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Entecavir and Peginterferon Alfa-2a in Adults With Hepatitis B e Antigen–Positive Immune-Tolerant Chronic Hepatitis B Virus Infection

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Monotherapy with interferon or nucleoside analog is generally not recommended during the immune-tolerant (IT) phase of chronic hepatitis B virus (HBV) infection. Recognition that high HBV DNA levels are associated with hepatocellular carcinoma has increased interest in treating HBV in the IT phase. Small pediatric studies reported efficacy with combination nucleoside analog and interferon therapy. The aim of this study was to evaluate the safety and efficacy of the combination of entecavir and peginterferon in adults in the IT phase of chronic HBV infection. Hepatitis B e antigen (HBeAg)–positive adults with HBV DNA > 10^7 IU/mL and alanine aminotransferase (ALT) ≤ 1.5 times the upper limit of normal (ULN) (male: ≤ 45, female: ≤ 30 U/L) received entecavir 0.5 mg daily for 8 weeks followed by the addition of peginterferon alfa-2a 180 µg/week to entecavir for an additional 40 weeks. The primary endpoint was HBeAg loss and HBV DNA ≤ 1,000 IU/mL 48 weeks after end of treatment (EOT). Among 28 participants from 11 sites, the median age was 37.2 (range: 22-61) years, 54% were male, and 96% were Asian. Nearly all were infected with genotype C (64%) or B (32%). Median baseline HBV DNA was 8.2 $\log_{10}$ IU/mL, and ALT was 0.9 times the ULN. Although one (4%) participant cleared HBeAg, none met the primary endpoint of both HBeAg loss AND HBV DNA ≤ 1,000 IU/mL 48 weeks post-EOT. ALT elevations > 5 times the ULN occurred in eight (29%) participants, and none were associated with icterus. Forty-eight weeks posttreatment, HBV DNA rebounded to baseline levels in all participants, including the participant who lost HBeAg, and ALT values returned to near baseline levels in all but four participants. *Conclusion*: A lead-in strategy of 8 weeks of entecavir followed by combination peginterferon and entecavir therapy for 40 weeks had limited efficacy in adults in the IT phase of chronic HBV infection and cannot be recommended. (Hepatology 2019;69:2338-2348).

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Hepatitis B virus (HBV) infection remains a significant global public health problem, with roughly 250 million persons chronically infected worldwide.[1] Chronic HBV infection may result in progressive liver disease that leads to cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC). The majority of individuals with chronic HBV acquire infection at birth or in early

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; anti-HBe, antibody to HBeAg; CI, confidence interval; EOF, end of follow-up; EOT, end of treatment; HBeAg, Hepatitis B e antigen; HBRN, Hepatitis B Research Network; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IA, immune-active; IT, immune-tolerant'; LLOD, lower limit of detection; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; SAE, serious AE; ULN, upper limit of normal.

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childhood. The course of chronic infection has been divided into clinical phases based on virological characteristics, liver enzyme levels, and hepatic histology.(2) The immune-tolerant (IT) phase is characterized by the presence of hepatitis B e antigen (HBeAg), very high HBV DNA levels (> 10^7 IU/mL) but normal or near-normal aminotransferases, and minimal or no inflammation on liver biopsy, suggesting that despite active viral replication, there is no immune response to the virus.(3) Most individuals in the IT phase are children or young adults who acquired infection by vertical transmission. The age of transition to the immune-active (IA) phase is variable depending on HBV genotype and possibly race but typically occurs in adolescence or early adulthood.(3) Entry into the IA phase is characterized by a rise in alanine aminotransferase (ALT) levels and active inflammation on biopsy with fluctuating levels of HBV DNA. Repeated ALT flares and progressive liver injury may occur with eventual clearance of HBeAg, often with the development of antibody to HBeAg (anti-HBe). After HBeAg clearance, patients may enter the immune control or inactive carrier (IC) phase of infection with low or undetectable levels of HBV DNA and normal ALT values, or they may progress to HBeAg-negative IA disease with ongoing active hepatitis and fluctuating HBV DNA levels despite clearance of HBeAg.(3)

National and international clinical practice guidelines recommend treatment for HBV infection during the IA phases (HBeAg positive or HBeAg negative) of infection and for patients with cirrhosis.(4,5) Treatment during the IT phase has not been recommended because of the presence of minimal liver disease and limited efficacy of interferon or nucleoside analog monotherapy.(4,5) With recognition that high HBV DNA levels and persistence of HBeAg are associated with an increased risk of HCC, and that during the IT phase, HBV-specific T cells are present and able to proliferate and produce cytokines,(6) there has been increased interest in treating HBV during the IT phase. A pilot study of 23 children in the IT phase treated with lamivudine for 8 weeks followed by the addition of interferon for 44 weeks showed HBeAg

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seroconversion in five (22%) and hepatitis B surface antigen (HBsAg) loss in four (17%) children.[7] Based on these promising results, the Hepatitis B Research Network (HBRN) developed a clinical trial to assess combination therapy using entecavir, a more potent antiviral agent than lamivudine, followed by the addition of peginterferon in adult patients in the IT phase of chronic HBV infection.

Patients and Methods

HBRN

The HBRN is an National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)–funded, multicenter, prospective study of chronic hepatitis B in both children and adults followed at 28 clinical sites in the United States and Canada, the goals of which are to better define the natural history, pathogenesis, and therapy of chronic HBV. The HBRN includes both a pediatric and an adult cohort study and has initiated three therapy trials: two trials of entecavir and peginterferon (one in children and one in adults) with IT chronic HBV and one trial of tenofovir and peginterferon in adults with IA chronic HBV. This report is a description of the design and results of the open-label, adult IT study carried out at 11 adult clinical sites in the United States and at one clinical site in Canada. The Data Coordinating Center for the HBRN was located at the University of Pittsburgh, Pittsburgh, PA.

PARTICIPANTS

Participants who met the following criteria were eligible for enrollment: at least 18 years old, HBsAg positive, HBeAg positive, HBV DNA > 10^7 IU/mL on two occasions greater than 12 weeks apart and within 6 weeks of enrollment, and three documented ALT values less than 1.5 times the upper limit of normal (ULN) over the year before study entry. The ULN differed for men (30 U/L) and women (20 U/L). Initially men with ALT ≤ 60 U/L and women with ALT ≤ 40 U/L were eligible; however, the study was amended to ensure all participants were truly in the IT phase to include only men with ALT ≤ 45 U/L and women with ALT ≤ 30 U/L. Participants more than 50 years old were required to have had a liver biopsy within 96 weeks of enrollment documenting a histologic activity index (HAI) score < 3 and fibrosis stage ≤ 1. Key exclusion criteria included a past or current history of hepatic decompensation (ascites, variceal bleeding, hepatic encephalopathy), HCC, solid organ or bone marrow transplantation, platelet count < 120,000/mm^3, direct bilirubin > 0.5 mg/dL, albumin < 3.5 g/L, International Normalized Ratio > 1.5, creatinine clearance < 50 mL/minute, human immunodeficiency virus (HIV), hepatitis D or hepatitis C virus coinfection, preexisting psychiatric or autoimmune disease, or any other medical condition potentially exacerbated by interferon therapy. A full list of inclusion and exclusion criteria is provided in Supporting Tables S1 and S2.

Originally designed as a randomized trial with a control arm, the protocol was revised to be a single-arm pilot treatment study because of low enrollment. Participants previously randomly assigned to the control group were offered the option of enrolling in the revised protocol, provided that they still met trial eligibility criteria. The protocol was approved by the HBRN Steering Committee and the institutional review boards (research ethics board in the case of the Toronto site) of the participating sites, and all participants provided written informed consent. The study was overseen by an independent data safety monitoring board appointed by the NIDDK to monitor the clinical studies of the HBRN. The ClinicalTrials.gov identifier was NCT01369199.

After amendment to a single-arm pilot treatment study, the sample size was reestimated based on desired precision of the point estimate of the primary outcome (i.e., assuming 25% would meet the primary endpoint and a 95% Clopper-Pearson confidence limit within ± 15% of the estimated percentage, the revised sample size was 40 participants).

TREATMENT

In the initial protocol, participants were randomly assigned to treatment or observation. After the protocol was revised, those in the control arm were offered the option to move to the treatment arm, and subsequent enrollees received open-label therapy. Treatment consisted of open-label entecavir 0.5 mg/day for 8 weeks followed by entecavir combined with peginterferon alfa-2a 180 μg/week for an additional 40 weeks. After starting therapy, study visits occurred at weeks

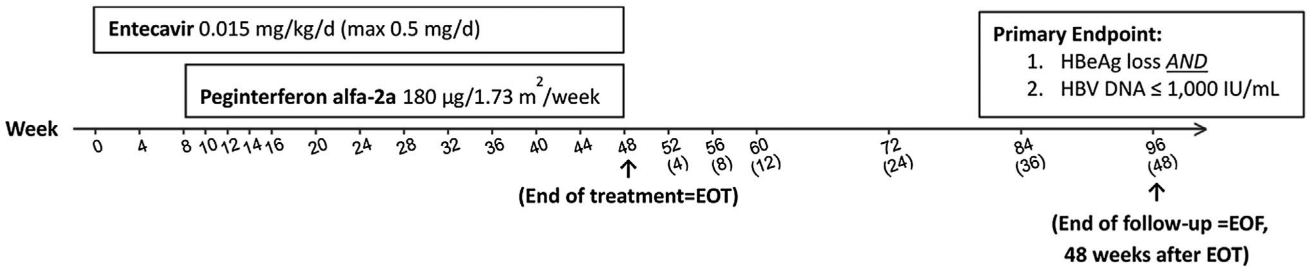

FIG. 1. Trial design. Entecavir 0.5 mg daily alone for 8 weeks followed by peginterferon alfa-2a 180 µg/week combined with entecavir for an additional 40 weeks. Endpoints were evaluated at the EOT (week 48) and 48 weeks after treatment completion (week 96). Weeks 52, 60, 72, and 96 correspond to 4, 12, 24, and 48 weeks after EOT, respectively, for those who discontinued treatment early.

8, 12, 16, 20, 24, 30, 36, 42, and 48, at which point all treatment was stopped and participants were followed for an additional 48 weeks with visits at weeks 52, 60, 72, and 96 (Fig. 1). Entecavir was provided by Bristol-Myers Squibb (New York, NY), and peginterferon alfa-2a was provided by Genentech (San Francisco, CA) under separate clinical trial agreements with the NIDDK.

ENDPOINTS

The primary efficacy endpoint was the loss of HBeAg and HBV DNA ≤ 1,000 IU/mL at the end of follow-up (EOF; 48 weeks following the end of treatment [EOT]). The primary safety endpoint was the frequency of adverse events (AEs) and serious AEs (SAEs). Secondary efficacy endpoints were assessed at the EOT (i.e., week 48 for those who completed treatment per protocol) and at the EOF (i.e. week 96 for those who completed the full 48 weeks of treatment). Secondary endpoints included loss of HBsAg, HBsAg seroconversion, loss of HBeAg, HBeAg seroconversion, ALT < 1.5 times the ULN, ALT normalization (male: < 30 U/L, female: < 20 U/L), HBV DNA ≤ 1,000 IU/mL, and HBV DNA < 20 IU/mL. Other *post hoc* endpoints included change in ALT and quantitative HBsAg from baseline. ALT elevations ≥ 2-fold the baseline were categorized by the height of elevation compared with the ULN using standard hepatotoxicity grading: grade 1 as > 1-3 times the ULN, grade 2 as > 3-5 times the ULN and grade 3 as > 5 times the ULN. Icteric ALT elevations are those associated with a bilirubin concentration > 2.5 mg/dL. Participants' maximum decline in quantitative HBsAg and HBeAg during therapy were calculated and categorized as ≤ 0.5 versus > 0.5 $\log_{10}$ IU/mL and ≤ 1.0 versus > 1.0 $\log_{10}$ IU/mL.

VIROLOGICAL ASSAYS

Study eligibility was based on qualitative HBeAg and HBsAg results performed at the individual sites. Efficacy endpoints were based on quantitative HBeAg and HBsAg (Elecsys; Roche Molecular Systems, Branchburg, NJ) assays performed at the HBRN central virology laboratory at the University of Washington, with lower limits of detection (LLODs) of 0.3 IU/mL for HBeAg and 0.05 IU/mL for HBsAg. HBV DNA testing was performed centrally using a real-time PCR assay (COBAS Ampliprep/COBAS TaqMan Test, version 2.0; Roche Molecular Systems) with a lower limit of quantification of 20 IU/mL and an LLOD of 10 IU/mL. HBV genotyping was performed at the Molecular Epidemiology and Bioinformatics Laboratory in the Division of Viral Hepatitis at the Centers for Disease Control and Prevention using mass spectrometry.[8]

STATISTICAL ANALYSIS

Descriptive statistics (median, range) and bootstrapped 95% confidence intervals (CIs) about medians are reported for continuous variables. Frequencies and percentages are reported for categorical variables. The primary efficacy analysis was performed including all enrolled participants. Participants who did not have primary endpoint data were considered as treatment failures. The percentage of participants achieving the efficacy endpoints was summarized with the point estimate and the 95% exact binomial CI. For safety endpoints, rates per person-year and 95% CIs about a Poisson variable are shown. For *post hoc* calculations of aggregate treatment response for HBV DNA, HBsAg, HBeAg, and ALT, participants who stopped therapy before the given time point or who did not have laboratory results were excluded (i.e., per-protocol analysis). One participant who was never treated with study medications was excluded for *post hoc* analyses. SAS 9.3 (SAS Institute, Cary, NC) and R 3.3.1 were used for statistical analysis and graphical display.

Results

PARTICIPANT CHARACTERISTICS

Of 58 patients screened, 29 met eligibility criteria and were enrolled in the trial. Three of these participants were randomly assigned to the control arm and two agreed to move to the treatment arm after the protocol amendment (both still met eligibility criteria), whereas the other participant refused consent, leaving 28 participants in the treatment arm. Reasons for exclusion included refused consent (n = 16); laboratory exclusion for HBV DNA; ALT level; platelet, hemoglobin, or neutrophil count (n = 13); and other medical condition (n = 1) (Supporting Fig. S1). Of the 28 who were to start treatment, 15 (54%) were male, 27 (96%) were Asian, the median age was 37.2 years (range: 22-61 years), and median body mass index was 22.4 (range: 18.0-28.3) kg/m^2. There were 18 (64%) individuals with genotype C, nine (32%) with genotype B, and one (4%) with genotype E infection. At baseline, the median HBV DNA level was 8.2 (range: 7.2-8.8) $\log_{10}$ IU/mL, and the median quantitative HBsAg level was 4.7 (range: 4.2-5.1) $\log_{10}$ IU/mL. Baseline ALT values ranged from 9 to 47 U/L or 0.5 to 1.6 times the ULN (median = 0.9 times the ULN), and 19 (68%) had values at or below the ULN. The median FIB-4 score was 0.7 (range: 0.4-2.3). One participant who was 61 years of age underwent liver biopsy and had an HAI = 1 and Ishak fibrosis = 0. Other baseline laboratory parameters are shown in Table 1.

TABLE 1. Baseline Characteristics

Characteristics	Total N = 28 n (%) or Median (Range)
Demographics	
Sex	
Male	15 (54)
Female	13 (46)
Race	
Black/African American	1 (4)
Asian	27 (96)
Age (years)	37.2 (22.2-61.2)
BMI (kg/m^2)	22.4 (18.0-28.3)
Virology and serology	
HBV genotype	
B	9 (32)
C	18 (64)
E	1 (4)
HBeAg (+)	28 (100)
HBV DNA ($\log_{10}$ IU/mL) - screening	8.3 (7.4-8.8)
HBV DNA ($\log_{10}$ IU/mL) - baseline	8.2 (7.2-8.8)
Quantitative HBeAg ($\log_{10}$ IU/mL)	3.3 (2.3-3.6)
Quantitative HBsAg ($\log_{10}$ IU/mL)	4.7 (4.2-5.1)
ALT (U/L): screening	21 (9-47*)
Males	32 (16-47*)
Females	18 (9-26)
ALT (U/L): baseline	21 (9-47)
Males	27 (14-47)
Females	15 (9-30)
AST (U/L)	20 (15-30)
FIB-4	0.7 (0.4-2.3)
INR	1.0 (0.9-1.1)
Total bilirubin (mg/dL)	0.6 (0.3-2.0)
Albumin (g/dL)	4.2 (3.5-4.8)
Platelets (x 10^3/mm^3)	242.5 (137-325)
White blood cells (x 10^3/mm^3)	5.0 (3.0-9.1)

*A male participant with ALT = 47 U/L was eligible for enrollment under the study protocol version 6.0 (ALT ≤ 60 U/L for males, ≤ 40 U/L for females). The study protocol version 7.0 was revised as ALT ≤ 45 U/L for males and ≤ 30 U/L for females.
Abbreviation: AST, aspartate aminotransferase.

TREATMENT OUTCOMES

One participant withdrew consent before the first dose of medication and was counted as failing to meet study efficacy endpoints; however, on-treatment results for this participant were excluded from calculations of summary statistics. Two additional participants were not available for the last follow-up visit, leaving 25 participants who completed 48-week post-treatment follow-up (Supporting Fig. S1). Among 28 participants, 27 adults initiated treatment and 20 (71%) completed the full 48 weeks of entecavir and 40 weeks of peginterferon treatment without dose reduction or early discontinuation. Four participants had at least one peginterferon dose reduction or interruption. Three participants discontinued one or both study medications. Two participants discontinued peginterferon early because of an AE, one at week 36 due to altered thyroid function and the other at week 35 due to a diagnosis of Graves disease. The average weekly dose was 177 μg (range: 90-180 μg) for the 24 participants who did not discontinue peginterferon early, representing 98% of the total expected dose.

PRIMARY EFFICACY ENDPOINT

No participant met the primary endpoint of both HBeAg loss and HBV DNA ≤ 1,000 IU/mL 48 weeks after treatment ended (week 96 for those who completed treatment) (Table 2, Fig. 2). One participant lost HBeAg at week 48 and remained HBeAg-negative, anti-HBe positive, and HBsAg positive at week 96. However, HBV DNA levels, which were suppressed to being undetectable by the EOT, rebounded to high levels (7.5 $\log_{10}$ IU/mL) by week 96, accompanied by an increase in ALT levels from 27 U/L at the EOT to 70 U/L at week 96. Viral sequencing confirmed the presence of the double Basal Core Promotor mutations (A1762T and G1764A) but the absence of the precore mutation (G1896A) at baseline and at last study follow-up. No posttrial follow-up was available for this individual.

One other participant had discrepant qualitative and quantitative HBeAg results throughout the study. At screening, the local qualitative HBeAg was positive, but the central quantitative HBeAg at baseline was below the limit of detection (< 0.3 IU/mL). Multiple results from both assays were unchanged, and this participant remained HBeAg positive by local qualitative testing but HBeAg negative by the central quantitative assay throughout the course of therapy and follow-up, with a similar HBV DNA curve during and after treatment as seen in other participants. This individual with discrepant qualitative and quantitative HBeAg results was not regarded as having HBeAg loss, and quantitative HBeAg results were not included in aggregate summary statistics for HBeAg (Table 2, Fig. 3C).

HBV DNA RESPONSE

Median $\log_{10}$ HBV DNA levels declined during the 8 weeks of entecavir monotherapy from 8.2 (range: 7.2-8.8) $\log_{10}$ IU/mL at baseline to 4.9 (range: 3.2-6.0) $\log_{10}$ IU/mL at week 8. At the EOT, HBV DNA levels were ≤ 1,000 IU/mL in all 26 participants who completed therapy and < 20 IU/mL in five participants (Table 2). Only one of these five participants had undetectable HBV DNA (< 10 IU/mL). No participants experienced on-treatment virological breakthrough. After stopping therapy, median HBV DNA levels rebounded to 8.3 (range: 6.9-9.0) $\log_{10}$ IU/mL by week 96 (Fig. 2A), and levels were within 0.3 $\log_{10}$ IU/mL of baseline values in all but four participants

TABLE 2. Efficacy Endpoints

	EOT		EOF	
	n = 28 (%)	95% CIs (%)	n = 28 (%)	95% CIs (%)
Endpoints				
HBsAg loss	0 (0)	(0.0-12.3)	0 (0)	(0.0-12.3)
HBsAg seroconversion	0 (0)	(0.0-12.3)	0 (0)	(0.0-12.3)
HBeAg loss*	1 (4)	(0.1-18.3)	1 (4)	(0.1-18.3)
HBeAg seroconversion	1 (4)	(0.1-18.3)	1 (4)	(0.1-18.3)
HBV DNA < 20 IU/mL	5 (18)	(6.1-36.9)	0 (0)	(0.0-12.3)
HBV DNA ≤ 1,000 IU/mL	26 (93)	(76.5-99.1)	0 (0)	(0.0-12.3)
ALT < 1 x ULN	11 (39)	(21.5-59.4)	13 (46)	(27.5-66.1)
ALT < 1.5 x ULN	16 (57)	(24.5-62.8)	21 (75)	(55.1-89.3)
Primary endpoint				
HBeAg loss* and HBV DNA ≤ 1,000 IU/mL	1 (4)	(0.1-18.3)	0 (0)	(0.0-12.3)

*The individual with discrepant qualitative (positive) and quantitative (negative) HBeAg results over time was not regarded as having HBeAg loss.
Analyses were performed using all enrolled participants.

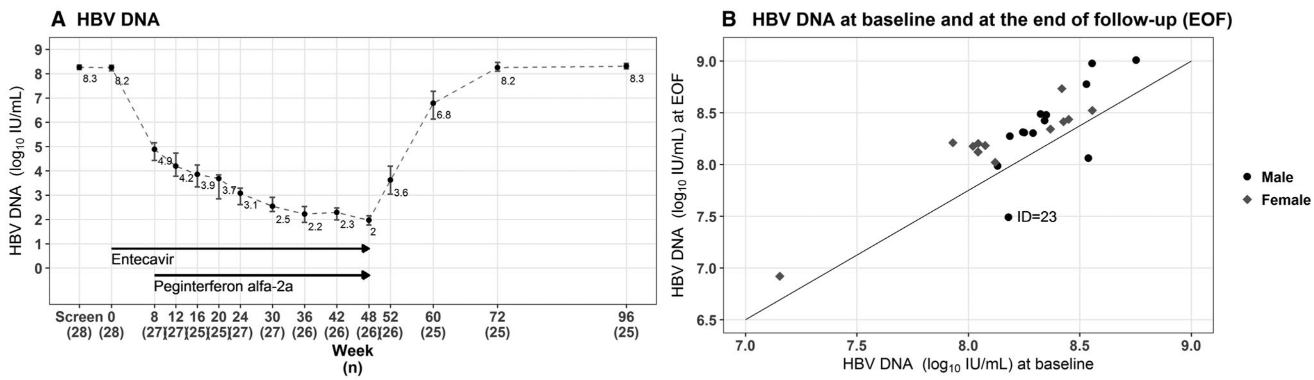

FIG. 2. HBV DNA levels. (A) Median and 95% CIs of HBV DNA levels are shown at baseline, during treatment, and after treatment. (B) HBV DNA levels at baseline are plotted against HBV DNA at the EOF (week 96), indicating that HBV DNA levels returned to near baseline levels (> 10^7 IU/mL) in all participants, including the individual who cleared HBeAg at the EOF.

(Fig. 2B). In the subject (identifier = 23) who became HBeAg negative, HBV DNA was 7.5 log_{10} IU/mL at week 96 compared with 8.2 log_{10} IU/mL at baseline (Fig. 2B).

HBeAg RESPONSE

Median quantitative HBeAg levels declined from 3.3 (range: 2.3-3.6) log_{10} IU/mL to 2.7 (range: LLOD-3.2) log_{10} IU/mL by week 48 and rebounded to 3.4 (range: LLOD-3.6) log_{10} IU/mL by week 96 (Fig. 3A). As noted, one participant experienced HBeAg seroconversion at week 48 of treatment that persisted to the EOF, but HBV DNA levels rebounded to near baseline levels with accompanying increase in ALT (Fig. 3C).

HBsAg RESPONSE

No participants cleared HBsAg during the study. Median quantitative HBsAg levels were similar at baseline (4.7 [range: 4.2-5.1] log_{10} IU/mL) and week 8 (4.7 [range: 4.1-5.1] log_{10} IU/mL) during entecavir monotherapy. After the addition of peginterferon, median HBsAg levels declined to 4.1 (range: 1.7-4.9) log_{10} IU/mL by week 48 but rebounded to 4.9 (range: 4.0-5.3) log_{10} IU/mL by the EOF (Fig. 3B). During therapy, the maximum HBsAg decline was 3.1 log_{10} IU/mL. Of the 27 treated participants, 19 (70%) had a more than 0.5 log_{10} decline and six (22%) had a more than 1.0 log_{10} decline in HBsAg levels.

ALT RESPONSES

None of the 27 participants who started therapy experienced an ALT elevation of 2-fold the baseline during lead-in entecavir monotherapy, whereas 19 (70%) had such an elevation during combination therapy, and these elevations were above 5 times the ULN in eight (30%). There were no icteric ALT elevations (bilirubin > 2.5 mg/dL), and treatment was not altered because of ALT increases. All ALT elevations resolved after completing treatment, and ALT levels returned to near baseline levels in most participants. (Fig. 4). In addition, seven grade 1 and four grade 2 ALT elevations were noted during combination therapy.

At the EOT, ALT levels decreased, and by EOF, 13 of 28 (46%) participants had normal ALT and 21 of 28 (75%) participants had values < 1.5 times the ULN (Table 2). At week 96, ALT was above 1.5 times the ULN in four of 25 subjects, but in no instance was ALT more than twice the baseline level (Fig. 4B). Three of these four were women with ALT values ranging from 32 to 37 U/L, compared with baseline values ranging from 15 to 30 U/L. The other participant was the person who had HBV DNA rebound despite HBeAg seroconversion and ALT of 70 U/L at week 96 compared with 37 U/L at baseline.

SAFETY

There were 42 treatment-emergent AEs (i.e., from first treatment to last follow-up) reported by 14

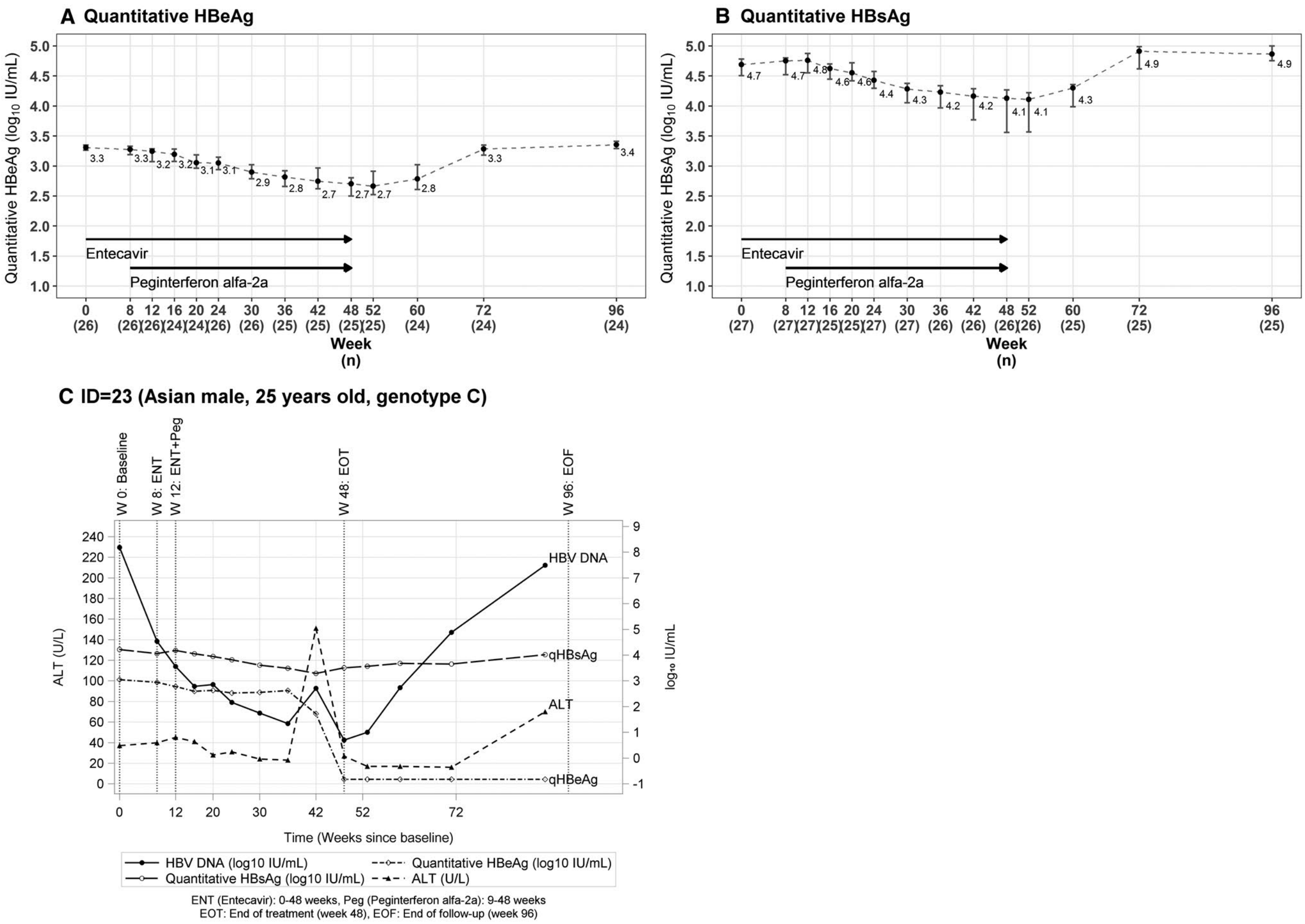

FIG. 3. (A) Quantitative HBeAg, (B) quantitative HBsAg, and (C) laboratory results over time in the participant who had HBeAg seroconversion. Quantitative (A) HBeAg and (B) HBsAg levels are shown before, during, and after treatment. (C) The pattern of ALT, HBV DNA, and quantitative HBsAg and HBeAg are shown in the individual who underwent HBeAg seroconversion on treatment. This patient stopped peginterferon at week 35 because of a diagnosis of Graves disease but continued entecavir until week 48. The decrease in HBeAg and HBsAg levels and sharp rise in ALT levels occurred after peginterferon was stopped and before entecavir was discontinued. The week 48 line indicates the end of entecavir treatment and the first time HBeAg was found to be negative.

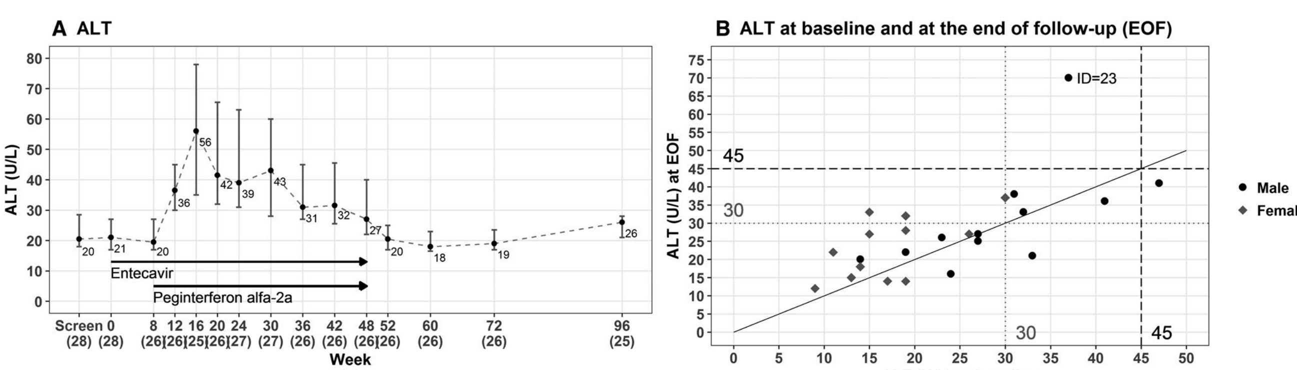

FIG. 4. ALT levels. (A) Median and 95% CI of ALT levels are shown before, during, and after treatment. (B) ALT levels at baseline are plotted against ALT levels at EOF (week 96), indicating that ALT levels returned to near baseline levels in most participants. ALT values of 1.5 times the ULN are indicated for men (45 U/L) in dashed lines and women (30 U/L) in dotted lines, respectively.

TABLE 3. Treatment-Emergent AEs by System (n = 42) Reported by 14 Participants

System	Number of AEs (%)
Dermatologic	10 (24)
Urological	6 (14)
Gastrointestinal	4 (10)
Constitutional symptom	4 (10)
Ear/nose/throat	4 (10)
Endocrine	3 (7)
Neurological	3 (7)
Infection	2 (5)
Musculoskeletal	2 (5)
Hematological	2 (5)
Ophthalmic	1 (2)
Severe respiratory	1 (2)

participants (Supporting Table S3A), none of which were scored as probably or definitely related to entecavir; in contrast, 14 (33%) were considered probably or definitely related to peginterferon (Supporting Table S3B,S3C). The treatment-emergent AE rate was 86 per 100 person-years (95% CI: 64-117 per 100 person-years). The treatment-emergent SAE rate was 2 per 100 person-years (95% CI: 0-15 per 100 person-years). Most AEs were mild (50%) or moderate (43%) in severity (Supporting Table S3B). The most common AEs were dermatologic (24%) and urological (14%) (Table 3). Three AEs led to three separate treatment interruptions of peginterferon in one participant. Two participants stopped peginterferon therapy due to an AE possibly related to peginterferon, one at week 36 due to altered thyroid function and the other at week 35 due to a specific diagnosis of Graves disease. Both participants continued entecavir. One SAE was reported during the off-treatment observation period, a case of malaria, considered to be unrelated to treatment.

Discussion

National and international guidelines do not recommend treatment during the IT phase of chronic HBV infection, largely because of the limited efficacy of currently available therapies.[4,5] This study evaluated adding peginterferon to entecavir for 40 weeks after 8 weeks of lead-in entecavir monotherapy. Only one of 28 (3.6%) participants achieved HBeAg seroconversion, and this individual had rebound in HBV DNA and ALT posttreatment, transitioning to the HBeAg-negative IA phase rather than to the IC state. No patients achieved off-therapy virological control or HBsAg loss.

Although the combination treatment was not effective, this trial provides some useful insights. The concept of immune tolerance was first proposed based on the observation that, despite high levels of viremia, patients in the IT phase have minimal or no hepatic inflammation, suggesting a lack of immune recognition or tolerance to the virus.[2] However, more in-depth characterization of the HBV-specific immune response in different phases of chronic HBV infection has challenged the concept of immune tolerance. Young patients in the IT phase have been shown to have similar or greater frequency and reactivity of HBV-specific T cells in the peripheral blood compared with older patients in the IA phase of disease.[9] Furthermore, viral diversity in young IT patients suggests immune selection pressure, questioning the concept of true tolerance to the virus.[10] Bertoletti and colleagues have noted that the main difference between the IT phase and the IA phase is not immune tolerance but the presence of an inflammatory milieu in the IA phase that drives nonspecific inflammation in response to recognition of HBV by T cells and have thus suggested renaming the IT phase as the "noninflammatory, high viremic" phase of disease.[6] This is reflected in the recent European Association for the Study of the Liver HBV clinical practice guidelines in which the authors proposed a nomenclature for the phases of chronic HBV infection and suggested that the IT phase be renamed "HBeAg-positive Chronic Infection" to distinguish it from the IA phase, which they proposed to rename "HBeAg-positive Chronic Hepatitis."[4] The data from our study are notable for the fact that despite a rapid rebound in HBV DNA levels after stopping entecavir and peginterferon to near baseline values, there was no associated hepatitis flare, with ALT values settling out at or near baseline levels. Continued follow-up showed that ALT values remained stable, indicating that the patients returned to the same "tolerant" or "noninflammatory" state posttreatment. This is similar to what was seen after stopping 4 years of nucleoside analog therapy in IT patients[11] and contrasts notably with studies of antiviral therapy withdrawal in HBeAg-positive patients who started therapy in the IA phase of disease, in whom there were reports of severe or even fatal withdrawal hepatitis.[12] These data suggest that the immune response to HBV is indeed different in patients in the IT and IA phase of chronic HBV infection. Studies of nucleoside analog withdrawal in HBeAg-negative patients have also reported ALT flares in 53%-76% of patients in association with viral rebound.[13-15]

Despite the lack of hepatitis flares after stopping treatment, ALT elevations to more than twice baseline occurred in 19 patients during peginterferon therapy, and levels rose to above 5 times the ULN in eight (30%) participants. Declines in quantitative HBsAg and HBeAg levels of at least 0.5 $\log_{10}$ IU/mL were also seen during combination therapy and were more common in those with ALT elevations, possibly suggesting that the ALT elevations were associated with viral control. Neither ALT flares nor declines in quantitative HBsAg or HBeAg levels occurred during the 8 weeks of entecavir monotherapy. These results are in keeping with previous data showing that HBsAg decline rarely occurs during nucleoside analog therapy but is commonly observed during peginterferon treatment.[16]

Previous studies of interferon therapy for adult IT patients showed negligible rates of HBeAg or HBsAg loss.[17] Trials of nucleoside therapy in the IT patients have shown that HBV DNA can be suppressed, but HBeAg and HBsAg loss are rare.[18] However, small trials in children of interferon treatment after a lead-in of 8 weeks of lamivudine reported HBeAg seroconversion rates of up to 39% and rates of HBsAg loss of up to 21%.[7,19] Part of the rationale for combining a nucleoside analog with interferon is the observation that viral suppression may lead to some degree of restoration of HBV-specific immune responses, which may then promote a more robust immune response driven by interferon.[20-22] We hypothesized that by using a more potent agent such as entecavir, we might achieve similar results in adult patients to those seen in children. A parallel HBRN study in children found that 3% had durable HBeAg and HBsAg clearance with undetectable HBV DNA and normal ALT. Although there were responses in children but none in adults, the CIs around the response rates were similar. Notably, in the one adult participant who cleared HBeAg and developed anti-HBe in our study, HBV DNA levels increased after stopping therapy, and ALT levels rose to 70 U/L at last follow-up. Although it is difficult to make strong inferences from one individual, it raises the possibility that therapy was actually harmful to this participant, leading to a loss of "tolerance" and the development of HBeAg-negative IA disease. Similarly, in a study of nucleoside analog monotherapy in 20 adult IT patients, HBeAg loss occurred in three patients after treatment cessation, and one developed HBeAg-negative active disease.[11]

This study has important limitations, most notably the lack of a control arm. The original protocol was designed to include an untreated control arm; however, recruitment was lower than anticipated, largely because of a low frequency of eligible IT patients in the HBRN sites, demonstrating the challenge of studying this population in North America. To gain some insight into the effect of therapy compared with the natural history of the IT phase, we evaluated the virological and clinical parameters of study participants before entering this trial while they were followed as part of the prospective HBRN cohort study. Because of variable duration of follow-up, it is difficult to make formal comparisons, but there were few to no changes in HBV DNA and HBsAg levels among the participants before starting therapy. Second, the overall sample size was smaller than planned; however, the very limited response rate makes it unlikely that a significant therapeutic effect was missed. Based on the CIs, a maximum of a 12% response rate could have been missed. Third, the study consisted of almost exclusively Asian patients with genotypes B and C; however, the IT phase is most frequently observed in Asians who acquire HBV perinatally. Fourth, the median age of participants was 37.2 years and eight (29%) were above 40 years of age, older than is often seen in the IT phase. Age was not an apparent determinant of response, but whether younger patients may have responded differently as suggested by the two responders in the HBRN trial in children is unknown. Fifth, it is possible that a longer duration of combination therapy may have led to greater responses, given that in many individuals, quantitative HBsAg levels were trending downward at the end of therapy; however, tolerability of peginterferon-based therapy for greater than a year is not likely to be a clinically attractive treatment strategy.

In conclusion, an 8-week lead-in of entecavir followed by the addition of 40 weeks of peginterferon was ineffective in adults in the IT phase of chronic HBV infection. The lack of hepatitis flare on rebound viremia after stopping antiviral therapy suggests that the immune response to HBV in the IT phase is truly distinct from that in the IA phase of disease. Understanding the mechanisms leading to tolerance or at least lack of inflammation may be key to developing therapeutic strategies for the IT phase of chronic HBV.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30417/suppinfo.