

# A Randomized, Controlled Trial of Combination Therapy for Chronic Hepatitis B: Comparing Pegylated Interferon- $\alpha$ 2b and Lamivudine with Lamivudine Alone

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**Background:** Conventional interferon and lamivudine monotherapy are unsatisfactory in treating hepatitis B virus (HBV) infection.

**Objective:** To evaluate the efficacy and safety of pegylated interferon- $\alpha$ 2b and lamivudine combination therapy for chronic hepatitis B.

**Design:** Randomized, controlled, open-label trial.

**Setting:** Outpatient clinic in a referral center.

**Participants:** 100 treatment-naive patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B and moderately elevated alanine aminotransferase levels.

**Measurement:** The primary end point was sustained virologic response (HBeAg seroconversion and HBV DNA level < 500 000 copies/mL) at 24 weeks after cessation of treatment.

**Intervention:** A staggered regimen of combination therapy with pegylated interferon- $\alpha$ 2b (1.5  $\mu$ g/kg of body weight per week; maximum, 100  $\mu$ g) given for 32 weeks plus lamivudine (100 mg daily) given for 52 weeks versus lamivudine (100 mg daily) monotherapy given for 52 weeks. Of the 100 participants, 96% completed treatment and 80% completed post-treatment follow-up.

**Results:** The rate of sustained virologic response was 36% for the combination treatment group and 14% for the lamivudine

monotherapy group (absolute difference, 22 percentage points [95% CI, 6 to 38 percentage points]). End-of-treatment outcomes showed that, compared with monotherapy, patients receiving combination therapy more often had virologic response (60% vs. 28% [absolute difference, 32 percentage points (CI, 14 to 50 percentage points)]); had more substantial reductions of HBV DNA (3.91  $\log_{10}$  copies/mL vs. 2.83  $\log_{10}$  copies/mL); and less often had lamivudine-resistant mutants (21% vs. 40%). The percentages of patients with normalization of alanine aminotransferase levels and histologic improvement did not differ. Adverse effects, such as transient influenza-like symptoms, alopecia, and local erythematous reactions, were more common with combination therapy.

**Limitations:** This study lacked a double-blind design and was conducted at 1 institution. Because of the staggered pegylated interferon-lamivudine regimen, patients assigned to combination therapy received treatment for 8 weeks longer than those assigned to monotherapy.

**Conclusions:** In patients with HBeAg-positive chronic hepatitis B, staggered combination treatment with pegylated interferon- $\alpha$ 2b and lamivudine may lead to a higher rate of virologic response than lamivudine monotherapy.

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Chronic hepatitis B virus (HBV) infection affects more than 300 million people globally (1). Patients who have HBV infection with positivity for hepatitis B e antigen (HBeAg) and persistently active disease have increased risks for progressive disease leading to liver cirrhosis and hepatocellular carcinoma (2). Conventional interferon treatment with injections given up to 3 times per week for 12 to 24 weeks may lead to HBeAg seroconversion in 33% of treated patients compared with 12% of untreated controls (3). The treatment response to conventional interferon treatment among Asian patients seems less satisfactory (4, 5). Pegylated interferon- $\alpha$ 2b is synthesized by bonding recombinant interferon- $\alpha$ 2b to polyethylene glycol and is given once weekly rather than 3 times weekly (6). The antiviral efficacy of pegylated interferon- $\alpha$ 2b for treating chronic hepatitis C is superior to that of conventional interferon (7, 8), but few studies have evaluated pegylated interferon in patients with chronic hepatitis B.

Lamivudine is an oral nucleoside analogue that effectively suppresses HBV replication (9, 10). Studies suggest that only 16% to 18% of patients treated with lamivudine

for 1 year develop HBeAg seroconversion (9–11). Extending lamivudine treatment for up to 4 years is associated with development of drug-resistant viral mutants in about 70% of patients (12). The durability of HBeAg seroconversion is estimated to be 46% to 64% up to 3 years after the cessation of lamivudine treatment (13–15).

Successful elimination of HBV depends on a durable immune clearance of the existing pool of intrahepatic HBV, particularly the closed covalent circular DNA (16). Combining an immunomodulator (such as interferon) and an antiviral agent (such as lamivudine) is an appealing ap-

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proach for treating chronic hepatitis B. However, past studies in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B showed conflicting results about the superiority of combination therapy over lamivudine monotherapy (11, 17–20). We evaluated whether the combination of pegylated interferon- $\alpha$ 2b and lamivudine improves antiviral efficacy and increases HBeAg seroconversion rates more than lamivudine monotherapy in patients with HBeAg-positive chronic hepatitis B and moderately elevated alanine aminotransferase (ALT) levels. Since extending interferon treatment from 16 to 32 weeks is associated with higher rates of HBeAg seroconversion (21), combination therapy includes pegylated interferon- $\alpha$ 2b given for 32 weeks.

## METHODS

### Patients

We recruited consecutive patients 18 to 65 years of age with chronic hepatitis B from the Hepatitis Clinic of the Prince of Wales Hospital, Hong Kong, China, a secondary referral center serving around 1 million people. All patients had been positive for hepatitis B surface antigen (HBsAg) for at least 6 months, were HBeAg-positive, and had a serum HBV DNA level of at least 500 000 copies/mL and an ALT level that was 1.3 to 5 times the upper limit of normal. We excluded patients who had decompensated liver disease or a history of interferon or antiviral agent use. Other exclusion criteria were co-infection with hepatitis C virus, hepatitis D virus, or HIV; history of hepatocellular carcinoma; other causes of liver disease, including autoimmune hepatitis; Wilson disease; hemochromatosis and  $\alpha_1$ -antitrypsin deficiency; serious medical or psychiatric illness; concurrent use of corticosteroid or immunosuppressive agents; and pregnancy. We conducted the study in accordance with the guidelines of the Declaration of Helsinki. The ethics committee of The Chinese University of Hong Kong approved the protocol, and all patients gave witnessed, written informed consent.

### Study Design

The study was a phase III, open-label, randomized trial. Within 4 weeks of screening for eligibility criteria, patients were randomly assigned to either combination therapy with pegylated interferon- $\alpha$ 2b (PegIntron, Schering-Plough Corp., Kenilworth, New Jersey) and lamivudine (Zeffix, GlaxoSmithKline, Middlesex, United Kingdom) or lamivudine monotherapy in a ratio of 1:1. We based study group assignment on a computer-generated list, and research staff who were not involved in patient management placed the random numbers in opaque envelopes. A research nurse prescribed study drugs after receiving the information about treatment allocation at the baseline visit.

Figure 1 shows the study design. Pegylated interferon- $\alpha$ 2b was given as a subcutaneous injection at a dosage of 1.5  $\mu$ g/kg of body weight per week for patients who

### Context

Few studies have evaluated combination therapies for chronic hepatitis B.

### Contribution

In this single-center, open-label trial, 100 patients with hepatitis B e antigen-positive chronic hepatitis B and moderately elevated alanine aminotransferase levels were randomly assigned to a staggered regimen of pegylated interferon- $\alpha$ 2b for 32 weeks plus lamivudine for 52 weeks or lamivudine monotherapy. Patients receiving combination therapy more often had virologic responses and less often developed lamivudine-resistant mutants than those receiving monotherapy. Transient influenza-like symptoms, alopecia, and local erythematous reactions were more common with combination therapy.

### Cautions

Patients assigned to combination therapy received treatment for 8 weeks longer than those assigned to monotherapy.

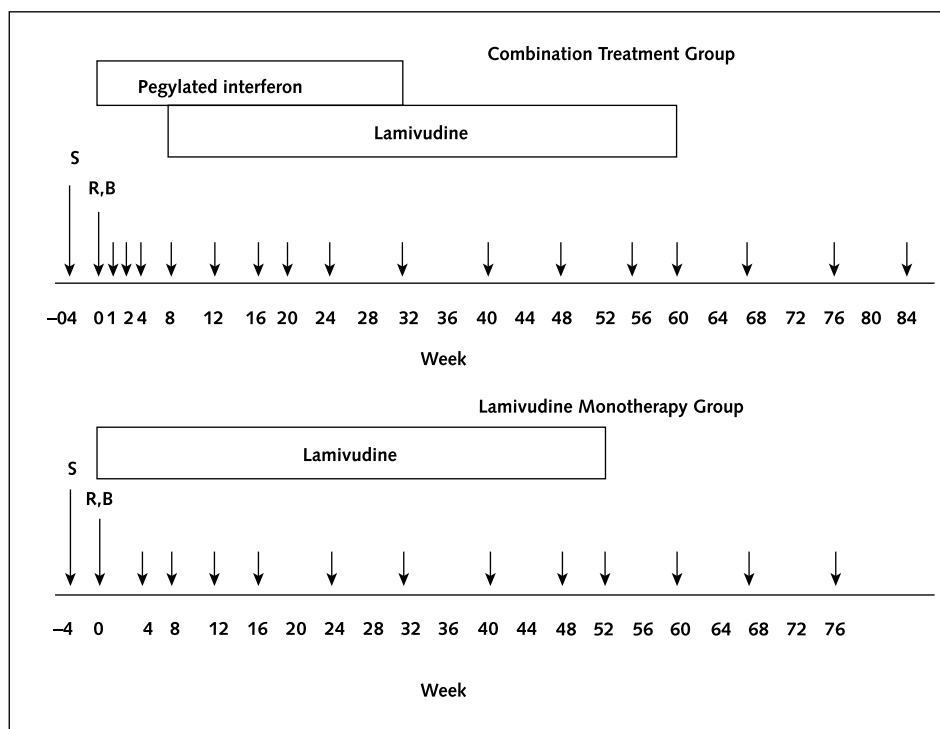
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weighed less than 65 kg or 100  $\mu$ g per week for patients who weighed more than 65 kg for 32 weeks (6). Lamivudine was administered as 100 mg orally daily for 52 weeks in both groups of patients. In patients receiving combination therapy, pegylated interferon- $\alpha$ 2b was administered 8 weeks before lamivudine was administered. Then both treatments were given in combination for 24 weeks, followed by lamivudine monotherapy for a further 28 weeks. Patients in the combination group were asked to return at weeks 1, 2, 4, 8, 12, 16, 20, 24, 32, 40, 52, and 60 (end of treatment). Patients in the lamivudine monotherapy group received lamivudine for 52 weeks and were asked to return for follow-up at weeks 4, 8, 12, 16, 24, 32, 40, 48, and 52 (end of treatment). We followed patients in both groups every 8 weeks in the post-treatment period for 24 more weeks. We gave open-label lamivudine treatment to patients who experienced severe post-treatment relapse of chronic hepatitis B (defined as an ALT level > 10 times the upper limit of normal and HBV DNA level > 500 000 copies/mL).

### Safety

The investigators interviewed patients for symptomatic adverse effects and closely monitored laboratory tests at each follow-up visit. They recorded symptoms and events that patients reported spontaneously, symptoms and events elicited in response to open-ended questions, and adverse effects observed at the follow-up visits. They assessed all adverse events on the likelihood of causality by the study drug or drugs. They assessed the severity of adverse events according to a preset table and classified the event as mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening (grade 4).

Figure 1. Study design.



Each arrow indicates a follow-up visit. B = baseline visit; R = randomization visit; S = screening visit.

The dosage of pegylated interferon was reduced, as necessary, according to the severity of the adverse events. The dosage was reduced from 100  $\mu\text{g}$  per week (or 1.5  $\mu\text{g}/\text{kg}$  per week if body weight < 65 kg) to 50  $\mu\text{g}$  per week (or 1.0  $\mu\text{g}/\text{kg}$  per week if body weight < 65 kg) for grade 3 adverse events (or sometimes grade 2 adverse events at the discretion of the investigator). The dosage could be further reduced to 25  $\mu\text{g}$  per week (or 0.5  $\mu\text{g}/\text{kg}$  per week if body weight < 65 kg) if the adverse event recurred despite initial dosage reduction. Pegylated interferon therapy was stopped in case of grade 4 adverse events. Patients receiving combination treatment were allowed to continue lamivudine if investigators thought that the adverse effect was related to pegylated interferon use. Lamivudine therapy was stopped if the adverse event persisted despite cessation of pegylated interferon therapy.

We tested serum for HBV DNA levels, HBeAg, and antibody to HBeAg (at baseline, then 8 weekly until the end of treatment, and weeks 8, 16, and 24 after treatment) and HBsAg and antibody to HBsAg (at baseline, end of treatment, and 24 weeks after treatment). We determined the presence of lamivudine-resistant mutations in the serum sample at the end of treatment. Liver biopsy was performed within 4 weeks before treatment began and at the end of treatment.

### Laboratory Assays

#### Serologic Assays

We tested for HBsAg and antibodies to hepatitis C virus, hepatitis D virus, and HIV by using commercially

available enzyme-linked immunosorbent assay kits (Abbott GmbH Diagnostika, Wiesbaden-Delkenheim, Germany). We measured HBeAg and antibody to HBeAg by using an enzyme-linked immunosorbent assay (Sanofi Diagnostics, Pasteur, France).

#### Virologic Assays

We based our sample size calculations and initial screening for eligibility on the DNA cross-linking assay (NAXCOR XLnt, NAXCOR, Menlo Park, California), which has a lower limit of detection of 500 000 copies/mL for quantification of HBV DNA (22). Since the TaqMan real-time polymerase chain reaction assay (Applied Biosystem, Foster City, California) became available in our laboratory, we used this assay to measure HBV DNA levels at baseline and in all follow-up visits (23, 24). The range of HBV DNA detection was from  $10^2$  to  $10^9$  copies/mL; the correlation coefficient of the standard curve was routinely greater than 0.990. We performed HBV genotyping by restriction fragment length polymorphism in a residual serum sample taken from each patient at their initial visit (25, 26). We determined the presence of lamivudine-resistant mutants by using the INNO-LiPA HBV DR line probe assay (Innogenetics N.V., Ghent, Belgium) according to the instruction of the manufacturer (27).

#### End Points

We defined virologic response as HBeAg seroconversion (that is, loss of HBeAg), detection of antibody to

HBeAg, and HBV DNA level less than 500 000 copies/mL and biochemical response as normalization of ALT level. We assessed both the virologic and biochemical end-of-treatment responses (week 52 for monotherapy and week 60 for combination therapy) and the sustained responses at 24 weeks after the end of treatment.

The primary end point was sustained virologic response. Secondary end points included sustained biochemical response, end-of-treatment virologic and biochemical responses, reduction in HBV DNA levels, and histologic improvement. We scored histologic necroinflammation by using the Knodell scoring system (score range, 0 to 18) and liver fibrosis by using the Ishak scoring system (score range, 0 to 6). The necroinflammation score was the sum of 3 histologic components: severity of periportal necrosis (score range, 0 to 10), intralobular necrosis (score range, 0 to 4), and portal inflammation (score range, 0 to 4). We considered a reduction of at least 2 points to be a clinically meaningful indicator of histologic changes. We regarded liver biopsy results with at least 3 portal tracts as sufficient for histologic scoring. One histopathologist, who was blinded to treatment assignments or the times at which the specimens were obtained, assessed all histologic specimens. The proportion of patients developing lamivudine-resistant mutant in the 2 treatment groups was also compared.

### Statistical Analysis

On the basis of the literature, the proportion of patients receiving lamivudine monotherapy who achieve sustained virologic response was around 15%. Since the reported sustained virologic response for combination of conventional interferon and lamivudine was up to 33% (17), we anticipated that the use of pegylated interferon and lamivudine combination treatment had superior efficacy and that the rate of sustained virologic response was 30% higher than that of lamivudine monotherapy (that is, sustained virologic response in combination groups vs. lamivudine monotherapy group, 45% vs. 15%). Given these estimated response rates, we calculated that 94 patients would be required to provide a power of 80% at an  $\alpha$  level of 0.05, allowing for a dropout rate of 10%.

We used a modified intention-to-treat analysis to assess treatment responses. In the analysis, we included all randomly assigned patients who received at least 1 dose of the study medication. For the assessment of virologic or biochemical response, we considered patients who withdrew before the end of treatment (week 52 for monotherapy and week 60 for combination therapy) or for whom data were missing at the end of treatment to have failed response. For analyses of sustained response, we regarded patients who were classified as having a failed response at the end of treatment due to early withdrawal or missing data as having treatment failure. Among patients assigned to combination therapy who stopped pegylated interferon treatment prematurely, we assessed virologic and biochemical responses if lamivudine treatment was contin-

ued to the end of treatment. For the analysis of adverse events, we included all patients who were randomly assigned to either treatment group and who received at least 1 dose of study medication.

We performed all statistical tests by using SPSS, version 11.0 (SPSS, Inc., Chicago, Illinois). We expressed continuous variables as the median (range). Hepatitis B virus DNA was logarithmically transformed for analysis. We compared continuous variables, including patient age, liver biochemistry,  $\log_{10}$  HBV DNA levels, and histologic scores, by using the Mann–Whitney U test. We compared categorical variables and the proportions of patients with virologic and biochemical responses, histologic improvements, lamivudine-resistant mutants, and adverse events by using the Pearson chi-square test or Fisher exact test, as appropriate. We compared the timing of HBeAg seroconversion by using Kaplan–Meier survival analysis. We used a logistic regression model to compare virologic response, with adjustment for baseline imbalance in ALT levels. A *P* value less than 0.05 was considered statistically significant. All statistical tests were 2-sided.

### Role of Funding Source

Schering-Plough Corp. supplied pegylated interferon- $\alpha$ 2b, and GlaxoSmithKline supplied lamivudine. The authors developed the study protocol and were responsible for data collection and progress of the study. The authors had full access to all of the study's data files and were responsible for statistical analysis, reporting of data, and manuscript submission. Representatives of the pharmaceutical companies that supplied the drugs did not comment on the manuscript before submission.

## RESULTS

### Study Sample

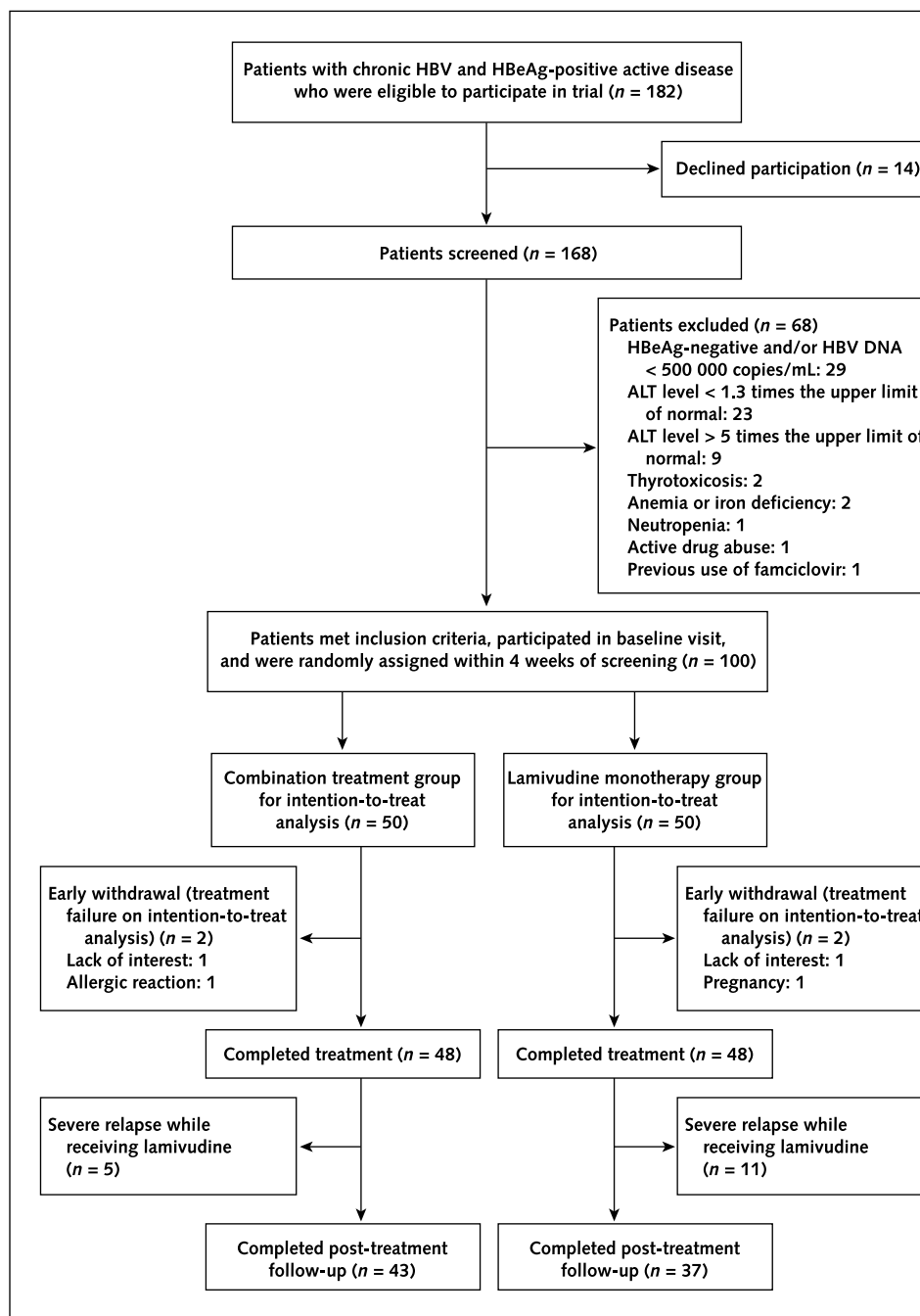
We screened 182 patients between April 2000 and March 2002 and enrolled 100 eligible patients (Figure 2). Most baseline characteristics were well matched between the 2 groups (Table 1). However, although all studied patients met inclusion criteria at the screening visit, several patients had either normal ALT levels or levels that were greater than 5 times the upper limit of normal at the baseline randomization visit. The median ALT levels of the combination group were higher than those of the lamivudine group ( $P = 0.02$ ), but the proportion of patients ( $P > 0.2$ ) in the different ALT categories (1 to 2 times, 2 to 5 times, or  $>5$  times the upper limit of normal) did not differ between groups.

Forty-eight patients (96%) in each treatment group completed treatment. No patients received any additional antiviral or immunomodulator treatment other than the study drugs. Also, no patients assigned to lamivudine monotherapy received pegylated interferon during the study.

### Virologic Response

On intention-to-treat analysis, 30 of 50 patients (60%) in the combination treatment group and 14 of 50

Figure 2. Flow of patients included at various stages of the trial.



Severe relapse after cessation of treatment was defined as alanine aminotransferase (*ALT*) level more than 10 times the upper limit of normal accompanied by hepatitis B virus (*HBV*) DNA level greater than 500 000 copies/mL. These patients were given open-label lamivudine. HBeAg = hepatitis B e antigen.

patients (28%) in the lamivudine monotherapy group showed virologic response at the end of treatment (absolute difference, 32 percentage points [95% CI, 14 to 50 percentage points];  $P = 0.001$ ). After adjustment for baseline *ALT* levels, the absolute difference in predicted probabilities of combination treatment and lamivudine monotherapy for end-of-treatment response was 31 percentage points (CI, 10 to 49 percentage points;  $P = 0.003$ ). All patients who had lost HBeAg developed antibodies to

HBeAg. One patient receiving combination treatment had lost HBsAg by the end of treatment. No patient receiving lamivudine monotherapy became HBsAg-negative. Five patients receiving combination therapy and 2 patients receiving lamivudine monotherapy became HBV DNA-negative by polymerase chain reaction assay. Among patients with baseline *ALT* levels less than 5 times the upper limit of normal, the end-of-treatment virologic response of the combination treatment group was still statistically signifi-

Table 1. Baseline Characteristics of the Studied Patients\*

Characteristic	Combination Therapy Group (n = 50)	Lamivudine Group (n = 50)
Age, y	32 (19–57)	34 (21–65)
Men, n (%)	31 (62)	36 (72)
Body weight, kg	64 (41–90)	68 (42–89)
BMI, kg/m <sup>2</sup>	22 (16–33)	25 (18–32)
ALT level, n (%)†		
Normal	2 (4)	3 (6)
1–2 times the upper limit of normal	13 (26)	21 (42)
2–5 times the upper limit of normal	27 (54)	20 (40)
5 times the upper limit of normal	8 (16)	6 (12)
ALT level, U/L	144 (48–1179)	119 (36–461)
HBeAg-positive, %	100	98‡
HBV DNA level > 500 000 copies/mL, %	100	100
HBV DNA level, log <sub>10</sub> copies/mL	8.04 (5.91–9.74)	7.67 (5.74–9.49)
HBV genotype, n (%)		
B	15 (30)	16 (32)
C	32 (64)	31 (64)
B and C	3 (6)	3 (6)
Histology§		
Necroinflammation score	5 (1–11)	5 (1–12)
Fibrosis score	1 (0–5)	1 (0–5)

\* Continuous variables are shown as the median (range). Patients met entry criteria at screening but not at baseline (continued on study as per protocol). ALT = alanine aminotransferase; BMI = body mass index; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

† Combination group: ALT level < 1.3 times upper limit of normal (n = 3 [6%]), ALT > 5 times upper limit of normal (n = 8 [16%]). Lamivudine group: ALT level < 1.3 times the upper limit of normal (n = 9 [18%]), ALT level > 5 times the upper limit of normal (n = 6 [12%]).

‡ 1 patient (2%) was HBeAg-negative.

§ Among 46 patients in the combination group and 47 patients in the lamivudine group with baseline liver histology data available.

cantly higher than the response of the lamivudine monotherapy group (25 of 42 [60%] patients vs. 12 of 44 [27%] patients [absolute difference, 32 percentage points (CI, 12 to 52 percentage points)]).

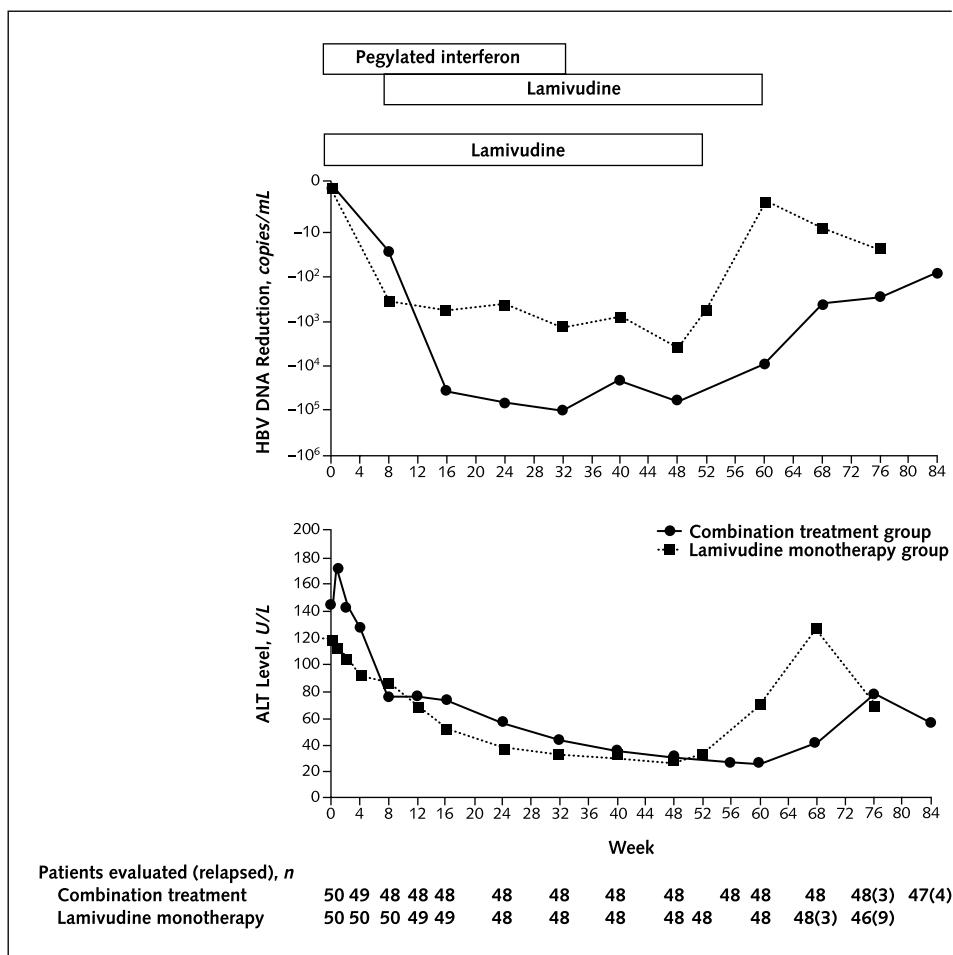
At the end of treatment, the median reduction in HBV DNA of patients who completed combination treatment and lamivudine monotherapy was 3.89 log<sub>10</sub> copies/mL (range, 1.59 to 6.35 log<sub>10</sub> copies/mL) and 2.74 log<sub>10</sub> copies/mL (range, -0.10 to 5.68 log<sub>10</sub> copies/mL), respectively (median difference, 1.24 log<sub>10</sub> copies/mL [CI, 0.78 to 1.66 log<sub>10</sub> copies/mL]) (Figure 3). Since the treatment duration in the combination treatment group was 8 weeks longer than the duration of treatment in the lamivudine monotherapy group, we also compared the changes in HBV DNA levels when patients in both groups finished 48 weeks of treatment. At 48 weeks, the reduction in HBV DNA level among patients receiving combination treatment and lamivudine monotherapy was 4.65 log<sub>10</sub> copies/mL (range, -0.84 to 7.83 log<sub>10</sub> copies/mL) and 3.62 log<sub>10</sub> copies/mL (range, 1.32 to 7.33 log<sub>10</sub> copies/mL), respectively (median difference, 1.10 log<sub>10</sub> copies/mL [CI, 0.55 to 1.65 log<sub>10</sub> copies/mL]). Fifty percent of the patients receiving combination treatment and 28% of those receiving lamivudine monotherapy had virologic response at week 48 (absolute difference, 22 percentage points [CI, 3 to 41 percentage points]), although all HBeAg seroconversion in lamivudine monotherapy group occurred on or before week 40.

Nine patients in the combination treatment group had HBeAg seroconversion at week 8 before the commencement of lamivudine therapy. After 24 weeks of lamivudine

treatment in both groups, 11 additional patients receiving combination treatment and 11 patients receiving lamivudine monotherapy had HBeAg seroconversion. More patients in the combination treatment group had HBeAg seroconversion in the last 28 weeks of extended lamivudine treatment (10 of 30 [33%] remaining patients) than in the lamivudine monotherapy group (3 of 39 [8%] remaining patients) (absolute difference, 25 percentage points [CI, 7 to 45 percentage points]). Among patients who developed HBeAg seroconversion during treatment, the median time for HBeAg seroconversion after commencement of treatment was 24 weeks (range, 8 to 60 weeks) in the combination treatment group and 24 weeks (range, 0 to 40 weeks) in the lamivudine monotherapy group (P = 0.13).

At 24 weeks after treatment, 18 patients (36%) in the combination treatment group and 7 patients (14%) in the lamivudine monotherapy group had sustained virologic response (absolute difference, 22 percentage points [CI, 6 to 38 percentage points]; P = 0.011). After adjustment for the baseline ALT levels, the absolute difference in predicted probabilities of combination treatment and lamivudine monotherapy for sustained virologic response was 22 percentage points (CI, 3 to 47 percentage points; P = 0.015). Among the subgroup of patients with baseline ALT levels less than 5 times the upper limit of normal, the predicted probability for sustained virologic response was still higher in the combination treatment group (15 of 42 patients [36% (CI, 21% to 50%)]) than in the lamivudine monotherapy group (6 of 44 patients [14% (CI, 4% to 24%)]) (absolute difference, 22 percentage points [CI, 4 to 40 percentage points]). Twelve of 30 (40%) patients who received combination treatment and 7 of 14

Figure 3. Serial median  $\log_{10}$  hepatitis B virus (HBV) DNA reduction (top) and serial median alanine aminotransferase (ALT) levels (bottom) among patients who received pegylated interferon and lamivudine combination treatment (solid line) versus patients who received lamivudine monotherapy (dotted line) from baseline to 24 weeks after treatment.



All randomly assigned patients were included in the analysis. The numbers of patients receiving open-label lamivudine treatment for hepatitis B relapse after cessation of studied drugs are presented. One patient in the combination treatment group and 2 patients in the lamivudine monotherapy group developed severe reactivation of hepatitis while receiving open-label lamivudine treatment and did not attend the follow-up visit at week 84 and week 76, respectively.

(50%) patients who received lamivudine monotherapy developed post-treatment viral relapse after an initial end-of-treatment response. Three patients receiving combination treatment and 2 patients receiving lamivudine treatment were negative for HBV DNA by polymerase chain reaction assay at 24 weeks after treatment.

### Biochemical Response

Figure 3 shows the ALT levels for patients in the 2 treatment groups. At the end of treatment, 45 patients (90%) receiving combination treatment and 39 patients (78%) receiving lamivudine monotherapy had normalization of ALT levels (absolute difference, 12 percentage points [CI, -2 to 26 percentage points]). At 24 weeks after treatment, more patients receiving combination treatment had sustained ALT level normalization compared with those receiving lamivudine monotherapy (50% vs. 30% [absolute difference, 20 percentage points (CI, 1 to 39 percentage points)]). Among those with sustained virologic

response, all except 1 patient who received combination treatment had sustained normalization of ALT levels (ALT level 1.1 times the upper limit of normal).

### Histologic Response

Paired liver biopsy specimens were available in 40 patients receiving combination treatment and 44 patients receiving lamivudine monotherapy. Eight patients had insufficient liver tissue for accurate grading in 1 of the paired biopsy specimens (7 patients at baseline and 1 patient at week 52), 3 patients declined liver biopsy after treatment because of personal reasons, 4 patients withdrew prematurely from the study (1 patient also had insufficient liver tissue at baseline biopsy), and 2 post-treatment liver biopsies were cancelled because of the hospital outbreak of severe acute respiratory syndrome in March 2003. Among the 16 patients without evaluable paired liver biopsy specimens, 6 patients (4 receiving combination treatment and 2 receiving lamivudine monotherapy) had end-of-treat-

ment virologic response and 2 patients (both receiving combination treatment) had sustained virologic response.

Twenty-four (60%) patients receiving combination treatment and 26 (59%) patients receiving lamivudine monotherapy had at least a 2-point increase in necroinflammatory score (absolute difference, 1 percentage point [CI, -20 to 22 percentage points]). Four (10%) patients receiving combination treatment and 4 (9%) receiving lamivudine monotherapy had at least a 2-point decrease in necroinflammatory score. Six (15%) and 4 (9%) patients receiving combination therapy and lamivudine monotherapy, respectively, had at least a 2-point increase in fibrosis scores (absolute difference, 6 percentage points [CI, -8 to 20 percentage points]), while 4 (10%) and 2 (5%) patients, respectively, had at least a 2-point decrease in fibrosis scores (absolute difference, 5 percentage points [CI, -6 to 17 percentage points]).

### Drug-Resistant Mutants

At the end of treatment, a higher proportion of patients receiving lamivudine monotherapy developed a lamivudine-resistant mutant (19 of 48 [40%] patients) than did those receiving combination treatment (10 of 48 [21%] patients) (absolute difference, 19 percentage points [CI, 8 to 37 percentage points]). Among patients receiving monotherapy, 7 had a lamivudine-resistant mutant and 12 had both wide-type and lamivudine-resistant mutants. Among patients receiving combination therapy, 5 had a resistant mutant and 5 had mixed wide-type and resistant mutants. Patients who developed lamivudine resistance tended to have higher pretreatment HBV DNA (Table 2). A higher proportion of patients who developed lamivudine resistance (9 of 29 [31%] patients) had HBV DNA greater than 500 000 copies/mL at the end of treatment than those who did not develop lamivudine resistance (7 of 67 [10%] patients) (absolute difference, 21 percentage points [CI, 2 to 39 percentage points]). However, the development of lamivudine resistance did not seem to mitigate the virologic, biochemical, and histologic response (Table 2).

### Safety

Most adverse symptoms and events were transient and were related to the use of pegylated interferon- $\alpha$ 2b (Table 3). Four (8%) patients receiving combination treatment had serious adverse events. One patient developed bipolar disorder requiring antidepressant therapy (week 21), 1 developed pulmonary tuberculosis with right pleural effusion requiring antituberculosis treatment (week 11), 1 developed thyrotoxicosis requiring propyluracil treatment (week 17), and 1 developed severe local reaction at the injection sites (week 8) that resolved spontaneously. Pegylated interferon- $\alpha$ 2b treatment was stopped in all 4 cases. Lamivudine treatment was continued in the first 3 cases until week 60, and we evaluated the treatment responses as per protocol. The fourth patient, who had received only 7 doses of pegylated interferon, withdrew from the study and was considered to have treatment failure. Five (10%) patients required reduction of dosage of pegylated interferon- $\alpha$ 2b to 50  $\mu$ g per week (if body weight > 65 kg) or 1.0  $\mu$ g/kg per week (if body weight < 65 kg) as per protocol because of anemia (1 patient), neutropenia (3 patients), and/or thrombocytopenia (4 patients). One patient had pegylated interferon withheld for 2 doses at weeks 4 and 5 because of severe hepatitis flare-up (ALT level, 1762 U/L) and resumed at full dose at week 6 when ALT level decreased to a lower level (242 U/L). No patient who received lamivudine monotherapy developed serious adverse event during treatment, and no patient had dosage adjustment for lamivudine.

Five (10%) patients in the combination group and 11 (23%) patients in the lamivudine monotherapy group developed severe post-treatment relapse of chronic hepatitis B (absolute difference, -13 percentage points [CI, -27 to 2 percentage points]). Two patients in lamivudine monotherapy group had post-treatment relapse leading to elevation of serum bilirubin levels to 82  $\mu$ mol/L (4.8 mg/dL) and 153  $\mu$ mol/L (9.0 mg/dL), respectively. Lamivudine treatment was resumed, and all patients responded. No patient died or required liver transplantation.

Table 2. Virologic, Biochemical, and Histologic Variables of Patients and the Development of Lamivudine-Resistant Mutation\*

Variable	Lamivudine-Resistant Mutant Present (n = 29)	Lamivudine-Resistant Mutant Absent (n = 67)	Adjusted P Value†
Ratio of patients with genotype B to those with genotype C, n:n	12:17	19:42‡	>0.2
Baseline HBV DNA, log <sub>10</sub> copies/mL	8.60 (6.46–9.77)	8.00 (5.74–10.20)	0.009
Baseline ALT level, U/L	135 (48–461)	126 (36–1179)	>0.2
Baseline necroinflammatory score	5 (2–12)	5 (1–11)	0.096
Baseline fibrosis score	1 (0–5)	1 (0–6)	>0.2
End-of-treatment virologic response, n (%)	12 (41)	32 (48)	>0.2
End-of-treatment biochemical response, n (%)	24 (83)	60 (90)	>0.2
Sustained virologic response, n (%)	8 (28)	17 (25)	>0.2
Sustained biochemical response, n (%)	8 (28)	32 (48)	>0.2

\* Continuous variables are shown as the median (range).

† P value adjusted for treatment group by logistic regression model.

‡ 6 patients had mixed genotypes B and C.

**Table 3. Common Adverse Events and Severe or Life-Threatening (Grade 3 or 4) Laboratory Toxicity during Treatment and at 24-Week Follow-up\***

Variable	Combination Therapy Group (n = 50)	Lamivudine Group (n = 50)	P Value
<b>Common adverse events, n (%)</b>			
Upper respiratory tract symptom†	37 (74)	19 (38)	0.001
Fever	36 (72)	2 (4)	<0.001
Alopecia	24 (48)	2 (4)	<0.001
Abdominal discomfort	22 (44)	13 (26)	0.093
Malaise	22 (44)	7 (14)	0.002
Headache	21 (42)	2 (4)	<0.001
Myalgia	13 (26)	2 (4)	0.006
Arthralgia	12 (24)	2 (4)	0.01
Reduced appetite	12 (24)	0 (0)	0.001
Local erythematous reaction	12 (24)	0 (0)	0.001
Allergic rashes	9 (18)	1 (2)	0.02
Dizziness	8 (16)	1 (2)	0.036
Vomiting or diarrhea	7 (14)	3 (6)	>0.2
Weight loss (>10%)	7 (14)	1 (2)	0.065
<b>Laboratory toxicity, n (%)</b>			
Increased ALT level	8 (16)	12 (24)	>0.2
Decreased phosphate level	2 (4)	1 (2)	>0.2
Decreased neutrophil count	1 (2)	0 (0)	>0.2
Increased creatine kinase level	0 (0)	1 (2)	>0.2
Increased alkaline phosphatase level	0 (0)	1 (2)	>0.2

\* Common adverse events were defined as those occurring in 5% or more of the patients in either group. ALT = alanine aminotransferase.

† Upper respiratory tract symptoms included cough, running nose, and sore throat.

## DISCUSSION

The antiviral effect of lamivudine, a nucleoside analogue, for treating chronic HBV infection is suboptimal. The drug is associated with a low HBeAg seroconversion rate, frequent post-treatment relapses, and development of drug resistance with extended treatment. Combination therapy with lamivudine and other nucleoside analogues (for example, adefovir dipivoxil and telbivudine) may not improve virologic response (28, 29), and combination therapy with lamivudine and interferon shows conflicting results (Table 4). In our study, we administered a combination of pegylated interferon- $\alpha$ 2b and lamivudine in a staggered manner. Our rationale for staggered treatment was that initial treatment with pegylated interferon- $\alpha$ 2b

probably enhances the immune clearance of intrahepatic HBV, including the closed covalent circular DNA, and that adding lamivudine at a later phase theoretically suppresses HBV replication and prevents reinfection of the hepatocytes. This particular hypothesis requires confirmation with studies of viral kinetics.

We found that patients with HBeAg-positive chronic hepatitis B and moderately elevated ALT levels had a higher rate of virologic response at the end of treatment with the staggered combination regimen (60%) than with monotherapy (28%). The rate of sustained HBeAg seroconversion 24 weeks after cessation of treatment was also higher with the staggered combination regimen (36%) than with monotherapy (14%). The end-of-treatment se-

**Table 4. Summary of Previous Randomized Trials on Interferon and Lamivudine Combination Treatment in Hepatitis B e Antigen-Positive Chronic Hepatitis B Virus**

Study, Year (Reference)	Study Location	Combination Treatment	Comparison Treatment	Response in Combination Treatment Group, n/n (%)*	Response in Comparison Group, n/n (%)*	P Value
Hason et al., 2003 (32)	Middle East	Interferon- $\alpha$ 2a $\times$ 16 wk + lamivudine $\times$ 48 wk†	Lamivudine $\times$ 48 wk	2/32 (6.2)	0/29 (0)	>0.2
Yalcin et al., 2003 (33)	Middle East	Interferon- $\alpha$ 2b + lamivudine $\times$ 12 mo	Interferon- $\alpha$ 2b $\times$ 12 mo	15/33 (45)	3/16 (19)	0.13
Barbaro et al., 2001 (17)	Mediterranean	Interferon- $\alpha$ 2b + lamivudine $\times$ 24 wk	Lamivudine $\times$ 52 wk	25/76 (33)	11/75 (15)	0.014
Schalm et al., 2000 (11)	North Europe North America	Interferon- $\alpha$ 2b $\times$ 16 wk Lamivudine $\times$ 24 wk‡	Interferon- $\alpha$ 2b $\times$ 16 wk Lamivudine $\times$ 52 wk	20/68 (29)	12/64 (19) 14/80 (18)§	0.12 0.10

\* Response defined as sustained hepatitis B e antigen seroconversion and suppressed hepatitis B virus DNA/total patients.

† Lamivudine started 4 weeks after the commencement of interferon therapy.

‡ Interferon started 8 weeks after the commencement of lamivudine therapy.

§ Response assessed at the end of lamivudine treatment for lamivudine monotherapy group.

roconversion rate for the staggered combination regimen was better than that reported for other antiviral treatment regimens (9–11, 17, 28–30). Although a previous study had suggested that pegylated interferon might lead to higher rates of sustained seroconversion than conventional interferon (31), we found a rate of sustained post-treatment seroconversion similar to that reported in patients using either a conventional interferon and lamivudine combination (Table 4) or conventional monotherapy (34). Because these studies involved various patient populations with different HBV genotypes and used varying treatment regimens and definitions of virologic response, head-to-head comparisons are still needed to establish whether combination therapy with pegylated interferon and lamivudine leads to similar or higher rates of sustained response than either pegylated interferon alone or conventional interferon with or without lamivudine.

We also found a relatively high rate (40%) of post-treatment viral relapse among patients with initial end-of-treatment responses. The high relapse rate could be related to short durations of either pegylated interferon- $\alpha$ 2b or lamivudine treatment. Extended lamivudine treatment after HBeAg seroconversion might improve the sustainability of virologic response (15, 35). However, despite suppression of HBV DNA in most patients and HBeAg seroconversion in almost half of the cases, we found lamivudine-resistant mutations in 21% of patients receiving combination treatment and 40% of patients receiving lamivudine monotherapy. The amount of lamivudine resistance that we found is higher than that reported in some series of Asian patients, perhaps because we used a highly sensitive line probe assay that could detect very low levels of mutants in a mixture with wild type (9–11, 27).

We found that patients in both treatment groups had biochemical and histologic improvements similar to those shown in previous studies with lamivudine treatment (9–11). We based histologic measurements on results of repeated liver biopsies that were performed in a sample of patients at the end of treatment. Histologic measurements taken several months after treatment might differ, particularly since patients in the lamivudine monotherapy group had higher post-treatment relapse rates than those in the combination therapy group.

Our trial has several limitations. First, we compared a staggered regimen of 60-week combination treatment with pegylated interferon and lamivudine versus 52-week treatment of lamivudine monotherapy. Under this study design, patients receiving combination treatment received a longer duration of antiviral treatment, which might affect the difference in clinical outcome. However, the antiviral effect of combination treatment at week 48 was still superior to that of lamivudine monotherapy and was similar to that of combination treatment at week 60. The extended therapy duration to 60 weeks did not seem to be a major factor affecting the virologic and clinical responses.

Second, since we did not include a pegylated interferon-

$\alpha$ 2b monotherapy group, we did not evaluate the additive benefit of lamivudine over pegylated interferon alone. The final results of a multicenter trial that compares the pegylated interferon and lamivudine combination with pegylated interferon monotherapy will shed light on this important issue (36). Third, the trial was not double-blinded since we did not use a placebo. However, no patients received antiviral or immunomodulator therapy other than the assigned drugs, and the end points were laboratory values that were probably not affected by patients' perceptions. Fourth, patients assigned to combination treatment had higher median ALT levels at baseline than patients assigned to monotherapy. We thought it unlikely that these relatively small differences in ALT levels accounted for the differences between groups in clinical outcomes, particularly since we confirmed a beneficial effect of combination treatment among the subgroup of patients with baseline ALT levels less than 5 times the upper limit of normal. Fifth, although we evaluated virologic response 24 weeks after treatment, chronic hepatitis B is a persistent disease. Studies with even longer follow-ups are needed to assess clinical outcomes and the durability of response to treatment.

In conclusion, we found that, in patients with HBeAg-positive chronic hepatitis B and moderately elevated ALT levels, combination treatment with pegylated interferon- $\alpha$ 2b and lamivudine was associated with higher rates of end-of-treatment and post-treatment HBeAg seroconversion, an increased potency of HBV suppression, and a lower incidence of lamivudine resistance than lamivudine monotherapy. We now need head-to-head comparisons to see whether this combination treatment leads to similar or higher rates of sustained response compared with either pegylated interferon alone or conventional interferon with or without lamivudine.

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