Clinical Infectious Diseases

MAJOR ARTICLE

Entecavir Plus Pegylated Interferon and Sequential Hepatitis B Virus Vaccination Increases Hepatitis B Surface Antigen Seroclearance: A Randomized Controlled Proof-of-Concept Study

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Background. Hepatitis B surface antigen (HBsAg) seroclearance is considered a functional cure for patients with chronic hepatitis B, but is rarely achievable with oral nucleos(t)ide analogues alone. We conducted a randomized controlled proof-of-concept trial to evaluate the impact of adding pegylated interferon (peg-IFN) alfa-2a plus sequential or concomitant hepatitis B virus (HBV) vaccination.

Methods. A total of 111 patients who achieved serum HBV DNA <20 IU/mL and quantitative HBsAg <3000 IU/mL with entecavir were randomly assigned (1:1:1) to the E + sVIP group (entecavir + peg-IFN alfa-2a [180 μg every week over 48 weeks] plus sequential HBV vaccination [20 μg of HBsAg on weeks 52, 56, 60, and 76]), the E + cVIP group (entecavir + peg-IFN alfa-2a + concomitant HBV vaccination [weeks 4, 8, 12, and 28]), or the control group (entecavir only). The primary endpoint was HBsAg seroclearance at week 100, and secondary endpoints included safety.

Results. No differences in baseline quantitative HBsAg were observed among the groups. The E + sVIP group in the intention-to-treat analysis showed a significantly higher chance of HBsAg seroclearance during week 100 than the control group (16.2% vs 0%; $P = .025$), but the E + cVIP group (5.4%) failed to reach a significant difference ($P = .54$). Adverse events were significantly more frequent in the E + sVIP (81.1%) and E + cVIP group (70.3%) than the control group (2.7%) (both $P < .0001$). However, the frequency of serious adverse events did not differ significantly among the 3 groups (2.7%, 5.4%, and 2.7%, respectively; $P = 1.00$).

Conclusions. Entecavir plus an additional peg-IFN alfa-2a treatment followed by sequential HBV vaccination under an intensified schedule significantly increases the chance of HBsAg seroclearance compared to entecavir alone.

Clinical Trials Registration. NCT02097004.

Keywords. hepatitis B virus; functional cure; therapeutic vaccination; nucleoside analogue.

Seroclearance of the hepatitis B virus (HBV) surface antigen (HBsAg) reflecting the transcriptional activity of covalently closed circular DNA (cccDNA) [1] is considered to be the closest event to a true cure for chronic hepatitis B (CHB) and has been termed a functional cure [2, 3]. However, potent nucleos(t)ide analogues (NAs) in our current antiviral armamentaria rarely achieve HBsAg seroclearance, although they sustain virological suppression during treatment. NAs only modestly reduce intrahepatic HBV DNA (by <2 $\log_{10}$ IU/mL during 2 years) and do not interfere with HBV cccDNA [4]. The annual rate of HBsAg seroclearance for NA-treated patients is 0.8% [5], and it is assumed that approximately 52 years of NA therapy is required for most patients to achieve HBsAg seroclearance considering HBsAg kinetics [6]. Therefore, lifelong NA treatment is generally necessary to minimize the risk of recurrent liver injury following replication of HBV.

Pegylated interferon (peg-IFN) alfa provides a higher chance for HBsAg loss at the rate of 3%–7% after 1 year of peg-IFN treatment compared to NAs [7, 8]. Recent randomized controlled trials investigated the effect of adding or switching to peg-IFN in patients with suppressed HBV in response to NA treatment rather than first-line peg-IFN monotherapy in NA-naive patients. The probabilities of HBsAg seroclearance ranged from 1.2% to 13.3% at the end of follow-up in those studies [9].

Therapeutic HBV vaccination may also have a role in HBsAg seroclearance. Preclinical studies have reported that a combined treatment with a conventional surface antigen vaccine and NA induces a more robust anti–woodchuck virus surface antigen response in the woodchuck model [10, 11]. In a human trial, recombinant HBV vaccination achieved an approximately 10% HBsAg seroconversion rate in the inactive carrier phase of CHB [12]. However, the effect of therapeutic vaccination with a

Received 13 February 2020; editorial decision 11 May 2020; accepted 14 June 2020; published online June 18, 2020.

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Clinical Infectious Diseases® 2020;XX(XX):1–10

DOI: 10.1093/cid/ciaa807

conventional HBV vaccine on HBsAg seroclearance in patients with NA-induced viral suppression has not been fully evaluated in humans.

In this randomized controlled proof-of-concept study, we assessed the efficacy and safety of a combined treatment with 3 currently available treatment options in clinical practice (ie, NA, peg-IFN alfa-2a, and conventional HBV vaccination), which have never been tested together to the seroclearance of HBsAg. Given that peg-IFN may attenuate B-cell function, which is essential to antibody formation following active vaccination [13], the efficacies of both concomitant peg-IFN plus HBV vaccination and peg-IFN treatment followed by sequential vaccination were studied.

METHODS

Patients

Patients with CHB whose serum HBV DNA was fully suppressed (<20 IU/mL) by entecavir treatment and serum quantitative HBsAg (qHBsAg) was <3000 IU/mL from a single tertiary referral center (Seoul National University Hospital, Seoul, Korea) were eligible for this study. The exclusion criteria included patients with decompensated liver function, decreased renal function, psychiatric disorder, and history of malignancy including hepatocellular carcinoma (HCC) within 5 years (Supplementary Methods).

Trial Design and Treatment

This study was a randomized controlled open-label proof-of-concept study and was conducted at a university-affiliated hospital (Seoul National University Hospital, Seoul, Korea). All eligible participants were randomly assigned in a 1:1:1 ratio to receive entecavir treatment plus peg-IFN alfa-2a therapy followed by sequential HBV vaccination on an intensified schedule (the E + sVIP group); entecavir treatment plus peg-IFN therapy with concomitant HBV vaccination on an intensified schedule (the E + cVIP group); or entecavir alone (the control group). A central web-based system using a computer-generated permuted block with a block size of 6 or 9 was used for random assignment.

Patients in the E + sVIP group received entecavir 0.5 mg per oral once daily and a subcutaneous injection of 180 μg peg-IFN alfa-2a every week for 48 weeks, followed by an intramuscular injection of recombinant HBV vaccine containing 20 μg HBsAg 4 times (at weeks 52, 56, 60, and 76). Patients in the E + cVIP group received entecavir 0.5 mg per oral once daily, a subcutaneous injection of 180 μg every week for 48 weeks, and a concomitant intramuscular injection of recombinant HBV vaccine containing 20 μg HBsAg 4 times (at weeks 4, 8, 12, and 28) during the peg-IFN treatment period. Patients in the control group received entecavir 0.5 mg per oral once daily (Figure 1). The study drugs (ie, entecavir, peg-IFN alfa-2a, and recombinant HBV vaccine) were kindly provided by Bristol-Myers Squibb (Princeton, New Jersey), Roche (Nutley, New Jersey), and LG Life Science (Seoul, Korea), respectively (Supplementary Methods).

All participants provided written informed consent before enrollment. This study protocol was approved by the Institutional Review Board of Seoul National University Hospital (Seoul, Korea). All methods and procedures associated with this study were conducted in accordance with Good Clinical Practice guidelines and accorded ethically with the principles of the Declaration of Helsinki and local laws.

Endpoints and Assessments

The primary endpoint was HBsAg seroclearance at week 100 (Supplementary Methods).

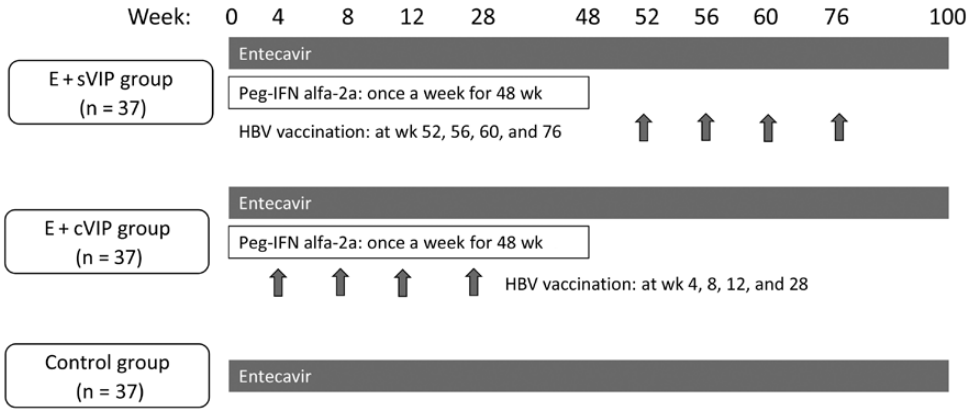

Figure 1. Study design. Patients in the E + sVIP group received entecavir, 0.5 mg per oral once daily and a subcutaneous injection of 180 μg pegylated interferon (peg-IFN) alfa-2a every week for 48 weeks, followed by an intramuscular injection of recombinant hepatitis B virus (HBV) vaccine containing 20 μg hepatitis B surface antigen (HBsAg) 4 times (at weeks 52, 56, 60, and 76). Patients in the E + cVIP group received entecavir 0.5 mg per oral once daily, a subcutaneous injection of 180 μg every week for 48 weeks, and a concomitant intramuscular injection of recombinant HBV vaccine containing 20 μg HBsAg 4 times (at weeks 4, 8, 12, and 28) during the peg-IFN treatment period. Patients in the control group received entecavir 0.5 mg per oral once daily.

The secondary endpoints included changes in the qHBsAg level from baseline to week 100; serum levels of qHBsAg, and anti-hepatitis B surface antibody (Supplementary Methods) at each visit; and safety. Adverse events (AEs), which were classified and graded according to the World Health Organization Adverse Reaction Terminology [14], were assessed from the time the patient provided written informed consent until the end of the study or dropout. Multiple occurrences of specific events were counted once per patient, and the event with the greatest severity was summarized.

Sample Size and Statistical Analysis

Sample size for the study was determined to capture the difference in the primary endpoint of HBsAg seroclearance at week 100 between the E + sVIP or E + cVIP and control groups. We expected that the primary endpoint would occur in 20% of the E + sVIP or E + cVIP group as (1) HBsAg seroclearance rate by therapeutic vaccination in the inactive carrier phase of CHB is approximately 10% [12]; (2) peg-IFN treatment reportedly induces HBsAg seroclearance in approximately 10% of NA-treated patients [12]; (3) and there might be an additive effect between HBV vaccination and peg-IFN treatment. In contrast, we expected that the HBsAg seroclearance rate would be 1% in the control group [5]. Assuming a 2-sided type I error of 0.05 and power of 80%, the potential loss to follow-up rate of 15%, and a randomization ratio for 1:1:1 among the E + sVIP group, the E + cVIP group, and the control group, 37 patients per each group were required to demonstrate a difference in the primary endpoint between the E + sVIP and control groups and between the E + cVIP and control groups. Therefore, a total of 111 patients were needed for this trial.

The efficacy outcomes were assessed according to the intention-to-treat principle. The proportion of HBsAg seroclearance was compared between groups using Fisher exact test. AEs were compared between the 2 study groups using the χ^2 test or Fisher exact test. A P value < .025 was considered significant with Bonferroni correction in comparing endpoints between the control group and either the E + sVIP or E + cVIP group. Otherwise, P < .05 was considered significant (Supplementary Methods). The statistical analysis was performed by independent statisticians using SAS software version 9.4 (SAS Institute, Cary, North Carolina).

RESULTS

Patients

A total of 127 participants were screened between 2 April 2014 and 1 April 2016; 111 eligible participants were randomly assigned to the E + sVIP group (n = 37), the E + cVIP group (n = 37), or the control group (n = 37). All randomized patients were included in both the efficacy and safety populations. During the study period, 4 patients in the E + sVIP group were withdrawn due to severe AEs (n = 2) or withdrew their consent (n = 4). Two patients in the E + cVIP group dropped out because of withdrawal of consent (Figure 2).

No differences were observed in the baseline characteristics between the E + sVIP and control groups and between the E + cVIP and control groups (Table 1). The median qHBsAg levels showed no intergroup difference (all P > .3). The proportion of patients with qHBsAg <250 IU/mL was also comparable among the 3 groups.

Efficacy

HBsAg Seroclearance at Week 100

Among the efficacy population, 8 patients achieved HBsAg seroclearance during week 100, a primary endpoint: 6 (16.2%) in the E + sVIP group, 2 (5.4%) in the E + cVIP group, and none in the control group. The chance of HBsAg seroclearance at week 100 was significantly higher in the E + sVIP group than in the control group (relative risk, 2.19 [95% confidence interval, 1.69–2.84]; P = .025). However, the E + cVIP group failed to increase the chance of HBsAg seroclearance compared to the control group (P = .54). Baseline qHBsAg titer was not associated with the probability of HBsAg seroclearance (per 1 $\log_{10}$ IU/mL: odds ratio, 1.00 [95% confidence interval, .99–1.02]; P = .45).

Serum Levels of qHBsAg at Each Visit

qHBsAg levels were comparable between the E + sVIP group and control group at baseline and week 4. However, the qHBsAg levels were significantly lower in the E + sVIP and E + cVIP groups than in the control group from week 12 to week 100 (Table 2; all P < .01).

Figure 3A demonstrates the relative change (percentage, vs baseline) in qHBsAg level at each visit. Interestingly, the qHBsAg levels for 7 of the 8 patients who achieved HBsAg seroclearance at week 100 (ie, 100% decrease at week 100) showed a decrease of >70% at week 24. In contrast, in the peg-IFN–treated groups (E + sVIP and E + cVIP), most patients who did not achieve the early reduction (>70% decrease at week 24 vs baseline) in qHBsAg (43 of 44 patients [97.7%]) failed to achieve HBsAg seroclearance at week 100 (Figure 3B). In the E + sVIP group, 13 (35.1%) experienced a decrease in qHBsAg that exceeded 70% at week 24. Thus, the relative qHBsAg decrease (%) at week 24 compared to the baseline value sensitively predicted HBsAg seroclearance at week 100 with a cutoff value of 70% (P = .014; sensitivity = 83.3% and negative predictive value = 95.8%). Seventeen patients (45.9%) in the E + cVIP group achieved a qHBsAg decrease >70% at week 24 compared to baseline and 2 achieved HBsAg seroclearance at week 100. In this group, the qHBsAg decrease of >70% was not associated with HBsAg seroclearance at week 100 (P = .20).

At week 48 (at the end of peg-IFN treatment in the E + sVIP and E + cVIP groups), 9 patients (24.3%) in the E + sVIP group,

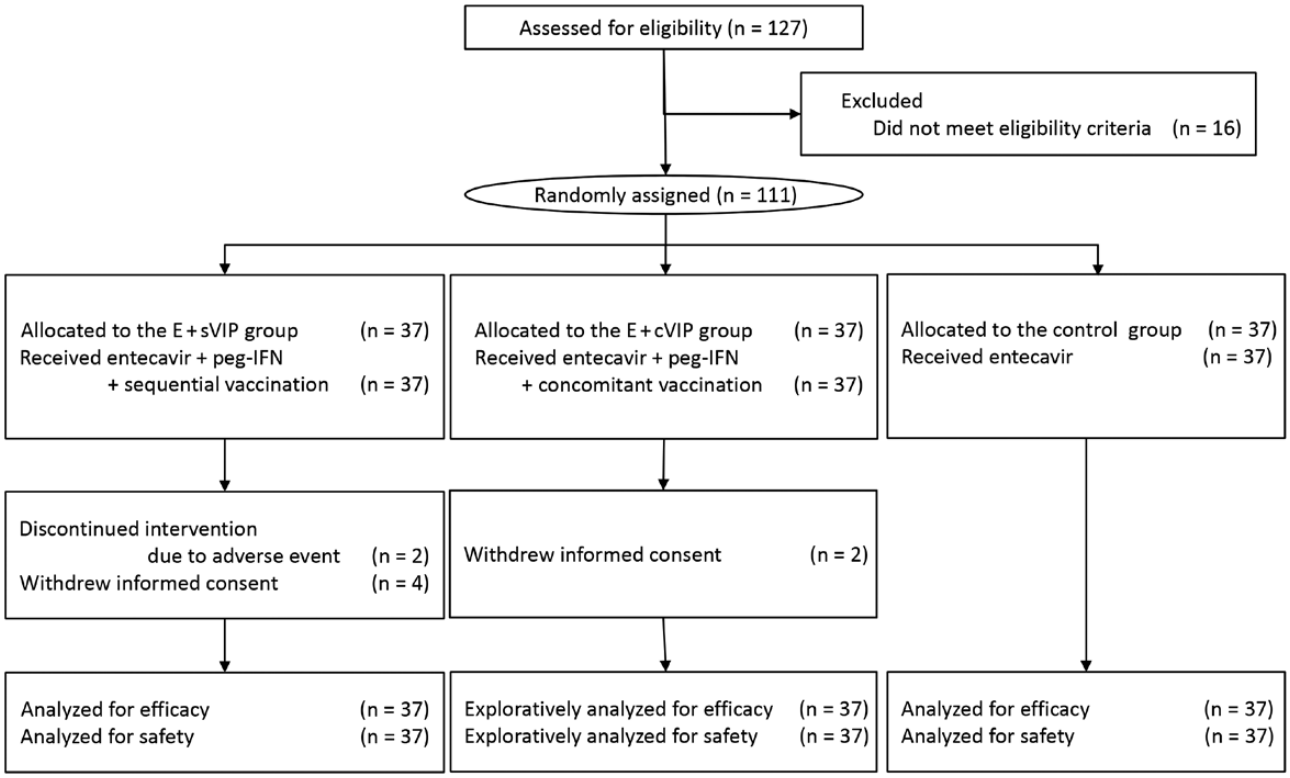

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) diagram. Of the enrolled patients, 16 did not meet eligibility criteria and were excluded from random assignment. Abbreviations: E + cVIP, entecavir plus pegylated interferon alfa-2a with concomitant hepatitis B virus vaccination; E + sVIP, entecavir plus pegylated interferon alfa-2a followed by sequential hepatitis B virus vaccination; peg-IFN, pegylated interferon.

6 (16.2%) in the E + cVIP group, and 1 (2.7%) in the control group achieved HBsAg seroclearance; there was no statistical difference between the E + sVIP and E + cVIP groups (P = .41). Of these patients, 3 in the E + sVIP group, 4 in the E + cVIP group, and 1 in the control group failed to maintain negative HBsAg up to week 100 (Figure 3A). In patients who achieved HBsAg seroclearance at week 48, the mean increase in qHBsAg after peg-IFN treatment (week 48 to week 100) was

Table 1. Baseline Characteristics

Variables	E + sVIP Group (n = 37)	E + cVIP Group (n = 37)	Control Group (n = 37)	*P* Value (E + sVIP vs Control)	*P* Value (E + sVIP vs Control)
Age, y	53.2 ± 9.1	51.9 ± 10.0	51.2 ± 9.8	.35[a]	.76[a]
Male sex, No. (%)	31 (83.8)	33 (89.2)	29 (78.4)	.55[b]	.21[b]
qHBsAg, IU/mL, median (IQR)	542.6 (2.2–2666)	688.8 (111–2937)	854.4 (10.7–2954)	.39[c]	.72[a]
qHBsAg <250 IU/mL, No. (%)	12 (32.3)	7 (18.9)	7 (18.9)	.86[b]	1.00[c]
Anti-HBs, IU/mL, median (IQR)	0.2 (0.0–6.1)	0.2 (0.0–53.6)	0.0 (0.0–1.0)	.16[c]	.25[a]
HBeAg-positive, No. (%)	3 (8.1)	2 (5.4)	0 (0.0)	1.00[b]	1.00[c]
Platelets, $\times 10^3/\mu L$	170.1 ± 53.3	173.3 ± 56.3	165.3 ± 52.2	.69[a]	.53[a]
PT, INR	1.02 ± 0.06	1.03 ± 0.06	1.02 ± 0.06	.78[a]	.46[a]
Albumin, g/dL	4.5 ± 0.2	4.4 ± 0.2	4.4 ± 0.3	.37[a]	.85[a]
ALP, IU/L	59.0 ± 14.2	67.5 ± 21.3	62.5 ± 16.2	.27[a]	.38[a]
AST, IU/L	24.4 ± 8.1	24.6 ± 8.6	23.8 ± 6.2	.85[a]	.77[a]
ALT, IU/L	27.1 ± 14.3	26.8 ± 14.8	27.1 ± 10.9	.60[a]	.51[a]

Data are expressed as mean ± standard deviation, median (RUQ), or No. (%).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBs, anti-hepatitis B surface antibody; AST, aspartate aminotransferase; E + cVIP, entecavir plus pegylated interferon alfa-2a with concomitant hepatitis B virus vaccination; E + sVIP, entecavir plus pegylated interferon alfa-2a followed by sequential hepatitis B virus vaccination; HBeAg, hepatitis B envelope antigen; INR, international normalized ratio; IQR, interquartile range; PT, prothrombin time; qHBsAg, quantitative hepatitis B virus surface antigen.

[a]By independent *t* test.

[b]By χ^2 test.

[c]By Wilcoxon rank-sum test.

Table 2. Changes of Quantitative Hepatitis B Surface Antigen Titer at Each Time Point

Time Point	E + sVIP (n = 37)	E + cVIP (n = 37)	Control (n = 37)	*P* Value[a] (E + sVIP vs Control)	*P* Value[a] (E + cVIP vs Control)
Baseline	542.6 (2.2–2662.0)	688.8 (111.1–2937.0)	854.4 (10.7–2954.0)	.15	.72
Week 4	462.0 (2.2–2576.0)	608.30 (86.9–2889.0)	788.7 (10.4–3074.0)	.13	.65
Week 12	343.0 (0–2576.0)	450.0 (0.4–3314.0)	782.1 (11.0–2561.0)	.01	.11
Week 24	162.1 (0–2932.0)	404.5 (0–3567.0)	782.3 (6.6–3283.0)	.0005	.02
Week 36	107.9 (0–2727.0)	362.4 (0–3000.0)	674.4 (7.1–2398.0)	.0001	.01
Week 48	93.2 (0–2057.0)	258.3 (0–2779.0)	697.2 (0–2594.0)	.0001	.005
Week 60	130.0 (0–1536.0)	310.7 (0–2695.0)	578.9 (1.0–2661.0)	.0001	.02
Week 72	130.0 (0–2007.0)	319.2 (0–2622.0)	605.4 (4.8–2205.0)	.0002	.03
Week 100	146.0 (0–2053.0)	306.7 (0–2207.0)	544.3 (2.2–2469.0)	.002	.06
P value[b] (week 100 vs baseline)	<.0001	<.0001	<.0001		

Data are expressed as median (range).

Abbreviations: E + cVIP, entecavir plus pegylated interferon alfa-2a with concomitant hepatitis B virus vaccination; E + sVIP, entecavir plus pegylated interferon alfa-2a followed by sequential hepatitis B virus vaccination.

[a]By Wilcoxon rank-sum test.

[b]By Wilcoxon signed-rank test.

significantly smaller in the E + sVIP group compared with the E + cVIP group (0.8 IU/mL vs 41.5 IU/mL; $P = .017$).

Changes in qHBsAg Level From Baseline to Week 100

The qHBsAg level was significantly lower at week 100 than at baseline in all 3 groups (all $P < .0001$; Table 2). The median qHBsAg level in the E + sVIP, E + cVIP, and control groups decreased by 396.6 IU/mL, 392.1 IU/mL, and 310.1 IU/mL, respectively. No significant difference was observed in the absolute magnitude of the decrease in qHBsAg between the E + sVIP and control groups ($P = .53$), although the relative decrease (%) in median qHBsAg was larger in the E + sVIP group than in the control group (−73.1% vs −36.3%).

Serum Anti-HBs Levels at Each Visit

Anti-HBs levels were comparable between the E + sVIP and control groups and between the E + cVIP and control groups from baseline until week 24 (all $P > .05$; Supplementary Table 1). Anti-HBs levels were significantly higher in the E + sVIP group than in the control group at weeks 36, 48, and 60 (all $P < .05$). However, statistical significance was lost by weeks 72 and 100.

Safety

Overall, AEs were observed in significantly more patients in the E + sVIP (81.1%) and E + cVIP (70.3%) groups than the control group (2.7%; both $P < .001$; Table 3). The dose of peg-IFN was reduced in 12 patients with cytopenia as per the study protocol (Supplementary Results). However, the risk of serious AEs was comparable between the E + sVIP and control groups (2.7% vs 2.7%; $P = 1.00$): 1 patient developed HCC in the E + sVIP group and 1 patient developed breast cancer in the control group, which were not related to the medication. A total of 51 AEs occurred in the E + sVIP group and 1 occurred in the control group. Most AEs observed in the E + sVIP group were mild (92.0%) and were related to the treatment medication (66.0% were definitely related to the treatment medication, 12.0% were probably related, and 2.0% were possibly related). The most common AE was injection site pain. Twenty-six patients (70.3%) in the E + cVIP group experienced at least 1 AE and 2 patients (5.4%) experienced a serious AE, which were comparable to the control group ($P = 1.00$): One patient developed HCC and another patient developed a gallbladder polyp, neither of which was related to the medication. Two patients who developed HCC had a previous history of HCC >5 years before study enrollment.

DISCUSSION

In this proof-of-concept trial, patients who received entecavir plus an additional peg-IFN alfa-2a treatment followed by sequential HBV vaccination on an intensified schedule (the E + sVIP group) after achieving complete viral suppression showed significantly higher probability of HBsAg seroclearance than patients who were administered entecavir alone. The probability of HBsAg seroclearance was as high as 16.2% in patients who received an additional peg-IFN treatment and sequential vaccination, in contrast to 0% of patients who continued entecavir treatment alone. Although overall AEs were significantly more frequent in the E + sVIP and E + cVIP groups than the control group, the risk of serious AEs was comparable among the 3 groups.

In the E + sVIP group, a >70% qHBsAg reduction from baseline to week 24 during the 48-week peg-IFN treatment could sensitively predict HBsAg seroclearance at week 100 with a high negative predictive value. Thus, failure to achieve early reduction of qHBsAg (ie, relative decrease of qHBsAg by >70% on week 24) might have a stopping role for additional peg-IFN on NA treatment to avoid unsuccessful peg-IFN treatment when we adopt peg-IFN plus sequential HBV vaccination. If we applied this stopping rule to the E + sVIP group, approximately two-thirds (64.9%) of the E + sVIP group might

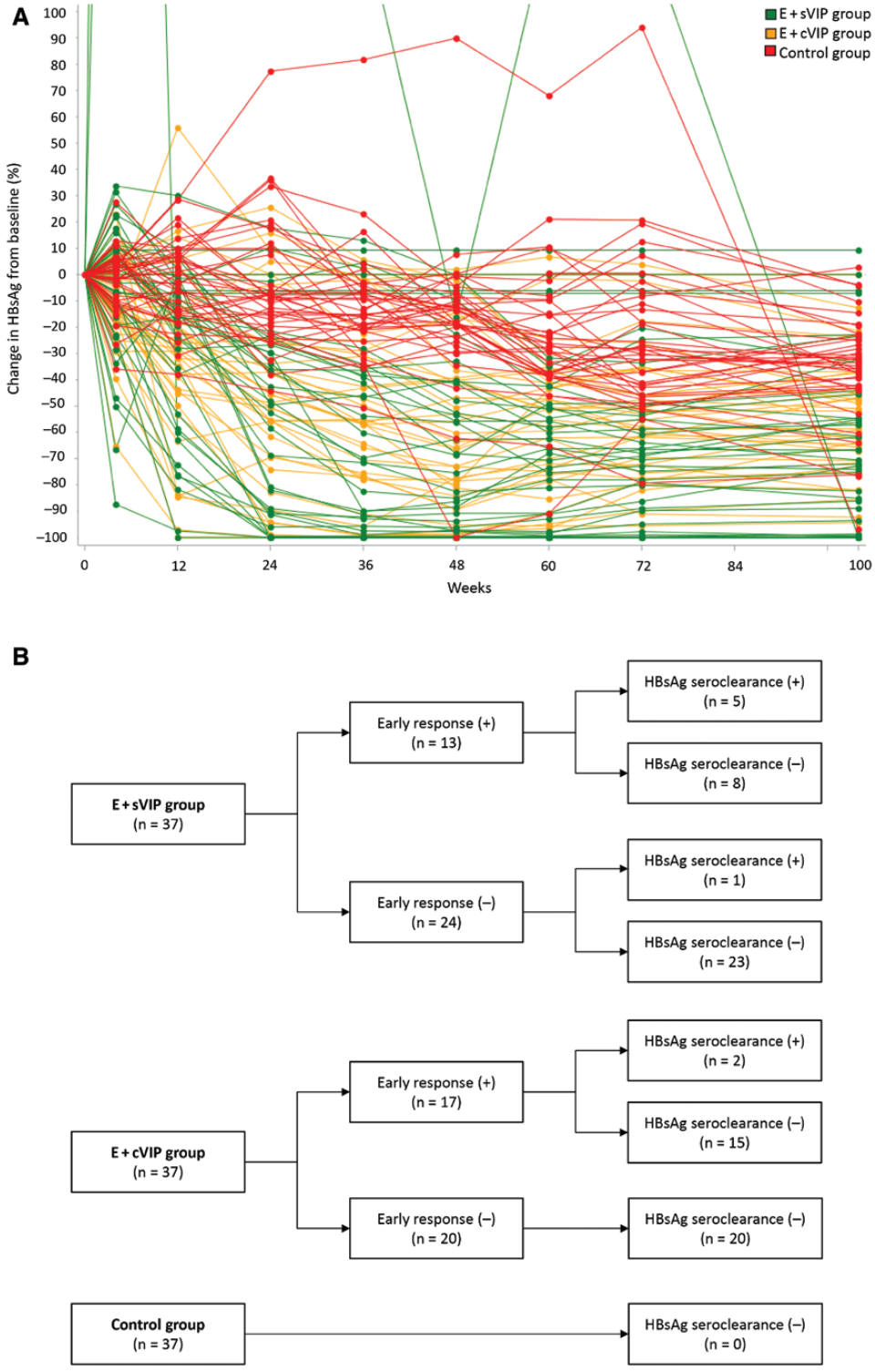

Figure 3. Treatment results of respective groups. *A*, Relative changes (%, vs baseline) in quantitative hepatitis B surface antigen (qHBsAg) level at each visit of respective patients of the entecavir plus pegylated interferon alfa-2a followed by sequential hepatitis B virus vaccination group (E + sVIP; green lines); entecavir plus pegylated interferon alfa-2a with concomitant hepatitis B virus vaccination group (E + cVIP; orange lines); and control group (red lines). *B*, HBsAg seroclearance results according to treatment group and the presence of early response (>70% reduction in HBsAg from baseline by week 24).

have stopped peg-IFN treatment during week 24, which may enhance the cost-effectiveness and decrease AEs of this treatment strategy.

Compared with recent trials using peg-IFN to achieve HBsAg seroclearance, the unique design of this study is the additional utilization of an intensified HBV vaccination schedule,

Table 3. Adverse Events (Safety Population)

Adverse Event	E + sVIP (n = 37)	E + cVIP (n = 37)	Control (n = 37)	*P* Value[a] (E + sVIP vs Control)	*P* Value[a] (E + cVIP vs Control)
Overall incidence (persons)	30 (81.1)	26 (70.3)	1 (2.7)	<.0001	<.0001
Neutropenia	3 (8.1)	3 (8.1)	0		
Pancytopenia	3 (8.1)	1 (2.7)	0		
Thrombocytopenia	1 (2.7)	1 (2.7)	0		
Injection site pain	15 (37.5)	17 (45.9)	NA		
Upper respiratory tract infection	1 (2.7)	0	0		
Weight decrease	2 (5.4)	0	0		
Hypophagia	2 (5.4)	0	0		
Headache	2 (5.4)	1 (2.7)	0		
Migraine	1 (2.7)	0	0		
Altered mood	1 (2.7)	0	0		
Alopecia	2 (5.4)	1 (2.7)	0		
Pruritus	3 (8.1)	4 (10.8)	0		
Contact urticaria	1 (2.7)	0	0		
Hepatocellular carcinoma	1 (2.7)	1 (2.7)	0		
Breast cancer	0	0	1 (2.7)		
Serious AEs	1 (2.7)	2 (5.4)	1 (2.7)	1.00	1.00
Overall AEs (No. of events)	50	51	1		
Severity				NA	NA
Mild	46 (92.0)	48 (94.1)	0		
Moderate	3 (6.0)	2 (3.9)	0		
Severe	1 (2.0)	1 (2.0)	1 (100.0)		
Causality				NA	NA
Definitely related	33 (66.0)	27 (52.9)	0		
Probably related	6 (12.0)	7 (13.7)	0		
Possibly related	1 (2.0)	4 (7.8)	0		
Unlikely	0	4 (7.8)	0		
Not related	10 (20.0)	19 (17.7)	1 (100.0)		

Data are presented as No. (%) unless otherwise indicated. Listed are AEs, classified and graded according to the World Health Organization Adverse Reaction Terminology [14], that were considered drug-related (definitely related, probably related, or possibly related), that were serious AEs regardless of relationship to drug, or that occurred in at least 3 patients in either study group regardless of relationship to drug.

Abbreviations: AE, adverse event; E + cVIP, entecavir plus pegylated interferon alfa-2a with concomitant hepatitis B virus vaccination; E + sVIP, entecavir plus pegylated interferon alfa-2a followed by sequential hepatitis B virus vaccination; NA, not applicable.

[a]By Fisher exact test.

based on results from preclinical and clinical studies [10–12, 15]. We evaluated whether the timing of therapeutic vaccination (during vs after peg-IFN treatment) would achieve a different HBsAg seroclearance result. Interestingly, at week 48 (the end of peg-IFN treatment), the probability of HBsAg negativity was not higher in the E + cVIP group compared with the E + sVIP group despite additional concomitant vaccination during peg-IFN treatment. The mean increase in qHBsAg after peg-IFN treatment (week 48 to week 100) was significantly smaller in the E + sVIP group than in the E + cVIP group, possibly due to sequential vaccination during this period. The E + cVIP group failed to increase the probability of HBsAg seroclearance compared with the control group, whereas the E + sVIP group showed a significantly greater probability of HBsAg seroclearance. These results can be explained by the observation that abundant HBsAg induces immune exhaustion and absorbs neutralizing antibodies [16–20]. Furthermore, concurrent peg-IFN induces B-cell dysfunction, which disturbs the effect of therapeutic vaccinations [13]. Therapeutic vaccination may therefore be effective in patients with sustained NA-induced viral suppression when the following 2 conditions are met: (1) sufficient early response to peg-IFN (>70% reduction in qHBsAg by week 24) and (2) sequential, but not concomitant, administration of HBV vaccination that may reduce qHBsAg more profoundly after peg-IFN treatment.

The E + sVIP group in our study demonstrated a relatively high probability of HBsAg seroclearance (16.2%) at the end of follow-up (week 100: 52 weeks after completing peg-IFN) compared with other groups treated with peg-IFN with/without NA in previous studies [21–26]. Among previous studies, a Chinese study showed the highest HBsAg-negative rate (15.3%) at the end of follow-up (week 144: 48 weeks after completion of peg-IFN) with switching to 96 weeks of peg-IFN treatment from NA treatment [23]. Considering that the longer duration of peg-IFN treatment is associated with more severe AEs and higher cost, our strategy of 48 weeks of peg-IFN alfa-2a followed by HBV vaccination might be more affordable and safer to achieve HBsAg seroclearance. Previous studies using 48

weeks of peg-IFN treatment with/without continuing NA treatment achieved only 0–9.7% of HBsAg seroclearance at the end of follow-up [21, 22, 25]. Moreover, as 99% of Korean patients with CHB are infected with genotype C HBV [27, 28] and genotype C results in a significantly worse treatment response to IFN [29], therapeutic vaccination may have additional effect on the peg-IFN–assisted HBsAg seroclearance.

The current study utilized 3 currently available treatment options (ie, NA, peg-IFN alfa-2a, and conventional HBV vaccination). Thus, this treatment strategy may have an advantage as it can be easily adapted to daily clinical practice. A number of preclinical and clinical studies are being conducted using novel anti-HBV strategies to cure HBV infection including inhibition of viral entry [30], destruction or functional silencing of cccDNA [31], RNA interference [32], modulation of nucleocapsid assembly [33], reduction of HBsAg secretion with nucleic acid polymers [34], and modulation of the immune checkpoint, such as programmed death-1 [35]. It is necessary to evaluate whether a novel agent alone or in combination with a currently available option is more effective to cure CHB.

In conclusion, entecavir plus an additional peg-IFN alfa-2a treatment followed by sequential HBV vaccination on an intensified schedule significantly increased the chance for HBsAg seroclearance compared to entecavir alone. This treatment strategy was associated with a higher frequency of overall AEs, which were mainly mild to moderate. The frequency of serious AEs was comparable between the E + sVIP and control groups.

These study results need to be validated in other countries where HBV has different genotypes. In addition, a large study directly comparing NA plus peg-IFN and sequential vaccination, NA plus peg-IFN without vaccination, and NA only is warranted. Since baseline qHBsAg titer has been reported as a predictor of HBsAg seroclearance during peg-IFN treatment [24], although it was not proven in the current study, stratification of patients according to baseline qHBsAg titer can be considered in a future study.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: J.-H. L., Y. J. K. Provision of study materials or patients: J.-H. L., Y. B. L., E. J. C., S. J. Y., J.-H. Y., and Y. J. K. Collection and assembly of data: J.-H. L., Y. B. L., E. J. C., S. J. Y., J.-H. Y., and Y. J. K. Data analysis and interpretation: J.-H. L. and Y. J. K. Manuscript writing: J.-H. L. Final approval of the manuscript: all authors. The study was designed by the principal academic investigator. Data were managed by investigators.

Financial support. This work was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health and Welfare, Republic of Korea (grant number HI16C1074); Bristol-Myers Squibb (research grant [grant number 0620133790, 2013-2468] and study drug); Roche (research grant [grant number 0620140140, 2014-0179] and study drug); and LG Life Science (study drug).

Potential conflicts of interest. J.-H. L. reports receiving lecture fees from GreenCross Cell, Daewoong Pharmaceuticals, and Gilead Korea. S. J. Y. reports lecture fees from Bayer HealthCare Pharmaceuticals. J.-H. Y. reports research grants from Bayer HealthCare Pharmaceuticals, Daewoong Pharmaceuticals, and Bukwang Pharmaceuticals. Y. J. K. reports research grants from Bristol-Myers Squibb, Roche, JW Creagene, Bukwang Pharmaceuticals, Handok Pharmaceuticals, Hanmi Pharmaceuticals, Yuhan Pharmaceuticals, Samjin Pharmaceuticals, and Pharmaking, and lecture fees from Bayer HealthCare Pharmaceuticals, Gilead Science, MSD Korea, Yuhan Pharmaceuticals, Samil Pharmaceuticals, CJ Pharmaceuticals, Bukwang Pharmaceuticals, and Handok Pharmaceuticals.

All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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