limit of normal.
*Only one patient received Lamivudine from Aug to Sep in 2011 (the exact start and end dates were unknown) before screening in 2020. †Calculated from patients who were ≥LLOQ (LLOQ=10 copies/mL for HBV RNA, 1,000 U/mL for HBcrAg). ‡Cutoff index value ≥1. §Calculated from patients who were HBeAg-positive.

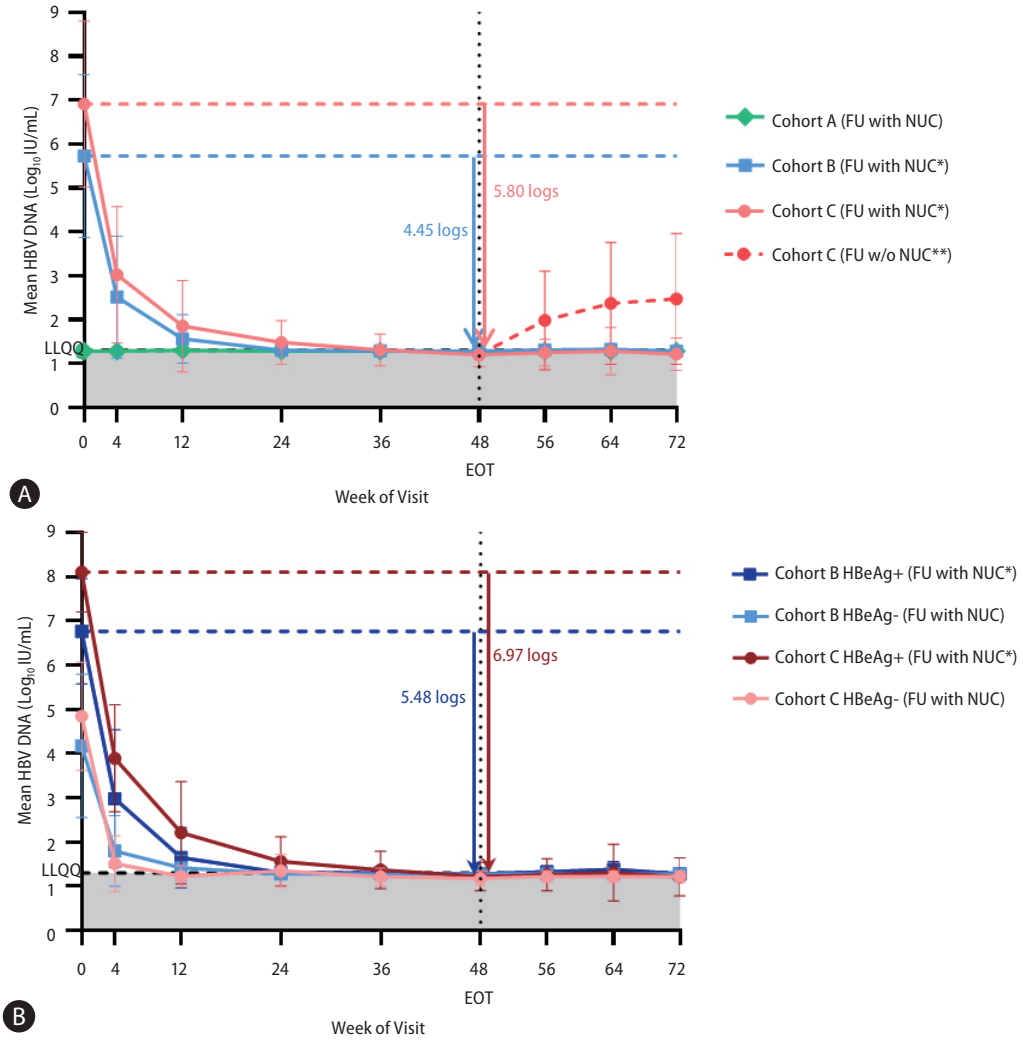

Figure 3. Mean HBV DNA levels over 72 weeks. (A) Three cohorts overall and (B) HBeAg-positive and HBeAg-negative subgroups of treatment-naïve patients in Cohorts B and C. *Excluded one non-compliant patient during the FU period. **One patient was retreated with NUC from Week 60. Error bars represent standard deviation. HBV, hepatitis B virus; EOT, end of treatment; FU, follow-up; NUC, nucleos(t)ide analogue; Peg-IFN, pegylated interferon.

gators' discretion and the remaining patient restarted NUC treatment from Week 60 with HBV DNA subsequently declining to below the LLOQ.

HBV RNA responses

Among the patients with quantifiable baseline HBV RNA, HBV RNA levels were suppressed to below the LLOQ at Week 48 in 93% (14/15), 100% (8/8) and 88% (22/25) of patients in Cohorts A, B and C, respectively. The mean (SD) 48-week declines in HBV RNA for patients with quantifiable baseline HBV RNA were 1.82 (1.05) $\log_{10}$ copies/mL in Cohort A, 3.45 (1.41) $\log_{10}$ copies/mL in Cohort B, and 4.20 (1.78) $\log_{10}$ copies/mL in Cohort C (Fig. 4). During the follow-up without linvencorvir, HBV RNA levels rebounded to approximately the baseline levels in Cohort A patients, but mean reductions from baseline of 2.16 (1.66) and 3.27 (1.71) $\log_{10}$ copies/mL were retained at Week 72 in Cohorts B and C patients, respectively. Patients in all three cohorts with unquantifiable baseline HBV RNA maintained HBV RNA levels below the LLOQ during the study treatment and NUC-alone follow-up periods.

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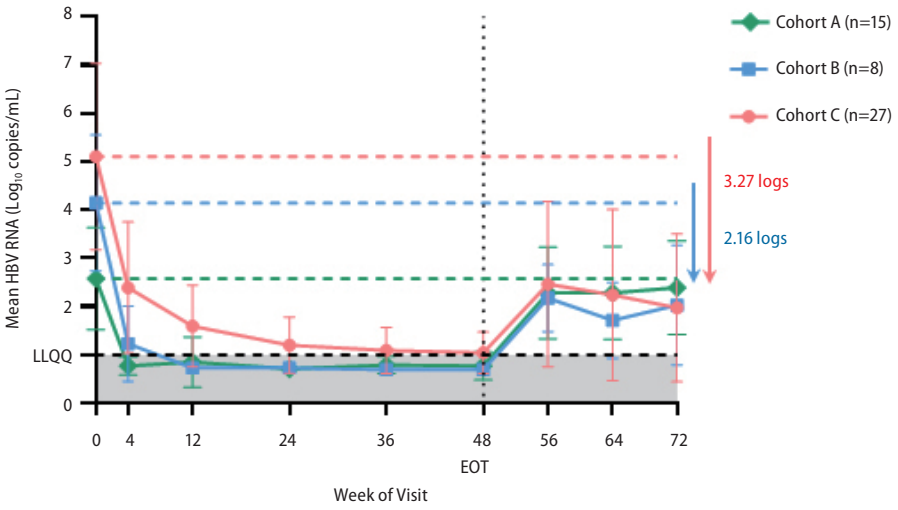

Figure 4. Mean HBV RNA levels over 72 weeks. Error bars represent standard deviation. HBV, hepatitis B virus; EOT, end of treatment; LLOQ, lower limit of quantification; NUC, nucleos(t)ide analogue; Peg-IFN, pegylated interferon.

HBsAg responses

No HBsAg loss or anti-HBs seroconversion occurred among patients completing the study. No apparent mean declines for Cohort A and B in HBsAg were observed during the study (Fig. 5A), but two HBeAg-positive patients in Cohort B had maximal HBsAg declines of 0.40–0.45 $\log_{10}$ IU/mL. In Cohort C, at Week 48, mean (SD) HBsAg decline was 1.39 (0.98) $\log_{10}$ IU/mL and numerically larger mean (SD) HBsAg declines occurred in HBeAg-positive and patients with baseline HBsAg ≥4 $\log_{10}$ IU/mL (1.64 [0.90] $\log_{10}$ IU/mL and 1.72 [0.88] $\log_{10}$ IU/mL, respectively). HBV genotype B and C patients achieved mean (SD) HBsAg declines of 1.35 (0.62) and 1.74 (1.13) $\log_{10}$ IU/mL from baseline levels of 3.80 (0.76) and 4.41 (0.91) $\log_{10}$ IU/mL, respectively (Table 2, Fig. 5A). At Week 48, 21% (6/28) and 68% (19/28) of patients achieved HBsAg levels <2 and 3 $\log_{10}$ IU/mL, respectively (Table 2). HBsAg declines were concurrent with treatment-emergent grade 2–4 ALT elevations which mostly occurred in treatment-naïve patients, with statistically significant positive correlations between graded ALT elevations and categorical maximal HBsAg declines (Spearman's rho 0.432, P=0.017 for Cohort C and 0.697, P=0.025 for Cohort B) (Supplementary Fig. 1).

HBeAg and HBcrAg responses

At Week 48, NUC-suppressed HBeAg-positive Cohort A patients had mean (SD) HBeAg decline of 0.23 (0.23) $\log_{10}$ IU/mL from 0.41 (0.75) $\log_{10}$ IU/mL at baseline. Treatment-naïve, HBeAg-positive Cohorts B and C patients had mean (SD) HBeAg declines of 1.48 (0.84) and 2.10 (0.90) $\log_{10}$ IU/mL, respectively (Fig. 5B). Among the HBeAg-positive treatment-naïve patients, 50% (3/6) and 39% (7/18) achieved HBeAg loss and anti-HBe seroconversion occurred in 17% (1/6) and 33% (6/18) in Cohorts B and C, respectively. At Week 48, HBcrAg levels declined from baseline by mean (SD) of 0.13 (0.24), 1.23 (0.76), and 1.76 (1.1) $\log_{10}$ U/mL in Cohorts A, B, and C, respectively (Fig. 5C). During the follow-up period, levels of HBeAg and HBcrAg were generally sustained in treatment-naïve patients.

Adverse events

AEs occurred in 69% (22/32) of NUC-suppressed patients in Cohort A, 90% (9/10) of treatment-naïve patients in Cohort B, and all 30 treatment-naïve patients in Cohort C (Table 3). Headache, pyrexia, and increased ALT levels were among the most commonly reported AEs. Increased ALT levels occurred mainly at Weeks 2–8, and resolved within 14 weeks with no accompanying bilirubin/indirect bilirubin increase, although a mild increase in bilirubin occurred in a NUC-suppressed patient who had pre-existing liver disease (cholestasis and Gilbert syndrome). Moreover, in all five patients with grade 4 ALT elevations, linvencorvir was interrupted per protocol, but no further ALT elevations occurred after re-administering. Most AEs were grades 1–2. Grade 3–4 AEs were reported in

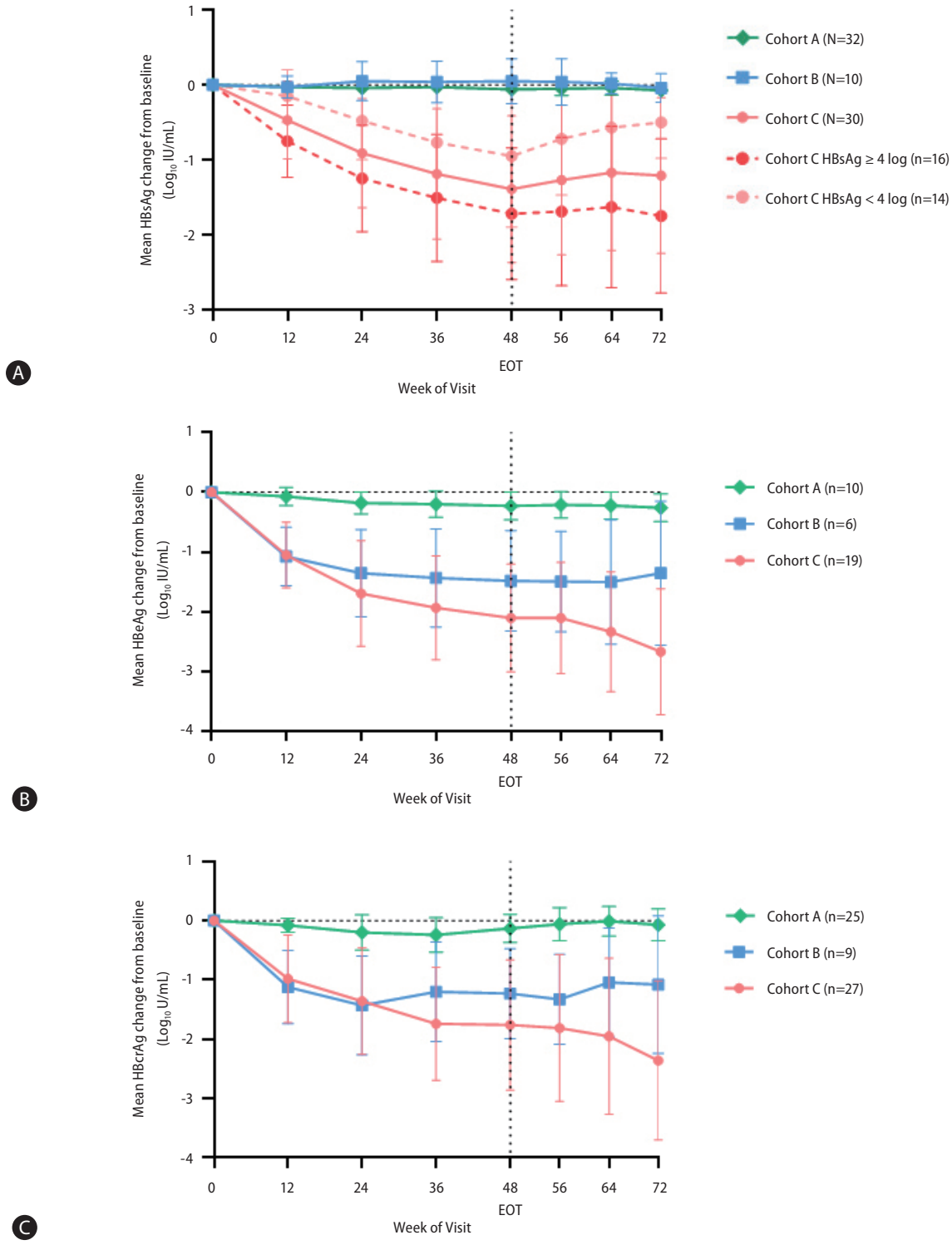

Figure 5. HBsAg (A), HBeAg (B) and HBcrAg (C) mean changes from baseline* over 72 weeks. *Patients with baseline value below the LLOQ were excluded from change from baseline analysis. HBsAg ≥4 log means baseline HBsAg level ≥4 $\log_{10}$ IU/mL; HBsAg <4 log means baseline HBsAg level <4 $\log_{10}$ IU/mL. Error bars represent standard deviation. EOT, end of treatment; HBsAg, hepatitis B surface antigen; HBcrAg, hepatitis B core-related antigen; LLOQ, lower limit of quantification; NUC, nucleos(t)ide analogue.

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Table 2. HBsAg levels in treatment-naïve patients in Cohort C

HBsAg	Linvencorvir+NUC+Peg-IFN-α (Cohort C)		
	Overall	HBeAg+	HBeAg-
Baseline	n=30	n=19	n=11
$\log_{10}$ IU/mL	3.96 (0.90)	4.40 (0.71)	3.19 (0.63)
<3 $\log_{10}$ IU/mL	6 (20%)	1 (5%)	5 (45%)
<2 $\log_{10}$ IU/mL	0	0	0
At Week 48	n=28	n=18	n=10
CFB, $\log_{10}$ IU/mL	–1.39 (0.98)	–1.64 (0.90)	–0.94 (0.99)
Genotype B*	–1.35 (0.62)	–1.30 (0.64)	–1.50 (0.62)
Genotype C†	–1.74 (1.13)	–2.08 (1.05)	–0.84 (0.91)
≥0.5 $\log_{10}$ IU/mL CFB	21 (75.0%)	16 (88.9%)	5 (50.0%)
>1.0 $\log_{10}$ IU/mL CFB	20 (71.4%)	15 (83.3%)	5 (50.0%)
>2.0 $\log_{10}$ IU/mL CFB	7 (25.0%)	5 (27.8%)	2 (20.0%)
<3 $\log_{10}$ IU/mL	19 (68%)	12 (61%)	8 (80%)
<2 $\log_{10}$ IU/mL	6 (21%)	2 (11%)	4 (40%)

Data are presented as mean (standard deviation) or number (%).
CFB, change from baseline; HBsAg, hepatitis B surface antigen; NUC, nucleos(t)ide analogue; Peg-IFN, pegylated interferon.
*Overall, n=12; HBeAg+, n=9; HBeAg-, n=3. †Overall, n=11; HBeAg+, n=8; HBeAg-, n=3.

four Cohort A patients (13%), two Cohort B patients (20%), and 11 Cohort C patients (37%). There were eight serious AEs and one death (due to malignant melanoma), none of which were related to linvencorvir. Most treatment-related AEs occurred in Cohort C: 74 related to linvencorvir, 25 related to NUC and 266 related to Peg-IFN-α. Four AEs were assessed as being related to linvencorvir in each of Cohorts A and B (Table 3). There were no trends of clinically significant changes in vital signs or ECG data.

Pharmacokinetics

Linvencorvir was rapidly absorbed and eliminated, with low accumulation of linvencorvir and its major metabolites in plasma after 48 weeks of dosing. The PK profiles of linvencorvir, with or without SoC (NUC with or without Peg-IFN-α), were considered similar. The plasma concentration of NUCs and Peg-IFNs remained stable during the study treatment period.

DISCUSSION

In this study, no patient achieved functional cure at 24 weeks post-48-week treatment with linvencorvir 600 mg/day plus NUC with or without Peg-IFN-α. Linvencorvir plus NUC with or without Peg-IFN-α did demonstrate potent suppression of HBV DNA and RNA. Linvencorvir plus NUC and PEG-IFN-α in treatment-naïve patients led to the greatest overall declines in HBV antigens. Linvencorvir was generally safe and well tolerated in combination with SoC.

HBV DNA was maintained below the LLOQ throughout the study in NUC-suppressed patients and was suppressed below the LLOQ in the majority of treatment-naïve patients, including HBeAg-positive patients with high viral load. Moreover, after linvencorvir cessation, HBV DNA generally remained suppressed by NUC monotherapy. While complete suppression of HBV DNA is an essential part of functional cure, 30% to 50% HBeAg positive and/or patients with high viral load cannot achieve HBV DNA<LLOQ with 1–3 years NUC monotherapy. Furthermore, some CHB patients may develop low-level viremia even with long-term NUC treatment.[7-9] For these NUC difficult-to-treat patients, addition of linvencorvir to NUC may be a potential therapeutic strategy. Larger and longer trials would be necessary to test this hypothesis.

Serum HBV RNA was suppressed to below the LLOQ in the majority of NUC-suppressed and treatment-naïve patients during study treatment, which reflected target engagement by linvencorvir. During the off-linvencorvir follow-up period, retained HBV RNA declines were only observed in treatment-

Table 3. Overview of AEs in NUC-suppressed (Cohort A) and treatment-naïve (Cohorts B and C) patients

	Linvencorvir+NUC		Linvencorvir+NUC+Peg-IFN-α
	Cohort A (n=32)	Cohort B (n=10)	Cohort C (n=30)
Patients with at least one AE, n (%)	22 (69%)	9 (90%)	30 (100)
Total number of AEs	110	48	468
Total number of treatment-related AEs	4	5	301
Linvencorvir	4	4	74
NUC	0	1	25
Peg-IFN	NA	NA	266
Most common AEs*, n (%)			
Headache	3 (9%)	2 (20%)	14 (47%)
Pyrexia	0	1 (10%)	18 (60%)
ALT increased	1 (3%)	1 (10%)	11 (37%)
Alopecia	0	1 (10%)	11 (37%)
Platelet count decreased	0	0	12 (40%)
Fatigue	1 (3%)	0	9 (30%)
AST elevation	1 (3%)	0	9 (30%)
Decreased appetite	0	0	10 (33%)
Patients with at least one, n (%)			
AE with fatal outcome	0	1 (10%)‡	0
SAE	1 (3%)†	1 (10%)‡	2 (7%)§
AE leading to withdrawal	0	0	2 (7%)¶
AE leading to Linvencorvir/Peg-IFN interruption‖ or modification	1 (3%)/NA	1 (10%)/NA	5 (17%)/12 (40%)
Related AE	3 (9%)	3 (30%)	30 (100%)
Related to Linvencorvir	3 (9%)	2 (20%)	21 (70%)
Related to NUC	0	1 (10%)	10 (33%)
Related to Peg-IFN	NA	NA	30 (100%)
Grade 3–4 AE	4 (13%)	2 (20%)	11 (37%)
Non-ALT elevation-associated Grade 3–4 AE	3 (9%)	1 (10%)	9 (30%)

AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable; NUC, nucleos(t)ide analogue; Peg-IFN, pegylated interferon; SAE, serious adverse event; ULN, upper limit of normal; URTI, upper respiratory tract infection.
*Occurring with ≥30% incidence in at least one cohort. †Gastroesophageal reflux disease onset on Day 364. ‡The patient died on Day 535 due to malignant melanoma (SAE) onset on Day 425, with unresolved cellulitis and lymphadenitis (SAE diagnosed on Day 446). §One patient had SAEs of URTI (Day 185) and cellulitis (Day 251); one patient had SAEs of hypersensitivity (Day 286) and dizziness (Day 472). ¶Peg-IFN-α. AEs: thyroid disorder; allergic dermatitis. ‖ Per protocol, patients with ALT >10×ULN should interrupt linvencorvir and Peg-IFN treatment (Cohort C only).

naïve patients, suggesting that initial HBV RNA declines in treatment-naïve patients may be more readily retained than secondary declines in NUC-suppressed patients. This partially sustained HBV RNA suppression, together with durable declines in HBcrAg and HBeAg, may indicate suppression of cccDNA transcriptional activity or a reduction in cccDNA levels,[24,25] which is rarely observed in NUC therapy alone.[26] Consistent with this hypothesis, CAMs have been shown in vitro to induce disassembly of nucleocapsids, thereby interfering with cccDNA reservoir establishment and replenishment.[26-28]

Linvencorvir showed little benefit in HBsAg reduction on top of NUC, however, when combined with Peg-IFN-α, HB-

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sAg declines were observed in treatment-naïve patients. Notably, the HBsAg mean decline observed in Cohort C was larger than it was in a previous study of TDF plus Peg-IFN combination therapy.[29] Moreover, HBsAg levels declined comparably in Cohort C patients with HBV genotypes C and B. It has been reported that HBsAg decline was significantly lower in patients with either HBV genotypes C or D than in patients with HBV genotypes A and B with one-year Peg-IFN plus NUC treatment.[30,31] However, due to the limited sample size, the baseline differences, and the lack of a control group of Peg-IFN plus NUC, any additional benefit to HBsAg reduction from linvencorvir on top of Peg-IFN and NUC needs to be confirmed.

There were no unexpected safety concerns when linvencorvir was administered in combination with NUC or NUC plus Peg-IFN-α. AEs occurring in patients receiving linvencorvir plus NUC and Peg-IFN-α were consistent with the safety profile of Peg-IFN-α. As the observations seen in the previous study with 4-week linvencorvir monotherapy,[20] transient treatment-emergent ALT elevations were observed almost exclusively in treatment-naïve patients but not in NUC-suppressed patients, and were accompanied by declining levels of viral antigens, including HBsAg. These ALT elevations are consistent with the natural history of CHB patients with active viral replication and are considered indicators of the host immune response against HBV rather than drug-induced liver injury.[32-34]

Limitations of this study include its non-randomized, non-controlled design with no stratification, as well as the small sample size. The small sample size and unbalanced baseline characteristics detract from the validity of subgroup analyses.

In conclusion, linvencorvir is generally safe and well tolerated when added to SoC therapy for CHB. Linvencorvir on top of SoC potently suppresses HBV replication, including in HBeAg-positive patients and those with high viral load, however limited benefit is shown towards HBsAg loss. Next-generation CAMs with higher potency and greater inhibitory activity towards cccDNA reservoir maintenance may result in different outcomes towards the achievement of functional cure in CHB patients.

Authors' contribution

All authors approved the final manuscript for submission. Jinlin Hou, Man-Fung Yuen contributed to the study design and conduct, data acquisition, data interpretation, and manuscript drafting and revision. Wen Zhang contributed to the data analyses, data interpretation, and manuscript drafting and revision. Qingyan Bo contributed to the study design, data analyses, data interpretation, and manuscript revision. Edward Gane and Wenhong Zhang contributed to the study design and conduct, data acquisition, data interpretation, and manuscript revision. Rozalina Balabanska, Jiming Zhang, Tien Huey Lim, Qing Xie, Chau-Ting Yeh, Sheng-Shun Yang, Xieer Liang, Piyawat Komolmit, Apinya Leerapun, Ting-Tsung Chang, Tsung-Hui Hu, Seng Gee Lim, Wan-Long Chuang and Barbara Leggett contributed to the study conduct, data acquisition, data interpretation, and manuscript revision. Zenghui Xue, Ethan Chan and Yuchen Zhang contributed to the data analyses, data interpretation, and manuscript revision. Qiaoqiao Xie, Xue Zhou and Miriam Triyatni contributed to the data interpretation, and manuscript revision. All authors reviewed and approved the final manuscript for submission.

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Conflicts of Interest

Jinlin Hou received grants from Bristol Myers Squibb and Johnson & Johnson; and declared other financial or non-financial interests with AbbVie, Arbutus, Bristol Myers Squibb, Gilead Sciences, Johnson & Johnson, and Hoffmann-La Roche.

Edward Gane is an advisory committee or review panel member for AbbVie, Arbutus, Arrowhead, Assembly Biosciences, Availa, Clear B Therapeutics, Dicerna, Finch Therapeutics, Gilead Sciences, Janssen, Novartis, Hoffmann-La Roche, and Vir Bio; and has received speaking and teaching fees from AbbVie, Aligos, DrugFarm, Enanta, Gilead, GlaxoSmith-Kline, Janssen, Merck, and Novartis.

Sheng-Shun Yang has received speaking fees from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Ipsen, and Merck Sharp & Dohme, and served as an advisory board member for AbbVie, Gilead Sciences, Hoffmann-La Roche, Sysmex, and Ipsen.

Seng Gee Lim received payment or honoraria for lectures from Gilead, Janssen, Hoffman-La Roche, Sysmex and GSK; served as advisory board member for Gliead, Abbot, Hoffmann-La Roche, GSK, Janssen, Sysmex, Abrutus, Assembly, and Grifols; served in leadership role in ICE-HBV, HBV Forum, AASLD Asia Pacific Advisory Board, and ANRS-MIE Advisory Board; and receipt of research support from Gilead, Abbot, Hoffmann-LaRoche, Sysmex, Fibronostics, and Merck.

Man-Fung Yuen serves as advisory board member/ consultant for and/or received research funding from AbbVie, Aligos Therarpeutics, AiCuris, Antios Therapeutics, Arrowhead Pharmaceuticals, Arbutus Biopharma, Assembly Biosciences, Bristol Myer Squibb, Bluejay Therapeutics, Clear B Therapeutics, Dicerna Pharmaceuticals, Finch Therapeutics, Fujirebio Incorporation, GlaxoSmithKline, Gilead Sciences, Immunocore, Janssen, Merck Sharp and Dohme, and Hoffmann-La Roche, Vir Biotechnology.

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Zenghui Xue, Ethan Chen, Yuchen Zhang, Qiaoqiao Xie and Wen Zhang are employees of Hoffmann-La Roche. Qingyan Bo and Xue Zhou were former employees of Hoffmann-La Roche.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

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