Gastroenterology 2016;150:134–144

Combination of Tenofovir Disoproxil Fumarate and Peginterferon α-2a Increases Loss of Hepatitis B Surface Antigen in Patients With Chronic Hepatitis B

Patrick Marcellin,[1,§] Sang Hoon Ahn,[2] Xiaoli Ma,[3] Florin A. Caruntu,[4] Won Young Tak,[5] Magdy Elkashab,[6] Wan-Long Chuang,[7] Seng-Gee Lim,[8] Fehmi Tabak,[9] Rajiv Mehta,[10] Joerg Petersen,[11] Graham R. Foster,[12] Lillian Lou,[13] Eduardo B. Martins,[14] Phillip Dinh,[14] Lanjia Lin,[14] Amoreena Corsa,[14] Prista Charuworn,[14] G. Mani Subramanian,[14] Hans Reiser,[14] Hendrick W. Reesink,[15] Scott Fung,[16] Simone I. Strasser,[17] Huy Trinh,[18] Maria Buti,[19] Giovanni B. Gaeta,[20] Aric J. Hui,[21] George Papatheodoridis,[22] Robert Flisiak,[23] and Henry L. Y. Chan,[§,24] on behalf of the Study 149 Investigators

[1]Service d'Hépatologie, Hôpital Beaujon, University Paris-Diderot, Inserm Centre de Recherche sur l'Inflammation, Clichy, France; [2]Division of Gastroenterology, Yonsei University College of Medicine, Seoul, South Korea; [3]Drexel University College of Medicine, Philadelphia, Pennsylvania; [4]National Institute for Infectious Diseases, "Matei Bals", Bucharest, Romania; [5]Kyungpook National University Hospital, Daegu, South Korea; [6]Toronto Liver Center, Toronto, Canada; [7]Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; [8]Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [9]University of Istanbul, Cerrahpasa Medical Faculty, Istanbul, Turkey; [10]Liver Clinic, Surat, India; [11]IFI Institute for Interdisciplinary Medicine at the Asklepios Klinik St. George, University of Hamburg, Hamburg, Germany; [12]Queen Mary University of London, London, United Kingdom; [13]Nexus Development, Palo Alto, California; [14]Gilead Sciences Inc, Foster City, California;[15]Academic Medical Center, Amsterdam, The Netherlands; [16]University of Toronto, Department of Medicine, Toronto General Hospital, Toronto, Canada; [17]AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital and University of Sydney, Sydney, Australia; [18]San Jose Gastroenterology, San Jose, California; [19]Hepatology Unit, Hospital Universitari Vall d'Hebron and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas del Instituto Carlos III, Barcelona, Spain; [20]Viral Hepatitis Unit, Department of Clinical and Experimental Medicine, Second University of Naples, Naples, Italy; [21]The Chinese University of Hong Kong, Alice Ho Miu Ling Nethersole Hospital, Hong Kong; [22]Athens University Medical School, "Laiko" General Hospital of Athens, Athens, Greece; [23]Department of Infectious Diseases and Hepatology, Medical University of Bialystok, Bialystok, Poland; and [24]Department of Medicine and Therapeutics and Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong

See editorial on page 32.

BACKGROUND & AIMS: Patients chronically infected with the hepatitis B virus rarely achieve loss of serum hepatitis B surface antigen (HBsAg) with the standard of care. We evaluated HBsAg loss in patients receiving the combination of tenofovir disoproxil fumarate (TDF) and peginterferon α-2a (peginterferon) for a finite duration in a randomized trial. **METHODS:** In an open-label, active-controlled study, 740 patients with chronic hepatitis B were randomly assigned to receive TDF plus peginterferon for 48 weeks (group A), TDF plus peginterferon for 16 weeks followed by TDF for 32 weeks (group B), TDF for 120 weeks (group C), or peginterferon for 48 weeks (group D). The primary end point was the proportion of patients with serum HBsAg loss at week 72. **RESULTS:** At week seventy-two, 9.1% of subjects in group A had HBsAg loss compared with 2.8% of subjects in group B, none of the subjects in group C, and 2.8% of subjects in group D. A significantly higher proportion of subjects in group A had HBsAg loss than in group C ($P < .001$) or group D ($P = .003$). However, the proportions of subjects with HBsAg loss did not differ significantly between group B and group C ($P = .466$) or group D ($P = .883$). HBsAg loss in group A occurred in hepatitis B e antigen–positive and hepatitis B e antigen–negative patients with all major viral genotypes. The incidence of common adverse events (including headache, alopecia, and pyrexia) and treatment discontinuation due to adverse events was similar among groups. **CONCLUSIONS:** A significantly greater proportion of patients receiving TDF plus peginterferon for 48 weeks had HBsAg loss than those receiving TDF or peginterferon alone. ClinicalTrials.gov ID NCT01277601.

Keywords: HBV; HBeAg Seroconversion; Virologic Response; Clinical Trial.

Up to 400 million people worldwide are chronically infected with hepatitis B virus (HBV).[1] Almost all newly infected adults are able to clear the virus without therapy, but 80%–90% of infants infected during the first

[§]Authors share co-senior authorship.

Abbreviations used in this paper: **ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; peginterferon, pegylated interferon-α; S/CO, sample/cutoff relative light units of quantitative HBsAg; TDF, tenofovir disoproxil fumarate.**

Most current article

0016-5085
http://dx.doi.org/10.1053/j.gastro.2015.09.043

year of life and 30%−50% of children infected by the age of 6 years develop chronic HBV infection.[2] Chronic infection is linked to the persistence of covalently closed circular HBV DNA within the nucleus of hepatocytes.[2] The presence of hepatitis B surface antigen (HBsAg) in the serum is a surrogate marker for covalently closed circular HBV DNA transcriptional activity.[2–5] Clearance of HBsAg from the serum is associated with a functional remission of chronic hepatitis B (CHB) and improved long-term outcomes.[6,7] HBsAg loss is therefore recognized as the optimal therapeutic goal.[1,8,9]

Standard treatment of patients with CHB involves either a finite course of pegylated interferon-α (peginterferon), which stimulates the natural immune response against the virus,[10] or an oral antiviral for an indefinite duration, which suppresses replication of HBV to undetectable levels.[1,8,9] However, HBsAg loss is uncommon with both treatment strategies. In the trials of peginterferon α-2a given for 48 weeks, only 4% of hepatitis B e antigen (HBeAg)-negative and 4% of HBeAg-positive had HBsAg loss 6 months after the end of treatment.[11,12] In the phase 3 trials of tenofovir disoproxil fumarate (TDF), the rate of HBsAg loss was only 3% in HBeAg-positive patients who received 48 weeks of treatment with TDF, and no HBeAg-negative patients lost HBsAg.[13]

As peginterferon and antivirals have different mechanisms of action, it has been hypothesized that combining the 2 drug classes could improve rates of HBsAg loss. Several trials have evaluated combination treatment with peginterferon and oral antivirals for patients with CHB, but the results are inconclusive.[11,12,14–18] None of these trials were designed to evaluate serum HBsAg loss as a primary end point. We, therefore, compared the efficacy and safety of TDF (a potent oral nucleotide antiviral with a high barrier to resistance) and peginterferon combination therapy with TDF and peginterferon alone in patients with CHB. We also included a combination regimen involving a short duration (16 weeks) of peginterferon to explore whether a peginterferon-sparing regimen affected rates of HBsAg loss.

Methods

Study Design

This was a randomized, open-label, active-controlled, multinational, superiority trial (ClinicalTrials.gov ID NCT01277601). Patients received TDF (300 mg once daily orally) and peginterferon α-2a (180 μg/week, subcutaneously) separately or concomitantly. Patients were randomly assigned 1:1:1:1 to 1 of 4 treatment groups: TDF plus peginterferon for 48 weeks (group A); TDF plus peginterferon for 16 weeks followed by 32 weeks of TDF alone (group B); TDF alone for 120 weeks (group C); or peginterferon alone for 48 weeks (group D). All groups were followed to week 120. The protocol is provided in the Supplementary Material. All authors had access to the data, assume responsibility for the integrity and completeness of the reported data, and approved the manuscript for submission.

During follow-up, any patient who developed either hepatic decompensation or virologic or biochemical relapse (Supplementary Material) was retreated with TDF monotherapy (300 mg once daily orally).

The study was approved by the Institutional Review Board or Independent Ethics Committee at each site and was conducted according to the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The investigators, participating institutions, and sponsor agreed to maintain confidentiality of the data.

Randomization was done centrally through an Interactive Voice Response System. The randomization sequence was generated by an independent company using a computer program. Randomization was stratified by screening HBeAg status and HBV genotype to form 10 strata (Supplementary Table 1).

Patients

Patients aged 18−75 years with CHB were enrolled at 139 sites in 19 countries (Australia, Canada, France, Germany, Greece, Hong Kong, India, Italy, The Netherlands, Poland, Portugal, Romania, Singapore, South Korea, Spain, Taiwan, Turkey, United Kingdom, and United States) from March 2011 to March 2013. Eligible patients were HBeAg-positive or HBeAg-negative and had not previously received treatment with interferon or nucleotide analogs. Patients who had received nucleoside analogs were eligible provided that they received the final dose at least 24 weeks before screening. HBeAg-positive patients were required to have HBV DNA levels $\geq$20,000 IU/mL and HBeAg-negative patients $\geq$2000 IU/mL. Men were required to have alanine aminotransferase (ALT) levels $>$54 U/L and $\leq$400 U/L, and women $>$36 U/L and $\leq$300 U/L. Patients with bridging fibrosis or cirrhosis documented within the previous 12 months were excluded to reduce the risk of liver decompensation associated with ALT flares. Full eligibility criteria are provided in the Supplementary Material. All patients signed an informed consent form before screening in accordance with regulatory and local ethics committee requirements.

Study Assessments

Study visit assessments included measurement of serum HBsAg (Architect assay; Abbott Diagnostics, lower limit of detection: 0.05 IU/mL) and serum HBV DNA (polymerase chain reaction-based m2000sp/m2000rt; Abbott Diagnostics, lower limit of quantification: 15 IU/mL) in addition to standard laboratory, clinical, and safety assessments as described in Supplementary Table 2. The primary end point, HBsAg loss, was determined using the Architect Qualitative II assay (Abbott Diagnostics, lower limit of detection 1 S/CO).

Viral resistance testing (Supplementary Material) was conducted in patients with HBV DNA $\geq$117 IU/mL at weeks 48 or 72 who had received at least 24 weeks of TDF.

All specified laboratory tests were performed at a central laboratory.

Outcomes

The primary efficacy end point was the proportion of patients with HBsAg loss at week 72 by Kaplan−Meier analysis: the primary hypothesis compared group A with groups C or D; the secondary hypothesis compared group B with groups C or D. Secondary end points at weeks 48 and 72 included anti-HBs seroconversion, HBeAg loss, anti-HBe seroconversion, HBsAg

decline from baseline, virologic response (HBV DNA <15 IU/mL) and normalization of ALT. Sustained response (defined as HBV DNA <2000 IU/mL and normalization of ALT) was also assessed at week 72. Other endpoints included retreatment with TDF during the follow-up period and overall safety.

Statistical Analysis

All patients who received at least one dose of study drug were included in the efficacy and safety analyses. In order to characterize the full safety profile within this study, all available data up to and beyond week 72 were included in the safety analysis.

In the primary analysis, the proportion of patients with HBsAg loss at week 72 was estimated by a Kaplan−Meier method. A predefined 2-state Markov analysis was also conducted to account for HBsAg seroreversion as this serologic aspect is not reflected in the Kaplan−Meier analysis. Details of the 2-state Markov analysis has been published previously.[13] Briefly, the Markov model allows transitions between response (HBsAg loss) and nonresponse (HBsAg seroreversion) during the time of interest, whereas the Kaplan−Meier method only allows the transition from nonresponse to response and ignores the transition from response to nonresponse. Data for patients without HBsAg loss were censored at the last timepoint observed. Comparisons between groups were conducted via stratified log-rank tests, by baseline HBeAg status and HBV genotype.

For the primary efficacy end point, multiplicity adjustments using a multistage gatekeeping approach[19] controlled the study-wise error rate at <.05. Movement from primary hypothesis to the secondary hypothesis is predicated on rejection of at least one null hypothesis in the primary hypothesis. For all other comparisons, unadjusted *P* values are presented. Overall, 180 patients per treatment group would provide the study with about 80% power to detect a 10% difference in HBsAg loss, assuming that the test group (either group A or group B) had an HBsAg loss rate of 13% and the control arm had an HBsAg loss rate of 3% for both the primary and secondary hypotheses. The power/sample size calculation was based on a 2-sided type I error rate of .025. The sample size was calculated based on a complementary log−log transformation of the fixed-point survival test. It was also confirmed using Fisher's exact test.

Associations between baseline and on-treatment variables with HBsAg loss during the 72-week period were examined via a post-hoc exploratory Cox regression analysis. Additional statistical details are provided in the protocol (Supplementary Material).

Results

Of the 1597 patients screened, 751 were randomized and 740 received treatment (Supplementary Figure 1; Supplementary Table 3). Most patients were male (66%), Asian (75%), HBeAg-positive (58%), and 42% had HBV genotype C (Table 1). The demographics and baseline clinical characteristics of the patients were balanced across the 4 treatment groups (Table 1).

At week 72, the Kaplan−Meier cumulative estimate of HBsAg loss was 9.1% for group A, 2.8% for group B, 0% for group C, and 2.8% for group D. The rate of HBsAg loss in group A was significantly higher than rates for groups C ($P < .001$) or D ($P = .003$), establishing superiority in achieving HBsAg loss for the combination regimen over monotherapy with either TDF or peginterferon (Figure 1; Table 2). HBsAg loss in group A occurred in both HBeAg-positive and HBeAg-negative patients and across all major viral genotypes, with the highest rate occurring in genotype A patients (Table 3; Supplementary Tables 4 and 5). The rate of HBsAg loss in group B did not significantly differ from that of groups C ($P = .466$) or group D ($P = .883$) (Table 2). A post-hoc exploratory comparison demonstrated a significantly higher rate of HBsAg loss in group A than in group B (unadjusted $P = .002$).

Between weeks 48 and 72, seven patients who had HBsAg loss on at least one study visit experienced HBsAg seroreversion (5 HBeAg-positive patients: 3 in group A [2 genotype A patients and 1 genotype B patient], 2 in group B [genotype A], and 2 HBeAg-negative patients: 1 in group A and 1 in group B [both genotype B]). Of these, 5 had achieved HBsAg loss at or after end of treatment at week 48. To assess the impact of HBsAg seroreversion on overall efficacy, we conducted a 2-state Markov model analysis that revealed statistical significance in the comparison between groups A and C, but not between groups A and D (Table 2).

The multivariable Cox regression analysis identified 2 baseline factors that were independently associated with HBsAg loss: HBV genotype A and assignment to treatment group A (TDF plus peginterferon for 48 weeks). Two on-treatment factors were found to be strongly associated with HBsAg loss: increase in ALT to >400 U/L (males) or >300 U/L (females) during the first 12 weeks of therapy and having >1 $\log_{10}$ decline in HBsAg from baseline to week 12 (Supplementary Tables 6 and 7).

The rate in group A was significantly higher than the rates for groups C ($P < .001$) and D ($P = .005$) (Table 2). The rate of anti-HBs seroconversion at week 72 for group B did not differ significantly from that of groups C ($P = .800$) or D ($P = .177$). A post-hoc exploratory comparison demonstrated a significantly higher rate of anti-HBs seroconversion in group A than in group B (unadjusted $P < .001$). Of the 17 patients who maintained HBsAg loss at week 72 with any treatment regimen, 15 (88%) achieved anti-HBs seroconversion.

Mean HBsAg decline from baseline to week 48 was significantly greater in group A (1.1 $\log_{10}$ IU/mL) than in groups B, C, and D (0.5, 0.3 and 0.8 $\log_{10}$ IU/mL, respectively, $P < .05$ for all comparisons vs group A) (Figure 2). Patients in group B who received only 16 weeks of peginterferon had smaller declines of HBsAg between weeks 16 and 48 than did patients in group A, who received 48 weeks of peginterferon. At week 72, in patients who had not been retreated with TDF, group A demonstrated significantly greater HBsAg decline compared with all other groups (1.3 vs 0.3, 0.4, 0.6 $\log_{10}$ IU/mL , respectively, $P < .05$ for all comparisons vs group A).

At week 48, rates of HBeAg loss and anti-HBe seroconversion were higher in group A than in groups C and D ($P < .05$); at week 72, rates of HBeAg loss and anti-HBe seroconversion in group A were only higher than group C

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline

Characteristics	Group A (n = 186) TDF + peginterferon for 48 wk	Group B (n = 184) TDF + peginterferon for 16 weeks, then TDF for 32 wk	Group C (n = 185) TDF for 120 wk	Group D (n = 185) peginterferon for 48 wk
Age, *y*, mean ± SD (range)	38 ± 16.7 (18–69)	37 ± 9. (18–62)	36 ± 10.9 (18–66)	38 ± 10.5 (18–64)
Body mass index				
Mean ± SD	24 ± 3.4	24 ± 4.0	25 ± 4.1	24 ± 4.0
Range	17–37	17–46	17–45	16–45
Male sex, n (%)	127 (68)	119 (65)	121 (65)	119 (64)
Race, n (%)				
Asian	142 (76)	134 (73)	141 (76)	137 (74)
Black[a]	5 (3)	3 (2)	4 (2)	6 (3)
White	36 (19)	45 (25)	39 (21)	41 (22)
Other	3 (2)	2 (1)	1 (1)	1 (1)
Region, n (%)				
North America	38 (20)	31 (17)	37 (20)	37 (20)
Europe	42 (23)	52 (28)	40 (22)	45 (24)
Australia/New Zealand	10 (5)	14 (8)	15 (8)	14 (8)
Asia	96 (52)	87 (47)	93 (50)	89 (48)
HBV genotype,[b] n (%)				
A	17 (9)	16 (9)	14 (8)	14 (8)
B	50 (27)	51 (28)	49 (27)	53 (29)
C	78 (42)	79 (43)	78 (42)	79 (43)
D	39 (21)	36 (20)	41 (22)	38 (21)
E–H	2 (1)	2 (1)	3 (2)	1 (1)
HBeAg-positive, n (%)	108 (58)	105 (57)	109 (59)	106 (57)
HBV DNA, log_{10} *IU/mL*, mean ± SD	7.1 ± 1.5	7.1 ± 1.5	7.0 ± 1.5	6.9 ± 1.6
HBsAg, log_{10} *IU/mL*, mean ± SD	3.9 ± 0.8	3.8 ± 0.9	3.9 ± 0.8	3.8 ± 0.8
ALT, *U/L*, mean ± SD	121 ± 181	112 ± 94	101 ± 68	107 ± 92
Prior HBV medication,[c] n (%)	9 (5)	5 (3)	11 (6)	8 (4)
Entecavir	5 (3)	3 (2)	4 (2)	2 (1)
Lamivudine	4 (2)	3 (2)	7 (4)	5 (3)
Clevudine	2 (1)	0	0	0
Telbivudine	0	1 (0·5)	2 (1)	2 (1)

[a]Black or African American.
[b]Five patients with mixed genotypes were randomized: 1 genotype B/C; 3 genotypes D/G, and 1 genotype A/D.
[c]Multiple responses possible.

($P < .05$) (Table 2). Groups A and B had similar rates of HBeAg loss and anti-HBe seroconversion at weeks 48 and 72. At week seventy-two, 5, 7, and 6 patients in groups A, B, and C, respectively, who had been retreated after stopping therapy at week 48 achieved HBeAg loss.

At week 48, suppression of HBV DNA to <15 IU/mL was achieved by 61%–71% of patients in all treatment groups that included TDF, compared with 21% of patients receiving peginterferon alone. HBV DNA frequently rebounded after week 48 in the absence of therapy (Table 2; Supplementary Table 8). At week 72, the proportion of patients with normalized ALT levels in group C (TDF monotherapy) was 73% as compared with 36%–37% of patients in the peginterferon-containing treatment groups ($P < .0001$). At week 72, rates of sustained response in patients who had stopped therapy and were not retreated were 23%, 22%, and 19% in groups A, B, and D, respectively; $P > .05$.

Of the 555 patients randomized to the groups with treatment ending at week forty-eight, 312 met the criteria for TDF retreatment: 100 patients (54%) from group A; 100 patients (54%) from group B, and 112 patients (61%) from group D. The reasons for retreatment are shown in Supplementary Table 9. None of the patients requiring TDF retreatment after week 48 achieved HBsAg loss at week 72.

By week 72, viral resistance testing had been conducted in 31 patients (4.3%) according to the protocol. Of these, 11 (35.5%) had no sequence changes in HBV polymerase/reverse transcriptase compared with baseline, 11 (35.5%) had unique polymorphic site changes, and 9 (29.0%) were unable to be genotyped because of low viral load (HBV DNA <172 IU/mL). No conserved site changes were observed in patients while receiving a TDF-containing regimen.

Study drug discontinuation due to adverse events occurred more frequently with peginterferon monotherapy (8%) than in the other groups (0–4%) (Table 4). The most common adverse events were headache, alopecia, pyrexia, and fatigue/malaise, which are all characteristic of peginterferon treatment. With the exception of fatigue/malaise, which was more frequently recorded in group A, most adverse events occurred at a similar frequency among the

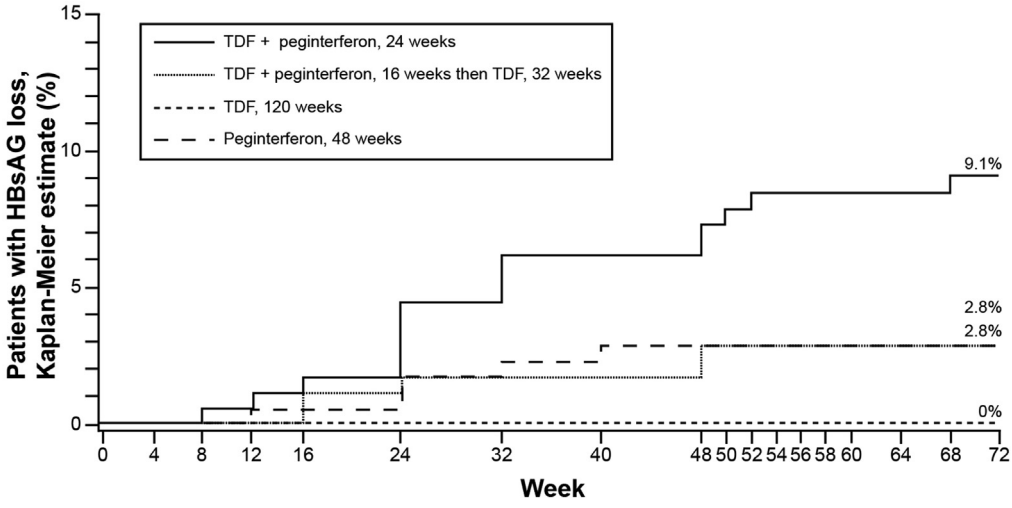


Figure 1. Effect of TDF and peginterferon as combination or monotherapy on HBsAg loss. The rate of HBsAg loss in group A was significantly higher than rates for groups C ($P < .001$) or D ($P=.003$), as well as group B (unadjusted $P < .001$). The rate of HBsAg loss in group B did not significantly differ from that of groups C ($P = .466$) or group D ($P = .883$).

combination therapy and peginterferon groups. The majority of serious adverse events were patients who experienced an increase in serum transaminases. These events occurred at a similar frequency in patients receiving combination therapy or peginterferon monotherapy, and were lowest in patients receiving TDF monotherapy (15, 15, 2, and 18 events for groups A−D, respectively).

After discontinuation of therapy at week 48 in groups A, B, and D, a similar proportion of patients receiving combination treatment and peginterferon monotherapy experienced ALT elevations >400 U/L (males) or >300 U/L (females). These transaminase elevations decreased with TDF retreatment. One patient in group B developed mild hepatic encephalopathy during a hepatic flare while not receiving treatment; symptoms resolved after initiation of TDF retreatment. One patient in group D decompensated after week 72 (more details in the Supplementary Material).

Discussion

In this study in HBeAg-positive and HBeAg-negative CHB patients without advanced liver disease, the rate of HBsAg loss at week 72 was significantly higher in patients receiving TDF plus peginterferon for 48 weeks than in those receiving monotherapy with either TDF or peginterferon. Patients receiving combination therapy with a shorter course of peginterferon (16 weeks) had a smaller decline in HBsAg levels and a lower rate of HBsAg loss than patients receiving 48 weeks of peginterferon plus TDF (post-hoc analysis, unadjusted). This study provides direct evidence that combining 2 agents with different mechanisms of action for a finite treatment duration can result in rates of serum HBsAg loss significantly higher than those obtained with current standard-of-care monotherapy regimens.

The mechanism of action by which the TDF and peginterferon combination induces HBsAg loss is not well understood. Active HBV replication can blunt the host immune response by disrupting various intracellular pathways, including those involved in antigen presentation and interferon-dependent signaling.[20] By rapidly reducing viral replication and serum viral protein levels, including HBsAg, TDF may improve responsiveness of the immune system to immune modulators, such as interferon.[21–23] The on-treatment ALT elevations observed during this trial, which were predictive of HBsAg loss appear to lend support to the importance of an immune modulator in eliciting the host response required for control of infection and HBsAg loss. This is also supported by the significantly lower rate of response in group B, which included only 16 weeks of peginterferon (included to explore whether limiting the duration of peginterferon affected rates of HBsAg loss). As would be expected, the level of HBsAg decline in group B was similar to that in group A until peginterferon was discontinued; however, upon discontinuation of peginterferon at week 16, HBsAg levels plateaued in group B so that few patients had achieved HBsAg loss at week 48. These data suggest that a peginterferon-sparing strategy is not sufficient to result in sustained immune control in most patients with hepatitis B. There was one case of hepatic encephalopathy and one case of ascites in patients who had received a short course of peginterferon. As there is a risk of liver decompensation in patents stopping peginterferon treatment, it is important that patients are screened carefully for underlying advanced fibrosis before considering such a treatment strategy.[1]

Although earlier trials combining lamivudine with peginterferon did not improve HBsAg loss rates over those observed with peginterferon alone,[11,12,24] subsequent studies have suggested that combination therapy may induce greater HBsAg decline, possibly improving rates of HBsAg loss.[16,25,26] However, these studies were limited by short duration, small sample size, and lack of control arms, as well as poor tolerability of the regimens studied.[27] Our study is the first to provide definitive evidence that patients receiving a potent oral agent, with high barrier to resistance, in combination with peginterferon can achieve higher rates of HBsAg loss than those receiving monotherapy, with no increase in adverse events compared with peginterferon alone.

Table 2. Efficacy Results at Weeks 48 and 72

Response	Group A (n = 186) TDF + peginterferon for 48 wk	Group B (n = 184) TDF + peginterferon for 16 weeks, TDF for 32 wk	Group C (n = 185) TDF for 120 wk	Group D (n = 185) peginterferon for 48 wk
HBsAg loss				
Kaplan–Meier estimate, %				
Wk 48	7.3	2.8	0	2.8
Wk 72	9.1	2.8	0	2.8
P values for wk 72				
vs group C	<.001	NS		
vs group D	.003	NS		
Markov 2-state estimate, %				
Wk 48	7.3	2.3		
Wk 72	6.8	1.1	0	2.8
P values for wk 72			0	2.8
vs group C	<.001	NS		
vs group D	NS	NS		
HBsAg seroconversion				
Kaplan–Meier estimate, %				
Wk 48	5.7	0.6	0	2.3
Wk 72	8.1	0.6	0	2.9
HBsAg change from baseline, log_{10} IU/mL[a]				
Wk 48	−1.1	−0.5	−0.3	-0.8
Wk 72	−1.3	−0.3	−0.4	-0.6
P values at wk 72				
vs group C	<.05	NS		
vs group D	<.05	NS		
HBeAg loss, n/N (%)[b]				
Wk 48	28/108 (25.9)	21/105 (20.0)	9/109 (8.3)	14/106 (13.5)
Wk 72	32/108 (29.6)	26/105 (24.8)	16/109 (14.7)	27/106 (25.5)
P values at wk 72				
vs group C	.009	NS		
vs group D	NS	NS		
HBeAg seroconversion, n/N (%)[b]				
Wk 48	25/108 (23.1)	20/105 (19.0)	9/109 (8.3)	13/106 (12.3)
Wk 72	27/108 (25.0)	25/105 (23.8)	14/109 (12.8)	26/106 (24.5)
P values at wk 72				
vs group C	.025	.051		
vs group D	NS	NS		
HBV DNA <15 IU/mL, n/N (%)[c]				
Wk 48	128/186 (68.8)	131/184 (71.2)	112/185 (60.5)	39/183 (21.3)
Wk 72	17/186 (9.1)	12/184 (6.5)	133/185 (71.9)	17/185 (9.2)
P values at wk 72				
vs group C	<.001	<.001		
vs group D	NS	NS		
ALT normalization – n/N (%)[c]				
Wk 48	70/172 (40.7)	118/172 (68.6)	111/176 (63.1)	53/173 (30.6)
Wk 72	63/172 (36.6)	62/172 (36.1)	128/176 (72.7)	64/173 (37.0)
Sustained response (HBV DNA <2000 IU/mL and ALT normalization), n/N (%)[c]				
Week 72	40/172 (23.3)	37/172 (21.5)	NA	32/173 (18.5)

NA, not applicable.
[a]Missing data at analyzed time point or data from all time points after the start of TDF retreatment were excluded from analysis.
[b]Patients with missing data at the analyzed time point were considered nonresponders at that analyzed end point.
[c]Patients with missing data at the analyzed time point or patients who started on TDF retreatment were considered nonresponders at that analyzed end point.

Table 3. Cumulative Hepatitis B Surface Antigen Loss Up To Week 72 According To Hepatitis B e Antigen Status and Genotype

	Group A (n = 186) TDF + peginterferon for 48 wk		Group B (n = 184) TDF + peginterferon for 16 weeks, then TDF for 32 wk		Group C (n = 185) TDF for 120 wk		Group D (n = 185) Peginterferon for 48 wk	
	Wk 48	Wk 72	Wk 48	Wk 72	Wk 48	Wk 72	Wk 48	Wk 72
HBeAg-positive (n = 428), n/N (%)								
Overall	7/108 (6.5)	10/108 (9.3)	3/105 (2.9)	4/105 (3.8)	0/109	0/109	4/106 (3.8)	4/106 (3.8)
Genotype A	2/8 (25.0)	3/8 (37.5)	1/8 (12.5)	2/8 (25.0)	0/6	0/6	0/5	0/5
Genotype B	2/27 (7.4)	3/27 (11.1)	1/26 (3.8)	1/26 (3.8)	0/25	0/25	2/26 (7.7)	2/26 (7.7)
Genotype C	2/57 (3.5)	3/57 (5.3)	0/57	0/57	0/59	0/59	1/58 (1.7)	1/58 (1.7)
Genotype D	1/15 (6.7)	1/15 (6.7)	1/13 (7.7)	1/13 (7.7)	0/17	0/17	1/16 (6.3)	1/16 (6.3)
Genotypes E–H	0/1	0/1	0/1	0/1	0/2	0/2	0/1	0/1
HBeAg-negative (n = 312), n/N (%)								
Overall	4/78 (5.1)	6/78 (5.1)	1/79 (1.3)	1/79 (1.3)	0/76	0/76	1/79 (1.3)	1/79 (1.3)
Genotype A	2/9 (22.2)	3/9 (33.3)	0/8	0/8	0/8	0/8	0/9	0/9
Genotype B	1/23 (4.3)	2/23 (8.7)	1/25 (4.0)	1/25 (4.0)	0/24	0/24	1/27 (3.7)	1/27 (3.7)
Genotype C	1/21 (4.8)	1/21 (4.8)	0/22	0/22	0/19	0/19	0/21	0/21
Genotype D	0/24	0/24	0/23	0/23	0/24	0/24	0/22	0/22
Genotypes E–H	0/1	0/1	0/1	0/1	0/1	0/1	0/0	0/0

NOTE. Overall, 7 patients seroreverted on or after wk 48; 5 HBeAg-positive patients (3 in group A: 2 genotype A patients and 1 genotype B patient, and 2 in group B: genotype A) and 2 HBeAg-negative patients (1 in group A: genotype B; 1 in group B: genotype B).

This study provides the proof-of-concept that combination therapy, unlike peginterferon or TDF monotherapies, can induce HBsAg loss at a similar frequency in both HBeAg-positive and HBeAg-negative patients and across all major genotypes. However, response is not entirely uniform and most patients do not achieve sustained immune control. Patients with certain baseline characteristics (eg, genotype A, HBeAg-positive status) tend to have higher rates of HBsAg loss. These results are consistent with previous studies of peginterferon monotherapy, where highest rates of HBsAg loss were achieved in genotype A patients[28] and in patients with higher ALT levels.[29,30] Indeed, the differences between baseline characteristics in the current study and the phase 3 peginterferon studies[11,12] could explain the slightly lower rate of HBsAg loss in this study than observed with peginterferon previously. For instance, more patients with lower ALT levels than the phase 3 peginterferon studies, a factor that has been associated with lower response rates to interferon.[1] Further work is needed to identify subgroups of patients who might benefit most from combination therapy, and to determine optimal duration of therapy that would minimize post-treatment HBsAg seroreversion. Five of the 7 patients receiving combination therapy who

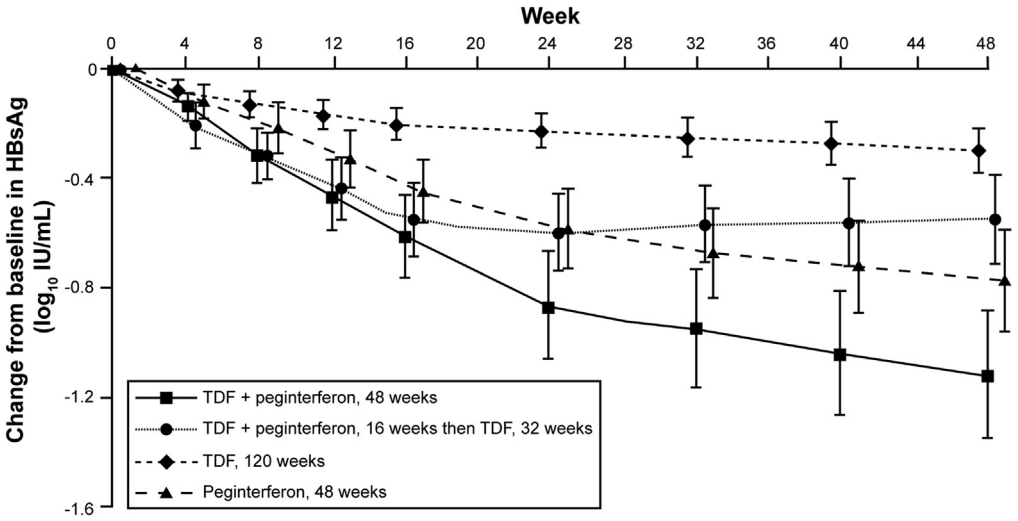


Figure 2. On-treatment HBsAg decline from baseline to week 48. Data shown are mean ± 95% CIs. Group A vs group B, $P < .001$; group A vs group C, $P < .001$; group A vs D, $P = .016$.

Table 4. Treatment Discontinuations and Adverse Events

Variable	Group A (n = 186) TDF + peginterferon for 48 wk		Group B (n = 184) TDF + peginterferon for 16 wk, then TDF for 32 wk		Group C (n =185) TDF for 120 wk	Group D (n = 185) peginterferon for 48 wk	
	All time points without TDF retreatment (n = 186)	All time points on TDF retreatment (n = 100)	All time points without TDF retreatment (n = 184)	All time points on TDF retreatment (n = 100)	Continued TDF treatment (n = 185)	All time points without TDF retreatment (n = 185)	All time points on TDF retreatment (n = 112)
Any nonserious adverse event, no. of patients (%)[a]	145 (78)	17 (17)	138 (75)	21 (21)	81 (44)	150 (81)	25 (22)
Any serious adverse event, no. of patients (%)	21 (11)	7 (7)	18 (10)	3 (3)	12 (7)	18 (10)	5 (5)
Discontinuation of treatment due to adverse events, n (%)	8 (4)	1	4 (2)	1 (1)	0 (0)	14 (8)	0
Common adverse events, no. of patients (%)[b]							
Headache	54 (29)	3 (3)	37 (20)	2 (2)	16 (9)	52 (28)	2 (2)
Alopecia	46 (25)	0	32 (17)	0	1 (1)	45 (24)	0
Pyrexia	39 (21)	1 (1)	36 (20)	0	7 (4)	43 (23)	2 (2)
Fatigue	40 (22)	1 (1)	33 (18)	4 (4)	20 (11)	41 (22)	5 (5)
Decreased appetite	23 (12)	0	36 (20)	2 (2)	2 (1)	18 (10)	0
Myalgia	29 (16)	0	36 (20)	1 (1)	2 (1)	36 (20	1 (1)
Nausea@	26 (14)	0	24 (13)	3 (3)	10 (5)	13 (7)	6 (5)
Pruritus	15 (8)	0	14 (8)	2 (2)	4 (2)	22 (12)	0
Asthenia	20 (11)	0	9 (5)	1 (1)	3 (2)	12 (7)	0
Malaise@	20 (11)	0	12 (7)	0	2 (1)	7 (4)	2 (2)
Dizziness	20 (11)	0	18 (10)	0	9 (5)	17 (9)	4 (4)
Rash	20 (11)	1 (1)	17 (9)	1 (1)	1 (1)	9 (5)	2 (2)
Diarrhea	13 (7)	0	10 (5)	0	9 (5)	19 (10)	4 (4)
Influenza-like illness	19 (10)	0	17 (9)	0	10 (5)	16 (9)	2 (2)
Insomnia	19 (10)	0	14 (8)	0	6 (3)	18 (10)	1 (1)
Psychiatric disorders[c]	14 (8)	0	3 (2)	0	2 (1)	9 (5)	3 (3)
Anxiety	8 (4)	0	2 (1)	0	0	7 (4)	1 (1)
Nasopharyngitis	5 (3)	4 (4)	16 (9)	2 (2)	18 (10)	6 (3)	1 (1)
Grade 3/4 laboratory abnormalities, no. of patients (%)							
Anemia	8 (4)	0	3 (2)	0	2 (1)	4 (2)	1 (1)
Lymphopenia	12 (6)	1 (1)	7 (4)	0	7 (4)	11 (6)	1 (1)
Neutropenia	30 (16)	0	21 (11)	2 (2)	2 (1)	27 (15)	1 (1)
Thrombocytopenia	3 (2)	0	4 (2)	0	0	10 (5)	0

Table 4. Continued

Variable	Group A (n = 186) TDF + peginterferon for 48 wk		Group B (n = 184) TDF + peginterferon for 16 wk, then TDF for 32 wk		Group C (n =185) TDF for 120 wk	Group D (n = 185) peginterferon for 48 wk	
	All time points without TDF retreatment (n = 186)	All time points on TDF retreatment (n = 100)	All time points without TDF retreatment (n = 184)	All time points on TDF retreatment (n = 100)	Continued TDF treatment (n = 185)	All time points without TDF retreatment (n = 185)	All time points on TDF retreatment (n = 112)
Patients with ALT >400 U/L (men) or >300 U/L (women), n/N (%)							
On-treatment	17/186 (9)	20/100 (20)	17/184 (9)	21/100 (21)	3/185 (2)	17/185 (9)	15/112 (13)
Off-treatment	25/186 (13)	NA	26/184 (14)	0/100	NA	14/185 (8)	NA

NA, not applicable.
[a]Includes nonserious adverse events occurring in ≥5% of patients.
[b]The listed events were reported in at least 10% of patients in any study group. Not retreated includes all patients who had not reinitiated TDF at the time point.
[c]Includes depression, depressed mood, and dysthymic disorders.

experienced HBsAg seroreversion after HBsAg loss had achieved HBsAg loss at the end or shortly after the end of their treatment and all seroconversions occurred within 3 months after the end of treatment. This suggests a possible need for extension of therapy in this group of responders to ensure durable HBsAg loss. The clinical relevance of these results is still to be established. The increased rate of HBsAg loss with combination therapy vs monotherapy is encouraging, especially in genotype A patients, but it is currently unclear whether the magnitude of this increase will be sufficient to change clinical practice. There is also the need to understand more about the most effective way of combining therapy. An alternative strategy, where peginterferon is added to oral antiviral therapy in virally suppressed patients has also demonstrated increased rates of HBsAg loss, which is sustained 24 weeks after stopping therapy, compared with oral antiviral therapy alone.[31] However, the lack of a peginterferon monotherapy treatment arm in this peginterferon add-on study by Bourliere and colleagues[31] means that it is not possible to conclude that this effect is greater than would be achieved without the oral agent.

Despite the fact that the observed rate of HBsAg loss in the study was lower than that assumed in the study design, reducing the power for comparison between groups A and D, statistical significance was still demonstrated between groups A and D. We do not believe the lack of power affects the interpretation of results. Because this study did not include patients with cirrhosis or bridging fibrosis, the extrapolation of results to these patients is not possible. Similarly, as only a small number of patients had been previously treated with nucleoside analogs (n = 33), there were insufficient data to assess the influence of prior HBV therapy on treatment outcomes. In addition, unlike previous studies, our study permitted TDF retreatment for patients who met certain criteria, such as increased HBV replication and serum ALT elevation. It is unclear if this management may have affected HBsAg decline and loss in this subpopulation. It is also important to note that this was an open-label, rather than a double-blind study. Finally, prolonged follow-up of patients who had not restarted TDF treatment would be required to determine the long-term benefits of response and durability of outcome.

In conclusion, combination therapy with TDF plus peginterferon for 48 weeks resulted in higher rates of HBsAg loss than either monotherapy. This provides support to the concept of combination therapy of finite duration for patients with CHB. Further studies are required to identify the optimal combination therapy regimen that would allow more patients to achieve and sustain HBsAg loss.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.09.043.

References

1. European Association for the Study of the Liver (EASL). EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57:167–185.
2. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008; 48:335–352.
3. Chan HL, Wong VW, Tse AM, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol 2007;5:1462–1468.
4. Moucari R, Mackiewicz V, Lada O, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009;49:1151–1157.
5. Martinot-Peignoux M, Lapalus M, Asselah T, et al. HBsAg quantification: useful for monitoring natural history and treatment outcome. Liver Int 2014;34(Suppl 1):97–107.
6. **Moucari R, Korevaar A**, Lada O, et al. High rates of HBsAg seroconversion in HBeAg-positive chronic hepatitis B patients responding to interferon: a long-term follow-up study. J Hepatol 2009;50:1084–1092.
7. Kim GA, Lim YS, An J, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut 2014; 63:1325–1332.
8. Liaw Y-F, Kao J-H, Piratvisuth T, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531–561.
9. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009;50:661–662.
10. Perrillo R. Benefits and risks of interferon therapy for hepatitis B. Hepatology 2009;49(5 Suppl):S103–S111.
11. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206–1217.
12. Lau GKK, Piratvisuth T, Luo KX, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682–2695.
13. Marcellin P, Heathcote E, Buti M, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442–2445.
14. Wursthorn K, Lutgehetmann M, Dandri M, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology 2006;44:675–684.
15. Chan HL-Y, Hui AY, Wong VW-S, et al. Long-term follow-up of peginterferon and lamivudine combination treatment in HBeAg-positive chronic hepatitis B. Hepatology 2005;41:1357–1364.
16. Takkenberg RB, Jansen L, de Niet A, et al. Baseline hepatitis B surface antigen (HBsAg) as predictor of sustained HBsAg loss in chronic hepatitis B patients treated with pegylated interferon-alpha2a and adefovir. Antivir Ther 2013;18:895–904.
17. Ning Q, Han M, Sun Y, et al. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). J Hepatol 2014;61:777–784.
18. Brouwer WP, Xie Q, Sonneveld MJ, et al. Adding peginterferon to entecavir for HBeAg-positive chronic hepatitis B: a multicentre randomized trial (ARES study). Hepatology 2015;61:1512–1522.
19. Dmitrienko A, Tamhane AC, Wiens BL. General multistage gatekeeping procedures. Biom J 2008; 50:667–677.
20. **Lütgehetmann M, Bornscheuer T**, Volz T, et al. Hepatitis B virus limits response of human hepatocytes to interferon-alpha in chimeric mice. Gastroenterology 2011;140:2074–2083.
21. Boni C, Laccabue D, Lampertico P, et al. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology 2012;143:963–973.
22. Tan AT, Hoang LT, Chin D, et al. Reduction of HBV replication prolongs the early immunological response to IFNalpha therapy. J Hepatol 2014;60:54–61.
23. Xu C, Guo H, Pan XB, et al. Interferons accelerate decay of replication-competent nucleocapsids of hepatitis B virus. J Virol 2010;84:9332–9340.
24. Janssen HL, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005;365:123–129.
25. Xie Q, Zhou H, Bai X, et al. A randomized, open-label clinical study of combined pegylated interferon alfa-2a (40KD) and entecavir treatment for hepatitis B "e" antigen-positive chronic hepatitis B. Clin Infect Dis 2014; 59:1714–1723.
26. Piccolo P, Lenci I, Demelia L, et al. A randomized controlled trial of pegylated interferon-alpha2a plus adefovir dipivoxil for hepatitis B e antigen-negative chronic hepatitis B. Antivir Ther 2009;14:1165–1174.
27. Marcellin P, Wursthorn K, Wedemeyer H, et al. Telbivudine plus pegylated interferon alfa-2a in a randomized study in chronic hepatitis B is associated with an unexpected high rate of peripheral neuropathy. J Hepatol 2015;62:41–47.
28. Janssen HL, Gerken G, Carreno V, et al. Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1999;30:238–243.
29. Perrillo RP, Schiff ER, Davis GL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. N Engl J Med 1990;323:295–301.
30. Lok AS, Wu PC, Lai CL, et al. A controlled trial of interferon with or without predisone priming for chronic hepatitis B. Gastroenterology 1992;102:2091–2097.
31. Bourliere M, Rabiega P, Ganne-Carrie N, et al. HBsAg clearance after addition of 48 weeks of PEGIFN in HBeAg negative CHB patients on nucleos(t)ide therapy with undetectable HBV DNA for at least one year: final results from ANRS-HB06 PEGAN study: multicenter,

randomized controlled phase III trial. J Hepatol 2015; 62(Suppl 2):S249.

Author names in bold designate shared co-first authorship.

Received June 11, 2015. Accepted September 30, 2015.

Reprint requests
Address requests for reprints to: Henry L. Y. Chan, MD, Department of Medicine and Therapeutics and Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong. e-mail: hlychan@cuhk.edu.hk; fax: +852 26373852.

Acknowledgments
The authors thank the patients and their families, as well as our medical writer, Dr Liesje Quine (Elements Communications) and medical editor, Fiona McGarvey (Elements Communications) and the following employees of Gilead Sciences: Nicole Ramza and Allison Fidelholtz for study conduct, Bryan Le for study data management, Kathryn Kitrinos for resistance analysis, Neby Bekele for statistical analyses, and David McNeel for critical review.

Conflicts of interest
These authors disclose the following: Patrick Marcellin: Speaker and investigator for Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead Sciences, Janssen, Merck, Roche, and Tibotec. Sang Hoon Ahn: Advisor and lecturer for Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, MSD, Novartis, Roche. Unrestricted research grants from Bristol-Myers Squibb, Gilead Sciences, and Roche. Florin A Caruntu: Advisor and speaker for AbbVie, Bristol-Myers Squibb, Gilead Sciences, GSK, Janssen, MSD, and Roche. Research grants from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Roche. Magdy Elkashab: CME speaker fees and Advisory Board consultation fees from Gilead Sciences. Wan-Long Chuang: Member of advisory board: AbbVie, Boehringer Ingelheim, Gilead Sciences, Novartis, Roche; Speaker: Bristol-Myer Squibb, Gilead Sciences, MSD, and Roche. Seng Gee Lim: Advisory Board member: AbbVie, Boehingher Ingelheim, Bristol-Myer Squibb, Gilead Sciences, MSD, Novartis, Pfizer, Vertex, Tobira; Speakers Bureau: Bristol-Myers Squibb, GSK, Novartis, Roche. Educational/research funding: Bristol-Myers Squibb, Gilead Sciences, MSD, Novartis. Fehmi Tabak: Local advisor for AbbVie, Bristol-Myers Squibb, Gilead Sciences, GSK, and speaker for AbbVie, Bristol-Myers Squibb, Gilead Sciences, GSK, and Janssen. Joerg Petersen: Grant/Research Support from Bristol-Myers Squibb, Novartis, Roche; research support from: AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead Sciences, Janssen, Merck, MSD, Roche, Siemens, Vertex; Consultant/Advisor for Abbott, AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim, GSK, Kedrion, Janssen, Merck, MSD, Novartis, Roche; Sponsored lectures: Abbott, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead Sciences, Kedrion, Janssen, Merck, MSD, Novartis, Roche. Graham R Foster: AbbVie, Boehringer Ingelheim, Bristol Myers Squibb, Gilead Sciences, Roche, Merck, Novartis, Tekmira. Lilian Lou: Stockholder of Gilead Sciences, Inc and former employee. Eduardo B Martins: Employee and stockholder of Gilead Sciences, Inc Philip Dinh: Employee and stockholder of Gilead Sciences, Inc Lanjia Lin: Employee and stockholder of Gilead Sciences, Inc Amy Corsa: Employee and stock holder of Gilead Sciences, Inc Prista Charuworn: Employee and stockholder of Gilead Sciences, Inc G. Mani Subramanian: Employee and stockholder of Gilead Sciences, Inc Hans Reiser: Employee and stock holder of Gilead Sciences, Inc Hendrick W Reesink: Consulting for AbbVie, Alnylam, Astex, Bristol-Myers Squibb, Gilead Sciences, GSK, Janssen-Cilag, Korean Green Cross, MSD, PRA-International, Regulus, Replicor, Roche, R-Pharm, Santaris; Research support from: AbbVie, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, MSD, PRA-International, Regulus, Replicor, Roche, Santaris. Scott Fung: Research grants, speaker and advisor for: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Roche; Research grant from Boehringer Ingelheim. Consultant: GSK. Simone I Strasser: Speaker and advisory board member for AbbVie, Bayer, Bristol-Myers Squibb, Gilead Sciences, Janssen, MSD/Merck, Norgine, Roche. Huy Trinh: Speaker, advisory board member, and research grants for/from Bristol-Myers Squibb and Gilead Sciences. Stockholder for Gilead Sciences Inc Maria Buti: Gilead Sciences, MSD and Novartis. Giovanni Gaeta: Speaker or advisor for: AbbVie, Bristol Myers Squibb, Gilead Sciences, Merck, Roche. George Papatheodoridis: Advisor/lecturer for Bristol-Myers Squibb, Gilead Sciences, Novartis and Roche; research grants from Bristol-Myers Squibb, Gilead Sciences and Roche. Robert Flisiak: Research grants from Bristol-Myers Squibb, Gilead Sciences, Janssen, MSD, Novartis, Roche. Advisory board member for AbbVie, Bristol-Myer Squibb, Boehringer-Ingelheim, Gilead Sciences, Janssen, MSD, Novartis, Roche. Speaker for AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, MSD, Roche. Henry L. Y. Chan: Advisor and speaker for AbbVie, Bristol-Myers Squibb, Gilead Sciences, Roche, MSD and Novartis; speaker for GSK, Echosens; unrestricted grant for HBV research from Roche. The remaining authors disclose no conflicts.

Funding
This study was supported by Gilead Sciences. The study was designed and conducted according to the protocol by the sponsor (Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data, monitored the study conduct, and performed the statistical analysis. All authors had access to the data and assume responsibility for the integrity and completeness of the reported data. All authors made substantial contributions to the conception or design of the study, acquisition, analysis, or interpretation of the data and were involved in the drafting of the manuscript. Phillip Dinh and Lanjia Lin provided statistical analysis. The final decision to submit the manuscript for publication was the responsibility of Patrick Marcellin and Henry L. Y. Chan. Writing support was provided by Dr Liesje Quine, Elements Communications Ltd, Westerham, Kent and funded by Gilead Sciences.

List of Investigators

The phase 4, randomized, open-label, active-controlled, superiority study to evaluate the efficacy and safety of TDF in combination with peginterferon α-2a (Pegasys; peginterferon) vs standard of care TDG monotherapy or peginterferon monotherapy for 48 weeks in noncirrhotic subjects with HBeAg-positive or HBeAg-negative CHB investigators are as follows:

Australia: Peter Angus, Wendy Cheng, Paul Desmond, Anouk Dev, Jacob George, Hugh Harley, Edmund Tse, Ian Kronborg, Alice Lee, Barbara Leggett, Miriam Levy, John Lubel, Graeme MacDonald, Gerard MacQuillan, Lindsay Mollison, Stuart Roberts, Joe Sasadeusz, Simone Strasser, Alan Wigg.

Canada: Curtis Cooper, Magdy Elkashab, Scott Fung, Peter WC Kwan, Samuel Lee, Mang Ma, Alnoor Ramji, Edward Tam, Florence Wong, Robert Bailey.

France: Jean-Didier Grange, Dominique Guyader, François Habersetzer, Patrick Marcellin, Ghassan Riachi, Didier Samuel.

Germany: Guido Gerken, Michael P Manns, Joerg Petersen, Eckart Schott, Marcus Schuchmann, Annette Grambihler, Stefan Zeuzem.

Greece: George Papatheodoridis, Maria Raptopoulou-Gigi.

Hong Kong: Henry Lik Yuen Chan, Aric Josun Hui, Tak Yin Owen Tsang, Man Fung Yuen.

India: Abhijit Chowdhury, Superna Pal, Subrat Kumar Acharya, Dr Shalimar, Dharmesh Kapoor, Sachin Palnitkar, Raj V Venugopal, Shrikant Mukewar, Shiv Kumar Sarin, Manoj K Sharma, Samir Shar, Parijat Gupte, Rajiv Mehta, Saumin Shah, VG Mohan Prasad.

Italy: Giovanni B Gaeta, Cinzia Balestrieri, Massimo Levrero, Mario Rizzetto.

Netherlands: Harry LA Janssen, Robert DeKnegt, Hendrik Reesink.

Poland: Robert Flisiak, Waldemar Halota, Andrzej Horban, Wlodzimierz Mazur, Maciej Jablkowski, Anna Piekarska, Krzysztof Tomasiewicz.

Portugal: Guilherme Macedo, Isabel Pedroto, Jose Velosa.

Romania: Florin A Caruntu, Emanoil Ceausu, Paul-Jurgen Porr, Adrian Streinu-Cercel, Adrian Goldis.

Singapore: Pik Eu Jason Chang, Seng Gee Lim, Eng Kiong Teo, Wei Lyn Yang.

South Korea: Si-Hyun Bae, Soon Koo Baik, Kwan Soo Byun, Sang Hoon Ahn, Jeong Heo, Seong Gyu Hwang, Hong Soo Kim, Yoon Jun Kim, So Young Kwon, June Sung Lee, Kwan Sik Lee, Nam Ik Han, Young-Suk Lim, Won Young Tak, Hyung Joon Yim, Young Kul Jung.

Spain: Raul Andrade, Maria Buti, Luis Morano, Juan Manuel Pascasio.

Taiwan: Ting-Tsung Chang, Chi-Yi Chen, Tzy-Yen Chen, Rong-Nan Chien, Wan-Long Chuang, Chao-Wei Hsu, Chi-Tan Hu, Jia-Horng Kao, Chuan-Mo Lee, Tzong-His Lee, Cheng-Yuan Peng, Wei-Wen Su, Sien-Sing Yang, Sheng-Shun Yang.

Turkey: Ramazan Idilman, Abdurrahman Kadayifçi, Orhan Sezgin, Ömer Fehmi Tabak.

United Kingdom: Kosh Agarwal, Graham Foster, David Mutimer.

United States: Ho Bae, Luis Balart, Nathan Shores, Marin William Moehlen, Terry Box, Juan Gallegos-Orozco, Kimberly Brown, Greg Galler, Raouf Hilal, Ira Jacobson, Hannah Lee, Xiaoli Ma, Albert Min, Ronald Nahass, Mindie Nguyen, Tuan Nguyen, Calvin Pan, James Park, Natarajan Ravendhran, Nancy Reau, Arun Sanyal, Mitchell Shiffman, Huy Trinh, John Vierling, John Hill.

Criteria for Retreatment

Patients in Groups A, B, or D who had stopped treatment at week 48 were monitored every 2 weeks for the first 12 weeks off therapy and then every 4 weeks for the subsequent 4 weeks to determine if they required prompt flare management by initiating/restarting TDF monotherapy. Patients initiated/restarted TDF if they met one of the following criteria:

- Increase of total bilirubin by >1.5 mg/dL (>25 μmol/L) from study baseline andconcurrent ALT >30 U/L for males and >19 U/L for females
- Increase in International Normalized Ratio (INR) >0.5 from study baseline or elevated INR ≥ 1.7, whichever is lowest, in subjects with adequate vitamin K therapy and concurrent ALT >30 U/L for males and >19 U/L for females
- Lactate levels greater than upper limit of normal (ULN) that were not a result of obvious nonhepatic etiology
- ALT >400 U/L in males or >300 U/L in females with or without associated symptoms
- Any ALT elevation associated with change in other laboratory parameters suggestive of worsening hepatic function
- HBV DNA >5865 IU/mL (2000 copies/mL) assessed at 24 weeks' off-treatment (irrespective of ALT level)
- Clinical or laboratory manifestations of hepatic decompensation
- In males, ALT >60 IU/L and ≤ 150 IU/L and in females ALT >38 U/L and ≤ 95 U/L persisting for 84 days and HBV DNA ≥ 15 IU/mL
- ALT >150 IU/L and ≤ 400 IU/L (male) or ALT >95 U/L and ≤ 300 U/L (female) persisting for ≥ 28 days

Patients requiring TDF retreatment continued treatment until week 120.

Patients with persistently elevated ALT levels with >60 U/L for males (but <400 U/L) or >38 U/L for females (but <300 U/L) with HBV DNA ≥ 15 IU/mL were followed up at least every 2 weeks until meeting a criterion for TDF reinitiation or until ALT returned to normal levels.

Randomization and Masking

Randomization was stratified by screening HBeAg status and HBV genotype, forming a total of 10 strata as shown in Supplementary Table 1. Subjects were allocated into 1 of the 4 treatment arms in the 1:1:1:1 ratio.

Eligibility Criteria

Inclusion Criteria

All patients had to meet the following inclusion criteria:

- Adult subjects (aged 18−75 years) with documented CHB (eg, positive for either serum HBsAg or HBV DNA for at least 6 months) before baseline
- Anti-HBV treatment-naïve subjects and subjects who have taken oral anti-HBV nucleoside therapy (lamivudine, telbivudine, clevudine, or entecavir) with the last dose at least 24 weeks before screening
- HBeAg-positive or HBeAg-negative
- HBV DNA $\geq$20,000 IU/mL for HBeAg-positive patients and HBV DNA $\geq$2000 IU/ml for HBeAg-negative patients
- ALT $>$54 U/L and $\leq$400 U/L for men and $>$36 U/L and $\leq$300 U/L for women
- Creatinine clearance (CLcr) $\geq$70 mL/min, as calculated by the Cockcroft−Gault equation (performed by the central laboratory)

Exclusion Criteria

Subjects who meet any of the following exclusion criteria were excluded from the study:

- Evidence of bridging fibrosis or cirrhosis at screening as confirmed by liver biopsy within 12 months before first visit. In countries where FibroScan is approved for clinical use, FibroScan ($<$8 kPa) could be used to verify the absence of bridging fibrosis and cirrhosis. Patients with FibroScan $>$8 kPa were excluded unless a biopsy within the 12 months before first visit confirmed the absence of bridging fibrosis and cirrhosis
- Evidence of decompensated liver disease defined as direct (conjugated) bilirubin $>1.2 \times$ ULN, prothrombin time $>1.2\times$ ULN, serum albumin $<$3.5 g/dL or history of clinical hepatic decompensation (eg, ascites, jaundice, encephalopathy, variceal hemorrhage). Patients with an isolated increase in direct bilirubin $>$6.1 μmol/L but $<$10.2 μmol/L, in the presence of a platelet count $>$100,000/mm^3 and normal INR may qualify for the trial
- Absolute neutrophil count $<$1500/mm^3, platelet $<$100,000/mm^3, hemoglobin $<$10 g/dL for female subjects or $<$11 g/dL for male subjects
- Evidence of hepatocellular carcinoma
- History of severe depression or known severe psychiatric disease
- History of significant renal disease (eg, nephrotic syndrome, renal dysgenesis, polycystic kidney disease, congenital nephrosis, acute tubular necrosis, other renal disease)
- Thyroid dysfunction
- History of autoimmune disease (eg, lupus, rheumatoid arthritis, autoimmune thyroiditis)
- Significant bone disease (eg, osteomalacia, chronic osteomyelitis, osteogenesis imperfecta, osteochrondroses, history of multiple bone fractures)
- Pregnant. A negative serum pregnancy test was required for female subjects of childbearing potential and all sexually active female subjects of childbearing potential had to agree to use a protocol-recommended method of contraception during heterosexual intercourse throughout the study and for 30 days after the last dose of study medication. Lactating female patients had to agree to discontinue nursing before initiation of the study
- Previous treatment with any form of interferons and anti-HBV nucleotides (TDF or adefovir dipivoxil)
- Co-infection with human immunodeficiency virus, hepatitis C virus, or hepatitis delta virus
- Ongoing therapy with any of the following: nephrotoxic agents; parenteral aminoglycoside antibiotics (eg, gentamicin, tobramycin, amikacin); cidofovir; cisplatin; foscarnet; intravenous amphotericin B; intravenous pentamidine; oral or intravenous ganciclovir; ciclosporin; tacrolimus; intravenous vancomycin; chronic daily nonsteroidal anti-inflammatory drug therapy; hepatotoxic agents (eg, anabolic steroids, isoniazid, itraconazole, ketoconazole, rifabutin, rifampin, and other agents with significant hepatotoxic potential); competitors of renal excretion (eg, probenecid); systemic chemotherapeutic agents; systemic corticosteroids; interleukin-2; and other immunomodulating agents and investigational agents (unless approved by the sponsor). Administration of any of these medications had to be discontinued at least 30 days before the baseline visit and for the duration of the study period
- Known hypersensitivity to the study investigational medicinal product, metabolites, or formulation excipients
- Any other condition (including alcohol or substance abuse) or prior therapy that, in the opinion of the investigator, would make the subject unsuitable for the study or unable to comply with dosing requirements

Study Visit Schedule

Study visits occurred every 4 weeks between week 4 and week 16 of treatment and then every 8 weeks from week 24 to week 48. After week 48, visits occurred every 2 weeks up to week 60, every 4 weeks up to week 72 and 12 weeks up to week 120. The assessments at each visit are shown in Supplementary Table 1.

Assessment of Adverse Events

All adverse events were assessed and recorded by the investigator as described in the protocol. A serious adverse event was defined as any adverse drug experience that resulted in death, a life-threatening situation, hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity. Further details are provided in the protocol. In addition, specific to this study, the following had to be reported as a serious adverse event:

- Serum ALT >2 × baseline levels and >400 U/L (male) or 300 U/L (female), with or without associated symptoms
- Confirmed ALT elevation (defined as 1-grade shift or 2 × previous value) associated with confirmed changes outside of the normal range in other laboratory parameters suggestive of worsening hepatic function (total bilirubin ≥2 mg/dL above baseline, INR ≥0.5 over baseline or elevated INR ≥1.7, whichever is lowest, abnormal serum albumin ≥1 g/dL below baseline or elevated serum lactate levels (if available), defined as >2× ULN per the Adult AIDS Clinical Trials Group guidelines
- Any clinical manifestations of hepatic decompensation (variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis)

Resistance Monitoring

Serum samples collected at each time point were stored for drug-resistance monitoring. Genotypic analyses were conducted by population sequencing at DDL Diagnostic Laboratories (Rijswijk, The Netherlands). Population sequencing of the HBV polymerase/reverse transcriptase (amino acids 1−344) was attempted for any patient on a TDF-containing regimen for at least 24 weeks who met the following criteria: HBV DNA ≥117 IU/mL at week 48 (groups A and B) or week 72 (group C) or their last visit on treatment before discontinuation. This includes patients that may have experienced a virologic blip or breakthrough at weeks 48 or week 72, or their last visit on treatment. Virologic blips or breakthroughs were defined as 1 or 2 visits, respectively, with HBV DNA ≥117 IU/mL after having HBV DNA <117 IU/mL or a 1 $\log_{10}$ or greater increase in HBV DNA from nadir.

Methodology for sequence analysis as well and the definition of conserved/polymorphic site changes have been summarized previously.[1]

Additional Statistical Methods

The study had 4 prespecified hypotheses, divided into 2 families. The first family consisted of 2 hypotheses to evaluate the full course of TDF plus peginterferon for 48 weeks against 2 monotherapies: groups A vs group C and group D. The second family consisted of 2 hypotheses to evaluate the shorter course of TDF plus peginterferon for 16 weeks followed by 32 weeks of TDF alone against 2 monotherapies: group B vs groups C and D.

$\pi_{A,B,C,D}$ denoted the probability of HBsAg loss in groups A, B, C, and D, respectively. The first family consisted of 2 hypotheses:

$$H_{110}: \pi_A \leq \pi_C \quad H_{111}: \pi_A > \pi_C$$
$$H_{120}: \pi_A \leq \pi_D \quad H_{121}: \pi_A > \pi_D$$

The second family consisted of 2 hypotheses:

$$H_{210}: \pi_B \leq \pi_C \quad H_{211}: \pi_B > \pi_C$$
$$H_{220}: \pi_B \leq \pi_D \quad H_{221}: \pi_B > \pi_D$$

Each family was tested using a Holm separable multiple testing procedure with truncation fraction $\gamma = 0.5$. The details are as follows:

1. In family 1, let P_{11} and P_{12} be the P-values corresponding to hypotheses H_{110} and H_{120}. Order the P values from smallest to largest and denote them $P_{(11)} \leq P_{(12)}$ and the corresponding hypotheses $H_{(110)}$ and $H_{(120)}$, respectively. Test these hypotheses using the Holm procedure at an $\alpha = 0.05$.
   a. If $P_{(11)} > \alpha/2$, then fail to reject $H_{(110)}$ and all testing stops.
   b. If $P_{(11)} \leq \alpha/2$, then reject $H_{(110)}$.
      i. If $P_{(12)} > 3\alpha/4$, then fail to reject $H_{(120)}$. Go to family 2
      ii. If $P_{(12)} \leq 3\alpha/4$, then reject $H_{(120)}$. Go to family 2.
2. In family 2, let P_{21} and P_{22} be the P-values corresponding to hypotheses H_{210} and H_{220}. Order the P values from smallest to largest and denote them $P_{(21)} \leq P_{(22)}$ and the corresponding hypotheses $H_{(210)}$ and $H_{(220)}$, respectively. Test these hypotheses using the Holm procedure at an $\alpha = \alpha_2$.
   a. If $P_{(21)} > \alpha_2/2$, then fail to reject $H_{(210)}$ and all testing stops.
   b. If $P_{(11)} \leq \alpha_2/2$, then reject $H_{(210)}$.
      i. If $P_{(12)} > \alpha_2$, then fail to reject $H_{(220)}$.
      ii. If $P_{(12)} \leq \alpha_2$, then reject $H_{(220)}$.

If both hypotheses in family 1 are rejected, then $\alpha_2 = 0.05$. If only one hypothesis is rejected in family 1, then $\alpha_2 = \alpha - [\gamma + (1\text{-}\gamma)^*(1/2)]\ \alpha = \alpha - (3/4)\ \alpha = (1/4)\ \alpha = 0.0125$.

Additional Safety Details

A 51-year-old Asian female, randomized to group B, was documented as having a decompensating liver event shortly after week 72, while on drug-free follow-up. At the time of the decompensating event, the patient had ALT $<5\times$ ULN with central laboratory INR of 1.37. She was started on diuretics for management of ascites. The patient qualified for inclusion in the study as the screening liver biopsy showed stage 0 fibrosis. There was evidence of more advanced liver disease: a baseline computed tomography scan showed changes consistent with liver cirrhosis, a baseline esophagogastroduodenoscopy revealed evidence of esophageal varices with baseline laboratory showing platelet of $100/mm^3$. A computed tomography scan conducted in week 40 indicated changes consistent with cirrhosis with splenomegaly: esophageal/paraesophageal/gastric varices and ascites.

Reference

1. Kitrinos KM, Corsa A, Liu Y, et al. No detectable resistance to tenofovir disoproxil fumarate after 6 years of therapy in patients with chronic hepatitis B. Hepatology 2014;59:434–442.

Supplementary Table 1. Randomization Strata

Strata	HBeAg status	HBV genotype
1	Positive	A
2	Positive	B
3	Positive	C
4	Positive	D
5	Positive	E–H
6	Negative	A
7	Negative	B
8	Negative	C
9	Negative	D
10	Negative	E–H

Supplementary Table 2. Study Procedures

Procedures	Screening Up to −15 d	Baseline d 1	Every 4 wk wk 4, 8, 12, 16 ±2 d	Every 8 wk wk 24, 32, 40, 48 ±3 d	Every 2 wk wk 50, 52, 54, 56, 58, 60 ±2 d	Every 4 wk wk 64, 68, 72 ±3 d	Every 12 wk wk 84 ±3 d	Every 12 wk wk 96 ±3 d	Every 12 wk wk 108 ±3 d	Every 12 wk wk 120 ±3 d	Early discontinuation
Written informed consent	X										
Medical HBV history, treatment history	X	X									
Vital signs, height, weight, physical examination	X	X	X	X	X	X	X	X	X	X	X
HIV, HCV, HDV, HBV DNA genotyping	X										
α-Fetoprotein	X			X (wk 24, 48)		X (wk72)		X (wk 96)		X (wk 120)	
TSH	X		X (wk 12)	X (wk 24, 32, 48)							
Hepatic ultrasound or computed tomography	X										
Biopsy or FibroScan	X										
HBV DNA quantification and HBsAg quantification	X	X	X	X	X	X	X	X	X	X	X

HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; TSH, thyroid-stimulating hormone.

Supplementary Table 3. Reasons for Screening Failures

Inclusion criterion failed or exclusion criterion met	Description	No. of patients[a]
Inclusion criterion 1	Adult aged 18−75 y with chronic hepatitis B (positive serum HBsAg ≥6 mo)	32
Inclusion criterion 2	Anti-HBV treatment-naïve subjects. Subjects who have taken <12 wk of oral anti-HBV nucleoside therapy with the last dose ≥24 wk before screening are also eligible	4
Inclusion criterion 3	Positive or negative for HBeAg	6
Inclusion criterion 4	HBV DNA $\geq 10^5$ copies/mL for HBeAg-negative patients and $\geq 10^6$ copies/mL for HBeAg-positive patients Amendment 4: HBV DNA ≥20,000 IU/mL Amendment 5: HBV DNA ≥20,000 IU/mL for HBeAg-positive and ≥2000 IU/mL for HBeAg-negative	252
Inclusion criterion 5	ALT >2× ULN and ≤10× ULN Amendment 2: ALT >60 U/L and ≤400 U/L for men and >40 U/L and ≤300 U/L for women Amendment 4: ALT >54 U/L and ≤400 U/L for men and >36 U/L and ≤300 U/L for women	493
Inclusion criterion 6	CLcr ≥70 mL/min, as calculated by the Cockcroft-Gault equation Amendment 2: CLcr ≥80 mL/min Amendment 4: CLcr ≥70 mL/min	79
Inclusion criterion 7	A negative serum pregnancy test is required for female subjects of childbearing potential	2
Inclusion criterion 8	All sexually active female subjects of childbearing potential must agree to use a protocol-recommended method of contraception during heterosexual intercourse throughout the study and for 30 d after the last dose of study medication	2
Inclusion criterion 9	Lactating female subjects must agree to discontinue nursing before initiation of study investigational medicinal product	0
Exclusion criterion 1	Known cirrhosis according to clinical evaluation at study entry and confirmation by recent USSD/CT scan/Fibroscan and/or liver histology within 6 mo Amendment 1: Evidence of bridging fibrosis or cirrhosis	114
Exclusion criterion 2	Decompensated liver disease defined as direct (conjugated) bilirubin >1.2× ULN, prothrombin time >1.2× ULN, serum albumin <3.5 g/dL, or history of clinical hepatic decompensation (eg, ascites, jaundice, encephalopathy, variceal hemorrhage)	69
Exclusion criterion 3	Absolute neutrophil count $<1500/mm^3$, platelet count $<100{,}000/mm^3$, hemoglobin <10 g/dL for female subjects or <11 g/dL for male subjects	42
Exclusion criterion 4	Evidence of hepatocellular carcinoma	2
Exclusion criterion 5	History of depression or known psychiatric disease Amendment 2: History of severe depression or known severe psychiatric disease	1
Exclusion criterion 6	History of significant renal disease (eg, nephrotic syndrome, renal dysgenesis, polycystic kidney disease, congenital nephrosis, acute tubular necrosis, other renal disease)	0
Exclusion criterion 7	Thyroid dysfunction	19
Exclusion criterion 8	History of autoimmune disease (eg, lupus, rheumatoid arthritis, autoimmune thyroiditis)	2
Exclusion criterion 9	Significant bone disease (eg, osteomalacia, chronic osteomyelitis, osteogenesis imperfecta, osteochrondroses, history of multiple bone fractures)	0
Exclusion criterion 10	Pregnant	0
Exclusion criterion 11	Previous treatment with any form of interferons and anti-HBV nucleotides	4
Exclusion criterion 12	Co-infection with human immunodeficiency virus, hepatitis C virus or hepatitis delta virus	22
Exclusion criterion 13	Ongoing therapy with nephrotoxic agents, competitors of renal excretion, systemic chemotherapeutic agents, systemic corticosteroids, interleukin-2 and other immunomodulating agents, investigational agents	4
Exclusion criterion 14	Known hypersensitivity to the study investigational medicinal product, metabolites or formulation excipients	0
Exclusion criterion 15	Any other condition (including alcohol or substance abuse) or prior therapy that, in the opinion of the Investigator, would make the subject unsuitable for the study or unable to comply with dosing requirements	3

CLcr, creatine clearance; CT, computed tomography.
[a]Note that some patients met more than one criterion.

Supplementary Table 4. Adherence[a] to Treatment

	Group A (n = 186) TDF + peginterferon for 48 wk	Group B (n = 184) TDF + peginterferon for 16 wk, then TDF for 32 wk	Group C (n = 185) TDF for 120 wk	Group D (n = 185) peginterferon for 48 wk
Adherence to peginterferon, %, mean ± SD	98.5 ± 4.5	96.9 ± 10.5	NA	98.8 ± 3.6
≥80% adherence to peginterferon, no. of patients (%)	184 (99.5)	177 (97.3)	NA	181 (98.9)
Adherence to TDF, %, mean ± SD	98.5 ± 3.3	98.4 ± 4.3	98.6 ± 3.4	NA
≥80% adherence to TDF, no. of patients (%)	182 (98.9)	182 (99.5)	183 (98.9)	NA

NA, not applicable.
[a]Adherence to TDF was calculated as a ratio of the tablets taken over the tablets prescribed. Adherence to peginterferon was calculated as a ratio of the syringes administered over the syringes prescribed.

Supplementary Table 5. Cumulative Hepatitis B Surface Antigen Loss by Hepatitis B e Antigen Status and Genotype at Week 48

Cumulative HBsAg loss up to wk 48	Group A (n = 186) TDF + peginterferon for 48 wk	Group B (n = 184) TDF + peginterferon for 16 wk, then TDF for 32 wk	Group C (n = 185) TDF for 120 wk	Group D (n = 185) Peginterferon for 48 wk
HBeAg-positive (n = 432)				
Overall, n (%)	9/108 (8.3)	4/106 (3.8)	0/110	4/108 (3.7)
Genotype, n/N (%)				
A	3/8 (37.5)	2/8 (25.0)	0/6	0/6
B	3/27 (11.1)	1/26 (3.8)	0/26	2/27 (7.4)
C	2/57 (3.5)	0/58	0/59	1/58 (1.7)
D	1/15 (6.7)	1/13 (7.7)	0/17	1/16 (6.3)
E–H	0/1	0/1	0/2	0/1
HBeAg-negative (n = 308)				
Overall, n/N (%)	4/78 (7.8)	1/78 (1.3)	0/75	1/77 (1.3)
Genotype, n/N (%)				
A	2/9 (22.2)	0/8	0/8	0/8
B	1/23 (4.3)	1/25 (4.0)	0/23	1/26 (3.8)
C	1/21 (4.8)	0/21	0/19	0/21
D	0/24	0/23	0/24	0/22
E–H	0/1	0/1	0/1	0/0

Supplementary Table 6. Cox Regression Analysis of Baseline Factors Associated With Hepatitis B Surface Antigen Loss

Comparison	No. of observations	Hazard ratio	Lower limit	Upper limit	*P* value	Overall *P* value
Univariate analysis						
≥30 years vs <30 years	740	0.88	0.38	2.02	.761	
Male vs female	740	1.72	0.69	4.28	.245	
Non-Asian vs Asian	740	1.62	0.72	3.64	.240	
Baseline body mass index	740	1.05	0.96	1.14	.295	
Baseline weight, *kg*	740	1.01	0.99	1.03	.429	
Baseline height, *m*	740	0.99	0.95	1.04	.755	
Baseline HBeAg status (positive vs negative)	740	0.6	0.26	1.38	.232	
Genotype	740					.002
B vs A		0.34	0.14	0.86	.024	
C vs A		0.11	0.04	0.33	<.001	
D vs A		0.15	0.04	0.48	.004	
C vs B		0.34	0.11	0.92	.049	
D vs B		0.44	0.11	1.35	.202	
D vs C		1.30	0.30	4.88	.714	
ALT <ULN vs >ULN, *U/L*	740	0	0		.992	
Baseline HBsAg, log_{10} *IU/mL*	740	1.04	0.65	1.66	.878	
Baseline HBV DNA, log_{10} *IU/mL*	740	1.02	0.8	1.31	.859	
Multivariate analysis						
Genotype	625					.001
B vs A		0.11	0.03	0.38	<.001	
C vs A		0.06	0.01	0.25	<.001	
C vs B		0.57	0.12	2.36	.458	
D vs A		0.13	0.02	0.54	.015	
D vs B		1.13	0.18	5.67	.892	
D vs C		1.97	0.32	10.53	.455	

Supplementary Table 7. Cox Regression Analysis of On-Treatment Factors Associated With Hepatitis B Surface Antigen Loss

Comparison	No. of observations	Hazard ratio	Lower limit	Upper limit	*P* value	Overall *P* value
Univariate analysis						
Treatment	740					.008
Group B vs group A		0.33	0.11	0.81	.029	
Group C vs group A		0.03	0.00	0.22	.018	
Group D vs group A		0.33	0.11	0.82	.030	
Group C vs group B		0.09	0.00	0.79	.115	
Group D vs group B		1.01	0.30	3.41	.989	
HBsAb <10 IU/mL vs HBsAb >10 IU/mL at wk 12	708	0.28	0.09	1.38	.061	
Change in HBsAg <1 log vs >1 log at wk 12	711	23.2	9.54	56.43	<.001	
HBV DNA <15 IU/mL vs HBV DNA >15 IU/mL at wk 12	709	2.94	1.25	6.93	.014	
HBsAg <2 log vs >2 log at wk 12	711	12.44	5.45	28.37	<.001	
HBsAg <3 long vs >3 log at wk 12	711	7.11	3.01	16.76	<.001	
ALT >400 U/L vs <400 U/L (males) or > 300 U/L vs <400 U/L (females) in first 12 wk	729	10.69	4.62	24.71	<.001	
Multivariate analysis						
Treatment	625					.029
Group B vs group A		0.27	0.08	0.80	.033	
Group C vs group A		0.09	0.00	0.74	.097	
Group C vs group B		0.32	0.00	3.34	.456	
Group D vs group A		0.26	0.07	0.77	.032	
Group D vs group B		0.96	0.22	4.10	.960	
Group D vs Group C		3.00	0.26	414.79	.479	
Decline in HBsAg >1 log vs <1 log at wk 12	625	7.8	2.05	29.22	.003	
ALT flare in first 12 wk vs no flare	625	4.65	1.54	14.46	.0009	

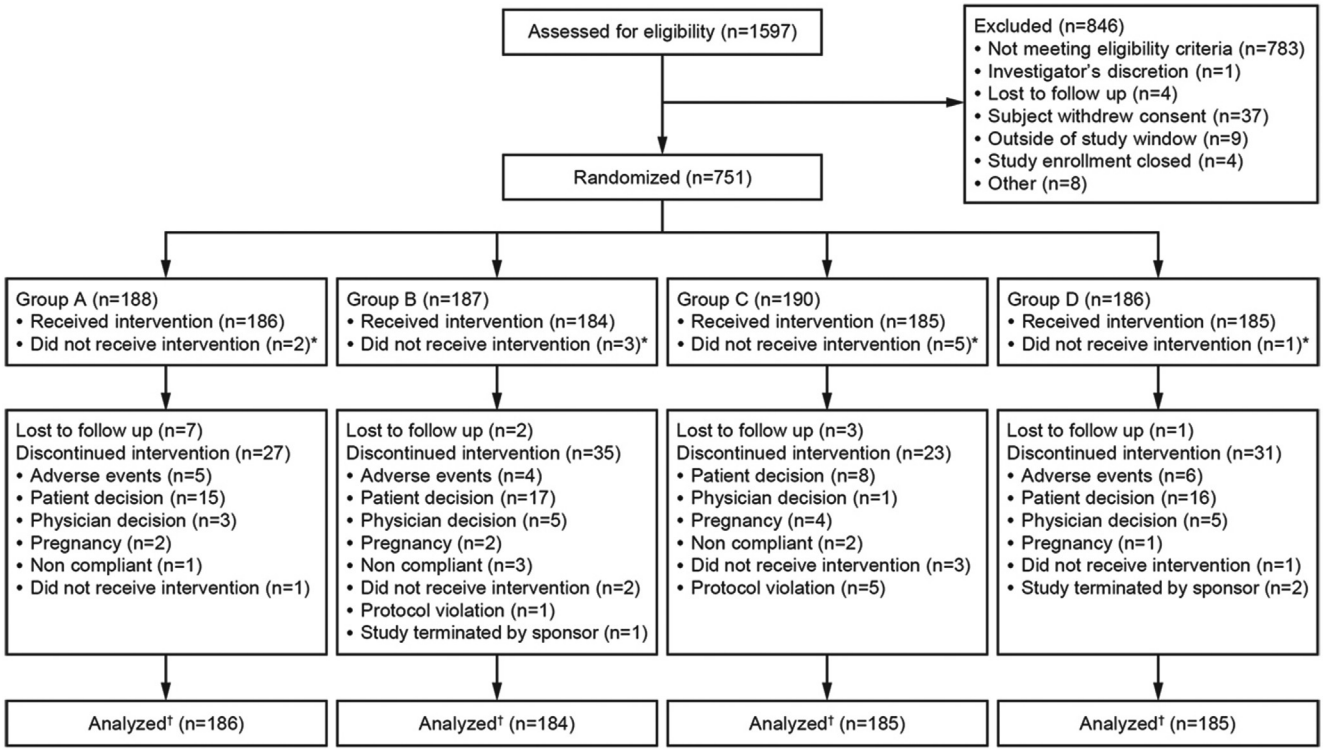
Supplementary Table 8. Proportion of Subjects With Hepatitis B Virus DNA <117 IU/mL (Protocol-Defined Secondary End Point)

HBV DNA <117 IU/mL	Group A (n = 186) TDF + peginterferon for 48 wk	Group B (n = 184) TDF + peginterferon for 16 wk, then TDF for 32 wk	Group C (n = 185) TDF for 120 wk	Group D (n = 185) Peginterferon for 48 wk
Wk 48, no. of patients (%)	160 (86.0)	153 (83.2)	149 (80.5)	61 (33.0)
Wk 72, no. of patients (%)	22 (11.8)	19 (10.3)	157 (84.9)	15 (8.1)

Supplementary Table 9. Reasons for Retreatment[a]

Retreatment criteria	Total no. of patients meeting retreatment criteria
Increase of total bilirubin by >1.5 mg/dL (>25 μmol/L) from study baseline and concurrent ALT >30 U/L for males and >19 U/L for females	1
Increase in INR >0.5 from study baseline or elevated INR ≥1.7, whichever is lowest, in subjects with adequate vitamin K therapy and concurrent ALT >30 U/L for males and >19 U/L for females	0
Lactate levels >ULN that were not a result of obvious nonhepatic etiology	9
ALT >400 U/L in males or >300 U/L in females with or without associated symptoms	65
Any ALT elevation associated with change in other laboratory parameters suggestive of worsening hepatic function	8
HBV DNA >5865 IU/mL (20,000 copies/mL) assessed at 24 wk off treatment (irrespective of ALT level)	178
Clinical or laboratory manifestations of hepatic decompensation	0
In males, ALT >60 IU/L and ≤150 IU/L and in females ALT >38 U/L and ≤95 U/L persisting for 84 d and HBV DNA ≥15 IU/mL	37
ALT >150 IU/L and ≤400 IU/L (male) or ALT >95 U/L and ≤300 U/L (female) persisting for ≥28 d	27
Unknown	3

[a]Note that some patients met more than one criterion.



Supplementary Figure 1. Patient disposition. *Eleven subjects were randomized but not dosed; 4 withdrew consent after randomization and reasons are not provided for the remaining 7. †The analyzed population represents patients included in the Kaplan−Meier estimate, the missing = failure analysis and the safety analysis.