Prior exposure to lamivudine increases entecavir-resistance risk in chronic hepatitis B patients without detectable lamivudine-resistance.

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List of Abbreviations:

ETV, entecavir; CHB, chronic hepatitis B; LAM, lamivudine; LAM-R, lamivudine-resistance; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; ETV-R, entecavir-resistance; IU, international unit; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; anti-HBe Ab, anti-hepatitis B e antibody; ALT, alanine aminotransferase; HR, hazard ratio; CI, confidence interval; TDF, tenofovir.

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Abstract

The efficacy of entecavir (ETV) in chronic hepatitis B (CHB) patients who were exposed to lamivudine (LAM), but had no detectable LAM-resistance (LAM-R) is not well evaluated. In this study, we aimed to evaluate whether the probability of developing genotypic resistance to ETV in LAM-exposed patients with/without LAM-R is comparable to that in antiviral-naïve patients. This retrospective cohort study included 500 consecutive patients with CHB who started ETV monotherapy at a single tertiary hospital in Korea. The patients were divided into three groups: NA-naïve patients (group 1, n=142), patients who were ever exposed to LAM with no currently or previously detected LAM-R (group 2, n=233), and patients with LAM-R when starting ETV (group 3, n=125). Overall median ETV treatment duration was 48.7 months. The probabilities of virologic breakthrough were significantly increased not only in group 3 (hazard ratio [HR]=14.4, P < 0.001) but also in group 2 (HR=5.0, P < 0.001), compared to group 1. Genotypic ETV-resistance (ETV-R) was more frequently developed in group 2 (HR=13.0, P = 0.013) as well as group 3 (HR=43.9, P < 0.001) than in group 1: the probabilities of developing ETV-R in groups 1, 2 and 3 were <1.0, 8.0, and 28.2%, respectively, at month 48. This study indicates that ETV-R occurred more frequently in LAM-exposed patients, even though they had no detectable LAM-R, than in NA-naïve patients. Therefore, LAM-exposed CHB patients, regardless of LAM-R, should be monitored more cautiously for the development of ETV-R during ETV monotherapy.

Keywords: lamivudine; entecavir; resistance; chronic hepatitis B
Entecavir (ETV) is an orally administered guanosine analogue that has been approved for treatment of chronic hepatitis B (CHB). In antiviral-naïve CHB patients, ETV has shown excellent antiviral efficacy with remarkably low probabilities of genotype resistance (1.2%) and virologic breakthrough (0.8%) for up to five years of treatment (1). In contrast to antiviral-naïve patients, the rates of genotypic resistance to ETV are much higher in patients with lamivudine (LAM)-resistance (LAM-R) (i.e., 51% of five-year cumulative probability) (1). Consequently, ETV is now recommended as one of the first-line therapeutic regimens for antiviral-naïve patients with CHB, but not for patients who developed LAM-R (2-4).

The emergence of LAM-R variants has been relatively frequent even in antiviral-naïve patients with CHB: approximately 20% of patients treated with LAM develop LAM-R at 1 year and 70–80% at 5 years of treatment (5-7). Although some selected cases that have succeeded in achieving treatment endpoints (i.e., HBeAg - seroconversion and/or maintained complete virologic suppression) could discontinue LAM without developing LAM-R (8, 9), the rates of durable response after cessation of LAM have been low (10, 11). Unfortunately, the retreatment strategies for virologic relapse in those cases are still indefinite. Since LAM was the first approved nucleoside analogue for the treatment of hepatitis B virus (HBV) infection and had been widely used as a first-line therapy for CHB before the introduction of more potent nucleos(t)ide analogues (NAs), including ETV (2), there are already a number of CHB...
patients who have been receiving LAM or who have experienced prior LAM treatment.

Although ETV may not be recommended to those who developed LAM-R variants, the applicability of ETV in those patients who were exposed to LAM, without previously or currently detected LAM-R variants remains unclear. In this study, therefore, we aimed to compare the risk of developing virologic breakthrough and genotypic resistance to ETV (ETV-R) in patients who were previously exposed to LAM without previous or current detectable LAM-R as compared to either patients with LAM-R or NA-naïve patients.
Methods and Materials

Patients

A retrospective longitudinal cohort study was performed in consecutive patients treated with ETV monotherapy for CHB between January 1, 2007 and November 5, 2010 at a single tertiary hospital (Seoul National University Hospital; Seoul, Republic of Korea). Among the patients, LAM-R was defined as a virologic breakthrough associated with genotypic resistance to LAM (i.e. rtL180M, rtL180V, rtM204I, rtM204V, and rtM204S). Virologic breakthrough was defined as at least a 1 log_{10} increase in serum HBV DNA (IU/mL), compared to the on-treatment nadir (12). Genotypic ETV-R referred to the detection of HBV variants with amino acid substitutions that conferred attenuated susceptibility to ETV (i.e. rtT184G, rtT184S, rtT184A, rtT184I, rtT184L, rtS202G, rtS202I and rtM250V) by direct sequencing method (13, 14). The patients with the following conditions were excluded from the study: co-infection with hepatitis C, hepatitis D, or human immunodeficiency virus; previous treatment for HBV with interferon α and NAs other than LAM, before and during ETV therapy; history of prior LAM-R without evidence of LAM-R at baseline; liver transplantation before and during the rescue therapy; a glomerular filtration rate < 50 mL/min, estimated by the Cockcroft-Gault equation; prior or current malignancy including hepatocellular carcinoma (HCC); and concomitant serious medical illness such as hematological disease and heart failure. The subjects who did not undergo the clinical and laboratory assessments described below were also excluded. The patients were
grouped into three groups: NA-naïve patients (group 1), patients who experienced LAM with no currently or previously detected LAM-R (group 2), and patients who had LAM-R at baseline (group 3).

**Follow-up and Endpoints**

All patients were followed every two to three months with routine biochemical liver function tests, hepatitis B e antigen (HBeAg) and antibody (anti-HBe Ab), and serum HBV DNA levels. Compliance with treatment was assessed by interview during every visit. Serum HBV DNA levels were quantified at baseline and at each follow-up visit with a low detection limit of approximately 20 IU/mL (15). HBV DNA was obtained from serum samples and the HBV polymerase gene was amplified using nested PCR. The Big-Dye terminator version 3.1 ready reaction cycle sequencing kit (Applied Biosystems, Foster City, CA) with an ABI Prism 3730 genetic analyzer (Perkin-Elmer, Foster City, CA) was used to perform the cycle sequencing reaction. Genotypic variants resulting in LAM-R and ETV-R were determined by direct sequencing analysis of serum samples obtained either when virologic breakthrough occurred during ETV treatment or at the time of starting ETV treatment in LAM-exposed patients (groups 2 and 3).

The primary endpoints of this study were the emergence of genotypic ETV-R variants and virologic breakthrough. The secondary endpoints included: i) biochemical response (normalization of serum alanine aminotransferase [ALT] level), and ii) complete virologic suppression (undetectable serum hepatitis B virus [HBV] DNA
by real-time polymerase chain reaction). The upper limit of normal ALT was defined as 30 IU/L for men and 19 IU/L for women (16).

The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital.

Statistical Analysis

Survival analysis was performed using the Kaplan-Meier method, the life table method, the Cox-regression model, or the Firth-based penalized logistic regression analysis to estimate and compare the time to the emergence of genotypic resistance, biochemical response, complete virologic suppression, and virologic breakthrough. Univariate and multivariate analyses were performed with Firth-based penalized logistic regression analysis. Variables with a $P < 0.05$ in univariate analysis or those with clinical implications were added to the multivariate logistic regression model to identify independent risk factors after adjusting for other variables. In multivariate Firth-based penalized logistic regression analysis, a stepwise method was used to select variables to be maintained in the final model: the conditional probabilities for stepwise entry and stepwise removal of a factor were 0.05 and 0.20, respectively.

Statistical analysis was performed with SPSS version 17.0 (SPSS Institute, Inc.; Chicago, IL), STATA version 10.0 (STATA Corp., College Station, TX) and SAS version 9.2 (SAS Institute Inc., Cary, NC). $P < 0.05$ was considered statistically significant.
Results

Baseline Characteristics

A total of 500 patients started ETV therapy during the study period. Their mean age was 53.1 ± 11.2 years; 332 patients (66.4%) were male. The median overall ETV treatment duration was 48.7 months (range, 10.4–84.3 months). A total of 142 patients were included in group 1 (NA-naïve patients), 233 in group 2 (LAM-exposed patients without prior or current LAM-R), and 125 in group 3 (patients with current LAM-R at baseline). Table 1 shows the baseline characteristics of these three groups.

One hundred fifteen patients (49.4%) in group 2 had discontinued LAM without LAM-R after achieving treatment endpoints (HBeAg-seroconversion and/or maintained complete virologic suppression). Seventy-six patients who experienced virologic breakthrough without LAM-R during prior LAM treatment in group 2 were treated with 1.0 mg/day of ETV, and remaining 157 patients in group 2 with 0.5 mg/day of ETV. All the patients in group 3 were treated with 1.0 mg/day of ETV.

The median overall LAM treatment duration of group 3 (38.3 months; range, 6.0–121.1 months) was longer than that of group 2 (23.5 months; range, 2.0–93.5 months) (P<0.001). Sixty-two patients (49.6%) had experienced virologic breakthrough during prior LAM treatment in group 3, which was significantly more frequent than that in the group 2 (76 patients; 32.6%) (P < 0.001).

Nineteen patients (8.2%) in group 2 and 9 patients (7.2%) in group 3 showed primary non-response at 3 months of LAM treatment, and there was no significant
difference between the groups. One hundred twenty-six patients (54.1%) in group 2 showed partial response at 6 months of LAM treatment, while 45 patients (36%) in group 3 showed partial response at 6 months of LAM treatment ($P = 0.006$). A total of 104 patients (44.6%) in group 2 achieved complete virologic suppression during prior LAM treatment, and median duration of continuing LAM after achieving complete virologic suppression was 18.3 months (range, 3.0–76.8 months). In group 3, only 19 patients (15.2%) had achieved complete virologic suppression during prior LAM treatment and median duration was 12.1 months (range, 4.0–26.8 months).

The median treatment-free duration (i.e. interval between cessation of LAM and initiation of ETV) of group 2 was 6.0 months (range, 0–54 months) and that of group 3 was 5.4 months (range, 0–25.2 months) ($P = 0.543$).

Among the included patients, 35 patients were evaluated for HBV genotype and all of them had genotype C HBV. Actually almost all of the patients with CHB in Korea (96–100%) have been known to have genotype C virus (17, 18).

**Biochemical, Virologic, and Serological Responses**

During the ETV treatment period, except for six patients with an initially normal ALT, the overall rate of biochemical response was 94.9% (355 of 494). The cumulative probabilities for biochemical responses at month 36 were 95% in group 1, 97% in group 2, and 94% in group 3, respectively. There was no significant difference among the three groups ($P = 0.830$).

Fig. 1A and Table 2 show the mean changes in the HBV DNA level at each time
The decrease in HBV DNA was significantly less prominent in group 3, compared to either group 1 or 2, at all the time points (all \( P < 0.001 \)). At month 36, group 1 showed significantly more profound HBV DNA suppression (\(-6.31 \pm 1.59 \log_{10} \) IU/mL) than group 2 (\(-5.09 \pm 2.80 \log_{10} \) IU/mL, \( P < 0.001 \)) as well as group 3 (\(-3.91 \pm 2.66 \log_{10} \) IU/mL, \( P < 0.001 \)) (Fig. 1A).

During the treatment period, complete virologic suppression with undetectable serum HBV DNA was achieved in 365 patients (73.0%). The cumulative probabilities of complete virologic suppression at month 36 were 72% in group 1, 34% in group 2, and 36% in group 3, respectively (Table 2). There was no statistically significant difference between groups 2 and 3 (hazard ratio [HR], 0.530; 95% confidence interval [CI], 0.271–1.039; \( P = 0.062 \)). Group 3 showed significantly lower probability of complete virologic suppression than either group 1 (HR, 0.077; 95% CI, 0.039–0.151; \( P < 0.001 \)) or group 2 (HR, 0.144; 95% CI, 0.086–0.241; \( P < 0.001 \)) (Fig. 1B).

Among the 199 patients positive for HBeAg at the time of initiating ETV therapy, 105 patients (52.8%) achieved HBeAg-seroconversion. The cumulative probabilities of the serological responses at month 36 were 86% in group 1, 83% in group 2, and 73% in group 3, respectively. There was no significant difference among the three groups (\( P = 0.111 \)).

**Virologic Breakthrough and ETV-Resistant Genotypic Variants**

Virologic breakthrough occurred in 84 patients (16.8%) during the treatment period. The cumulative probabilities of virologic breakthrough at months 36 and 48...
were respectively as follows: 1% and 3% in group 1; 3% and 10% in group 2; and 15% and 32% in group 3 (Table 2). Both LAM-exposed groups [group 2 (HR, 5.007; 95% CI, 1.916–13.083; \( P < 0.001 \)) and group 3 (HR, 14.368; 95% CI, 5.470–37.739; \( P < 0.001 \)))] showed significantly more frequent virologic breakthrough than the NA-naive group (group 1). Group 3 showed significantly more frequent virologic breakthrough than group 2 (HR, 2.870; 95% CI, 1.719–4.789; \( P < 0.001 \)) (Fig. 2A).

Genotypic ETV-R were documented in 50 patients (10%) during the treatment period and all ETV-R variants were accompanied by LAM-R. Univariate and subsequent multivariate analyses showed that exposure to LAM was an independent predictor of genotypic resistance to ETV. Compared to group 1, both group 2 (HR, 13.039; 95% CI, 1.721–98.777; \( P = 0.013 \)) and group 3 (HR, 43.885; 95% CI, 5.871–328.021; \( P < 0.001 \)) showed significantly higher risk of developing ETV-R, after adjustment for HBeAg status (Table 3). ETV-R was not found in group 1. The cumulative probabilities of the ETV-R variant at months 6, 12, 24, 36 and 48 were respectively as follows: <1, <1, 1, 2 and 8% in group 2; and 2, 3, 11, 16 and 28% in group 3 (Fig. 2B). Table 2 summarizes the efficacy and breakthrough for each group.

### Predictors for the Development of Genotypic Resistance in Group 2

Since no ETV-R variant occurred in group 1 (NA-naive patients) and international guidelines no longer recommend ETV monotherapy for patients in group 3 (patients who had LAM-R), (2, 3) we tried to determine the pre- and on-treatment predictors for the emergence of ETV-R variant in group 2 patients (i.e., those who experienced LAM,
but had no prior or current LAM-R variant).

Among the 233 patients included in group 2, 19 patients developed ETV-R variant and the shortest time to developing a variant was 33.3 months (range, 33.8–48 months). Thirteen of them had rtS202G variants, 5 had rtT184I and one had both rtS202G and rtT184I variants. With ETV therapy, 215 patients (92.3%) included in group 2 showed primary responses, defined as a $\geq 1$ log$_{10}$ decrease in serum HBV DNA (IU/mL) within 3 months of antiviral therapy (16), and 188 patients (81.0%) achieved complete virologic suppressions within 12 months. Univariate and multivariate analysis showed that complete virologic suppression within 12 months of ETV treatment was a sole independent predictor of developing ETV-R variant (HR, 0.019; 95% CI, 0.004–0.087; $P < 0.001$) (Fig.3); the other factors including HBeAg status, baseline serum HBV DNA levels and duration of prior LAM treatment or interruption of NA were not (Table 4). There was significant interaction between complete virologic suppression during prior LAM treatment and complete virologic suppression within 12 months of ETV treatment ($P < 0.001$).

Response to Prior LAM Treatment and ETV-R during ETV Treatment

We performed subgroup analysis of patients who were exposed to prior LAM treatment (groups 2 and 3). Among the patients who experienced virologic breakthrough during previous LAM treatment, ETV-R was less frequent in group 2 (16 of 76, 21.0% than in group 3 (26 of 62, 43.5%) at month 48 ($P = 0.002$). Among patients who had achieved complete virologic suppression for more than 1
year with prior LAM treatment, ETV-R occurred less frequently in group 2 (2 of 104, 1.9%) than in group 3 (2 of 19, 10.5%) at month 48 ($P < 0.001$).
Antiviral efficacy of ETV in LAM-exposed patients without LAM-R had not yet been well evaluated. Therefore, there are no current guidelines regarding the use of antiviral agents in LAM-exposed patients with no detectable LAM-R, and who were frequently treated as antiviral naïve patients in clinical practice. Although a previous study showed that cumulative probability of achieving virologic response during ETV therapy was slightly decreased in LAM-experienced patients without LAM-R as compared to LAM-naïve patients (19), there were no data comparing virologic breakthrough and genotypic resistance between LAM-experienced patients and NA-naïve patients.

This is the first study to examine the risk of developing ETV-R during ETV therapy in patients who were exposed to prior LAM treatment without detectable LAM-R (group 2), as compared to either NA-naïve patients (group 1) or patients with LAM-R (group 3). The results clearly demonstrated that ETV-R was significantly more frequent in LAM-exposed patients without prior/current LAM-R than in NA-naïve patients, but slightly less frequent compared to patients having current LAM-R. LAM-exposed patients (both groups 2 and 3) showed significantly less reduction of serum HBV DNA level and higher risk of virologic breakthrough than LAM-naïve patients. This study also indicated that developing ETV-R in LAM-exposed patients was significantly related to failure to achieve complete virologic suppression within 12 months of ETV treatment, which in turn was significantly related to achieving complete virologic suppression during prior LAM treatment.
In this study, ETV showed excellent antiviral efficacy in antiviral-naïve patients (group 1) without developing any virologic breakthrough or genotypic resistant variant for up to 84.3 months of median treatment duration, which is consistent with the findings of previous studies (20-22). In contrast, in LAM-experienced patients with current LAM-R (group 3), probabilities of developing virologic breakthrough and ETV-R at month 48 were as high as 32 and 28%, respectively, in spite of the higher dose of ETV (1.0 mg/day); this is also comparable to the results of previous reports (23, 24). Surprisingly, LAM-exposed patients without prior or current LAM-R (group 2) also revealed relatively high probabilities of developing virologic breakthrough and ETV-R at month 48 (10 and 8%, respectively) during long-term ETV treatment. As shown in subgroup analysis, even among the patients without LAM-R who had complete virologic suppression for more than 1 year with LAM treatment, 1.9% of patients developed ETV-R after 2 years of ETV treatment. Thus, sustained complete virologic suppression with prior LAM treatment failed to assure developing no ETV-R. HBV may exist in the form of quasi-species in CHB patients (25) and antiviral-resistant strains sometimes cannot be detected in time due to the limitation of test sensitivity, especially when its proportion is less than 20% in the pool of viral quasi-species (13, 26). The sensitivity of direct sequencing is reported as 43.2–66.7% (27-29). Thus, theoretically, there might have been a small number of LAM-resistant strains although tests failed to detect them at the time of initiating ETV treatment. Once LAM-resistant variants have been developed, they do not disappear but are archived and retained in the virus population (30). During ETV treatment, those
inferior LAM-resistant strains would readily become predominant strains by positive selection by ETV, since they are less susceptible to ETV (31, 32). According to the “two-hit” theory, this positive selection of LAM-resistant strains by ETV acts as the first hit, and the second hit of additional variant in these selected strains could easily occur to establish ETV-R (13). Surprisingly, ETV-R occurred even in a patient who was exposed to LAM for only 2 months. ETV-R developed in patients with as long as 6 months since LAM cessation and occurred during more than 33.3 months of ETV treatment in group 2. These findings collectively indicated that short duration of LAM treatment may be enough to select LAM-resistant strains which could survive even after discontinuation of LAM to affect the long-term efficacy of subsequent antiviral therapy.

This study provides another important clinical implication that prior antiviral treatment with low-potency drugs (i.e., LAM) may significantly affect the risk of developing strains resistant to the next antiviral treatment with ETV, a highly potent drug, even if there was no evidence of preexisting genotypic resistance to prior drugs. In this study, moreover, some patients who stopped low-potency NA treatment even after reaching treatment endpoints (HBeAg-seroconversion and/or maintained complete virologic suppression) experienced resistance to subsequent ETV, which has great implications. This finding again highlights the importance of using not low-potent NAs but highly potency NAs (e.g., ETV and tenofovir disoproxil fumarate [TDF]) for the initial treatment of CHB. A previous Japanese study reported that LAM-to-ETV switching therapy may be feasible, since there was neither virologic breakthrough nor
ETV-R during 2 years of ETV treatment and since LAM-to-ETV switching therapy was significantly superior to continuing LAM therapy in terms of virologic breakthrough (33). Compared to our study, that study had shorter follow-up duration (median, 20 months vs. 48.8 months) and approximately two-thirds of the ETV-R variants were detected after 2 years of ETV therapy in group 2 of our study. Therefore, the conclusion of the Japanese study should not be translated into excellent long-term efficacy of switching therapy from low-potency drugs to ETV. Close monitoring of serum DNA level and genotypic ETV-R variant may be required during ETV treatment in LAM-experienced patients, even though they never developed LAM-R. In addition, monotherapy with TDF, which is not cross-resistant to LAM, or combination therapy with nucleoside and nucleotide analogues (e.g., adefovir/LAM, TDF/LAM, TDF/ETV, and TDF/emtricitabine), rather than ETV monotherapy, could be useful for previously or currently LAM-exposed cases, especially those who failed to achieve complete virologic suppression either during prior LAM treatment or at 12 months of ETV treatment, since those regimens may suppress LAM-R variants more effectively. Our results suggest that the addition of a more potent drug that does not show cross-resistance (i.e., adding TDF to LAM or telbivudine, or adding ETV to adefovir) may be more beneficial than switching therapy in those cases who had suboptimal treatment response with low genetic barrier drug (e.g., LAM, adefovir, or telbivudine). It should also be questioned whether ETV could be one of the drugs of choice for LAM-exposed patients as well as for NA-naïve patients, since only 2 months of prior exposure to LAM triggered LAM-resistant strains in our study. Considering that a number of patients have been exposed to LAM, further
prospective studies on proper treatment strategy in LAM-exposed patients may be required to establish treatment guidelines of CHB. Especially, regarding safety and cost of therapy, further study is warranted to evaluate whether monotherapy with TDF that shares no cross resistance with LAM might be a good treatment option in patients exposed to LAM.

In conclusion, this study indicates that prior exposure to LAM treatment, even though the patients did not exhibit prior/current LAM-R, is significantly related to a high risk of the emergence of ETV-R during long-term ETV treatment. More attention should be paid to those LAM-experienced patients who are currently treated with ETV regardless of prior/current LAM-R, and it could be judicious to treat the high risk patients, who were previously treated with LAM but failed to achieve complete virologic suppression, with the combination or more potent regimens rather than ETV monotherapy. In addition, the importance of therapy with highly potent antivirals (i.e., ETV and TDF) from the first line therapy of CHB patients cannot be overemphasized.
References


Figure Legends

**Fig. 1.** Efficacy with ETV therapy. (A) Changes in mean log values of the serum HBV DNA levels from baseline during ETV therapy. The decrease in HBV DNA was significantly less prominent in group 3, compared to either group 1 or 2, at all the time points (all \( P < 0.001 \)). An independent sample \( t \) test was used for the statistical analysis at each time point. (B) Cumulative incidence of complete virologic suppression (undetectable serum HBV DNA). Analysis was done by the Kaplan-Meier analysis method; (\( P < 0.001 \) by log rank test). Group 3 showed significantly lower probability of complete virologic suppression than either group 1 or group 2.

**Fig. 2.** Breakthrough with ETV therapy. (A) Cumulative incidence of virologic breakthrough during ETV treatment using Kaplan-Meier curve (all \( P < 0.001 \) by log rank test). LAM-exposed groups (both group 2 and 3) showed significantly more frequent virologic breakthrough than the NA-naïve group (group 1). Group 3 showed significantly more frequent virologic breakthrough than group 2. (B) Cumulative incidence of emergence of ETV-R with ETV therapy using Kaplan-Meier curve (all \( P < 0.001 \) by log rank test). Compared to group 1, both group 2 and 3 showed significantly higher risk of developing ETV-R.

**Fig. 3.** Impact of the complete virologic suppression within 12 months (CVS 12) during ETV treatment on the development of ETV-R in group 2 patients. Patients with CVS 12 had significantly lower probability of developing ETV-R (\( P < 0.001 \) by log rank test).
# Tables

## Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=142)</th>
<th>Group 2 (n=233)</th>
<th>Group 3 (n=125)</th>
<th>P</th>
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<tr>
<td>Mean age, yeara</td>
<td>51.6 ± 12.1</td>
<td>54.3 ± 10.8</td>
<td>52.6 ± 10.8</td>
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<td>Male sex, n (%)</td>
<td>92 (64.8%)</td>
<td>145 (62.2%)</td>
<td>85 (68.0%)</td>
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<td>Liver cirrhosis, n (%)</td>
<td>73 (51.4%)</td>
<td>115 (49.4%)</td>
<td>54 (43.2%)</td>
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<td>HBeAg-positive, n (%)</td>
<td>54 (38.0%)</td>
<td>83 (35.6%)</td>
<td>62 (49.6%)</td>
<td>0.063</td>
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<td>Median HBV DNA, log10 IU/mL (range)</td>
<td>6.67 (2.07–9.81)</td>
<td>5.39 (3.70–9.81)</td>
<td>6.03 (4.32–9.81)</td>
<td>0.578</td>
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<td>ALT &gt; 2×ULN, n (%)</td>
<td>119 (83.8%)</td>
<td>202 (86.7%)</td>
<td>100 (80.0%)</td>
<td>0.702</td>
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<tr>
<td>Median prior LAM treatment duration, months (range)</td>
<td>NA</td>
<td>23.5 (2.0–93.5)</td>
<td>38.3 (6.0–121.1)</td>
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<td>Median ETV treatment duration, months (range)</td>
<td>45.2 (21.9–80.7)</td>
<td>54.7 (19.1–84.3)</td>
<td>41.7 (10.4–83.1)</td>
<td>0.165</td>
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*Data are given as the mean ± standard deviation. Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal; NA, not applicable.
Table 2. Treatment responses during entecavir therapy

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<th>Outcome</th>
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<th>Group 2</th>
<th>Group 3</th>
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<td>Reduction of HBV DNA (log_{10} IU/mL), mean ± SD</td>
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<tr>
<td>Month 3</td>
<td>−4.28 ± 1.82</td>
<td>−2.57 ± 2.54</td>
<td>−2.63 ± 2.12</td>
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<td>Month 6</td>
<td>−5.29 ± 1.61</td>
<td>−3.59 ± 2.96</td>
<td>−3.32 ± 2.18</td>
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<td>Month 12</td>
<td>−5.71 ± 1.40</td>
<td>−3.93 ± 3.24</td>
<td>−3.66 ± 2.49</td>
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<td>Month 24</td>
<td>−5.89 ± 1.52</td>
<td>−5.02 ± 2.72</td>
<td>−3.74 ± 2.47</td>
</tr>
<tr>
<td>Month 36</td>
<td>−6.13 ± 1.59</td>
<td>−5.09 ± 2.80</td>
<td>−3.91 ± 2.66</td>
</tr>
<tr>
<td>Complete virologic suppression, cumulative incidence (No. of patients-at-risk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 12</td>
<td>48% (73)</td>
<td>15% (188)</td>
<td>18% (87)</td>
</tr>
<tr>
<td>Month 24</td>
<td>60% (53)</td>
<td>24% (165)</td>
<td>25% (60)</td>
</tr>
<tr>
<td>Month 36</td>
<td>72% (33)</td>
<td>34% (136)</td>
<td>36% (34)</td>
</tr>
<tr>
<td>Virologic breakthrough, cumulative incidence (No. of patients-at-risk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 12</td>
<td>0% (136)</td>
<td>0% (222)</td>
<td>3% (111)</td>
</tr>
<tr>
<td>Month 24</td>
<td>1% (129)</td>
<td>1% (209)</td>
<td>7% (98)</td>
</tr>
<tr>
<td>Month 36</td>
<td>1% (101)</td>
<td>3% (179)</td>
<td>15% (80)</td>
</tr>
<tr>
<td>Month 48</td>
<td>3% (59)</td>
<td>10% (42)</td>
<td>32% (52)</td>
</tr>
<tr>
<td>Genotypic resistant to ETV, cumulative incidence (No. of patients-at-risk)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 12</td>
<td>0% (73)</td>
<td>0% (56)</td>
<td>3% (58)</td>
</tr>
<tr>
<td>Month 24</td>
<td>0% (53)</td>
<td>1% (40)</td>
<td>11% (44)</td>
</tr>
<tr>
<td>Month 36</td>
<td>0% (33)</td>
<td>2% (27)</td>
<td>16% (31)</td>
</tr>
<tr>
<td>Month 48</td>
<td>0% (25)</td>
<td>8% (18)</td>
<td>28% (16)</td>
</tr>
</tbody>
</table>

*Data are given as cumulative incidence as a percentage with the number of patients at risk. Abbreviations: HBV, hepatitis B virus; SD, standard deviation; HBeAg, hepatitis B e antigen; No., number.*
Table 3. The independent risk factors for genotypic resistance to entecavir

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Treatment group</td>
<td>1.000 (reference)</td>
<td>1.000 (reference)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>12.519 (1.657–94.567)</td>
</tr>
<tr>
<td>2</td>
<td>45.500 (6.241–346.474)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV DNA, Log10 IU/mL</td>
<td>1.203 (0.946–1.531)</td>
<td>0.132</td>
</tr>
<tr>
<td>Presence of LC</td>
<td>0.665 (0.324–1.363)</td>
<td>0.265</td>
</tr>
<tr>
<td>HBeAg positivity</td>
<td>2.625 (1.266–5.443)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval, HBV, hepatitis B virus; LC, liver cirrhosis; HBeAg, hepatitis B e antigen.
Table 4. The independent risk factors for genotypic resistance to entecavir in group 2

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Initial HBV DNA, Log10 IU/mL</td>
<td>1.281 (1.045–1.569)</td>
<td>0.017</td>
</tr>
<tr>
<td>Presence of LC</td>
<td>0.409 (0.151–1.104)</td>
<td>0.078</td>
</tr>
<tr>
<td>HBeAg positivity</td>
<td>3.794 (1.449–9.931)</td>
<td>0.007</td>
</tr>
<tr>
<td>Duration of prior LAM treatment</td>
<td>0.991 (0.967–1.016)</td>
<td>0.485</td>
</tr>
<tr>
<td>Duration of NA interruption</td>
<td>0.998 (0.993–1.004)</td>
<td>0.533</td>
</tr>
<tr>
<td>Complete virologic suppression</td>
<td>0.018 (0.005–0.068)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>within 12 months of ETV treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; LC, liver cirrhosis; HBeAg, hepatitis B e antigen; LAM, lamivudine; NA, nucleos(t)ide analogue; ETV, entecavir.