

Hepatitis B virus Resistance to Antivirals: Clinical Implications and Management

Fabien Zoulim, INSERM U 271, Lyon, France



[Print this page](#)

Introduction

Therapy of chronic hepatitis B remains a major clinical challenge. During many years interferon alpha was the only medication available. Although it was shown to be effective in inhibiting viral replication and inducing sustained HBe seroconversion in patients with favorable predictive factors (high ALT levels, low HBV DNA levels, absence of immune deficiency), many patients infected with wild type strains are non responders and those infected with pre-core mutants often relapsed after treatment withdrawal (1, 2). The development of specific inhibitors belonging to the class of nucleoside analogs that inhibit the HBV polymerase has provided new hope in the therapy of chronic hepatitis B (3, 4). Lamivudine, a L-dideoxycytidine analog, was developed and registered worldwide because of its initial beneficial antiviral effect accompanied by improvement of liver enzymes and liver histology (5, 6). However, due to the slow kinetics of viral clearance and the spontaneous genome variability, long-term treatment is associated with the selection of drug resistant mutants (7, 8). Although, there are some controversies regarding the clinical severity associated with lamivudine resistance, this has clearly slowed down the enthusiasm around lamivudine therapy. Fortunately, new inhibitors with different mechanism of action and resistance profiles have been discovered and are being evaluated in experimental systems and in clinical trials. Some of these compounds provide new treatment options as a rescue of lamivudine resistance, or for de novo treatment. These different issues will be discussed in this manuscript.

Diagnosis of Drug Resistance

As a practical guide for clinicians, viral resistance to antiviral therapy is defined by the rise in viral DNA levels in serum during therapy, also defined as phenotypic resistance, and the selection of a mutation in the viral polymerase gene, also defined as genotypic resistance (9, 10). This obviously implies that patient's compliance to therapy is correct, which should be checked regularly with the patient. Usually, plasma concentration of lamivudine in resistant patients is similar to that of patients without drug resistance (9). Up to now, the rise in HBV DNA levels when a patient is taking his treatment is sufficient to diagnose viral resistance. However, with the development of new drugs, it may be important in the future for clinicians to obtain data on viral genome sequence to adapt treatment to the virological situation and avoid cross-resistance.

As this is discussed in Dr Locarnini's manuscript, viral genome sequence analysis will be important to identify resistant strains to new drugs that will be defined by the selection of a given mutant that was not detectable in the major viral species prior to therapy and not known in the consensus sequences derived from data banks (Locarnini et al, J Hepatol, this issue). A more detailed definition of phenotypic resistance is required in vitro in tissue culture, especially when a new mutation is identified. Up to now, the definition of phenotypic resistance relied on the re-positivation of HBV DNA detected by hybridization assays, that have a lower limit of detection of approximately 10^5 - 10^6 copies/ml, after an initial drop at the beginning of therapy. With the development of new quantitative assays for the detection of viral DNA that are more sensitive and have a lower limit of detection equal to or below 10^3 copies/ml, it will be important to define the magnitude of the rise in viral DNA that may have clinical consequences (9). The definition of genotypic resistance is expected to change rapidly as new assays for viral genome sequence analysis will become available (line probe assays, DNA chip, etc...) and will improve our knowledge of spontaneous viral genome variability and selection of mutants by antiviral treatments (9, 11).

Interestingly, it was also observed in non responders a profile of primary resistance defined by the absence of decline of HBV DNA down to the limit of detection of hybridization assays or by the lack of a significant drop in viral DNA using the more sensitive PCR assays. This was first observed in patients receiving famciclovir without the selection of viral mutants or genotype, suggesting that this phenomenon is due to a defect of metabolism of the drug in its active metabolite in the infected liver (12). Rare observations have also been made with lamivudine administration (9).

Prediction of viral resistance to lamivudine

Results of clinical trials of lamivudine for chronic hepatitis B have identified pretreatment factors predictive of drug resistance : high body mass index, high liver inflammatory score index on liver biopsy examination, and high serum ALT levels (5, 6).

Furthermore in our studies of patients presenting lamivudine resistance, we could show that pre-treatment ALT levels higher than 3 times the upper limit of normal value, HBeAg positivity, HBV DNA levels higher than 1495 Meq/mL, and previous treatment with famciclovir, are predictive factors for a more rapid selection of drug resistant mutants (9, 13). This was not surprising as HBeAg positivity and high HBV DNA levels are markers of high viral replication which predisposes to the generation of viral genome mutations. The high ALT levels reflects hepatocyte lysis and subsequent cell turn over which are thought to be critical factors involved in the spread of the mutants in the infected liver (14). Furthermore, famciclovir administration may pre-select for a B domain mutation that acts as a compensatory mutation for the C domain mutation conferring resistance to lamivudine, explaining the rapid selection of the double mutation when the patient received sequential treatment by famciclovir followed by lamivudine (13, 15). In another study, the incidence of YMDD mutant was 76% at year 3 of treatment in HBeAg positive patients and only 10% at year 2.5 of therapy in HBeAg negative patients (16). This was confirmed in an independent study that showed that the rapidity of selection of YMDD mutant during therapy depends on pre-treatment ALT and HBV DNA levels as well as on the pre-core sequence status (9).

On the other hand, several studies have identified the following factors that predict viral resistance after the beginning of therapy. Despite the decrease in HBV DNA titer, when it remains higher than 10^3 copies/ml (Cobas Amplicor HBV Monitor test, Roche diagnostics) after 6 months of lamivudine administration, 62.3% of patients subsequently developed drug resistance while only 13% of those with HBV DNA levels lower than 10^3 had developed resistance (17). Moreover, several studies showed that using highly sensitive assays for the detection of viral polymerase gene mutations it is possible to detect drug resistant mutants several months prior to the rise in viral DNA titers (9) (and Lok et al, J Clin Microbiol in press, 2002).

Clinical severity of lamivudine resistance

As HBV is not cytopathogenic by itself and liver damage results from the immune attack of the infected hepatocytes, drug resistant virus - induced relapse of viral replication during therapy is not always associated with a rise in ALT levels. In the large phase III trials of lamivudine therapy for 12 months, the incidence of drug resistant mutants corresponding to the selection of mutations in the YMDD motif of the viral polymerase, was 14% in the Asian study, 31% and 32 % in the European and American studies (5, 6, 18). There was no difference in ALT levels in patients with wild type polymerase sequence and those with the mutation in the YMDD motif. After lamivudine cessation the incidence of polymerase mutants was analyzed in one study which showed a decrease from 31% at the end of treatment to 21% 12 weeks later, suggesting the re-emergence of wild type virus (18). In the Asian study there was no significant increase in ALT levels in these patients, nor was there a deterioration in liver histology (6). In the US study, 43% patients with YMDD mutation presented an histologic response compared to 63% patients with wild type sequence (5). As these polymerase mutants are not cytopathogenic by themselves, as is wild type virus, the impact of these mutants on immune mediated liver injury needs to be carefully assessed in longer clinical survey which may find a slow and progressive deterioration of liver histology in these patients.

In one study of extended lamivudine therapy, the incidence of YMDD mutants was 14% at week 52 and 38%

at week 104. Median HBV DNA in these patients was lower than pre-treatment levels. The same was true for ALT levels. Interestingly, 25% of these patients developed an ALT flare ($> 2xULN$), but ALT decreased in all patients that were maintained on lamivudine therapy. HBeAg seroconversion was achieved in 23% of these patients who continued lamivudine therapy, including HBV DNA loss and normalization of ALT levels by week 104 (19). However, the rate of HBeAg seroconversion in patients with lamivudine resistance was much lower in other studies ranging from 0 to 1.25 % (9, 17). In rare cases, the selection of lamivudine resistant strains was observed after anti-HBe seroconversion (17, 20).

In another study, 51 patients receiving an extended lamivudine monotherapy were followed for 3 years (21). The incidence of YMDD mutants was 53% at week 156. 27% of these patients achieved HBeAg seroconversion following the detection of the YMDD mutants. On the other hand 56% of the patients who never presented a YMDD mutant achieved HBeAg seroconversion. Median HBV DNA and ALT levels remained below the pre-treatment values over the 3 years of treatment. Nine patients with a polymerase mutant had a liver biopsy sampling and examination at week 156; 5 showed an improvement compared to baseline, 2 had no change and 2 had a deterioration.

Furthermore, some studies showed the possibility of clinical deterioration in these patients with ALT flares (22), that were more frequent in patients with pre-core mutant infection (9, 23). In these studies, clinical deterioration with ALT flares, or worsening of liver histology has been observed. The longitudinal study of 32 patients with YMDD mutants after at least 104 weeks of lamivudine therapy showed that 93.7% of them showed elevation of ALT levels, 40.6% experienced acute exacerbation within 4 to 94 weeks after the emergence of lamivudine resistant mutants. This incidence of acute exacerbation was much higher than in patients without YMDD mutants (4.3%). Acute exacerbation was accompanied by hepatic decompensation in 3 patients who subsequently recovered after HBe seroconversion. 75% of the patients with acute exacerbation showed a subsequent HBe seroconversion (22).

In patients with pre-core mutant infection, lamivudine resistance may be associated in approximately 50% of cases with acute exacerbation of the liver disease, that may lead in some cases to end-stage liver failure (9, 23).

In the setting of liver transplantation, a study showed that 6/14 patients with lamivudine resistance presented a clinical deterioration of their liver functions (24), while other case reports described the occurrence of rapidly evolving liver failure (25). In these patients with severe resistance, add-on therapy with adefovir, a nucleotide analogue which is active on both wild type and lamivudine resistant strains, provides a new treatment option to rescue these patients(26, 27).

From all these studies, it appears clear that lamivudine resistance may result in a significant number of cases in the deterioration of liver disease. This should be weighed against the beneficial effect of lamivudine and the risk of the natural history of the disease.

Treatment of lamivudine resistance Famciclovir was evaluated in lamivudine resistant patients. However, as we learned thereafter from in vitro phenotypic studies that these viral strains are cross-resistant with both famciclovir and lamivudine (28), this treatment strategy was not successful (9).

As treatment options for lamivudine resistance are available only since recently, lamivudine withdrawal has been tried in patients with drug resistance. This led to the re-emergence of wild type sequence but retreatment led to a more rapid reappearance of the drug resistant mutant, as this was previously shown with famciclovir therapy (9, 13, 15, 16, 29). Therefore famciclovir resistant mutants clearly predispose to lamivudine resistance either in add-on or switching strategies. In patients that are resistant to lamivudine, although treatment withdrawal is followed by a take-over by wild type virus, re-introduction of lamivudine is followed by a faster emergence of pre-selected lamivudine resistant viral strains.

Prior to the availability of adefovir dipivoxil, interferon alfa therapy has been tried to manage lamivudine resistance in patients with progressing liver disease. Our group has observed that add-on interferon alpha therapy with daily administration may control viral replication and liver disease activity (9). This may serve as a basis to evaluate in further trials the efficacy of pegylated interferon alpha in this indication.

More importantly, results of in vitro evaluation showed that adefovir inhibits the viral reverse transcriptase mutants that are resistant to lamivudine with IC50s comparable to that against the wild type polymerase viral strains (28, 30, 31). Several studies have shown that, in patients presenting with lamivudine resistance, administration of adefovir dipivoxil clearly induces a decrease in serum HBV DNA levels. The first observations were made in the liver and kidney transplant settings, and in HIV-HBV coinfecting patients (26, 27, 32). Then, clinical trials of adefovir dipivoxil administration either in addition to lamivudine or after a switch from lamivudine to adefovir dipivoxil showed that adefovir dipivoxil exhibit a significant antiviral effect with a 2 to 3 log drop in viremia levels together with an improvement in liver enzymes. After 24 weeks, there was no difference in the evolution of viral load after treatment modification between the two treatment groups (lamivudine plus adefovir dipivoxil versus adefovir dipivoxil alone) (Xiong et al, EASL 2002, Abstract, J Hepatol). However, since these are preliminary data, the optimal treatment regimen of lamivudine resistance is not known currently. It remains to see whether one treatment arm may induce a more rapid normalization of liver enzymes or liver functions, or whether the dynamic of replication of viral mutants may be different depending on the treatment option. Moreover, it will be important to see whether the kinetics of viral clearance are the same in patients with complex viral heterogeneity mixing pre-core mutants and lamivudine resistant mutants. Furthermore, it was shown that administration of Adefovir dipivoxil for up to 3 years does not select for viral polymerase resistant mutants (Delaney et al, EASL 2001, Abstract, J. Hepatol).

Other case reports have shown the limited efficacy of other antivirals such as ganciclovir, foscavir etc... Emtricitabine and clevudine, both L-pyrimidine analogs, were shown in in vitro studies to be inactive against lamivudine resistant strains which should limit their clinical utility in the rescue of lamivudine resistance(28, 33). It will be important to see the antiviral efficacy of entecavir, a purine analog, and of LdT in patients with lamivudine resistance(34, 35).

Prevention of lamivudine resistance

The most important issue, when starting antiviral therapy, is to prevent drug resistance. As a rationale, the use of inhibitors with different mechanism of action and different resistance profiles should be favoured in clinical trials to obtain an additive or synergistic antiviral effect to limit the risk of emergence of drug resistant strains (3). One study showed a potential beneficial effect of the association of famciclovir and lamivudine by accelerating the kinetics of viral clearance compared to lamivudine monotherapy (36). However, the long-term effect of the combination on the selection of drug resistant mutants was not studied. Trials combining lamivudine with adefovir dipivoxil have started, but other combinations such as clevudine and emtricitabine (37), or others with entecavir, etc... may follow the same rationale. This will be the next important step of the management of chronic hepatitis B. Results of trials associating or combining sequentially lamivudine and interferon alpha are discussed in another manuscript in this issue of J. Hepatol (Schalm et al). Some studies but not all, suggest that the addition of interferon alpha to lamivudine either in association (38) or sequentially (39) may decrease the rate of lamivudine resistance. This should be confirmed in further independent trials.

As lamivudine resistant strains are detected several weeks to several months prior to the rise in viral DNA or ALT levels, another strategy that may be used while awaiting for the results of the above mentioned trials and the general approval of adefovir dipivoxil, is to monitor closely viral resistance based on viral load and viral genome analysis (9) (Lok et al, J Clin Microbiol, in press). This should allow clinicians to adapt antiviral treatment by adding interferon alpha or adefovir dipivoxil in compassionate use programs, once viral resistance is detected and before the exacerbation of the liver disease.

Conclusion and Perspectives

HBV resistance to lamivudine is a frequent and significant therapeutical problem. With the results of clinical trials and independent studies, clinicians have now a better knowledge of the incidence of drug resistance and its clinical consequence. There are still many clinical questions to be answered regarding the predictive factors of lamivudine resistance development and of the severity associated with viral resistance. New drugs are being evaluated in experimental models and clinical trials. Among them, Adefovir dipivoxil is the more advanced in clinical development and represents a very promising inhibitor that has been assessed in phase

III trials and should be registered soon. It has an interesting antiviral profile with i) a potent activity against lamivudine resistant strains which is useful as a rescue treatment of lamivudine resistance, ii) and the absence of selection of drug resistant strains up to 3 years of therapy. As new progress has been made, we have now not only the capacity to control lamivudine resistance but also many tools to detect and prevent treatment failure. In this view, it will be important to determine the best strategy to prevent drug resistance using combinations of nucleoside analogues such as lamivudine, adefovir, and others, with immunostimulatory approaches based on pegylated interferon alfa, new therapeutic vaccines or others.

Figures

Figure 1

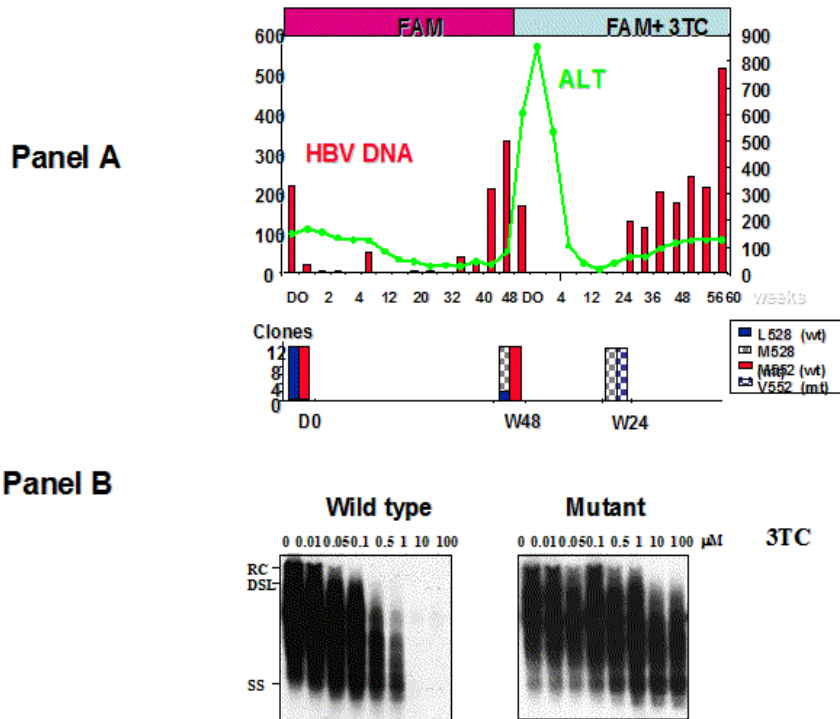
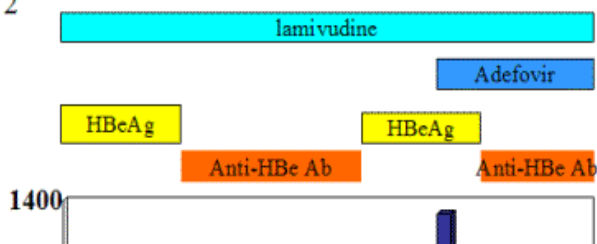


Figure 1. Selection of cross resistant viral strains with famciclovir therapy followed by famciclovir and lamivudine combination.

Panel A shows the evolution of viremia and ALT levels in a chronic hepatitis B patient who was nonresponder to interferon alphas and received famciclovir. This was associated by an initial virological response followed by a viral breakthrough associated with the selection of L528M mutation in the viral polymerase gene. Treatment was adapted with the addition of lamivudine which again induced a virological response but was then associated with a rapid viral breakthrough. This was due to the rapid selection of a double polymerase mutation L528M + M552V which was detected by clonal analysis prior to the rise in HBV DNA levels.

In panel B, cloning of viral polymerase sequences of pre-treatment strains and of strains resistant to the combination treatment in pCMV-HBV vectors showed that the pre-treatment strain was sensitive to lamivudine but that the double mutant was indeed resistant to lamivudine.

Figure 2



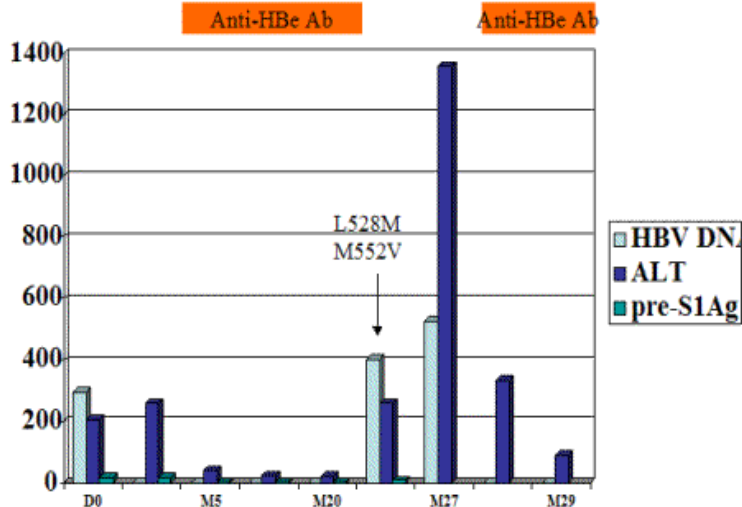


Figure 2. Selection of lamivudine resistance and rescue by adefovir dipivoxil administration.

The figure shows the evolution of ALT and virological markers in a patient who responded to lamivudine, seroconverted to anti-HBeAb, but then presented a viral breakthrough associated with HBeAg reversion and an acute exacerbation of liver disease which was rescued by the addition of adefovir dipivoxil.

REFERENCES

1. Perillo R, Schiff E, Davis G, Bodenheimer HC, Lindsay K, Payne J, Dienstag JL, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N. Engl. J. Med.* 1990;323:295-301.
2. Brunetto M, Giarin M, Saracco G, Oliveri F, Calvo P, Capra G, Randone A, et al. Hepatitis B virus unable to secrete e antigen and response to interferon in chronic hepatitis B. *Gastroenterology* 1993;105:845-850.
3. Zoulim F. Evaluation of novel strategies to combat hepatitis B virus targeting wild-type and drug-resistant mutants in experimental models. *Antivir Chem Chemother* 2001;12:131-142.
4. Zoulim F. Therapy of chronic hepatitis B virus infection: inhibition of the viral polymerase and other antiviral strategies [In Process Citation]. *Antiviral Res* 1999;44:1-30.
5. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, et al. Lamivudine as Initial Treatment for Chronic Hepatitis B in the United States. *N Engl J Med* 1999;341:1256-1263.
6. Lai CL, Chine RW, Leung NWY, Chang TT, Guan R, Tai DI, Ng KY, et al. A one year trial of lamivudine for chronic hepatitis B. *N. Engl. J. Med.* 1998;339:61-68.
7. Zoulim F, Trépo C. Drug therapy for chronic hepatitis B: antiviral efficacy and influence of hepatitis B virus polymerase mutations on the outcome of therapy. *J. Hepatol.* 1998;29:151-168.
8. Lewin SR, Ribeiro RM, Walters T, Lau GK, Bowden S, Locarnini S, Perelson AS. Analysis of hepatitis B viral load decline under potent therapy: Complex decay profiles observed. *Hepatology* 2001;34:1012-1020.
9. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, Johnson M, et al. Early Detection of Viral Resistance by Determination of Hepatitis B Virus Polymerase Mutations in Patients Treated by Lamivudine for Chronic Hepatitis B. *Hepatology* 2000;32:1078-1088.
10. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis b: 2000-summary of a workshop. *Gastroenterology* 2001;120:1828-1853.
11. Stuyver L, Van Geyt C, De Gendt S, Van Reybroeck G, Zoulim F, Leroux-Roels G, Rossau R. Line Probe Assay for Monitoring Drug Resistance in Hepatitis B Virus- Infected Patients during Antiviral Therapy. *J Clin Microbiol* 2000;38:702-707.
12. Pichoud C, Seignères B, Wang Z, Trepo C, Zoulim F. Transient selection of a hepatitis B virus polymerase gene mutant associated with a decreased replication capacity and famciclovir resistance. *Hepatology* 1999;29:230-237.
13. Seigneres B, Pichoud C, Ahmed SS, Hantz O, Trepo C, Zoulim F. Evolution of Hepatitis B Virus Polymerase Gene Sequence during Famciclovir Therapy for Chronic Hepatitis B. *J Infect Dis* 2000;181:1221-1233.
14. Zhou T, Saputelli J, Aldrich CE, Deslauriers M, Condreay LD, Mason WS. Emergence of drug-resistant populations of woodchuck hepatitis virus in woodchucks treated with the antiviral nucleoside lamivudine [In Process Citation]. *Antimicrob Agents Chemother* 1999;43:1947-1954.
15. Mutimer D, Pillay D, Cook P, Ratcliffe D, O'Donnell K, Dowling D, Shaw J, et al. Selection of multiresistant hepatitis B virus during sequential nucleoside-analogue therapy. *J Infect Dis* 2000;181:713-716.
16. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, et al. Long-term therapy of chronic hepatitis B with lamivudine [see comments]. *Hepatology* 2000;32:828-834.
17. Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough

in patients receiving prolonged lamivudine therapy. *Hepatology* 2001;34:785-791.

18. Schalm SW, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, Dhillon A, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial [see comments]. *Gut* 2000;46:562-568.
19. Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, et al. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group [see comments]. *Gastroenterology* 2000;119:172-180.
20. Pichoud C, Berby F, Stuyver L, Petit MA, Trepo C, Zoulim F. Persistence of viral replication after anti-HBe seroconversion during antiviral therapy for chronic hepatitis B. *J Hepatol* 2000;32:307-316.
21. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Lim SG, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001;33:1527-1532.
22. 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 [see comments]. *Hepatology* 1999;30:567-572.
23. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2000;32:847-851.
24. Perrillo R, Rakela J, Dienstag J, Levy G, Martin P, Wright T, Caldwell S, et al. Multicenter study of lamivudine therapy for hepatitis B after liver transplantation. Lamivudine Transplant Group. *Hepatology* 1999;29:1581-1586.
25. de Man RA, Bartholomeusz AI, Niesters HGM, Zondervan PE, Locarnini SA. The sequential occurrence of viral mutations in a liver transplant recipient re-infected with hepatitis B: hepatitis B immune globulin escape, famciclovir non-response, followed by lamivudine resistance resulting in graft loss. *J. Hepatol.* 1998;29:669-675.
26. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, Gutfreund K, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000;32:129-134.
27. Benhamou Y, Bochet M, Thibault V, Calvez V, Fievet MH, Vig P, Gibbs CS, et al. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. *Lancet* 2001;358:718-723.
28. Delaney WEt, Edwards R, Colledge D, Shaw T, Torresi J, Miller TG, Isom HC, et al. Cross-resistance testing of antihepadnaviral compounds using novel recombinant baculoviruses which encode drug-resistant strains of hepatitis B virus. *Antimicrob Agents Chemother* 2001;45:1705-1713.
29. Lau GK, Tsiang M, Hou J, Yuen S, Carman WF, Zhang L, Gibbs CS, et al. Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study [see comments]. *Hepatology* 2000;32:394-399.
30. Seigneres B, Aguesse-Germon S, Pichoud C, Vuillermoz I, Jamard C, Trepo C, Zoulim F. Duck hepatitis B virus polymerase gene mutants associated with resistance to lamivudine have a decreased replication capacity in vitro and in vivo. *J Hepatol* 2001;34:114-122.
31. Xiong X, Flores C, Yang H, Toole JJ, Gibbs CS. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. *Hepatology* 1998;28:1669-1673.
32. Peters MG, Singer G, Howard T, Jacobsmeyer S, Xiong X, Gibbs CS, Lamy P, et al. Fulminant hepatic failure resulting from lamivudine-resistant hepatitis B virus in a renal transplant recipient: durable response after orthotopic liver transplantation on adefovir dipivoxil and hepatitis B immune globulin. *Transplantation* 1999;68:1912-1914.
33. Seignères B, Pichoud C, Furman P, Trépo C, Zoulim F. Inhibitory activity of new nucleoside analogs on wild type and YMDD mutants of the hepadnavirus reverse transcriptase. *Hepatology* 2002;in press.
34. Standing DN, Bridges EG, Placidi L, Faraj A, Loi AG, Pierra C, Dukhan D, et al. Antiviral beta-L-nucleosides specific for hepatitis B virus infection. *Antivir Chem Chemother* 2001;12 Suppl 1:119-129.
35. Colonna RJ, Genovesi EV, Medina I, Lamb L, Durham SK, Huang ML, Corey L, et al. Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. *J Infect Dis* 2001;184:1236-1245.
36. Lai CL, Yuen MF, Cheng CC, Wong WM, Cheng TK, Lai YP. An open comparative study of lamivudine and famciclovir in the treatment of chronic hepatitis B infection. *Hepatology* 1998;28:Abstract 622, p 318A.
37. Gish RG, Leung NW, Wright TL, Trinh H, Lang W, Kessler HA, Fang L, et al. Dose range study of pharmacokinetics, safety, and preliminary antiviral activity of emtricitabine in adults with hepatitis B virus infection. *Antimicrob Agents Chemother* 2002;46:1734-1740.
38. Santantonio T, Anna Niro G, Sinisi E, Leandro G, Insalata M, Guastadisegni A, Facciorusso D, et al. Lamivudine/interferon combination therapy in anti-HBe positive chronic hepatitis B patients: a controlled pilot study. *J Hepatol* 2002;36:799-804.
39. Serfaty L, Thabut D, Zoulim F, Andreani T, Chazouilleres O, Carbonell N, Loria A, et al. Sequential treatment with lamivudine and interferon monotherapies in patients with chronic hepatitis B not responding to interferon alone: results of a pilot study. *Hepatology* 2001;34:573-577.