

## Predicting response to peginterferon alfa-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B

**Authors:** F Bonino[1], P Marcellin[2], G K K Lau[3], S Hadziyannis[4], R Jin[5], T Piratvisuth[6], G Germanidis[7], C Yurdaydin[8], M Diago[9], S Gurel[10], M-Y Lai[11], M R Brunetto[12], P Farci[13], M Popescu[14], P McCloud[15] for the Peginterferon Alfa-2a HBeAg-Negative Chronic Hepatitis B Study Group

**Affiliations:** [1] University of Pisa and Foundation IRCCS, Policlinico di Milano, Italy; [2]Service d'Hépatologie, INSERM Unite 481 and Centre de Recherches Claude Bernard sur les Hépatite Virales, Hôpital Beaujon, Clichy, France; [3]Queen Mary Hospital, The University of Hong Kong, Hong Kong, China; [4]Department of Medicine and Hepatology, Henry Dunant Hospital, Athens, Greece; [5]Digestive Disease Department, Beijing You An Hospital, Beijing, China; [6]Department of Medicine, Prince of Songkla University, Songkla, Thailand; [7]Papageorgiou General Hospital, Pathology Clinic, Thessalonika, Greece; [8]University of Ankara, Faculty of Medicine, Ankara, Turkey; [9]Hospital General Universitario de Valencia, Valencia, Spain; [10]University of Uludag, Faculty of Medicine, Bursa, Turkey; [11]National Taiwan University Hospital, Internal Medicine, Taipei, Taiwan, ROC; [12]Azienda Ospedaliera Universitaria Pisana, Pisa, Italy; [13]Universita di Cagliari, Cagliari, Italy; [14]Roche, Basel, Switzerland; [15]Roche, Dee Why, Australia

**Correspondence to:** Professor F Bonino, Scientific Director, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Policlinico, Via Francesco Sforza 28, 20122 Milano, Italy

Tel: +39 (02) 55038377; E-mail: [bonino@med-club.com](mailto:bonino@med-club.com)

**Keywords:** predictor, antiviral agents, post-treatment, chronic hepatitis B, multivariate

**Word count:** 3706

**Abbreviations:** ALT, alanine aminotransferase; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B s antigen; HBV, hepatitis B virus; HAI, Histologic Activity Index; ULN, upper limit of normal

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Heart editions and any other BMJ PGL products to exploit all subsidiary rights, as set out in our licence (<http://gut.bmjournals.com/misc/ifora/licenceform.shtml>).

### ABSTRACT

**Background and aims:** In a trial of patients with hepatitis B e antigen (HBeAg)-negative chronic hepatitis B, 24-week post-treatment biochemical and virologic response rates with peginterferon alfa-2a ± lamivudine were significantly higher than with lamivudine alone. The effect of pre-treatment factors on post-treatment responses has been investigated.

**Patients and methods:** Multivariate analyses were performed using available data from 518 patients treated with peginterferon alfa-2a monotherapy± lamivudine, or lamivudine monotherapy. A post-treatment response was defined as alanine aminotransferase (ALT) normalisation and hepatitis B virus (HBV) DNA <20,000 copies/mL.

**Results:** In logistic regression analyses across all treatment arms, peginterferon alfa-2a (± lamivudine) therapy, younger age, female gender, high baseline ALT, low baseline HBV DNA and HBV genotype were identified as significant predictors of combined response 24 weeks post-treatment. In the peginterferon alfa-2a and lamivudine monotherapy arms, patients infected with genotypes B or C had a higher chance of response than genotype D infected patients ( $p < 0.001$ ), the latter responding better to combination than peginterferon alfa-2a monotherapy ( $p = 0.015$ ). At 1 year post-treatment, response rates by intention-to-treat analysis were 19.2% for peginterferon alfa-2a, 19.0% for combination, and 10.0% for lamivudine-treatment groups, with genotypes B or C associated with a sustained combined response to peginterferon alfa-2a ± lamivudine therapy.

**Conclusions:** Baseline ALT and HBV DNA levels, patient age, gender, and infecting HBV genotype significantly influenced combined response 24 weeks post-treatment, in patients

treated with peginterferon alfa-2a and/or lamivudine. At 1 year post-treatment HBV genotype was significantly predictive of efficacy for patients treated with peginterferon alfa-2a ± lamivudine.

## INTRODUCTION

Chronic hepatitis B virus (HBV) infection continues to be a major global health problem with more than 400 million cases worldwide and is a significant cause of cirrhosis and hepatocellular carcinoma.[1] Hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) represents the late phase of the infection where the HBV population is predominantly made up of HBV precore/basic core promoter variants that reduce or abolish the expression of HBeAg.[2][3] Spontaneous sustained remissions are rare in patients with HBeAg-negative CHB and the disease is characterised by persistent viral replication, progressive liver damage, and early development of cirrhosis.[4][5][6] HBeAg-negative CHB is particularly prevalent in the Mediterranean region and Asia. Nevertheless, recent studies indicate that HBeAg-negative disease may be more common than previously suspected and that its prevalence is increasing worldwide.[2][3]

Until recently, conventional interferon alfa and lamivudine were the only drugs specifically licensed for the treatment of CHB. However, in patients with HBeAg-negative CHB, both drugs are associated with high rates of relapse upon treatment discontinuation and poor sustained response rates.[7][8][9][10][11] Furthermore, conventional interferon alfa has a suboptimal pharmacokinetic profile that necessitates an inconvenient dosing regimen (three-times weekly). Lamivudine is hindered by the development of drug resistance, which increases with prolonged use.[12][13] In the past few years, the nucleotide analogue, adefovir, has been approved as a treatment option for CHB, although continuous administration is required in patients with HBeAg-negative disease due to rapid biochemical, virologic, and histologic rebound upon adefovir withdrawal.[14] Continuous adefovir therapy is also associated with increased drug resistance, albeit at a lower cumulative rate than lamivudine.[15] Peginterferon alfa-2a, which was also recently approved for the treatment of CHB, has been shown to provide significantly higher post-treatment response rates compared with lamivudine in patients with HBeAg-negative CHB.[16] Peginterferon alfa-2a, which has a dual immunomodulatory and antiviral activity, has also proved more effective than both conventional interferon and lamivudine in patients with HBeAg-positive disease.[17][18]

To date, investigations of predictors of response have generally focused on patients with HBeAg-positive CHB. In patients with this phase of the disease, low serum HBV DNA levels and high serum alanine aminotransferase (ALT) levels at baseline have been shown to be independently associated with improved responses to conventional interferon alfa.[19][20][21][22] Likewise, in patients receiving lamivudine, high baseline ALT was identified as the most important predictor of HBeAg loss by multivariate modelling.[23] Higher rates of HBeAg clearance after conventional interferon alfa therapy have been reported for Asian patients infected with HBV genotype B compared with genotype C.[22][24] However, HBV genotype seems not to influence HBeAg responses to lamivudine, at least in Chinese patients.[25] At present, no detailed analysis of predictors of response has been performed in patients with HBeAg-negative CHB. Accordingly, in the current study we report predictors of combined response to peginterferon alfa-2a monotherapy, peginterferon alfa-2a plus lamivudine combination therapy and lamivudine monotherapy using data from a large multinational, randomised trial involving 537 patients with HBeAg-negative disease.[16]

## PATIENTS AND METHODS

The patients and results of the study of peginterferon alfa-2a monotherapy versus peginterferon alfa-2a plus lamivudine versus lamivudine monotherapy in HBeAg-negative CHB have been described elsewhere.[16] All patients gave written informed consent. All patients were positive for hepatitis B s antigen (HBsAg) and anti-HBe antibody, but negative for HBeAg. Entry criteria included an HBV DNA level >100,000 copies/mL and an ALT level >1 times, but ≤10 times the upper limit of normal (ULN; 30 IU/L in this study). All included patients had CHB status confirmed by liver biopsy within the 12 months prior to randomisation. Previous treatment for CHB was allowed, but not within the 6 months prior to the study. Patients were randomly assigned in a 1:1:1 ratio to receive 48 weeks of either peginterferon alfa-2a (40KD) (PEGASYS, Roche, Basel, Switzerland) 180 µg once weekly plus oral placebo once daily; peginterferon alfa-2a 180 µg once weekly plus lamivudine 100 mg once daily (Epivir-HBV/Zeffix, GlaxoSmithKline, Greenford, UK); or lamivudine 100 mg once daily. The co-primary endpoints of the study were ALT normalisation and HBV DNA <20,000 copies/mL, measured 24 weeks post-treatment (week 72). All study centres involved in the initial clinical trial were invited to participate in a roll-over long-term observational study to assess the durability of response. For this analysis, patients were assessed 1 year after the end of treatment using the same efficacy measures as at 24 weeks post-treatment.

### Virologic, biochemical and histologic measurements

Serum HBV DNA was measured at a central laboratory using the COBAS AMPLICOR HBV MONITOR® (Roche Diagnostics, Branchburg, NJ, USA). Serum ALT was measured at local laboratories in accordance with common standardised procedures. Baseline biopsy specimens were scored using the modified Histologic Activity Index (HAI) score [26] by an independent histopathologist who was unaware of the patient's treatment assignment.

### Definition of response and relapse

In the present analyses, a post-treatment combined response was defined as both ALT normalisation and HBV DNA <20,000 copies/mL, 24 weeks after the end of the treatment period. Patients with missing values 24 weeks post-treatment were considered as treatment failures, i.e. no combined response. A sustained virologic response was defined as HBV DNA <20,000 copies/mL at the end-of-treatment and 24 weeks post-treatment; post-treatment virologic relapse was defined as HBV DNA <20,000 copies/mL at the end-of-treatment, but >20,000 copies/mL 24 weeks post-treatment.

### Statistics

Multiple logistic regression with prospectively defined covariates of interest was used to determine pre-treatment factors predictive of a combined response 24 weeks post-treatment. We did not formally use any of the automatic selection procedures: forward-entry, backward elimination, stepwise or best-subset selection. The predictors included in the model were all pre-specified, except that *region* was eliminated because of the confounding with genotype. The parameters of the logistic regression were fitted with the maximum likelihood procedure, and all statistical tests were Wald tests.

The pre-treatment factors included were age, gender, genotype, ethnicity, bodyweight, HAI score, serum ALT (screening and baseline), and serum HBV DNA (baseline). Gender, genotype and ethnicity were included as categorical variables; age,

bodyweight, HAI score, serum ALT, and serum HBV DNA were included as continuous variables. Those pre-treatment factors found to be significant in the overall logistic regression model were assessed in stratified analyses to show rates of response across different categories of these characteristics. Additional comparative logistic regression analyses were performed to investigate pre-treatment and on-treatment factors associated with sustained virologic response and post-treatment virologic relapse. The statistical analysis software used was SAS (SAS Institute, Cary, NC) version 8.0.

## RESULTS

### Effect of pre-treatment factors on combined response 24 weeks post-treatment (data from the initial study)

As previously reported,[16] the percentage of patients achieving a combined response 24 weeks post-treatment was significantly higher with peginterferon alfa-2a monotherapy (36% [63/177]) or peginterferon alfa-2a plus lamivudine combination therapy (38% [68/179]) than with lamivudine monotherapy (23% [42/181];  $p=0.011$  and  $p=0.002$ , respectively).

Of the 537 patients analysed using the intent-to-treat principle in the original study,[16] only those of Asian or Caucasian origin and singularly infected with HBV genotype A, B, C or D were included in the overall logistic regression analysis ( $n=518$ ); 19 patients were excluded as they were of non-Caucasian/non-Asian origin and/or were infected with a mixed HBV genotype population or HBV genotypes E, G or H.

### Multivariate analyses

In the initial multivariate analysis of individual pre-treatment factors, baseline HBV DNA, baseline ALT, treatment arm, age, and gender were significant predictors of a combined response in patients receiving peginterferon alfa-2a monotherapy, peginterferon alfa-2a plus lamivudine or lamivudine monotherapy; genotype, ethnicity, bodyweight, and screening ALT were not significant. Because ethnicity and genotype were highly correlated and could potentially have a confounding effect on one another, multivariate analysis was performed with ethnicity excluded from the logistic regression model. The baseline variables found to be significant predictors of response in the initial model (i.e. baseline HBV DNA, baseline ALT, age, gender, and type of treatment) remained significant (Table 1).

In addition, genotype was also identified as a significant predictor of combined response 24 weeks post-treatment ( $p<0.001$ ). The odds ratios from the logistic regression analysis indicated that a 10-fold higher baseline ALT level (i.e. 500 IU/L versus 50 IU/L) increased the odds of combined response 3.7 fold, and that female patients had around twice the odds of achieving a combined response compared with male patients. The multivariate analysis also confirmed that the rate of combined response was significantly higher with peginterferon alfa-2a monotherapy ( $p=0.014$ ; odds ratio [OR]: 1.84) and peginterferon alfa-2a plus lamivudine ( $p=0.002$ ; OR: 2.19) than with lamivudine, even after adjusting for the effect of the other pre-treatment variables included in the model. Thus, those patients treated with peginterferon alfa-2a ( $\pm$  lamivudine) had around twice the odds of achieving a combined response 24 weeks post-treatment compared with those treated with lamivudine monotherapy.

**Table 1** Logistic regression analysis on pre-treatment factors and sustained combined response\*– multivariate analyses of all treatment arms ( $n=518$ )

| Pre-treatment Factor <sup>†</sup> | Comparison | Odds Ratio <sup>‡</sup><br>[95% CI] | p value |
|-----------------------------------|------------|-------------------------------------|---------|
|-----------------------------------|------------|-------------------------------------|---------|

|   |   |                             |                |
|---|---|-----------------------------|----------------|
| Age   | 10 year decrease                              | 1.26 [1.0–1.5]              | 0.018          |
| Gender  | Female vs male                                | 1.93 [1.1–3.4]              | 0.022          |
| Bodyweight  | 10 kg increase                                | 1.10 [0.9–1.3]              | 0.273          |
| Baseline ALT  | 1 log <sub>10</sub> unit (IU/L) increase      | 3.69 [1.7–8.1]              | 0.001          |
| HBV DNA   | 1 log <sub>10</sub> unit (copies/mL) decrease | 1.28 [1.1–1.4]              | <0.001         |
| HBV Genotype  | A vs B  | 0.42 [0.1–1.2]              | 0.097          |
|   | A vs C  | 0.33 [0.1–0.9]              | 0.030          |
|   | A vs D  | 0.97 [0.3–2.7]              | 0.958          |
|   | B vs C  | 0.79 [0.5–1.3]              | 0.344          |
|   | B vs D  | 2.31 [1.3–4.2]              | 0.006          |
|   | C vs D  | 2.9 [1.7–5.0]               | <0.001         |
|   | Treatment                                     | PEG-IFN $\alpha$ -2a vs LAM | 1.84 [1.1–3.0] |
| PEG-IFN $\alpha$ -2a + LAM vs LAM   |   | 2.19 [1.3–3.6]              | 0.002          |
| PEG-IFN $\alpha$ -2a vs PEG-IFN $\alpha$ -2a + LAM  |   | 1.19 [0.8–1.9]              | 0.460          |
| Treatment by HBV genotype interaction   | -   | -                           | 0.018          |
| *Combined ALT normalisation and HBV DNA <20,000 copies/mL after 48 weeks of treatment and 24 weeks of treatment-free follow-up. |   |                             |                |
| †ALT at screening was included in the model but was not a significant predictor of response (p=0.952)                           |   |                             |                |
| ‡Each odds ratio adjusted for other variables in model  |   |                             |                |
| LAM, lamivudine; PEG-IFN $\alpha$ -2a, peginterferon alfa-2a; vs, versus  |   |                             |                |

Of the 518 patients evaluated in the overall logistic regression model, 400 patients had available baseline HAI scores. Using data from these 400 patients in a separate multivariate analysis including all prospectively defined pre-treatment variables except ethnicity, baseline HAI score was not a significant predictor of combined response 24 weeks post-treatment (p=0.936).

### Stratified analysis by significantly predictive factors

As shown in [Figure 1](#), Panel A, rates of combined response tended to be higher in patients with higher baseline ALT levels, regardless of treatment. The influence of baseline ALT on combined response was particularly noticeable in the two peginterferon alfa-2a-containing arms, with a combined response occurring in almost half of the patients with ALT values >5xULN. While HBV DNA was a significant predictor in the multivariate analysis, baseline HBV DNA was not a significant predictor by univariate analysis (p=0.088) ([Figure 1](#), Panel B). Female and male patients responded equally well to peginterferon alfa-2a monotherapy ([Figure 1](#), Panel C). However, in the combination therapy and lamivudine monotherapy arms, combined response rates were higher in female patients than male patients. Patients under 40 years of age had higher combined response rates than patients over 40 years old, regardless of which treatment they received ([Figure 1](#), Panel D). Patients treated with peginterferon alfa-2a ( $\pm$  lamivudine) achieved higher rates of combined response than patients treated with lamivudine monotherapy, irrespective of baseline ALT, HBV DNA, gender, or age.

### Effect of HBV genotype on combined response 24 weeks post-treatment

In the overall logistic regression model, genotype was identified as a significant predictor of combined response by multivariate analysis (p<0.001) (Table 1). Furthermore, in this

model, a statistically significant interaction between treatment arm and HBV genotype was identified ( $p=0.018$ ). This interaction indicated that the response to treatment was not uniform across the four HBV genotypes A, B, C and D, and that patterns of response to the three study drugs differed according to genotype.

When the logistic regression analysis was conducted in a subgroup of 346 patients receiving either peginterferon alfa-2a monotherapy or lamivudine monotherapy, the interaction between treatment arm and genotype was no longer significant ( $p=0.637$ ). This model therefore indicated that rates of combined response were higher with peginterferon alfa-2a than with lamivudine, regardless of genotype ( $p=0.012$ ; OR: 1.9; 95% CI: 1.2–3.2). The multivariate analysis also showed that genotype clearly influenced response in patients treated with peginterferon alfa-2a or lamivudine monotherapy as the chance of a combined response was greater in patients infected with genotypes B or C versus genotype D ( $p<0.001$ ; OR: 5.9; 95% CI: 2.7–13.1, and  $p<0.001$ ; OR: 4.6; 95% CI: 2.2–9.5, respectively). This finding was also reflected in stratified analyses were patients infected with genotype D had substantially lower rates of combined response than patients with genotypes B or C (16% [9/55] versus 44% [19/43] and 49% [31/63] with peginterferon alfa-2a; 11% [7/63] versus 39% [19/49] and 26% [15/57] with lamivudine, respectively).

When the multivariate model was conducted in 342 patients treated with peginterferon alfa-2a monotherapy or peginterferon plus lamivudine, the interaction between treatment arm and genotype remained significant ( $p=0.027$ ). This interaction occurred because of a different pattern of response to the two peginterferon alfa-2a regimens among patients infected with genotypes B or D ([Figure 2](#)); in patients with genotype B the rates of combined response were 44% for peginterferon alfa-2a monotherapy and 22% for peginterferon alfa-2a plus lamivudine, while in patients with genotype D the rates of combined response were 16% for peginterferon alfa-2a monotherapy and 37% for peginterferon alfa-2a plus lamivudine. After adjusting for the effects of gender, age, bodyweight, screening ALT, baseline ALT, and baseline HBV DNA, the comparison of peginterferon alfa-2a plus lamivudine versus peginterferon alfa-2a monotherapy in HBV genotype D infected patients resulted in an OR of 3.5 (95% CI: 1.3–9.1,  $p=0.015$ ). In patients with HBV genotype B, the same comparison resulted in an OR of 0.4 (95% CI 0.1–1.2,  $p=0.097$ ).

### **Factors associated with sustained virologic response and post-treatment virologic relapse in patients receiving peginterferon alfa-2a-containing treatment**

Comparative analyses were carried out using data from 294 of 308 patients treated with peginterferon alfa-2a ( $\pm$  lamivudine) who achieved a virologic response (HBV DNA  $<20,000$  copies/mL) at the end of the 48-week treatment period; 14 patients of non-Asian or non-Caucasian origin and/or not singularly infected with HBV genotype A, B, C or D were excluded from the analyses. In total, 139 (47%) of these 294 patients had sustained HBV DNA  $<20,000$  copies/mL 24 weeks post-treatment; the remaining 155 patients had HBV DNA  $>20,000$  copies/mL 24 weeks post-treatment and were considered to have had a virologic relapse. Table 2 shows pre-treatment and on-treatment characteristics for patients with a sustained virologic response versus those with virologic relapse. Logistic regression showed that patient age, HBV genotype, and HBV DNA level at the end-of-treatment (week 48) were significantly associated with post-treatment durability of response by univariate analysis. In the multivariate analysis, age, baseline, and end-of-treatment viral load, and HBV genotype were the only factors significantly influencing post-treatment durability of response. This means that a sustained virologic response to peginterferon alfa-2a ( $\pm$

lamivudine) was more likely in younger patients ( $p < 0.024$ ; OR: 1.3 per 10 year decrease; 95% CI: 1.0–1.7), patients with lower baseline HBV DNA levels ( $p < 0.01$ ; OR: 1.2 per 1 log decrease; 95% CI: 1.0–1.4), and patients with lower end-of-treatment HBV DNA levels ( $p < 0.029$ ; OR: 2.9 per 1 log decrease; 95% CI: 1.1–7.7). In addition, the multivariate analysis showed that genotype C infected patients had a higher chance of a sustained virologic response after peginterferon alfa-2a ( $\pm$  lamivudine) therapy than genotype D infected patients ( $p < 0.001$ ; OR: 3.3; 95% CI: 1.7–6.5).

**Table 2** Pre-treatment and on-treatment characteristics in patients with sustained virologic response or virologic relapse – patients treated with peginterferon alfa-2a monotherapy or peginterferon alfa-2a plus lamivudine

| Characteristic   | Sustained virologic response (n=139) | Virologic relapse (n=155)        | p value* univariate | p value* multivariate |
|--|--------------------------------------|----------------------------------|---------------------|-----------------------|
| Treatment<br>PEG-IFN $\alpha$ -2a<br>PEG-IFN $\alpha$ -2a + LAM                  | 47%<br>53%                           | 46%<br>54%                       | 0.77                | 0.54                  |
| Age (years)<br>Mean $\pm$ SD   | 37.9 $\pm$ 10.6                      | 42.0 $\pm$ 11.3                  | 0.002               | 0.024                 |
| Gender<br>Male<br>Female   | 82%<br>18%                           | 83%<br>17%                       | 0.78                | 0.40                  |
| Bodyweight<br>Mean $\pm$ SD  | 68.7 $\pm$ 12.7                      | 70.9 $\pm$ 13.0                  | 0.14                | 0.36                  |
| Baseline HAI score<br>Mean $\pm$ SD  | 8.6 $\pm$ 3.5                        | 8.8 $\pm$ 3.5                    | 0.66 <sup>†</sup>   | 0.66 <sup>†</sup>     |
| Baseline ALT<br>Mean (IU/L) $\pm$ SD<br>>5 x ULN<br>>2–5 x ULN<br>$\leq$ 2 x ULN | 103 $\pm$ 96<br>19%<br>38%<br>43%    | 88 $\pm$ 66<br>15%<br>39%<br>46% | 0.12                | 0.23                  |
| ALT level at week 48<br>>2 x ULN<br>$\leq$ 2 x ULN                               | 15%<br>85%                           | 12%<br>88%                       | 0.59                | 0.36                  |
| Peak ALT level weeks<br>0–48 (IU/L)<br>Mean $\pm$ SD                             | 51 $\pm$ 110                         | 53 $\pm$ 69                      | 0.28                | 0.26                  |
| Baseline HBV DNA<br>(log <sub>10</sub> copies/mL)<br>Mean $\pm$ SD               | 7.1 $\pm$ 2.0                        | 7.5 $\pm$ 1.8                    | 0.15                | 0.01                  |

|                                      |     |     |        |       |
|--------------------------------------|-----|-----|--------|-------|
| HBV DNA at week 48<br>≤400 copies/mL | 95% | 83% | 0.008  | 0.03  |
| >400–20,000 copies/mL                | 5%  | 18% |        |       |
| HBV Genotype                         |     |     | <0.001 | 0.006 |
| A                                    | 4%  | 7%  |        |       |
| B                                    | 24% | 26% |        |       |
| C                                    | 50% | 28% |        |       |
| D                                    | 21% | 39% |        |       |

\*Rates presented are actual rates for each subgroup. P values are based on univariate or multivariate logistic regression models.

†p values are from a separate logistic regression model performed on 254 patients with available baseline HAI scores and including all other characteristics listed above

ALT, alanine aminotransferase; HAI, Histological Activity Index; HBV, hepatitis B virus; LAM, lamivudine; PEG-IFN $\alpha$ -2a, peginterferon alfa-2a; ULN, upper limit of normal range

### Effect of pre-treatment factors on combined response 1 year post-treatment (data from the long-term follow-up study)

In total, 304 of the original 537 patients (57%) analysed in the initial study participated in the roll-over observational study to assess the durability of response to treatment, including 111 (63%) of those originally treated with peginterferon alfa-2a monotherapy, 111 (62%) of those treated with peginterferon alfa-2a plus lamivudine, and 82 (45%) of those treated with lamivudine alone.[27].

As with the patients analysed 24 weeks post-treatment, only those infected with HBV genotypes A, B, C, and D were included in the analysis at the later time of assessment. The rates of combined response observed 1 year post-treatment were similar to those measured 24 weeks post-treatment, in all three treatment groups who participated in the long-term study. The proportion of patients achieving <20,000 copies/mL and normal ALT at 1 year post-treatment was 33.3% (34/102; 95% CI 24.3–43.4) for those originally in the peginterferon monotherapy group, 32.7% (34/104; 95% CI 23.8 – 42.6) for those in the peginterferon + lamivudine combination therapy group, and 24.0% (18/75; 95% CI 14.9 – 35.3) for those in the lamivudine group. Applying intention-to-treat (ITT) analysis, where patients with missing data at 1 year follow-up were deemed to be non responders, the response rates for the three treatment groups were 19.2% (34/177; 95% CI 13.1 – 25.3), 19.0% (34/179; 95% CI : 13.0 – 25.0) and 10% (18/181; 95% CI : 5.3 – 14.6), respectively.

Data from the multivariate analysis using the original ITT population confirmed that a combined virologic and biochemical response 1 year post-treatment was more likely to be achieved with peginterferon alfa-2a monotherapy ( $p=0.0223$ ; OR 2.11) and peginterferon alfa-2a + lamivudine ( $p=0.0185$ ; OR 2.16) than with lamivudine monotherapy (Table 3). These odds ratios indicated that patients treated with peginterferon alfa-2a with or without lamivudine had approximately twice the potential of achieving a combined response 1 year post-treatment than did those treated with lamivudine alone. In addition, genotype was a significant predictor of response 1 year post-treatment in logistic regression analysis. Also, patients infected with HBV genotypes B ( $p=0.0033$ ) or C ( $p<0.0001$ ) had a better chance of achieving a combined response 1 year post-treatment than did genotype D-infected patients (Table 3). On the other hand, age, gender, weight, baseline ALT and baseline HBV

DNA were not significant predictors of combined response to treatment 1 year post-treatment cessation (Table 3).

**Table 3:** Logistic regression analysis\* on pre-treatment factors and sustained combined response<sup>†</sup> at year 1 post treatment follow-up – multivariate analysis of all treatment arms (n=518)

| Factor                            | Odds Ratio (95% CI)  | p value |
|-----------------------------------|----------------------|---------|
| Age (10 yrs increase)             | 0.80<br>(0.63, 1.02) | 0.0687  |
| Gender (M vs F)                   | 0.68<br>(0.34, 1.37) | 0.2828  |
| Weight (10 kg increase)           | 1.03<br>(0.81, 1.30) | 0.8271  |
| ALT BL                            | 1.00<br>(1.00, 1.00) | 0.3970  |
| Log <sub>10</sub> HBV DNA BL      | 1.06<br>(0.93, 1.21) | 0.3612  |
| Genotype (A vs D)                 | 2.58<br>(0.73, 9.20) | 0.1432  |
| Genotype (B vs D)                 | 3.69<br>(1.54, 8.79) | 0.0033  |
| Genotype (C vs D)                 | 5.46<br>(2.46, 12.1) | <0.0001 |
| PEG-IFN $\alpha$ -2a vs. LAM      | 2.11<br>(1.11, 4.01) | 0.0223  |
| PEG-IFN $\alpha$ -2a +LAM vs. LAM | 2.16<br>(1.14, 4.10) | 0.0185  |

\*ITT analysis based on original patient population where patients with missing data were treated as non-responders  
<sup>†</sup>Combined response: normal ALT and DNA $\leq$ 20,000 cp/mL; only patients with genotype A B C or D were included.  
 ALT, alanine aminotransferase; LAM, lamivudine; PEG-IFN $\alpha$ -2a, peginterferon alfa-2a

## DISCUSSION

The current study describes the first detailed analysis of predictors of response in HBeAg-negative CHB. In multivariate analyses of patients treated with peginterferon alfa-2a alone, lamivudine alone, or the two in combination, high baseline ALT, low baseline HBV DNA, younger age, and female gender were identified as significant predictors of a combined ALT and HBV DNA response 24 weeks post-treatment. The multivariate models also showed that response rates, both 24 weeks and 1 year post-treatment, were significantly higher with peginterferon alfa-2a monotherapy than with lamivudine monotherapy, even after adjusting for the effect of the relevant baseline factors.

Baseline ALT levels had a strong effect on combined response after 48 weeks of treatment and 24-week post-treatment follow-up, with patients in the highest baseline ALT category (>5xULN) achieving the highest response rates, regardless of which treatment they received. This effect was particularly noticeable with the peginterferon alfa-2a-containing regimens, with almost one half of the patients with baseline ALT >5xULN achieving a combined response 24 weeks post-treatment. The effect of lower baseline HBV

DNA levels on combined response was most evident in the peginterferon alfa-2a plus lamivudine and lamivudine monotherapy arms. Brunetto *et al* have previously identified three biochemical profiles in HBeAg-negative CHB: 1) intermittent ALT flares with intervening periods of normal liver enzyme; 2) continuous ALT elevation without flares; and 3) intermittent ALT flares superimposed on continuous ALT elevation.[6]

The higher rate of response seen in patients with elevated ALT at the moment of treatment initiation in our study might suggest that biochemical reactivation of disease activity may potentially represent a suitable time for starting therapy in such patients, and should be explored further. The data indicate that in patients with HBeAg-negative CHB and elevated ALT (with the possible exception of those with compensated cirrhosis), treatment with peginterferon alfa-2a is both efficacious and safe, provided that the patients are carefully selected and monitored throughout treatment.

In this study, combined response rates 24 weeks post-treatment were consistently higher in female patients compared with male patients. This was most apparent in those treated with combination therapy or lamivudine alone. When combined response rates were stratified by patient age, the highest rates were seen in younger patients, irrespective of which treatment they received. This finding supports data from a previous study suggesting that younger patients treated with conventional interferon alfa have a higher probability of survival compared with older patients.[28]

Genotype was also identified as a significant predictor of combined response. The influence of genotype was particularly apparent in patients treated with peginterferon alfa-2a or lamivudine monotherapy, with those infected with HBV genotype B or C having higher rates of combined response compared with those infected with genotype D. In contrast to the overall findings of the study, which did not document a benefit of the combination regimen over peginterferon alfa-2a monotherapy, the combination of peginterferon alfa-2a and lamivudine seems to be beneficial to patients with genotype D. Genotype D patients receiving combination therapy had substantially higher rates of combined response than genotype D patients receiving peginterferon alfa-2a monotherapy. This apparent benefit of the combination regimen versus peginterferon alfa-2a monotherapy was not seen in patients infected with the other genotypes. However, this observation agrees with those of a recently published study by Colombatto *et al*. [30] where bio-mathematical modelling revealed a higher reduction of HBV-infected cells at the end of therapy among patients with genotype D who were receiving combination therapy.

Since only a small number of patients infected with genotype D participated in the long-term part of the study, it was not possible to confirm the benefits of the combination regimen in this sub-group of patients at the 1 year post-treatment time point. In summary, as these findings are based on relatively few patients and could be the result of random variation, additional comparative data are required to confirm if combination therapy is beneficial in the subset of HBeAg-negative CHB patients specifically infected with HBV genotype D.

Analysis of the subset of patients who achieved a virologic response (HBV DNA <20,000 copies/mL) at the end-of-treatment with peginterferon alfa-2a ( $\pm$  lamivudine) revealed that older patients and those with higher baseline and/or end-of-treatment HBV DNA levels were more likely to experience relapse than younger patients or those with lower HBV DNA levels. Our finding that higher baseline and end-of-treatment HBV DNA levels were associated with relapse concurs with previous studies of conventional interferon alone or in combination with lamivudine. [9][29] In these patients with virologic response at the end-of-treatment, HBV genotype was also seen to influence the durability of response

to peginterferon alfa-2a-containing therapy with patients infected with HBV genotype D more likely to relapse post-treatment than genotype C infected patients.

One of the limitations of our study is that predictor analyses using the entire study population was only possible based on efficacy outcomes assessed 24 weeks post-treatment. An analysis of the efficacy results 1 year post-treatment was based on a subgroup of patients participating in the long-term study, and those not participating were regarded as non-responders. For this reason, it cannot be ruled out that a selection bias might have affected the results seen in this subpopulation at this time point post-treatment.

In conclusion, the current analyses show that baseline ALT and HBV DNA levels, as well as patient age and gender, are factors which might influence post-treatment response. HBV genotype emerges as a new predictive factor in HBeAg-negative CHB, with genotype D being associated with a poorer response to treatment than other genotypes. With verification in future trials, these data on predictors of sustained response in patients with HBeAg-negative CHB may prove useful in clinical practice by allowing the selection of the most appropriate treatment strategy based on individual patient characteristics. The role of HBV genotype in tailoring treatment regimens deserves further investigation.

## FIGURE LEGENDS

**Figure 1** Combined response (ALT normalisation and HBV DNA <20,000 copies/mL) 24 weeks post-treatment by baseline ALT (Panel A), HBV DNA (Panel B), gender (Panel C) and age (Panel D). Vertical bars represent 95% CI.

**Figure 2** Combined response (ALT normalisation and HBV DNA <20,000 copies/mL) 24 weeks post-treatment by HBV genotype in patients treated with peginterferon alfa-2a monotherapy, peginterferon alfa-2a plus lamivudine, or lamivudine. Vertical bars represent 95% CI.

## SPONSOR DETAILS

Supported by a research grant from Roche, Basel, Switzerland.

### Competing interests:

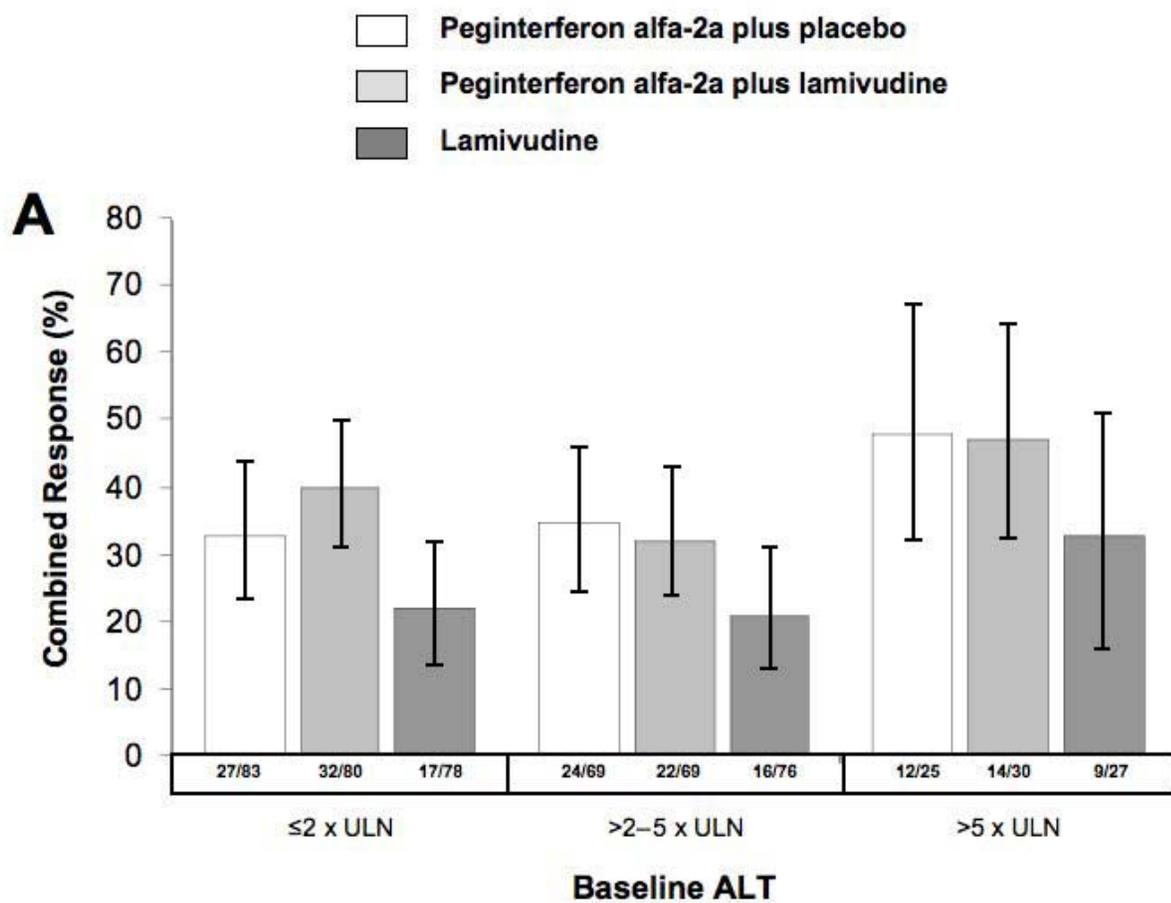
- F. Bonino has received reimbursement from Roche for attending symposia, speaking at symposium, undertaking clinical research, organising educational activities and consulting. F. Bonino has acted as an expert witness on study through publication of data.
- M. Brunetto has received reimbursement from Roche for attending symposia, speaking at symposium and undertaking clinical research. M. Brunetto has acted as an expert witness on study through publication of data.
- M. Diago has received fees for speaking at a Roche symposium.
- P. Farci has received reimbursement for consulting for Roche.
- G. Germanidis has received reimbursement from Roche for attending congresses and research funding.
- S. Gurel declared no competing interests.

- S. Hadziyannis has received reimbursement from Roche for consulting and for speaking, and has received funding for research from Roche.
- M. Y. Lai declared no competing interests.
- G. K. K. Lau has received fees for attending and speaking at a Roche symposium.
- P. Marcellin has received fees and reimbursement for symposia and for consulting from Roche.
- P. McCloud is an employee of Roche Products Pty Ltd.
- T. Piratvisuth has received reimbursement from Roche for speaking at symposium and undertaking clinical research.
- M. Popescu is an employee of Roche, Basel, Switzerland,
- C. Yurdaydin is on the advisory board of Roche and is a member of the Roche speaker's bureau.

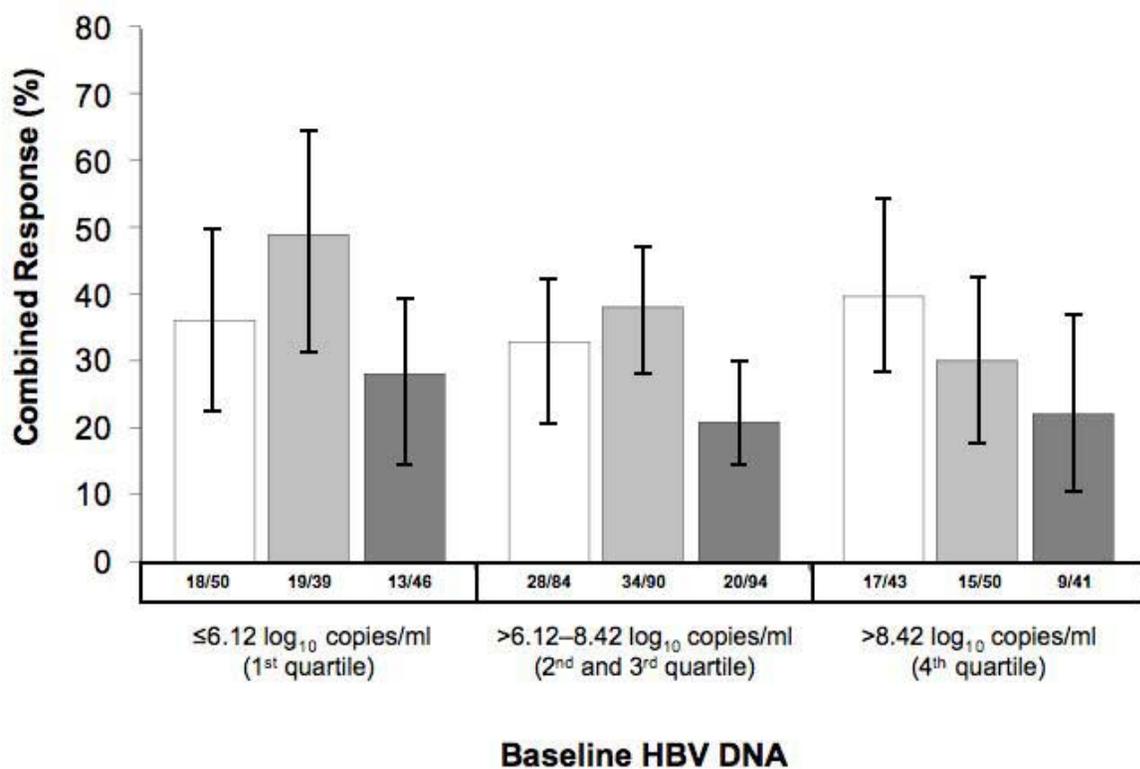
## REFERENCES

- 1 Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004;11:97-107.
- 2 Funk ML, Rosenberg DM, Lok ASF. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. *J Viral Hepat* 2002;9:52-61.
- 3 Chu CJ, Keeffe EB, Han SH, *et al*. Prevalence of HBV precore/core promoter variants in the United States. *Hepatology* 2003;38:619-28.
- 4 Bonino F, Rosina F, Rizzetto M, *et al*. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. *Gastroenterology* 1986;90:1268-73.
- 5 Hadziyannis SJ. Hepatitis B e antigen negative chronic hepatitis B: from clinical recognition to pathogenesis and treatment. *Viral Hep Rev* 1995;1:7-36.
- 6 Brunetto MR, Oliveri F, Coco B, *et al*. Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. *J Hepatol* 2002;36:263-70.
- 7 Brunetto MR, Oliveri F, Rocca G, *et al*. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis B e antigen. *Hepatology* 1989;10:198-202.
- 8 Oliveri F, Santantonio T, Bellati G, *et al*. Long term response to therapy of chronic anti-HBe-positive hepatitis B is poor independent of type and schedule of interferon. *Am J Gastroenterol* 1999;94:1366-72.
- 9 Manesis EK, Hadziyannis SJ. Interferon a treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. *Gastroenterology* 2001;121:101-9.
- 10 Tassopoulos NC, Volpes R, Pastore G, *et al*. Post lamivudine treatment follow up of patients with HBeAg negative chronic hepatitis B. *J Hepatol* 1999;30 (suppl 1):117.
- 11 Santantonio T, Mazzola M, Iacovazzi T, *et al*. Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine. *J Hepatol* 2000;32:300-6.
- 12 Liaw YF, Chien RN, Yeh CT, *et al*. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 1999;30:567-72.
- 13 Hadziyannis SJ, Papatheodoridis GV, Dimou E, *et al*. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2000;32:847-51.

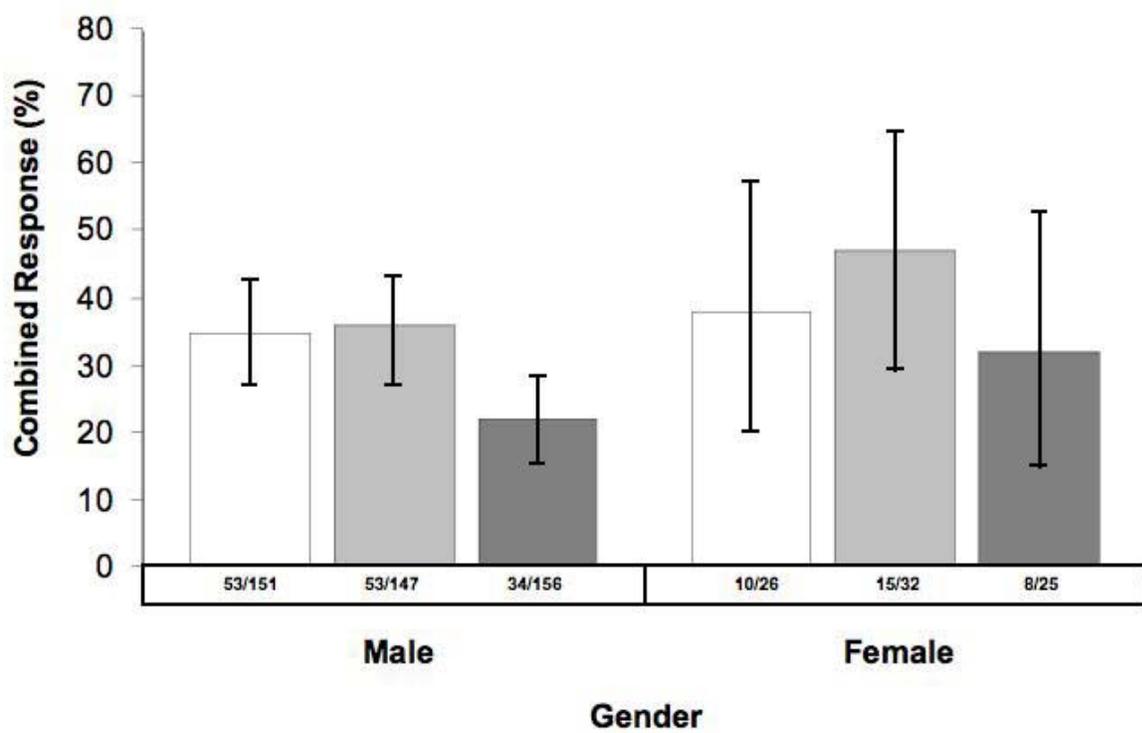
- 14 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, *et al.* Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005;352:2673-81.
- 15 Locarnini S, Qi X, Arterburn S, *et al.* Incidence and predictors of emergence of adefovir resistant HBV during four years of adefovir dipivoxil (ADV) therapy for patients with chronic hepatitis B (CHB). *J Hepatol* 2005;42 (suppl 1):17A.
- 16 Marcellin P, Lau GKK, 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;351:1206-17.
- 17 Cooksley WGE, Piratvisuth T, Lee S-D, *et al.* Peginterferon a-2a (40KD): An advance in the treatment of HBeAg-positive chronic hepatitis B. *J Viral Hepat* 2003;10:298-305.
- 18 Lau GKK, Piratvisuth T, Luo K-X, *et al.* Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005;352:2682-95.
- 19 Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? A statistical analysis of predictive factors. *Hepatology* 1989;10(5):761-3.
- 20 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. The Hepatitis Interventional Therapy Group. *N Engl J Med* 1990;323:295-301.
- 21 Lok AS, Wu PC, Lai CL, *et al.* A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterology* 1992;102:2091-7.
- 22 Wai CT, Chu CJ, Hussain M, *et al.* HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002;36:1425-30.
- 23 Perrillo RP, Lai CL, Liaw YF, *et al.* Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 2002;36:186-94.
- 24 Kao JH, Wu NH, Chen PJ, *et al.* Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000;33:998-1002.
- 25 Chan HL, Wong ML, Hui AY, *et al.* Hepatitis B virus genotype has no impact on hepatitis B e antigen seroconversion after lamivudine treatment. *World J Gastroenterol* 2003;9:2695-7.
- 26 Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-9.
- 27 Marcellin P, Lau GKK, Bonino F, *et al.* Sustained response to peginterferon alfa-2a (40KD) (PEGASYS®) in HBeAg-negative chronic hepatitis B. 1-year follow-up data from a large, randomized multinational study. *J Hepatol* 2005;42:A512.
- 28 Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2001;34:306-13.
- 29 Yurdaydin C, Bozkaya H, Cetinkaya H, *et al.* Lamivudine vs lamivudine and interferon combination treatment of HBeAg(-) chronic hepatitis B. *J Viral Hepat* 2005;12:262-8.
- 30 Colombatto P, Civitano L, Bizzarri R, *et al.* A multiphase model of the dynamics of HBV infection in HBeAg-negative patients during pegylated interferon alfa-2a, lamivudine and combination therapy. *Antivir Ther* 2006;11:197-212.



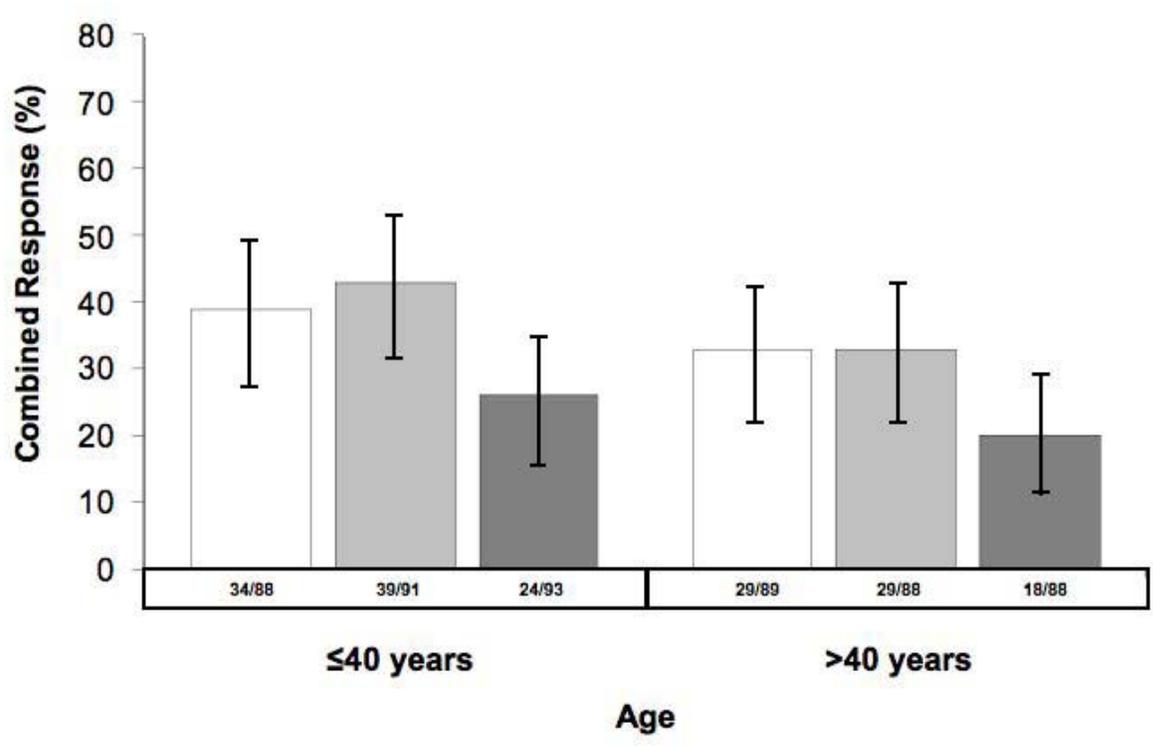
**B**



**C**



**D**



**Figure 2**

