

# Long-term alpha interferon and lamivudine combination therapy in non-responder patients with anti-HBe-positive chronic hepatitis B: Results of an open, controlled trial

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## INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a major worldwide public health problem. Over 300 million people are affected by the disease<sup>[1]</sup>, which is associated with high mortality and morbidity due to the associated risk of cirrhosis and hepatocellular carcinoma<sup>[2]</sup>. In addition, HBV infection is becoming an increasingly relevant problem for transplanted and immunodeficient patients, who are prone to develop more severe, accelerated liver disease<sup>[3]</sup>.

Until recently, interferon- $\alpha$  (IFN) was the only drug approved throughout the world for chronic HBV infection<sup>[4,5]</sup>. Unfortunately, relapse with return of viremia and hepatitis occurs in up to 50 % of cases<sup>[6]</sup>. The hepatitis B s antigen (HBsAg) may become negative in months to years after the end of treatment, suggesting total viral clearance<sup>[7]</sup>. IFN is less effective in the presence of HBeAg negativity<sup>[8]</sup>, or in patients who have already developed cirrhosis or have had recurrence of HBV after a hepatic allograft.

Lamivudine (3TC) is the first potentially non-cytotoxic<sup>[9,10]</sup> oral nucleoside analogue approved for the treatment of chronic hepatitis B. It suppresses viral DNA replication by means of chain termination, and competitively inhibits viral polymerase, but does not act on supercoiled DNA. For these reasons, lamivudine rapidly reduces serum HBV DNA, enhances transaminase normalization, and improves the histological picture<sup>[11-13]</sup>. But only 16 % achieve full HBeAg seroconversion, and after suspension of therapy serum HBV DNA levels and transaminases generally return to pretreatment values<sup>[14]</sup>. Furthermore, after clearance of HBV DNA, mutations in the sequence of the YMDD locus of the HBV RNA-dependent DNA polymerase appear in 15 % of cases after one year of therapy, leading to recurrence of viremia<sup>[15]</sup>. Onset of resistance generally occurs after six months to several years of lamivudine therapy<sup>[16]</sup>.

Combination therapy with IFN and lamivudine is being investigated in order to take advantage of the drugs' different mechanisms of action<sup>[17-21]</sup>. However, the results of short-term treatment studies have been not altogether encouraging<sup>[18]</sup>. The aim of the present study was to investigate whether long-term combination therapy was safe and well tolerated, and whether it could provide additional therapeutic benefits with respect to either of the single drug regimens. In particular, we set out to evaluate efficacy in terms of primary and sustained viremia and transaminase responses. We also assessed histological response, incidence of YMDD-variant HBV, and safety.

## Abstract

**AIM:** To investigate the safety and efficacy of long-term combination therapy with alpha interferon and lamivudine in non-responsive patients with anti-HBe-positive chronic hepatitis B.

**METHODS:** 34 patients received combination treatment (1 month lamivudine, 12 month lamivudine+interferon, 6 month lamivudine), 24 received lamivudine (12 months), 24 received interferon (12 months). Interferon was administered at 6 MU tiw and lamivudine at 100 mg orally once daily. Patients were followed up for 6 months after treatment.

**RESULTS:** At the end of treatment, HBV DNA negativity rates were 88 % with lamivudine+interferon, 99 % with lamivudine and 55 % with interferon, ( $P=0.004$ , combination therapy vs. interferon, and  $P=0.001$  lamivudine vs. interferon), and serum transaminase normalization rates were 84 %, 91 % and 53 % ( $P=0.01$  combination therapy vs. interferon, and  $P=0.012$  lamivudine vs. interferon). Six months later, HBV DNA negativity rates were 44 % with lamivudine+interferon, 33 % with lamivudine and 25 % with interferon, and serum transaminase normalization rates were 61 %, 42 % and 45 %, respectively, without statistical significance. No YMDD variants were observed with lamivudine+interferon (vs. 12 % with lamivudine). The combination therapy appeared to be safe.

**CONCLUSION:** Although viral clearance and transaminase normalization are slower with long-term lamivudine+interferon than that with lamivudine alone, the combination regimen seems to provide more lasting benefits and to protect against the appearance of YMDD variants. Studies with other regimens regarding sequence and duration are needed.

## MATERIALS AND METHODS

### Patients

This open trial concerned 82 adult Italian patients with chronic anti-HBe positive hepatitis B (68 males, 14 females) enrolled between February 1997 and February 1999 who had either not responded to or had relapsed after previous interferon treatment. The experimental protocol was carried out in accordance with the Helsinki Declaration, and was approved by the Ethics Review Committee, and all patients gave written informed consent to participate in a trial involving long-term treatment either with interferon plus lamivudine or with lamivudine or interferon alone.

Inclusion criteria for the study were: age between 18 and 70 years, detectable hepatitis B surface antigen (HBsAg) in serum at the time of screening and for at least six months before the start of the study, serum HBV DNA levels of at least 5 pg/ml at screening, raised alanine transaminase values within three months before the start of therapy. Exclusion criteria were: presence of co-infection with hepatitis C and D virus or HIV, signs of hepatic decompensation or hepatocellular carcinoma, other possible causes of chronic liver damage (i.e. alcohol abuse, hemochromatosis, Wilson's disease,  $\alpha$ 1-antitrypsin deficiency, autoimmune diseases, drug-induced hepatotoxicity and congestive heart failure), assumption of immunosuppressive or antiviral therapy within six months before the study. Needle biopsy performed in all cases within 2 years of the study, showed that 33 patients had a Scheuer score for fibrosis  $>3$ .

The patients were divided into three groups: group A ( $n=34$ ) for combination treatment, group B ( $n=24$ ) for treatment with lamivudine alone, group C ( $n=24$ ) for treatment with interferon alone.

### Treatment protocols and study design

Patients in group A received 100 mg/day of lamivudine (Glaxo-Wellcome Inc, Research Triangle Park, NC, USA) orally for one month, followed by lamivudine (100 mg/day) in association with six million units three times per week of natural interferon (Wellferon, Glaxo-Wellcome Inc, Research Triangle Park, NC, USA) for twelve months, and then lamivudine (100 mg/day) alone for six months. Patients in group B received lamivudine (100 mg/day) orally for twelve months. Patients in group C received six million units three times per week of natural interferon for twelve months. Interferon was administered subcutaneously by the patients after adequate training. Patients were followed up after the end treatment for twenty-four weeks. All patients had a liver biopsy within 2 years of entering the study, and were requested to have another at the end of treatment. Hepatitis flare up was defined as an increase in serum ALT during therapy and classified as mild, moderate or severe ( $>1.5$ , 5, 10 times respectively) according to the preceding ALT value.

The main end point of the study was to evaluate the end-of-treatment and sustained response, defined as HBV DNA loss and serum ALT normalization at the end of therapy and follow-up, respectively. Secondary variables included histological response, incidence of YMDD-variant HBV, and safety.

### Laboratory methods

Blood samples were obtained immediately before therapy, after 15 days, and then every month during treatment and follow-up. Routine biochemical tests and blood cell counts were performed. HBsAg, HBeAg, and anti-HBs and anti-HBe antibodies were determined by enzyme immunoassay. Serum HBV DNA was measured quantitatively by a solution-hybridization assay (Abbott, Chicago, USA) with a lower limit of 3.0 pg/ml of serum.

Presence of the YMDD variant was evaluated by a restriction

fragment length polymorphism assay in all ends of treatment samples and in cases where initial loss of HBV DNA was followed by a return to positivity, regardless of transaminase values. The biopsy specimens were scored according to the Scheuer histological activity index<sup>[22]</sup>.

### Statistical analysis

Baseline characteristics of the patients were presented as mean values  $\pm$  standard error (SE). Statistical evaluations were conducted according to the intention-to-treat procedure. The Chi-square test or the Fisher's exact test was used to compare differences in proportion between treatment groups. Continuous data were analyzed using Kruskal-Wallis test and Mann-Whitney test. A two-tailed  $P$  value of less than 0.05 was considered to be statistically significant.

## RESULTS

### Study population

All treatment arms were well matched with regard to baseline characteristics (Table 1). Of the 82 patients, 79 completed the treatment schedule, while the remaining 3 dropped out of the study: one group A patient withdrew, one group B patient and one group C patient experienced depression.

**Table 1** Baseline characteristics of patients

| Characteristics | Lamivudine+<br>interferon<br>( $n=34$ ) | Lamivudine<br>alone<br>( $n=24$ ) | Interferon<br>alone<br>( $n=24$ ) |
|-----------------|---|-----------------------------------|-----------------------------------|
| Age (year)      | 44.0 $\pm$ 2.0                          | 48.7 $\pm$ 2.4                    | 44.4 $\pm$ 2.5                    |
| Sex (m/f)       | 30/4                                    | 18/6                              | 20/4                              |
| ALT (U/l)       | 151 $\pm$ 21                            | 160 $\pm$ 39                      | 130 $\pm$ 17                      |
| AST (U/l)       | 81 $\pm$ 10                             | 111 $\pm$ 37                      | 66 $\pm$ 10                       |
| GT (U/l)        | 48 $\pm$ 3                              | 57 $\pm$ 6                        | 47 $\pm$ 3                        |
| Albumin (gr/dl) | 3.94 $\pm$ 0.04                         | 3.97 $\pm$ 0.04                   | 4.01 $\pm$ 0.03                   |
| HBV DNA (pg/ml) | 60 $\pm$ 25                             | 85 $\pm$ 43                       | 73 $\pm$ 36                       |
| Histology:      |   |                                   |                                   |
| Cirrhosis       | 13 (38%)                                | 10 (42%)                          | 10 (42%)                          |
| Severe activity | 10 (29%)                                | 7 (29%)                           | 9 (37%)                           |

$P$  value was not significant for all comparisons.

### Virological response (Figure 1)

**End-of-treatment response** HBV DNA negativity was observed at the end of treatment in 30 (88 %) patients who received combination therapy (i.e. group A), 23 (99 %) who had lamivudine alone (i.e. group B), and 13 (55 %) who had interferon alone (i.e. group C). HBV DNA negativity was achieved in week 12, 4 and 32, respectively, in the three groups. Two differences in primary response among the three groups were highly significant [combination vs. interferon:  $OR$  6.34 (95 % CI: 1.7-23 %),  $P=0.004$ ; lamivudine vs. interferon:  $OR$  19.46 (95 % CI: 3.3-113 %),  $P=0.001$ ].

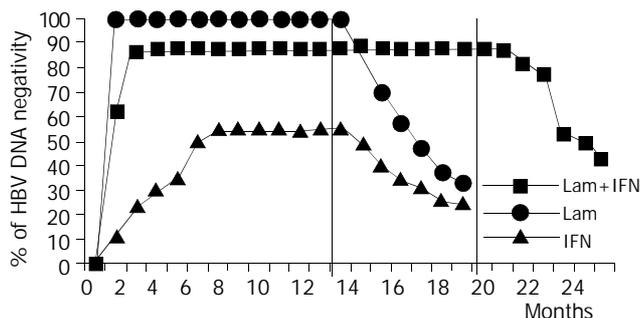
**Sustained response** A total of 15 (44 %) patients in group A, 8 (33 %) in group B and 6 (25 %) in group C were HBV DNA negative at the end of the follow-up. None of the differences in sustained virological response among the three groups reached statistical significance.

### Biochemical response (Figure 2)

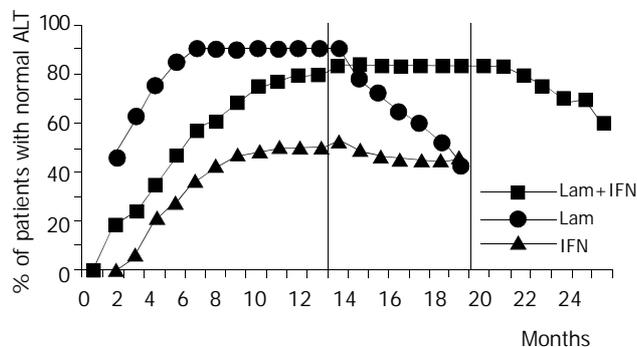
**End-of-treatment response** Serum transaminases became normal at the end of treatment in 29 (84 %) patients in group A, 21 (91 %) in group B and 13 (53 %) in group C [combination vs. interferon:  $OR$  4.9 (95 % CI: 1.4-17 %),  $P=0.01$ ; lamivudine vs. interferon:  $OR$  5.92 (95 % CI: 1.28-25.3 %),  $P=0.012$ ]. Normalization was achieved in week 20, 8 and 38, respectively,

in the three groups. A mild flare-up without bilirubin alteration was observed only in 8 group A patients. In all cases, serum transaminase normalization was preceded by a rise of HBV DNA at 2-8 months of therapy. The flare-up was always followed 1-2 months later by serum HBV DNA negativization and transaminase normalization. None of these patients had a YMDD variant.

**Sustained response** Sustained serum transaminase normalization was observed in 21 (61 %) patients in group A, 10 (42 %) in group B and 11 (45 %) in group C. None of the differences in sustained transaminase response among the three groups had statistical significance. Only 4 of the 8 patients who experienced a flare-up during combined therapy maintained their primary response.



**Figure 1** Virological response. Percentages of patients with HBV DNA negativity related either to the treatment or to the follow-up times. The vertical lines were drawn at the end of each treatment (19 months for interferon and lamivudine in combination, and 12 months for lamivudine and interferon monotherapy).



**Figure 2** Biochemical response. Percentages of patients with normal ALT levels related either to the treatment or to the follow-up times. The vertical lines were drawn at the end of each treatment (19 months for interferon and lamivudine in combination, and 12 months for lamivudine and interferon monotherapy).

**Secondary criteria of efficacy**

**Histological findings** Paired pre- and post-treatment liver biopsies were available for 22 (65 %), 15 (62 %) and 14 (58 %) patients in groups A, B and C, respectively. None of the post-treatment biopsies showed a worsening or unchanged histological picture. Significant improvements in necro-inflammatory activity in parenchyma ( $P < 0.01$ ) and portal space ( $P < 0.01$ ) were observed in each group. Significant improvements in fibrosis were found in groups A and B ( $P < 0.05$ ), but not in group C.

**Incidence of YMDD variant HBV** YMDD variants of HBV were not detected in any of the patients who received combination or IFN therapy. 3 of the 24 (12 %) patients treated with lamivudine alone had a YMDD variant after 6-9 months of therapy. In particular, two had a YVDD variant and the other

had a YIDD variant. They continued lamivudine therapy and were monitored weekly. After a progressive rise of serum HBV DNA, two of the patients showed 5 times of elevation of transaminases (with respect to the upper limit) and another showed 1.5 times of elevation. However, after the emergence of the variant, the HBV DNA and transaminase levels always remained lower than the baseline.

**Safety**

The whole treatment program was completed by 33 (98 %), 23 (96 %) and 23 (94 %) patients in groups A, B and C, respectively. No patient received reduced dosage but we directly stopped treatment. The side effects of the combination regimen were generally similar to those of interferon treatment. In addition, among the 34 patients in group A, symptomatic and reversible rises in serum levels of amylase, lipase and creatinine phosphokinase were recorded in 9 (28 %), 2 (5 %) and 1 (3 %) cases, respectively. Worsening of pre-existing hypertriglyceridemia was recorded in 2 (7 %) cases. By comparison, among the 24 patients in group B, lamivudine therapy was associated with an asymptomatic and reversible rise in amylase, lipase or creatinine phosphokinase in 6 (25 %), 1 (4 %) and 1 (4 %), respectively. No case of hepatocellular carcinoma, liver decompensation or death was recorded, and no patient requested orthotopic liver transplantation (Table 2).

**Table 2** Adverse events documented during treatment

| Adverse events          | Lamivudine+ interferon (n=34), n (%) | Lamivudine alone (n=24), n (%) | Interferon alone (n=24), n (%) |
|-------------------------|--------------------------------------|--------------------------------|--------------------------------|
| Influenza-like symptoms | 24 (70%)                             | none                           | 19 (79%)                       |
| Hair loss               | 8 (23%)                              | none                           | 9 (38%)                        |
| Weight loss             | 5 (16%)                              | none                           | 4 (15%)                        |
| Depression              | none                                 | 1 (4%)                         | 1 (6%)                         |
| Low white-cell count    | 19 (56%)                             | none                           | 14 (59%)                       |
| Low platelet count      | 14 (42%)                             | none                           | 10 (44%)                       |
| Amylase rise            | 9 (28%)                              | 6 (25%)                        | none                           |
| Lipase rise             | 2 (5%)                               | 1 (4%)                         | none                           |
| CPK rise                | 1 (3%)                               | 1 (4%)                         | none                           |
| Hypertriglyceridemia    | 2 (7%)                               | none                           | none                           |

**DISCUSSION**

The development of effective treatment strategies for patients with HBV remains a major clinical challenge. We investigated the possible benefits of a long-term interferon/lamivudine combination treatment regimen in a cohort of patients who had already received unsuccessful treatment with interferon alone. The effects of this long-term combination regimen were compared with single-agent treatment with either interferon or lamivudine in three similar groups of patients. In regard to virological response, we found that at the end of therapy, HBV DNA had become undetectable in 88 % of the patients receiving combination treatment, as compared with 99 % and 55 % of those who had single-line therapy with lamivudine or interferon, respectively. These differences in end-of-treatment response were statistically significant in comparison of the combination therapy and lamivudine monotherapy with interferon monotherapy. The analysis of sustained response showed that 44 % patients in the combination therapy group maintained DNA clearance as compared with only 33 % in the lamivudine group and 25 % in the interferon group. Although these differences did not have statistical significance, there was a trend in favor of the combination regimen. Similarly, although the end-of-treatment transaminase normalization rate was significantly higher in the combination

treatment (84 %) group and in the lamivudine group of patients (91 %) than that in interferon (53 %) group, the sustained response showed a non-significant trend in favor of the combination regimen (61 % in the combination group vs. 42 % and 45 % with lamivudine or interferon alone, respectively).

As has been reported for short-term treatment<sup>[23]</sup>, during our long-term combination therapy regimen there was a progressive viral decline, whereas with single-line lamivudine therapy the HBV DNA levels leveled off. Our data are in keeping with the concept that lamivudine therapy is more rapid and effective on virological and biochemical response than interferon alone or even in combination with lamivudine. However, after discontinuation of therapy, virological and biochemical relapses were considerably more common with lamivudine alone than those with the combination regimen (even though this difference did not actually reach statistical significance). Moreover, there was a trend suggesting that combination therapy delays relapse. The non-significant status of these trends could be due to the relatively small cohort sizes.

Among the two monotherapy options, interferon appeared less effective than lamivudine. In our study, interferon provided less end-of-treatment responses than lamivudine, and no advantages in terms of sustained response. The relatively poor capacity of interferon monotherapy to promote viral clearance may be explained by an inability to induce an efficient immune response.

After about 5 months of combination therapy, we observed a mild flare-up in 8 patients following a rise in serum HBV DNA. The flare-up was always followed, after a further one to two months of therapy by a return to serum HBV DNA negativity and normalization of transaminases. They may have been caused by enhanced immune responses prompted by increased mutant viral replication other than YMDD, which however were unable to permanently eradicate the virus.

As regards the secondary criteria of response, all the patients who underwent a post-treatment biopsy derived histological benefit in terms of necro-inflammatory activity in parenchyma and in portal space, regardless of their serological response. However, in keeping with other reports<sup>[24]</sup>, interferon monotherapy provided no improvement in fibrosis.

YMDD HBV variants were not detected in any of the patients who received combination therapy or interferon alone. After 6-9 months of lamivudine monotherapy, 3 (12 %) patients had YMDD, a rate similar to that reported in Asian patients. Interferon therapy seemed to exert a protective effect against the emergence of variants, probably because it lowers viral replication and enhances immunological response. In any case, in the three affected patients, the serum HBV DNA levels did not return completely to pretreatment levels. Thus, the mutant strains of HBV DNA that break through during prolonged therapy with lamivudine may not be as replicative-efficient or as pathogenic as wild-type strains. As in other reports<sup>[25]</sup>, we found that 3 to 4 months after lamivudine treatment was stopped, there was disappearance of this HBV mutant and re-emergence of the wild type virus. Honkoop *et al.*<sup>[26]</sup> observed acute exacerbation of chronic HBV infection after withdrawal of lamivudine therapy and concluded that the relationship between the development of "lamivudine-withdrawal hepatitis" and the lamivudine-resistant mutant virus was currently unclear, since both the emergence of a lamivudine resistant viral mutant and the resurgence of wild-type virus after withdrawal of therapy were able to induce severe hepatitis exacerbations. Studies of rechallenge with lamivudine have yet to be reported, and the significance of YMDD variant is being evaluated in the ongoing follow-up clinical trials.

As regards safety, the combination therapy was well tolerated and no serious side effects were observed. The side

effects observed during the course of the long-term combination regimen were those that were commonly associated with the two drugs. As expected, in the two monotherapy groups, the incidence of drug-related adverse events was much lower with lamivudine than that with interferon.

In conclusion, these results suggest that for patients who have already been unsuccessfully treated with interferon, long-term combination therapy may have some advantages over single-line treatment with lamivudine or interferon<sup>[27]</sup>. Other studies with other regimens regarding dose, sequence and duration of interferon and lamivudine are needed. Finally, correct staging of patients according to the particular phases in the natural history of chronic hepatitis B appears to be important to personalise patients' management. It is possible that combinations of two<sup>[28,29]</sup> or more nucleoside analogs may reduce the development of viral resistance, increase overall response rates, decrease adverse events. In general, multi-drug therapies<sup>[30]</sup> that take advantage of different modes of action concurrently may provide a more appropriate approach for particular subgroups of patients.

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