Pegylated IFN-α 2b added to ongoing lamivudine therapy in patients with lamivudine-resistant chronic hepatitis B

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Abstract

AIM: To investigate the role of pegylated-interferon (IFN) α-2b in the management of patients with lamivudine-resistant chronic hepatitis B.

METHODS: Twenty consecutive anti-HBe positive patients were treated with pegylated IFN α-2b (100 μg sc once weekly) for 12 mo. There was no interruption in lamivudine therapy. Hematology, liver biochemistry, serum HBV DNA levels were detected by PCR, and vital signs were also assessed. Liver histology was assessed in some patients at entry and at wk 52 for comparison.

RESULTS: Nine patients (45%) had a partial virological end-treatment response; seven patients (35%) showed complete virological end-treatment response. Eight patients (40%) showed biochemical end-treatment response. There was a trend for higher virological response rates in patients who had previously responded to IFN and relapsed compared to IFN non-responders (four out of seven patients vs none out of six patients, respectively; P = 0.1). Patients without virological end-treatment response showed significant worsening of fibrosis [median score 2 (range, 1 to 3) vs median score 3 (range, 1 to 4)], in the first and second biopsy respectively (P = 0.014), whereas necroinflammatory activity was not significantly affected. Patients with complete or partial virological end-treatment response did not show any significant changes in histological findings, possibly due to the small number of patients with paired biopsies (n = 5). Nevertheless, after 12 mo of follow-up, only one patient (5%) showed sustained virological response and only 2 patients (10%) showed sustained biochemical response. Two patients (10%) discontinued pegylated IFN both after 6 mo of treatment due to flu-like symptoms.

CONCLUSION: Pegylated IFNα-2b, when added to ongoing lamivudine therapy in patients with lamivudine-resistant chronic hepatitis B, induces sustained responses only in a small minority of cases.

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Key words: Pegylated interferon; Lamivudine resistance; HBeAg negative chronic hepatitis B; Adefovir

INTRODUCTION

Lamivudine has been a major breakthrough in the care of patients with chronic hepatitis B (CHB). Nevertheless, almost all patients with HBeAg-negative CHB, which accounts for the majority of patients in Greece, require long-term therapy with lamivudine to maintain a response. This strategy is associated with the frequent emergence of viral resistance with reported rates of 10%-27% at 1 year, 40%-56% at 2 years and 67% at 3 years of treatment, in a previous study in 77 patients with anti-HBe-positive CHB we have found resistance rates of 1.6% at 9 mo, 3.3% at 12 mo, 12.7% at 15 mo, 15% at 18 mo and 31% at 48 mo. Once lamivudine resistance occurs, patients may experience an attenuation of the initial clinical, virologic and histologic benefits of therapy. The wide use of lamivudine in CHB during the last 5-6 years has resulted in the constant increase of patients with lamivudine-resistant mutants. Their treatment may be the most pressing task for the current anti-HBV strategies in clinical practice. Adefovir dipivoxil is the only approved agent that has been...
Table 1 Baseline demographic and clinical features of the study population

| No | Sex | Age (yr) | t (prior lamivudine therapy) mo | Lamivudine resistance mutation | HBV DNA level (10^9 copies/L) | ALT level (times the ULN) |
|----|-----|---------|--------------------------------|-------------------------------|-------------------------------|--------------------------|
| 1  | M   | 53      | 26                             | L180M + M204V                 | 9.36                          | 1.3                      |
| 2  | M   | 55      | 14                             | L180M + M204I                 | 6.56                          | 6                       |
| 3  | M   | 63      | 18                             | L180M + M204I                 | 7.23                          | 7                       |
| 4  | M   | 53      | 25                             | L180M + M204V                 | 6.68                          | 2.75                    |
| 5  | F   | 55      | 19                             | M204I                         | 6.57                          | 8.5                     |
| 6  | M   | 63      | 11                             | L180M + M204V                 | 6.58                          | 9                       |
| 7  | M   | 62      | 10                             | M204I                         | 8.16                          | 1.3                     |
| 8  | M   | 47      | 24                             | L180M + M204V                 | 7.23                          | 2                       |
| 9  | M   | 53      | 18                             | L180M + M204I                 | 6.86                          | 2.5                     |
| 10 | M   | 46      | 16                             | L180M + M204I                 | 7.98                          | 10                      |
| 11 | M   | 66      | 24                             | L180M + M204V                 | 6.00                          | 1.5                     |
| 12 | F   | 48      | 22                             | L180M + M204I                 | 7.60                          | 3                       |
| 13 | M   | 54      | 21                             | L180M + M204V                 | 9.30                          | 7                       |
| 14 | M   | 60      | 41                             | L180M + M204I                 | 7.51                          | 3.8                     |
| 15 | M   | 38      | 29                             | L180M + M204V                 | 6.26                          | 2.5                     |
| 16 | M   | 61      | 22                             | L180M + M204I                 | 6.57                          | 5                       |
| 17 | M   | 62      | 8                              | M204I                         | 7.43                          | 3.5                     |
| 18 | M   | 61      | 25                             | L180M + M204V                 | 6.98                          | 7                       |
| 19 | M   | 65      | 12                             | L180M + M204I                 | 7.26                          | 5                       |
| 20 | M   | 54      | 12                             | M204I                         | 7.55                          | 2                       |

shown to be effective in this setting, whilst entecavir is also a potential candidate[11-13]. The efficacy of interferon-α (IFN) therapy has not yet been evaluated in any well-designed study in these patients, and therefore no conclusions can be drawn.

IFN-α has a dual mode of action, antiviral via inhibition of viral replication, and immunomodulatory via enhancement of the immunological response of the host against the virus[14]. Pegylation of interferon leads to improved pharmacokinetic and pharmacodynamic profiles, which translated to superior efficacy, compared with conventional, nonpegylated IFN, in the treatment of chronic hepatitis C, and more recently, CHB[15-20]. Two modulations of pegylated IFN (PEG-IFN) are currently being used in clinical practice, namely PEG-IFN-α 2b and PEG-IFN-α 2a. This study was designed to explore the role of PEG-IFN-α 2b in the management of patients with CHB with lamivudine-resistant HBV.

MATERIALS AND METHODS

Subjects

Between November 1999 and February 2003, a total of 20 consecutive anti-HBe positive patients [18 males (90%)], with a median age of 54 (range, 38-66) years, were enrolled in this prospective study. Patients eligible for the study were aged 18 years and older, hepatitis B surface antigen (HBsAg) positive, and receiving ongoing lamivudine therapy for CHB for at least 6 mo at the time of screening; median duration of prior lamivudine therapy was 20 (range, 8-41) mo. All patients were HBeAg negative and antiHBe positive (both at the beginning of prior lamivudine therapy and at the beginning of the present study), genotype D, and had confirmed HBV polymerase gene mutation within the YMDD motif by DNA sequencing (Trugene HBV genotyping, Visible Genetics Inc); lamivudine resistance mutations are shown in Table 1. Patients were required to have a screening HBV DNA level >10^9 copies/L (Amplicor HBV-DNA Monitor Test; Roche Diagnostics, Branchburg, NJ, USA, with a sensitivity of 400 × 10^3 copies/L) as well as elevated serum alanine aminotransferase (ALT) levels >1.2 times the upper limit of normal (ULN) on at least 2 occasions at least 1 mo apart within the preceding 6 mo. The exclusion criteria are as follows: absolute neutrophil count ≤ 10^9 cells/L; hemoglobin ≤ 100 or ≤ 90 g/L (males or females, respectively); platelet count < 50 × 10^9/L; prior treatment with interferon or other immunomodulatory therapies within the 6 mo preceding study screening; serious concurrent medical conditions, including other concurrent liver diseases; coinfection with hepatitis C virus or hepatitis delta virus or human immunodeficiency virus; current alcohol or substance use; and pregnancy and/or lactation. None of the patients had liver cirrhosis.

Amongst the 20 patients enrolled, 7 (35%) were naive to IFN and 13 (65%) had been previously treated with IFN 5 MU sc three times weekly for at least 12 mo (before receiving lamivudine); six of the latter had shown no response and seven had responded to IFN (i.e. had shown both reduction in serum HBV DNA level to <10^6 copies/L and normalization of ALT level at the end of IFN administration) but relapsed after discontinuing therapy.

Methods

Patients were treated with pegylated interferon α-2b (100 μg sc once weekly) for 12 mo. There was no interruption in lamivudine therapy, even after the cessation of PEG-IFN-α 2b. Patients were evaluated every month. At each visit, any untoward medical occurrences, regardless of causality, were recorded as adverse events. Hematology, liver biochemistry, serum HBV DNA levels, and vital signs were also assessed. Liver histology was assessed in 13 patients at entry and at wk 52 for histological comparison; the rest of the patients denied a liver biopsy. A single pathologist, who was blinded to the sequence of the biopsies, evaluated all
Statistical analysis

All data were analyzed using the statistical package SPSS (version 10.0; SPSS Inc., Chicago, IL). The population analyzed included all patients who received at least one dose of study medication. The Mann-Whitney and Chi-square tests were used for comparisons of quantitative and qualitative variables respectively. The Wilcoxon Signed rank test was used to test the effect of PEG-IFN-α 2b on histological findings in the patients who underwent paired liver biopsies. In all cases, a 2-tailed $P$ value less than 0.05 was considered statistically significant.

RESULTS

Baseline demographic and clinical features of the study population are presented in detail in Table 1. At baseline, median HBV DNA level was 7230 log$_10$ copies/L (range, 6000-9360 log$_{10}$ copies/L) and median ALT level was 3.5 (range, 1.3-10) times the ULN. Changes in median HBV DNA titers during PEG-IFN-α 2b therapy are shown in Figure 1. After 52 wk of treatment, 9 patients (45%) had a partial virological end-treatment response. The median change from baseline in serum HBV DNA levels was -7000 (range, -9400 to 7600) log$_{10}$ copies/L. Seven patients (35%) showed complete virological end-treatment response. In these patients HBV DNA became undetectable after a median of 9 (range, 3 to 12) mo. Eight patients (40%) showed biochemical end-treatment response. In these patients normalization of ALT levels occurred after a median time of 9 (range, 3 to 12) mo (Figure 2). Overall, serum ALT level decreased over 52 wk in 16 patients (80%). The median ALT level at baseline was 3.5 times the ULN; by wk 52, this had declined to 2.2 times the ULN.

None of the baseline demographic and clinical features predicted virological (partial or complete) or biochemical end-treatment responses. Nevertheless, it should be noted that none of the six patients who had not responded to prior IFN treatment showed complete virological end-treatment response, while four out of the seven patients who had relapsed after an initial response to IFN showed complete virological end-treatment response ($P=0.1$). The findings in the 13 patients in whom biopsies were performed are shown in Table 2 and in Figure 3. Patients without virological end-treatment response showed significant worsening of fibrosis ($P=0.014$) in the second biopsy, whereas necroinflammatory activity was not significantly affected. Patients with complete or partial virological end-treatment response did not show any significant changes in histological findings, possibly due to the small number of patients with paired biopsies ($n=5$). Likewise, patients without biochemical end-treatment response showed significant worsening of fibrosis ($P=0.014$) in the second biopsy, whereas necroinflammatory activity was not significantly affected. Also, patients with biochemical end-treatment response did not show any significant changes in histological findings, possibly due to the small number of patients with paired biopsies ($n=3$).

During follow up, HBV-DNA reappeared in six out of the seven patients who had shown complete virological end-treatment response, giving an overall sustained virological response rate of 5%. The median time to HBV-DNA re-emergence was 5 (range, 1 to 12) mo. Changes in median
HBV DNA titers during follow-up are shown in Figure 1 (patients’ data are censored at the time of commencement of treatment with adefovir dipivoxil). Two patients (10%) had a sustained biochemical response while in the remaining 6 patients with biochemical end-treatment response, ALT became abnormal after a median of 4 (range, 1 to 12) mo. Changes in median ALT levels during follow-up are shown in Figure 2 (patients’ data are censored at the time of commencement of treatment with adefovip dipoxil). Two patients (10%) discontinued PEG-IFN both after 6 mo of treatment and both due to flu-like symptoms (fatigue, low-grade fever, arthralgia and headache). Neither of these patients showed virological or biochemical end-treatment response; in fact, one of them showed a rise in HBV-DNA levels and both showed a rise in ALT levels at 52 wk. PEG-IFN was well-tolerated in all other patients and none of them required dose reduction or interruption of therapy. There were instances of hepatic decompensation during the study.

### DISCUSSION

Richman[21] has recently defined an antiviral drug as one that selects for resistance. Antiviral drug resistance depends on the viral mutation frequency, intrinsic mutability of the antiviral target site, the selective pressure exerted by the drug, and the magnitude and rate of virus replication. In particular, lamivudine resistance is due to mutations within the YMDD motif in the major catalytic region C of the HBV polymerase gene[22]. Viral resistance is clinically expressed by the virological breakthrough phenomenon, defined as the reappearance of serum HBV DNA after an initial clearance of viraemia despite the continuation of therapy[23]. The emergence of resistance has a negative impact on the efficacy of therapy in CHB patients, since virological breakthroughs are almost invariably followed by increasing viraemia levels, culminating in biochemical breakthroughs, which ultimately have an adverse effect on liver histology[24].

It is clear that the possible adverse effects of YMDD mutants do cast a concern. Rescue therapies for patients with worsening liver disease caused by lamivudine-resistant mutants are being evaluated. Until recently, treatment options for these patients have been limited to continuation or cessation of lamivudine therapy. Continuation of lamivudine aims to further suppress or to prevent the return of wild-type HBV which is more replicative competent than the YMDD mutant[25]. However, this strategy seems ineffective. Lamivudine withdrawal results in re-emergence of wild-type HBV within 3–4 mo[24]. Therefore, acute exacerbations of liver disease might ensue and could, although uncommon, result in hepatic decompensation or acute liver failure[25]. Adefovir dipivoxil effectively inhibits replication of YMDD mutants resistant to lamivudine and hence averts the resultant disease; entecavir has also shown promising results[26–28]. Nevertheless, neither of these novel nucleoside analogues was licensed in Greece during the study period.

There is a paucity of data regarding the role of IFN in the treatment of lamivudine-resistant HBV. Interferon-α has multiple sites of action in the viral life cycle and may be effective against lamivudine-resistant virus[29]. Recently, lamivudine was found to restore cytotoxic T-cell responses in patients with CHB, and, therefore, it may augment the immunomodulatory activity of IFN[25]. Hence, there would be a rationale in treating patients with lamivudine-resistant HBV mutants with IFN. Pegylation is the attachment of a polyethylene glycol (PEG) molecule to the base IFN molecule resulting in effective concentrations of IFN throughout the dosing interval and substantially reduced peak-to-trough ratio, in contrast to conventional IFN, which yields only intermittent drug exposure; pegylation also allows for once weekly frequency of administration[30]. Pegylated IFN has been shown to be highly active against wild-type HBV infection, both HBeAg-positive[17,22,23] and HBeAg-negative[29]. Complete virological end-treatment response was achieved by 35% of our patients. Therefore, this study confirms that PEG-IFN-α 2b is also active against lamivudine-resistant HBV. Of note, complete virological end-treatment response was achieved by 63% of patients with wild-type HBeAg-negative CHB in a recent landmark study[29]. The small number of patients included in the present report might per se preclude direct comparisons of efficacy between these two studies; however, different patients’ characteristics might have also accounted for the apparently inferior results of PEG-IFN therapy in our study. Even though no pretreatment factor

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Table 2: Histological findings in 13 patients with biopsy performed [median (range)]

| Virological end-treatment response | Necroinflammatory activity | Fibrosis score | Necroinflammatory activity | Fibrosis score |
|-----------------------------------|---------------------------|---------------|---------------------------|---------------|
| None                              | 3                         | 2             | 2                         | 3             |
| (n=8)                             | (2-3)                     | (2-3)         | (2-3)                     | (2-3)         |
| Partial                           | 2.5                       | 2.5           | 2.5                       | 2.5           |
| (n=2)                             | (2-3)                     | (2-3)         | (2-3)                     | (2-3)         |
| Complete                          | 2                         | 2             | 1                         | 1             |
| (n=3)                             | (2-3)                     | (2-2)         | (1-2)                     | (1-2)         |

Figure 3: Changes in median inflammation and fibrosis scores according to end of treatment virological response.
has been found to be reliably associated with a response to IFN in HBeAg-negative CHB\textsuperscript{[1]}, it must be mentioned that our patients were older and had higher ALT and lower HBV-DNA levels at baseline than the patients in the aforesaid study\textsuperscript{[20]}. Of course, it is also possible that PEG-IFN might not be as effective in lamivudine-resistant as in wild-type HBV strains, but this has to be investigated further in large-scale studies.

Patients with HBeAg-negative CHB receiving IFN retreatment respond as well as naive ones, irrespective of the outcome of the initial treatment\textsuperscript{[20]}. In accordance with this, in our study, prior IFN treatment, as well as its outcome, was not associated with the efficacy of PEG-IFN-α 2b. Nevertheless, this could be attributed to the limited number of patients studied, since there was a trend for higher virological response rates in patients who had previously responded to IFN and relapsed compared to IFN non-responders ($P=0.1$).

In patients with HBeAg-negative CHB, the 12 mo sustained response rates to IFN treatment vary from 10% to 47% (average 24%)\textsuperscript{[1]}, sustained 6 mo biochemical and complete virological response rates with PEG-IFN rise up to 59% and 19%, respectively\textsuperscript{[20]}. The low percentage of sustained biochemical and complete virological response (10% and 5% respectively) in our patients is of concern and renders PEG-IFN-α 2b rather unattractive for patients with lamivudine-resistant CHB. Nevertheless, the already mentioned differences in patients’ characteristics between studies and the inherent limitation of the small number of patients included in our report might have contributed to these discrepant findings. Furthermore, it must be pointed out that the time point of evaluation of the sustained response in our study was at 12 mo after treatment completion compared to 6 mo in the wild-type HBV study\textsuperscript{[3]}, and this should be taken into account when comparing our results with the latter ones. However, the issue of differing activity of IFN in lamivudine-resistant compared to wild-type HBV strains definitely needs to be addressed.

In conclusion, this study shows that, 52 wk of treatment with PEG-IFN-α 2b, when added to ongoing lamivudine therapy in patients with lamivudine-resistant CHB, induces sustained responses only in a small minority of cases. Therefore, other treatment strategies should be considered for these patients, possibly including more prolonged or earlier (when the viral load is less than 10\textsuperscript{5} copies/L) administration of PEG-IFN-α 2b.

REFERENCES

1. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000--summary of a workshop. Gastroenterology 2001; 120: 1828-1853

2. Hadziyannis SJ. Hepatitis B e antigen negative chronic hepatitis B: from clinical recognition to pathogenesis and treatment. Viral Hepat Rev 1995; 1: 7-36

3. Tassopoulos NC, Volpes R, Pastore G, Heathcote J, Buti M, Goldin RD, Hawley S, Barber J, Condreay L, Gray DF. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. Lamivudine Precore Mutant Study Group. Hepatology 1999; 29: 889-896

4. Lok AS, Hussain M, Cursano C, Margotti M, Gramenzi A, Grazi GL, Jovine E, Benardi M, Andreone P. Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. Hepatology 2000; 32: 1145-1153

5. Buti M, Cotrina M, Jardi R, de Castro EC, Rodriguez-Frias F, Sanchez-Avila F, Esteban R, Guardia J. Two years of lamivudine therapy in anti-HBe-positive patients with chronic hepatitis B. J Viral Hepat 2001; 8: 270-275

6. Papadoterdidis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthroughs under long-term lamivudine in HBV e antigen-negative precore mutant HBV liver disease. Hepatology 2002; 36: 219-226

7. Hadziyannis SJ, Papadoterdidis GV, Dimou E, Laras A, Papadimitropoulos V. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2000; 32: 847-851

8. Vassiliadis T, Satsaioura K, Saveriadis A, Kolokotroni D, Voutsas A, Giouleme O, Nilolaidis N, Balaska K, Orfanou E, Evgenidis N. Long-term lamivudine therapy in patients with precore mutant HBV-related liver disease. J Hepatol 2002; 36(Suppl 1): 94A

9. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiiff ER. Histological outcome during long-term lamivudine therapy. Gastroenterology 2003; 124: 105-117

10. Papadoterdidis GV, Dimou E, Papadimitropoulos V. Nucleoside analogues for chronic hepatitis B: antiviral efficacy and viral resistance. Am J Gastroenterol 2002; 97: 1618-1628

11. Perrillo R, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, Moonat A, Gardner S, Woessner M, Bourne E, Brosart CL, Schiiff E. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. Gastroenterology 2004; 126: 81-90

12. Peters MG, Hann Hw Hw, Martin P, Heathcote EJ, Buggis P, Rubin R, Bourliere M, Kowdley K, Trepo C, Gray DF, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosart CL. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004; 126: 91-101

13. Tassopoulos N, Hadziyannis S, Giancaria J, Rizzetto M, Schiiff ER, Pastore G, Rutkiewicz V, Thomas N, Denisky G, Joshi S. Entecavir is effective in treating patients with chronic hepatitis B who have failed lamivudine therapy (abstract). Hepatology 2001; 34: 340A

14. Craxi A, Cooksley WG. Pegylated interferons for chronic hepatitis B. Antiviral Res 2003; 60: 87-89

15. Cooksley WG, Piratvisuth T, Lee SD, Mahachai V, Chao YC, Tanwander T, Chutapatii A, Chang WY, Zahn FE, Pluck N. Peginterferon alfa-2a (40 KDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. J Viral Hepat 2003; 10: 298-305

16. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C. 2002–June 10-12, 2002. Hepatology 2002; 36(Suppl 1): S3-20

17. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Filiask R, McCloud P, Pluck N. Peginterferon alfa-2a (40 KDa) in chronic hepatitis B: 2002; 126: S3-20

18. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca Us, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 2005; 365: 123-129

19. Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, Chan FK, Hung LC, Lee YT, Tam JS, Lam CW, Sung JJ. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alfa2b and lamivudine with lamivudine alone. Ann Intern Med 2005; 142: 240-250

20. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin

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R, Lu ZM, Piratvisuth T, Germanidis G, Yurdaydin C, Diago M, Gurel S, Lai MY, Button P, Pluck N. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004; 351: 1206-1217

21 Richman DD. The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. Hepatology 2000; 32: 866-867

22 Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, Brown N, Condreay LD. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 1998; 27: 1670-1677

23 Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001; 34: 1225-1241

24 Chayama K, Suzuki Y, Kobayashi M, Kobayashi M, Tsubota A, Hashimoto M, Miyano Y, Koike H, Kobayashi M, Koida I, Arase Y, Saitoh S, Murashima N, Ikeda K, Kumada H. Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. Hepatology 1998; 27: 1711-1716

25 Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999; 30: 567-572

26 Rang A, Günther S, Will H. Effect of interferon alpha on hepatitis B virus replication and gene expression in transiently transfected human hepatoma cells. J Hepatol 1999; 31: 791-799

27 Boni C, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, Cavalli A, Urbani S, Boehme R, Panbianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology 2001; 33: 963-971

28 Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001; 121: 101-109

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