CLINICAL—LIVER, PANCREAS, AND BILIARY TRACT

Sustained Response of Hepatitis B e Antigen-Negative Patients 3 Years After Treatment with Peginterferon Alfa-2a

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This article has an accompanying continuing medical education activity on page 2388. Learning Objective: Upon completion of this exercise, successful learners will be able to assess the likelihood of sustained response, 3 years post-treatment in patients with HBeAg-negative chronic hepatitis B treated with peginterferon alfa-2a, and will be able to utilize this knowledge when assessing the potential treatment options and deciding upon the most appropriate course of management for their patients with chronic hepatitis B.

See CME quiz on page 2388.

BACKGROUND & AIMS: Patients with hepatitis B e antigen (HBeAg)–negative chronic hepatitis B treated with peginterferon alfa-2a with or without lamivudine achieve significantly higher 6-month posttreatment rates of response compared with those treated with lamivudine alone. The durability of ≤3-year posttreatment response was investigated in this study. **METHODS:** Patients received peginterferon alfa-2a only (180 μg once weekly; n = 177), in combination with lamivudine (100 mg daily; n = 179) or lamivudine alone (n = 181) for 48 weeks. A total of 315 patients (116, 114, and 85, respectively) participated in this posttreatment observational study. **RESULTS:** Three years after treatment, the percentage of patients with normal alanine aminotransferase (ATL) was higher for patients treated with peginterferon alfa-2a (31%) than with lamivudine (18%; $P = 0.032$). Similarly, 28% of patients treated with peginterferon had hepatitis B virus (HBV) DNA levels ≤ 10,000 copies/mL versus 15% of patients treated with lamivudine ($P = .039$). Peginterferon alfa-2a treatment and high baseline ALT level were independent baseline predictors of long-term virologic response ($P = .040$ and $P = .01$, respectively). Of the patients who had been treated with a peginterferon alfa-2a–containing regimen, 8.7% cleared hepatitis B surface antigen (HBsAg; 44% of those with undetectable HBV at 3-year posttreatment follow-up) compared with none treated with lamivudine alone. **CONCLUSIONS: Biochemical and virologic responses were sustained for ≤3 years in approximately 25% of patients given a 48-week course of peginterferon alfa-2a, with or without lamivudine. The increased rate of HBsAg clearance in patients with HBeAg-negative chronic hepatitis B supports the use of peginterferon alfa-2a as a first-line treatment.**

Hepatitis B e antigen (HBeAg)–negative chronic hepatitis B (CHB), which is characterized by progressive liver damage,[1] results from chronic infection with hepatitis B virus (HBV) variants that have mutations in the precore/core promoter region that reduce or abolish HBeAg expression.[2–4] The disease has a poor prognosis, with only rare incidences of spontaneous remission; its prevalence is increasing throughout the world.[3,5,6]

Rates of sustained treatment response are generally poor because of the high probability of relapse, particularly after nucleos(t)ide analog therapy.[7–9] Interferon alfa and nucleos(t)ide analogs have been recommended as first-line therapy for HBeAg-negative CHB.[10,11] Although sustained response after conventional interferon alfa

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; ULN, upper limit of normal.

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therapy is associated with increased rates of hepatitis B surface antigen (HBsAg) seroconversion and significantly improved rates of overall and complication-free survival,[9,10] its pharmacokinetic profile necessitates thrice weekly dosing. Suppression of HBV DNA by nucleos(t)ide analogs requires continued administration; however, their prolonged use is associated with the development of drug resistance.[7,12,13]

Peginterferon alfa-2a has shown significantly higher posttreatment response rates compared with lamivudine, both as monotherapy and when used in combination with lamivudine in a recent large, multicenter, randomized study of patients with HBeAg-negative CHB.[14] The proportion of patients with normalization of alanine aminotransferase (ALT) levels and HBV DNA levels ≤ 20,000 copies/mL 6 months after a 48-week course of therapy was 44% and 29%, respectively, among those who received lamivudine, 59% and 43%, respectively, in the peginterferon alfa-2a monotherapy group ($P = .004$ and $P = .007$ vs lamivudine).

The durability of response to conventional interferon alfa therapy in HBeAg-negative CHB is reported to be approximately 20%–25%,[10] with most patients who experience biochemical or virologic relapse doing so within the first year of follow-up after treatment.[8,15–17] Because the long-term response to peginterferon alfa-2a beyond 6 months of follow-up has not been described, this report examines the response 3 years after treatment in patients with HBeAg-negative CHB treated with peginterferon alfa-2a, with or without lamivudine.

Materials and Methods

The trial was designed by the sponsor (F. Hoffmann-La Roche, Basel, Switzerland) and the principal academic investigators. Clinical data were collected by the peginterferon alfa-2a HBeAg-negative CHB Study Group (listed in Appendix 1). The data were managed by the sponsor and the academic investigators; the sponsor performed the statistical analysis. The academic investigators had unrestricted access to the data and were actively involved in the analysis and interpretation and the writing of the manuscript. The principal authors were responsible for the decision to publish the results. One academic author (Dr Marcellin) and one industry employee (Mr Wu) vouch for the completeness and accuracy of the data.

Study Design and Patients

Initial study. In the initial study (WV16241), 537 patients were randomly assigned 1:1:1 to receive 180 μg/wk peginterferon alfa-2a (Pegasys; F. Hoffmann-La Roche) plus oral placebo daily (n = 177), peginterferon alfa-2a 180 μg/wk plus 100 mg/d lamivudine (Epivir-HBV/Zeffix; GlaxoSmithKline, Brentford, United Kingdom; n = 179), or 100 mg/d lamivudine (n = 181) for 48 weeks.[14]

Adult patients were eligible for the initial study if they had been negative for HBeAg and positive for anti-HBe and HBsAg for ≥ 6 months; had an HBV DNA level > 100,000 copies/mL, and a serum ALT > 1 but ≤ 10 times the upper limit of normal (ULN) range (upper limit of normal range, 30 IU/mL) at least once during the 1-month screening period; and had findings on liver biopsy within the previous 24 months consistent with the presence of CHB, with evidence of prominent necroinflammatory activity.[14]

The coprimary efficacy outcomes were normalization of ALT to below the ULN and suppression of HBV DNA < 20,000 copies/mL (3571 IU/mL),[18] 6 months after treatment (week 72).

Long-term follow-up study. All participating centers in the initial study were invited to participate in a separate, stand-alone, long-term observational follow-up protocol (long-term study). All patients who were randomly assigned, received treatment, and returned for follow-up in the initial study were eligible for the long-term study. Patients receiving either registered or investigational therapy for CHB during the follow-up period were excluded from the follow-up phase and were regarded as nonresponders. Similarly, patients who had an ALT value > 2 times ULN or HBV DNA levels > 100,000 copies/mL 12 months after treatment discontinued the study and were considered to be nonresponders in the analyses.

Interim analyses were planned a priori for each of the yearly follow-up visits, ie, at 1-year intervals after treatment in the initial study.

The study was conducted according to the guidelines of the Declaration of Helsinki and with the principles of Good Clinical Practice and was approved by local ethics committees. All patients gave written informed consent.

Efficacy Parameters

ALT was measured at local laboratories in accordance with standard procedures. Serum HBV DNA and HBsAg were measured at 1 of 3 central laboratories using the COBAS AMPLICOR HBV MONITOR (Roche Diagnostics, Branchburg, NJ); HBsAg and anti-HBs antibodies were measured with the ABBOTT ARCHITECT assay (Abbott Laboratories, Abbott Park, IL).

The primary efficacy parameters defined for the long-term follow-up study were (1) normalization of ALT (≤ 30 IU/L) and (2) suppression of HBV DNA levels to ≤ 20,000 copies/mL. Because of recent insight into the clinical relevance of viral cutoff values, HBV DNA suppression to ≤ 10,000 copies/mL was also investigated. This level corresponds to that accepted by the most recent guidelines on CHB treatment;[10] therefore, these results are shown here in detail rather than those for HBV DNA ≤ 20,000 copies/mL. Secondary efficacy parameters included suppression of HBV DNA to ≤ 400 copies/mL, as well as the proportion of patients with

HBsAg clearance and HBsAg seroconversion (clearance of HBsAg and presence of anti-HBs).

Efficacy analyses were based on (1) the long-term follow-up study population, defined as all patients who participated in the long-term observational study and (2) the initial study population, defined as all patients participating in the initial interventional study. Patients who were discontinued from the protocol because they had initiated a new HBV treatment or had experienced a biochemical or virologic breakthrough as defined by the protocol, as well as those with missing data, were considered in the analyses based on both study populations as not having achieved a response for any of the efficacy endpoints.

In addition to the above-mentioned parameters measured at each follow-up visit, any intervening or ongoing significant clinical events related to CHB were documented.

Statistical Analysis

A descriptive summary of the data was prepared for each of the yearly follow-up visits. Multiple logistic regression was used to identify potential pretreatment or on-treatment factors predictive of an HBV DNA response ≤ 10,000 copies/mL 6 months after treatment and 3 years after treatment. Factors included in the model were sex, age, ethnicity, body weight, serum HBV DNA at baseline, HBV genotype, serum ALT at baseline, fibrosis stage, and type of study treatment. In a separate model, HBV DNA and ALT at treatment weeks 12, 24, and 48 were also explored. The statistical analysis software used was SAS (SAS Institute, Cary, NC) version 8.0. *P* values were calculated with the Cochran-Mantel-Haenszel or Fisher's exact test when appropriate.

This article presents the data collected up to 3 years after completion of study treatment for those patients who entered the long-term study.

Results

Of the 54 centers involved in the initial study, 42 participated in the long-term study, contributing 315 (59%) of the original 537 patients (Table 1). Significantly more patients who received peginterferon alfa-2a with or without lamivudine (66% and 64%, respectively) than lamivudine alone (47%; $P < .01$ vs peginterferon alfa-2a with or without lamivudine) in the initial study went on to participate in the long-term study.

Biochemical and Virologic Response Up to 3 Years After Treatment

Cross-sectional analysis. The patient disposition and overview of results at end of treatment, 6 months after treatment, and 1, 2, and 3 years after treatment are shown in Table 2. Overall, the analysis has shown significantly higher rates of response to peginterferon alfa-2a than to lamivudine 3 years after end of treatment, with approximately one-fifth of the patients treated with peginterferon alfa-2a achieving a biochemical or virologic response.

Three years after the end of treatment 36 patients treated with peginterferon alfa-2a monotherapy had normal ALT (31% of the long-term study and 20% of the initial study participants) compared with 15 patients treated with lamivudine (18% of the long-term study and 8% of the initial study participants; $P = .032$ and $P = .001$, respectively) (Table 2).

HBV DNA levels ≤10,000 copies/mL were observed in 32 patients (28% of the long-term study and 18% of the initial study participants) who had received peginterferon alfa-2a, compared with 13 of the patients treated with lamivudine (15% of the long-term study and 7% of the initial study participants, $P = .039$ and $P = .002$, respectively) (Table 2).

A total of 35 patients (30% of the long-term study population) had HBV DNA < 20,000 copies/mL at 3 years after treatment compared with 31 (27%) of the combination treatment group and 13 (15%) of the lamivudine group ($P = 0.032$ and $P = 0.036$, respectively). The percentage of patients with biochemical or virologic response at the 1-, 2-, and 3-year follow-up assessments after treatment is shown in Figure 1*A–C*.

Advanced fibrosis (stages 3 and 4) was present in 55 of the 230 patients treated with peginterferon alfa 2a enrolled in the follow-up study. The rates of response 3 years after treatment for patients with and without advanced fibrosis were 25% and 26%, respectively, for HBV DNA ≤ 10,000 copies/mL (18% and 15% for HBV DNA ≤ 400 copies/mL), suggesting virologic response was not affected by the histologic stage of disease.

Longitudinal analysis. Because the natural history of HBeAg-negative CHB is characterized by fluctuations in ALT and HBV DNA levels, we considered it important to look closely at these over time in individual patients during long-term follow-up. Individual HBV DNA profiles at year 1, 2, and 3 follow-up assessments after treatment for patients who responded to peginterferon alfa-2a alone or in combination with lamivudine or lamivudine alone are shown in Figure 2. Individual ALT profiles at these time points are available as Supplementary Material.

Of all patients treated with peginterferon alfa-2a participating in the long-term study, 52 (23%) had a longitudinal HBV DNA response; 33 patients (14%) had documented HBV DNA levels ≤ 10,000 copies/mL at each of the 1-, 2-, and 3-year posttreatment assessments; 19 patients had HBV DNA ≤ 10,000 copies/mL at 2 of the 3 study visits between 1 and 3 years after treatment, whereas data from one study visit were missing.

In contrast, 9% of the lamivudine-treated patients participating in the long-term study had a longitudinal HBV DNA response as defined above, 5% had documented HBV DNA levels ≤ 10,000 copies/mL at each of the 1-, 2-, and 3-year assessment after treatment ($P = .008$ and $P =$

Table 1. Characteristics of Patients in the Initial Study* and in the Long-Term Follow-Up Study

	Peginterferon alfa 2a		Peginterferon alfa 2a + lamivudine		Lamivudine	
	Initial study (n = 177)	LT study (n = 116)	Initial study (n = 179)	LT study (n = 114)	Initial study (n = 181)	LT study (n = 85)
Age, mean/median, *y*	40/41	40/41	41/40	40/39	40/40	39/39
Sex, female/male, %	15/85	16/84	18/82	18/82	14/86	13/87
Race, %						
White	37	25	36	30	38	32
Oriental	61	73	63	71	62	68
Other	2	2	1	0	0	0
Weight, mean, *kg*	71.0	69.8	69.7	68.7	70.6	72.0
HBV genotype, %						
A	6	6	6	7	4	6
B	24	28	23	29	27	33
C	36	43	39	40	32	34
D	31	20	30	21	35	26
Other (E, H, B/C, D/G)	3	3	3	3	2	1
HBV DNA at before treatment start, log_{10} *cp/mL*						
Mean	7.1	7.1	7.3	7.2	7.2	6.9
Median	7.0	7.0	7.2	7.0	7.0	6.8
Range	2.3–12.0	2.3–12.0	2.7–12.0	3.1–12.0	2.8–12.0	3.5–12.0
ALT before treatment start, *IU/L*						
Mean	94.4	86.5	90.8	88.2	105.7	85.1
Median	61.5	60.4	64.2	63.1	71.6	87.2
Range	10.2–508	10.2–454	11.3–514	16.5–514	9.8–1051	18.0–643
Presence of bridging fibrosis or cirrhosis before treatment start, n (%)	54 (31)	33 (28)	40 (22)	22 (20)	53 (29)	16 (19)
Use of lamivudine/NAs before study treatment, n (%)	7 (4)	5 (4)	15 (8)	9 (8)	9 (5)	3 (4)
Use of conventional IFN before study treatment, n (%)	11 (6)	6 (5)	18 (10)	11 (10)	14 (8)	6 (7)
Response 6 months after treatment (end of initial study)						
ALT normal, %	59	66	60	65	44	54
HBV DNA ≤ 20,000 cp/mL, %	43	48	44	52	29	39
Combined response (ALT normal and HBV DNA ≤ 20,000 cp/mL), %	36	40	38	45	23	31
HBV DNA ≤ 400 copies/mL, %	19	23	20	24	7	8

cp, copies; IFN, interferon; LT, long-term; NA, nucleos(t)ide analog.

.018, respectively, vs rates with peginterferon alfa-2a with or without lamivudine). Individual HBV DNA profiles for lamivudine-treated patients are available as Supplementary Material.

A total of 45 patients treated with peginterferon alfa-2a had ALT levels constantly ≤ 30 U/L at all 3 follow-up visits (or 20% of all patients participating in the long-term follow-up study). An additional 27 patients (12%) had ALT levels ≤ 30 IU/L at 2 of the 3 follow-up visits and a missing value at the remaining visit. Among the lamivudine-treated patients only 6 patients had ALT levels constantly ≤ 30 U/L at all 3 yearly follow-up visits (or 7%; $P = .008$ vs peginterferon alfa-2a).

In conclusion, among the long-term study participants 23% of the patients treated with peginterferon alfa-2a had a longitudinal HBV DNA response compared with 9% of the lamivudine-treated patients ($P = .008$) (Figure 3). The rates of longitudinal ALT response were 31% and 19% for peginterferon alfa-2a and lamivudine, respectively ($P = .029$) (Figure 3).

Predictors of a Virologic Response

The baseline factors associated with a virologic response (≤10,000 copies/mL) at 6 months and 3 years after treatment are shown in Figure 4. Treatment with peginterferon alfa-2a and baseline ALT level were factors significantly and independently associated with a virologic response 3 years after treatment ($P = 0.04$ for monotherapy; $P = 0.090$ for combination therapy versus lamivudine; $P = 0.01$ for higher vs lower ALT levels). HBV DNA at baseline and age were significant predictors of virologic response 6 months after treatment but did not reach the significance level of 0.05 as independent predictors of response 3 years after treatment (Figure 4).

Table 2. Patient Disposition and Overview of Response at End of Treatment, 6 Months After Treatment, and 1, 2, and 3 Years After Treatment

Initial study (48 weeks of treatment and 24 weeks of follow-up)	PegIFNα-2a (n = 177)		PegIFNα-2a + lamivudine (n = 179)		Lamivudine (n = 181)	
	n	%	n	%	n	%
End of treatment						
ALT < 1 time ULN	67	38	87	49	132	73
HBV DNA ≤ 20,000 cp/mL	144	81	164	92	154	85
HBV DNA ≤ 400 cp/mL	112	63	156	87	113	73
6 months after treatment						
ALT < 1 times ULN	105	59	107	60	80	44
HBV DNA ≤ 20,000 cp/mL	76	43	79	44	53	29
HBV DNA ≤ 400 cp/mL	34	19	35	20	12	7
HBsAg loss	7	5	5	3	0	0

	PegIFNα-2a			PegIFNα-2a + lamivudine			Lamivudine		
Entered long-term follow-up study	n	Follow-up study population, % (n = 116)	Initial study population, % (n = 177)	n	Follow-up study population, % (n = 114)	Initial study population, % (n = 179)	n	Follow-up study population, % (n = 85)	Initial study population, % (n = 181)
1 year after treatment									
ALT<1 time ULN	58	50	33	51	45	28	28	33	15
HBV DNA ≤ 10,000 cp/mL	35	30	20	35	31	20	17	20	9
HBV DNA ≤ 400 cp/mL	17	15	10	14	12	8	6	7	3
HBsAg loss	4	3	2	5	4[a]	3	0	0	0
2 years after treatment									
ALT<1 time ULN	37	32	21	32	28[a]	18	17	20	9
HBV DNA ≤ 10,000 cp/mL	32	28	18	24	21	13	10	12	6
HBV DNA ≤ 400 cp/mL	18	16	10	13	11	7	3	4	2
HBsAg loss	7	6	4	6	5*	3	0	0	0
3 years after treatment									
ALT<1 time ULN	36	31[b]	20[c]	35	31[a]	20[d]	15	18	8
HBV DNA ≤ 10,000 cp/mL	32	28[e]	18[f]	28	25[g]	16[h]	13	15	7
HBV DNA ≤ 400 cp/mL	21	18[i]	12[j]	15	13[k]	8[l]	5	6	3
HBsAg loss	9	8[m]	5[n]	9	8[o]	5[p]	0	0	0

Cp indicates copies; PegIFNα-2a, peginterferon alfa-2a.
[a]PegIFNα-2a plus lamivudine versus lamivudine, 3 years after treatment, $P = .036$.
[b,c]PegIFNα-2a versus lamivudine, 3 years after treatment: [b]$P = .032$; [c]$P = .001$. [d]PegIFNα-2a plus lamivudine versus lamivudine, 3 years after treatment, $P = .002$.
[e,f]PegIFNα-2a versus lamivudine, 3 years after treatment: [e]$P = .039$; [f]$P = .002$.
[g,h]PegIFNα-2a plus lamivudine versus lamivudine, 3 years after treatment: [g]$P = .111$; [h]$P = .012$.
[i,j]PegIFNα-2a versus lamivudine, 3 years after treatment: [i]$P = .011$; [j]$P < .001$.
[k,l]PegIFNα-2a plus lamivudine versus lamivudine, 3 years after treatment: [k]$P = .092$; [l]$P = .020$.
[m,n]PegIFNα-2a versus lamivudine, 3 years after treatment: [m]$P = .001$; [n]$P = .002$.
[o,p]PegIFNα-2a plus lamivudine versus lamivudine, 3 years after treatment: [o]$P = .011$; [p]$P = .002$.

No on-treatment factors were clinically useful predictors of response to peginterferon alfa-2a 3 years after treatment (data not shown). HBV DNA suppression to ≤ 400 copies/mL 6 months after treatment was a good indicator of 3-year posttreatment response. Of the 69 patients treated with peginterferon alfa-2a with HBV DNA ≤ 400 copies/mL 6 months after treatment, 15 (22%) did not participate in the long-term follow-up study. Among the 54 patients who did participate, 46% (25/54) had HBV DNA levels below the detection limit 3 years after treatment, whereas 7% (4/54) had HBV DNA levels of 401–10,000 copies/mL. Nine patients had missing values for HBV DNA 3 years after treatment, of which 3 had undetectable HBV DNA 2 years after treatment, and 6 had an undetectable HBV DNA value 1 year after treatment and also a missing value 2 years after treatment. Thirty percent of the patients with HBV DNA ≤ 400 copies/mL 6 months after treatment experienced virologic breakthrough documented by HBV DNA levels > 10,000 copies/mL at one of the subsequent follow-up visits. Thirty percent of the 54 patients treated with peginterferon alfa-2a who achieved HBV DNA ≤ 400 copies/mL 6 months after treatment had cleared HBsAg by the 3-year posttreatment assessment. Of the 181 patients treated with lamivudine, 12 had HBV DNA ≤ 400 copies/mL 6 months after treatment. Of these 12 patients, only one returned for subsequent assessment, with an HBV DNA level of 1,399 copies/mL 3 years after treatment.

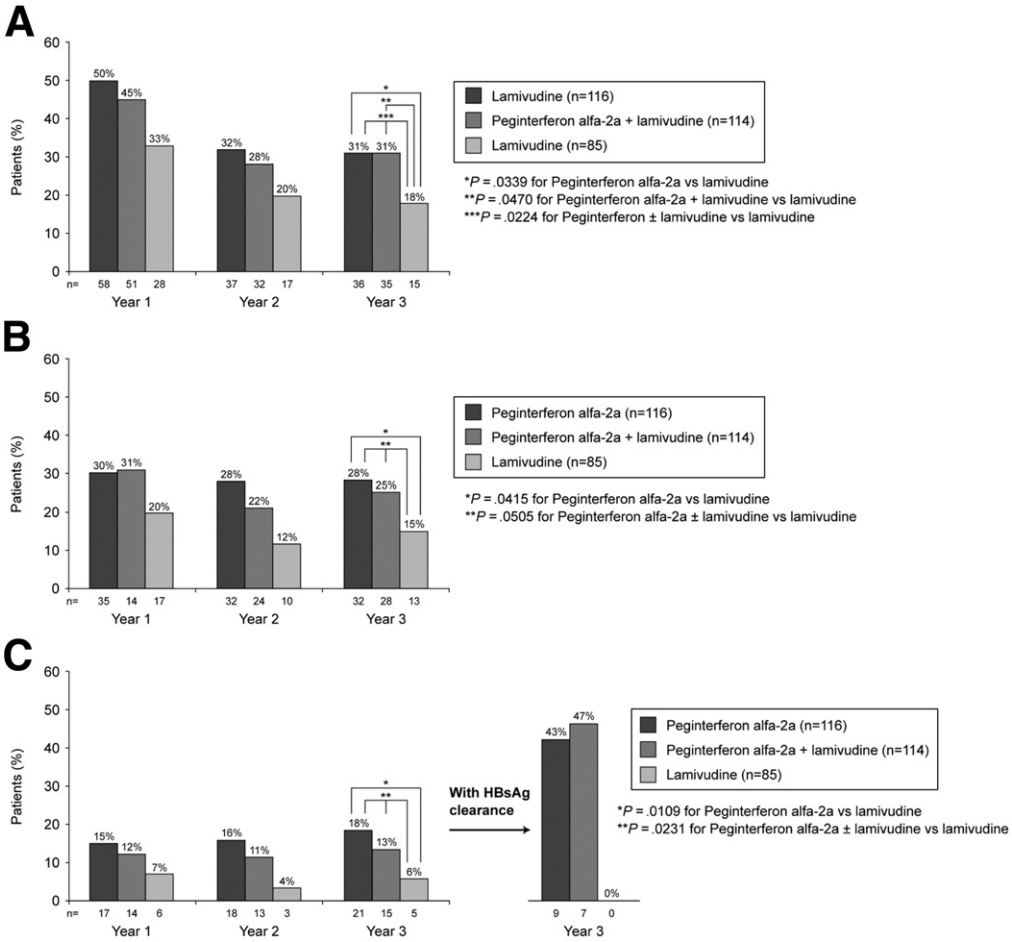


Figure 1. Long-term (A) biochemical (ALT normal) and virologic response (B) HBV DNA ≤ 10,000 copies/mL and (C) ≤400 copies/mL. Data are expressed as percentages of patients participating in the long-term observational study.

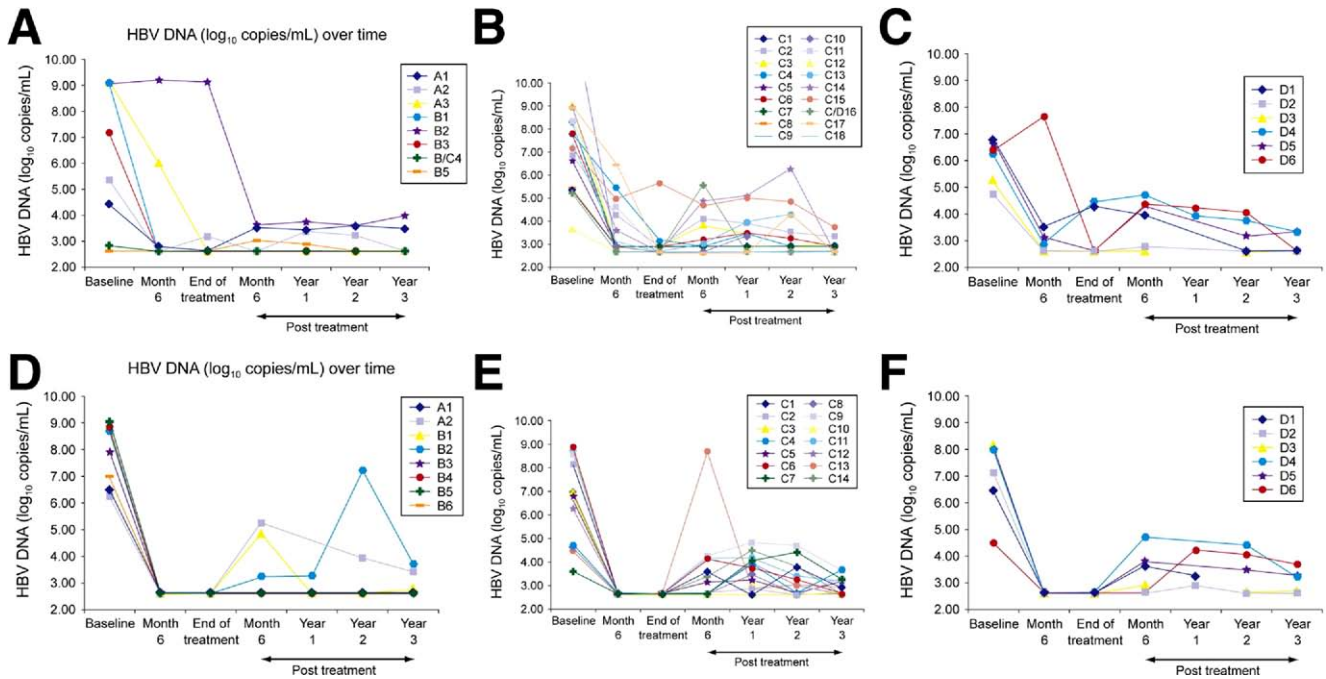


Figure 2. HBV DNA profiles of individual patients achieving a virologic response (HBV DNA ≤ 10,000 copies/mL) 3 years after completion of treatment with peginterferon alfa-2a monotherapy (A–C; n = 32) or peginterferon alfa-2a in combination with lamivudine (D–F; n = 28) The letters A to D on each key, indicate the HBV genotype for each patient.

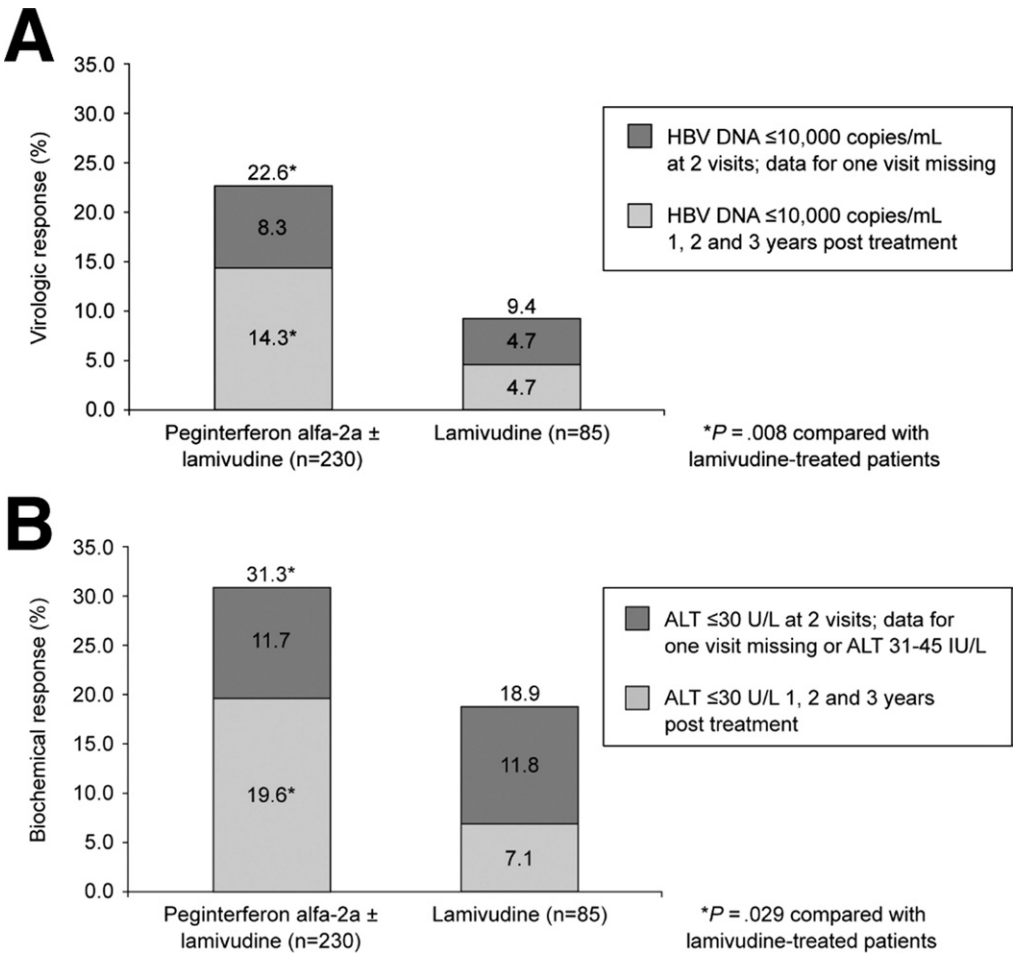


Figure 3. Longitudinal virologic (A) and biochemical (B) response in patients participating in the long-term follow-up study.

HBsAg Response Up to 3 Years After Treatment

The percentage of patients treated with peginterferon alfa-2a clearing HBsAg increased over the 3-year follow-up period (Table 2). A total of 20 patients (8.7% of the long-term follow-up population and 5.6% of the initial study population) who had been treated with a regimen containing peginterferon alfa-2a and followed in the long-term study cleared HBsAg: 18 patients were HBsAg negative 3 years after treatment; 2 patients had cleared HBsAg by the 1- and 2-year posttreatment assessments, respectively, but did not return for the subsequent follow-up visits. Eight of the 20 patients had detectable HBs antibodies.

Six months after treatment, at the end of the initial study, 12 patients had experienced HBsAg clearance. Of these 12 patients, 10 participated in the long-term study. Three years after the end of treatment a total of 18 patients (8%) had HBsAg clearance: 9 of the 10 patients who had already experienced HBsAg clearance 6 months after treatment and 9 patients who had cleared HBsAg later during follow-up (2, 3, and 4 patients had documented HBsAg negativity for the first time 1, 2, and 3 years after treatment, respectively). Two additional patients, for whom further follow-up data are not available, had negative HBsAg 1 and 2 years after treatment. One patient who was HBsAg negative 6 months after treatment tested HBsAg positive 1 year after treatment and was then lost to follow-up. In summary, a total of 20 patients (8.7%) had HBsAg clearance at the last long-term follow-up visit, whereas one patient who cleared HBsAg during the initial study experienced relapse during the follow-up period.

Eighteen of the patients who cleared HBsAg had undetectable HBV DNA levels at the end of treatment; one patient had a low level of HBV DNA at this time (644 copies/mL); the other patient did not have HBV DNA measured at the end of treatment but had undetectable HBV DNA 4 weeks after the end of treatment. HBV DNA levels remained undetectable in most of the patients throughout the 3-year follow-up period; low levels of HBV DNA (between 465 and 14,000 copies/mL) were detected on occasion in 5 patients. At the 3-year follow-up evaluation after treatment, the HBV DNA levels were ≤ 400 copies/mL in 17 of the 20 patients who cleared HBsAg; in one patient the HBV DNA level was 1,331 copies/mL; data were unavailable for 2 patients.

Of the 181 patients treated with peginterferon alfa-2a (with or without lamivudine) who had HBV DNA ≤ 400

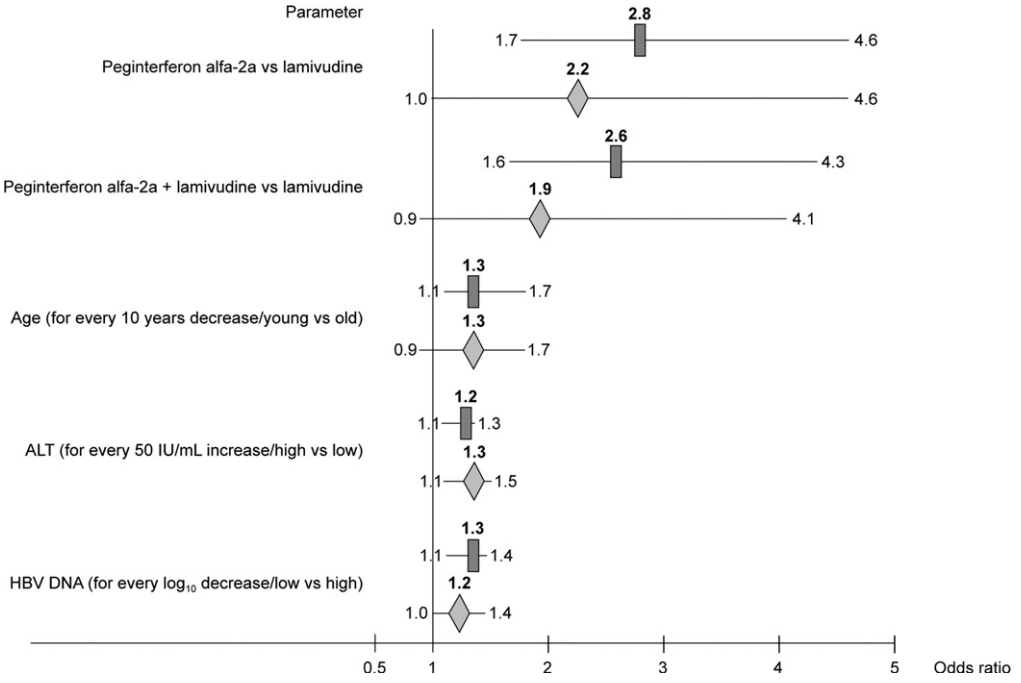


Figure 4. Predictors of virologic response (HBV DNA ≤ 10,000 copies/mL) at 6 months and 3 years after treatment. Results from multivariate logistic regression analysis. Solid bars represent odds ratios (ORs) for achieving a virologic response 6 months after treatment. Diamonds represent OR for achieving a virologic response 3 years after treatment. Horizontal lines represent 95% confidence intervals of the ORs. In addition to the variables presented in the figure, the following variables were included in the logistic regression model but were not significantly associated with virologic response: sex, race, body weight, HBV genotype, fibrosis stage. Five hundred and seventeen and 315 patients were included in the models predicting virologic response at 6 months and 3 years after treatment, respectively.

copies/mL at the end of treatment, 17 (9.4%) had cleared HBsAg at the 3-year posttreatment assessment. In contrast, only 1 (2%) of the 49 patients treated with peginterferon alfa-2a who had HBV DNA > 400 copies/mL at the end of treatment had cleared HBsAg 3 years after the end of treatment. A high proportion (44%) of patients treated with peginterferon alfa-2a with undetectable HBV DNA 3 years after treatment had also cleared HBsAg, compared with none of those who received lamivudine alone.

HBsAg clearance was observed in patients treated with peginterferon alfa-2a infected with all major HBV genotypes: 3 genotype A (20%), 4 genotype B (6%), 9 genotype C (9%), 3 genotype D (6%), and 1 mixed genotype C/D (2%). HBsAg clearance was not observed in any patient treated with lamivudine monotherapy ($P = 0.008$ for peginterferon alfa-2a with or without lamivudine vs lamivudine).

Clinical Events During Long-Term Follow-Up

No cases of hepatic decompensation were reported during the long-term follow-up period. However, most patients with insufficient therapeutic response were ineligible for the follow-up study because they would have been considered to need therapeutic intervention. Two CHB-related deaths, because of hepatocellular carcinoma, were reported; one in a patient treated with peginterferon alfa-2a plus lamivudine who died 3.5 years after study start and one in a patient treated with lamivudine alone who died 3.9 years after study start. Although both patients had a virologic and biochemical response 6 months after treatment, both had advanced fibrosis at study entry, and neither cleared HBsAg. Only these 2 patients, of the 71 patients with advanced fibrosis or cirrhosis (fibrosis stage 3 or 4) at study entry and who subsequently joined the long-term study, developed hepatocellular carcinoma. Viral load and ALT levels were elevated in both of these 2 patients during long-term follow-up.

Withdrawals From the Long-Term Study

In total, 210 of the 315 patients who entered the long-term follow-up study withdrew during the follow-up phase (72, 75, and 63 patients in the peginterferon alfa-2a, combination, and lamivudine arms, respectively). The most common reasons given for withdrawal from the study were insufficient therapeutic response (145/210 patients, 69%) and failure to return (42/210 patients, 20%). These patients were considered as nonresponders for all efficacy parameter evaluations.

Discussion

The long-term data reported here show that 26% of patients treated with peginterferon alfa-2a, with or without lamivudine, and followed during a 3-year period achieved a virologic response with HBV DNA levels ≤ 10,000 copies/mL 3 years after treatment. This is an HBV DNA level accepted by the latest treatment guidelines as an appropriate marker of response.[10] The demographics of the current study (average age approximately 40 years and 60% of patients of Asian origin)[14] are similar to the large-scale REVEAL study that showed that this level of replication is associated with a marked reduction in the risk of hepatocellular carcinoma in untreated patients.[19] In addition, this level of replication is associated with reduced risk of progressive liver disease.[20]

Because fluctuations in ALT and HBV DNA levels are characteristic of the natural course of HBeAg-negative dis-

ease, it is important to note that, among the long-term study participants receiving peginterferon alfa-2a, longitudinal HBV DNA response (≤ 10,000 copies/mL) documented at 1, 2, and 3 years after treatment was 14% (9% if all patients in the initial study were included). Similarly, longitudinal biochemical response (ALT ≤ 30 IU/mL) at 1, 2, and 3 years after treatment was 20% for patients in the follow-up study and 13% if all patients in the initial study were included. The initial study showed higher rates of response to peginterferon alfa-2a than to lamivudine. These findings were confirmed in the long-term study.

In accordance with previous studies in patients treated with conventional interferon alfa,[8,15–17] virologic relapse after cessation of treatment with peginterferon alfa-2a in the current study occurred mainly within the first year after treatment. Among patients treated with peginterferon alfa-2a experiencing HBV DNA rebound, relapse occurred within 1 year after treatment in 80% of patients. Patients who have sustained a response to peginterferon alfa-2a 1 year after treatment were likely to sustain this response in the longer term. However, because patients were followed at yearly intervals, it is possible that transient increases in HBV DNA levels occurring between these assessments may have been missed.

One of the limitations of our study was that the long-term follow-up enrolled only a proportion of the patients who participated in the initial study introducing the risk of selection bias.[14] The participation rate of 59% was similar to the one recently reported by Buster et al[21] (65%) in a long-term follow-up study after treatment with peginterferon alfa-2b in patients with HBeAg-positive CHB. Because the follow-up study included proportionally more patients receiving a peginterferon alfa-2a based regimen than lamivudine alone, it is possible that data derived from this analysis may overestimate the "true" rates of response if patients who were more likely to respond entered the long-term study. Applying the conservative assumption that patients who did not participate in the long-term follow-up study did not achieve a virologic response, nevertheless, supports one of the main conclusions of the study: significantly more patients treated with peginterferon alfa-2a achieved a protocol-defined biochemical or virologic response 3 years after treatment than those who received lamivudine alone. However, this conservative approach will probably result in underestimating the rate of response because no patients were enrolled in the long-term study from >20% of study centers. This is supported by the observation that a considerable number of patients who did not participate in the long-term study had responses 6 months after treatment (undetectable HBV DNA levels, HBsAg loss), that are associated with sustained long-term response.

Another limitation of this study was the high number of patients who failed to return during long-term follow-up. Most patients who did not return did so because of viral reactivation requiring antiviral therapy, and these patients were classified as not having achieved a response in the analysis.

The sustained long-term effects of peginterferon alfa-2a treatment are reflected by the continual increase in the proportion of patients who achieved HBsAg clearance during the posttreatment period and are in agreement with previous observations on long-term follow-up of patients treated with conventional interferon alfa; approximately 10% of patients treated for 6–12 months achieved HBsAg clearance[2,22] and 15% of those treated for 24 months and followed for >5 years.[23] Rates of HBsAg clearance of 71% have been reported 11 years after the end of treatment in patients with CHB disease who responded to therapy.[24] Our observations suggest that potent HBV DNA suppression to ≤ 400 copies/mL at the end of peginterferon alfa-2a treatment appears to be required but is not sufficient for subsequent clearance of HBsAg. The rate of HBsAg clearance was high (44%) in patients treated with peginterferon alfa-2a with undetectable HBV DNA 3 years after treatment. Our findings in HBeAg-negative disease reflect those reported recently in HBeAg-positive disease after a 52-week course of peginterferon alfa-2b with or without lamivudine whereby 11% of patients cleared HBsAg after a mean follow-up period of 3 years.[21] In the HBeAg-positive study, HBsAg clearance was significantly more common in patients infected with HBV genotype A than in those with other genotypes. In contrast, in our HBeAg-negative patients, HBsAg clearance did not appear to be genotype related.

The lack of HBsAg clearance seen in our study in patients treated solely with the nucleos(t)ide analog lamivudine has also been observed for patients with HBeAg-negative disease treated with newer anti-HBV agents such as adefovir, entecavir, and tenofovir.[25–27] In a recent large study of entecavir, only 1 of 325 patients with HBeAg-negative disease treated for 48 weeks achieved HBsAg clearance, although 90% of patients had HBV DNA suppressed to < 300 copies/mL at this time.[26] HBsAg clearance after treatment with the newer more potent nucleos(t)ide analogs appears to be more readily achievable in patients with HBeAg-positive disease. HBsAg clearance was reported in 6% of HBeAg-positive patients after 2 years of treatment with tenofovir[28] compared with <1% in HBeAg-negative patients with the same treatment duration.[29] It is currently unclear whether HBsAg clearance during treatment with nucleotside analogs is durable if treatment is stopped.

The sustained posttreatment response mechanism after a course of treatment with peginterferon alfa-2a remains unclear. However, in addition to its antiviral action, peginterferon alfa-2a exerts an immunomodulatory activity, which may contribute to the observed long-term effects—particularly the ability to induce, via activation of a virus-specific immune response, HBsAg seroconversion.[7,30–34] An analysis of end treatment HBsAg levels in a subgroup of patients in the long-term study reported here and their association with response to peginterferon alfa-2a 3 years posttreatment have now been published.[35] This, coupled with the

observation of early on-treatment HBsAg decline during peginterferon alfa-2a therapy, suggests that monitoring HBsAg levels may be a helpful predictor of posttreatment response.[36–37]

In summary, a 48-week course of peginterferon alfa-2a, alone or in combination with lamivudine, has been shown to induce biochemical or virologic responses that were sustained 3 years after treatment in approximately 25% of patients participating in the long-term follow-up study. A high rate (44%) of HBsAg clearance was achieved among patients who sustained suppression of HBV DNA to undetectable levels 3 years after treatment. The ability to induce HBsAg clearance—an outcome that is associated with long-term complication-free survival—supports the use of peginterferon alfa-2a as a first-line treatment of HBeAg-negative disease, avoiding the need for long-term therapy and the associated risks of developing drug resistance in those patients who achieve and maintain their response.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2009.03.006.

Appendix 1. Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group

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The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and analysis.

Conflicts of interest

The authors disclose the following: Dr Marcellin has received consulting and lecture fees from Roche, and grants from Roche, Dr Bonino has received consulting fees from Roche; Dr Lau received consulting fees from Roche, and GlaxoSmithKline, and lecture fees from Roche, and GlaxoSmithKline, Dr Farci has received consulting fees from Roche; Dr Yurdaydin, Dr Piratvisuth, Dr Jin, Dr Gurel, Dr Hadziyannis, and Dr Lu have received grant support from Roche. Mr Wu and Dr Popescu report being employees of Roche.

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Supplementary Material

Longitudinal Analysis

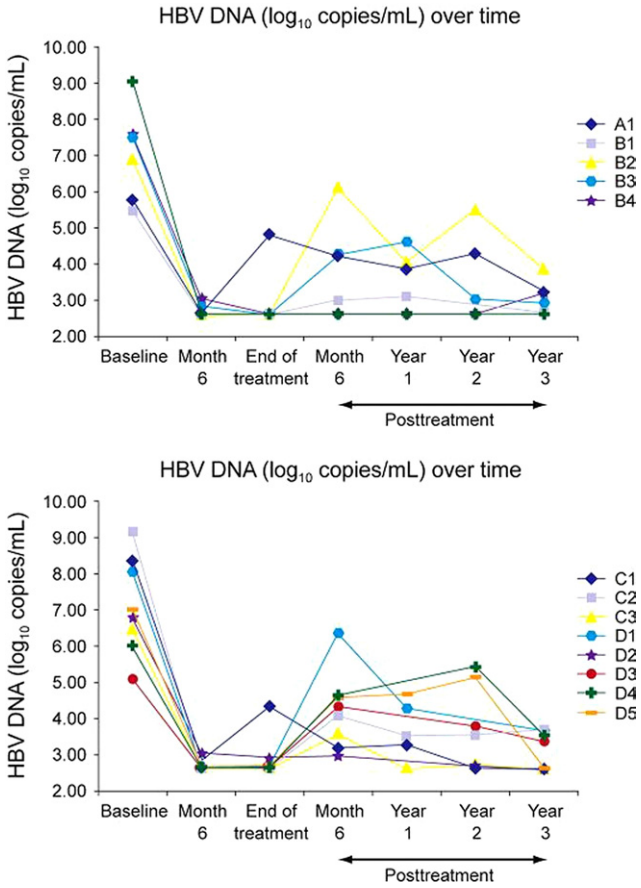
Of the patients treated with peginterferon alfa-2a, 135 patients (or 38% of patients entering the initial study) had HBV DNA levels ≤ 10,000 copies/mL 6 months after treatment. Of these, 31 (23%) did not participate in the long-term follow-up study. Of the 104 patients participating in the long-term study, 47 patients either experienced relapse, defined as HBV DNA ≤ 10,000 copies/mL at least once during the follow-up period (44 patients) or had missing values (3 patients) at all 3 follow-up visits. Relapse occurred most frequently early after the end of treatment with 35, 7, and 2 patients having levels of HBV DNA ≤ 10,000 copies/mL detected for the first time at 1, 2, and 3 years after treatment, respectively. Of the remaining 57 patients, 29 had HBV DNA levels ≤ 10,000 copies/mL at all 3 follow-up visits; 16 had a documented response at 2 of the 3 follow-up visits and a missing value at one visit; 12 patients had documented HBV DNA response at only 1 of the visits with missing values for the remaining 2 visits. Thus, 45 patients had a sustained longitudinal HBV DNA response, defined as levels ≤ 10,000 copies/mL documented at least at 2 of the 3 study visits between 1 and 3 years after treatment. In contrast, of the 126 patients treated with peginterferon alfa-2a who had HBV DNA levels ≤ 10,000 copies/mL 6 months after treatment, only 7 patients had HBV DNA levels suppressed ≤ 10,000 copies/mL during subsequent follow-up. In summary, 52 patients (or 23% of all patients treated with peginterferon alfa-2a participating in the long-term study) had a sustained longitudinal HBV DNA response as defined above; 33 patients (or 14%) had documented HBV DNA levels ≤ 10,000 copies/mL at each of 1-, 2-, and 3-year posttreatment assessments.

Among the 85 patients treated with lamivudine who participated in the long-term study, 77 patients had either a documented relapse (n = 61), had missing values at all 3 follow-up visits (n = 10), or missing values at 2 and 3 years after treatment (n = 6). Eight patients (6 of the 21 patients with HBV DNA ≤ 10,000 copies/mL and 2 of the 64 patients with HBV DNA > 10,000 copies/mL 6 months after treatment) had a sustained longitudinal response. Thus, 9% of the patients treated with lamivudine participating in the long-term study had a sustained longitudinal HBV DNA response as defined above, 5% had documented HBV DNA levels ≤ 10,000 copies/mL at each of 1-, 2-, and 3-year posttreatment assessments (P = 0.008 and P = .018, respectively, vs rates with peginterferon alfa-2a with or without lamivudine). Individual HBV DNA responses in patients treated with lamivudine are shown in Supplementary Figure 1.

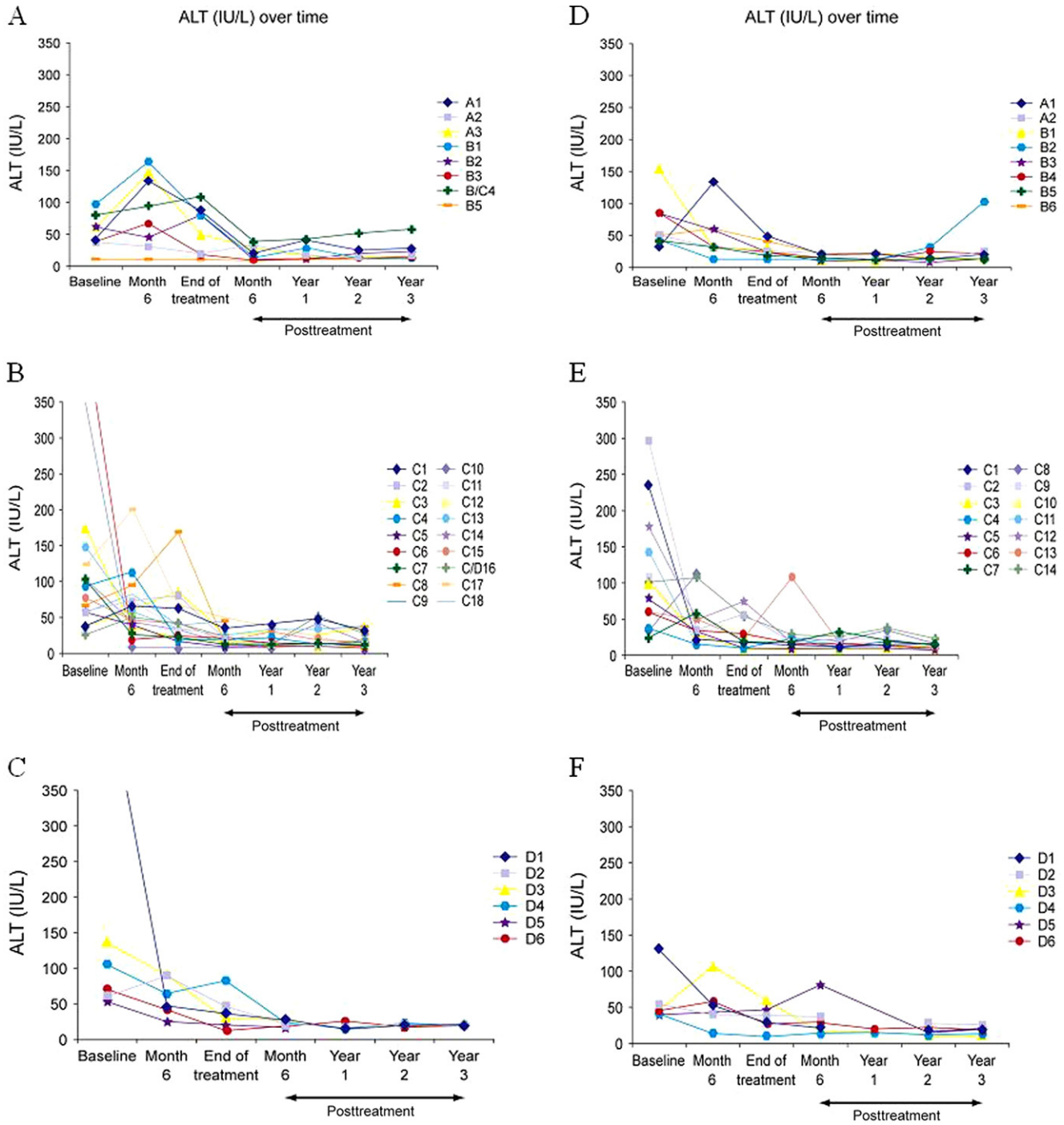
Of the patients treated with peginterferon alfa-2a, 212 patients (or 60% of patients entering the initial study) had ALT levels ≤ 30 U/L 6 months after treatment. Of these, 62 (29%) did not participate in the long-term follow-up study. Of the 150 patients participating in the long-term study, 41 patients had ALT levels constantly ≤30 U/L at each of the 1-, 2-, and 3-year posttreatment assessment; 23 patients had ALT levels ≤ 30 U/L at 2 of the 3 follow-up assessments and either a missing value or an ALT level of 30–45 U/L at the remaining visit. Thirty-six patients had documented ALT elevations >45 U/L at least once (26 patients experienced ALT relapse 1 year after treatment, 7 patients at 2 years, and 3 patients at 3 years after treatment); 50 patients had missing values for ≥2 visits. Among the 80 patients with ALT > 30 U/L 6 months after treatment only 4 patients had ALT levels constantly ≤ 30 U/L at all 3 yearly follow-up visits; a further 4 patients had ALT levels ≤ 30 U/L at 2 of the 3 follow-up visits and either a missing value or ALT levels of 30–45 U/L at the remaining visit. In summary, a total of 45 patients treated with peginterferon alfa-2a had ALT levels constantly ≤ 30 U/L at all 3 follow-up visits (20% of patients participating in the long-term follow-up study). An additional 19 patients (8%), who could also be considered to have a longitudinal ALT response, had ALT levels ≤ 30 U/L at 2 of the 3 follow-up visits and a missing value at the remaining visit. Individual ALT profiles of patients treated with peginterferon alfa-2a are shown in Supplementary Figure 2.

Of the 85 patients treated with lamivudine participating in the long-term follow-up study, only 6 patients had ALT levels constantly ≤ 30 U/L at all 3 yearly follow-up visits (7% of all patients participating in the long-term follow-up study, P = .008 vs peginterferon alfa-2a). An additional 10 patients (12%), who could also be considered to have a longitudinal ALT response, had ALT levels ≤ 30 U/L at 2 of the 3 follow-up visits and either a missing value (19 patients) or ALT levels between 30 and 45 U/L at the remaining visit. Individual ALT profiles of patients treated with lamivudine are shown in Supplementary Figure 3.

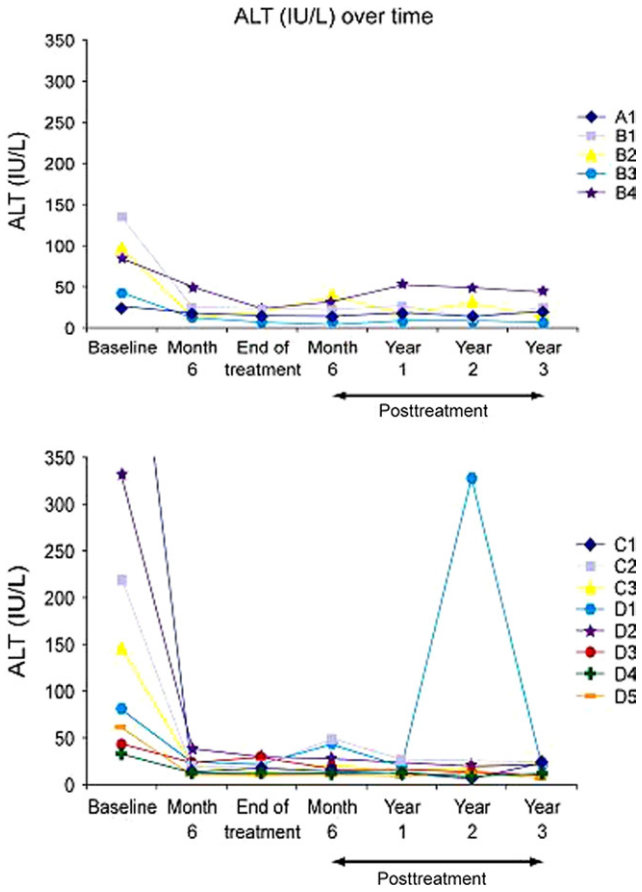
Overall, among the long-term study participants 23% of the patients treated with peginterferon alfa-2a had a sustained longitudinal HBV DNA response compared with 9% of the patients treated with lamivudine (P = .008) (Figure 3, main document). The rates of longitudinal ALT response were 31% and 19% for peginterferon alfa-2a and lamivudine, respectively (P = .029) (Figure 3, main document).



Supplementary Figure 1. HBV DNA profiles of individual patients achieving a virologic response (HBV DNA ≤ 10,000 copies/mL) 3 years after completion of treatment with lamivudine monotherapy (n = 13). The letters A to D in each key indicate the HBV genotype for each patient.



Supplementary Figure 2. ALT level profiles of individual patients achieving a virologic response (HBV DNA ≤ 10,000 copies/mL) 3 years after completion of treatment with peginterferon alfa-2a monotherapy (A–C; n = 32) or peginterferon alfa-2a in combination with lamivudine (D–F; n = 28). The letters A–D in the key indicate the HBV genotype for each patient.



Supplementary Figure 3. ALT profiles of individual patients achieving a virologic response (HBV DNA ≤ 10,000 copies/mL) 3 years after completion of treatment with lamivudine monotherapy; n = 13). The letters A to D in each key indicate the HBV genotype for each patient.