

Efficacy of Adefovir-Based Combination Therapy for Patients with Lamivudine- and Entecavir-Resistant Chronic Hepatitis B Virus Infection

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Treatment strategies for entecavir (ETV)-resistant chronic hepatitis B (CHB) patients are not yet well established. The aim of this study was to evaluate overall antiviral efficacy and to compare the efficacy of combination therapy with adefovir (ADV) plus nucleoside analogues (lamivudine [LAM], telbivudine [LdT], or ETV) in patients infected with LAM- and ETV-resistant hepatitis B virus (HBV) variants. Virologic, biochemical, and serologic responses during combination therapy with ADV plus nucleoside analogues were assessed. Propensity score analysis was used to select a matched group of patients for the comparison of rescue therapy regimens. A total of 67 consecutive patients were analyzed. Complete virologic suppression was achieved in 27 patients. The overall cumulative incidence of complete virologic suppression at month 24 was 47.4%: 44.3% in the LAM or LdT plus ADV group and 51.4% in the group given ETV and ADV. There was no significant difference between these two groups ($P = 0.234$). The cumulative incidences of complete virologic suppression were still comparable between the two groups selected and matched using the propensity score model ($P = 0.419$). Virologic breakthrough was observed in 9 patients, and rtA181V substitution was newly detected in one patient. Hepatitis B e antigen (HBeAg) negativity and lower baseline HBV DNA level were associated with complete virologic suppression in univariate analysis. In multivariate analysis, lower baseline HBV DNA level remained an independent predictor. In conclusion, combination therapy with ADV plus nucleoside analogues fails to show sufficient antiviral efficacy in CHB patients with resistance to both LAM and ETV. Further study is warranted to evaluate the efficacy of a more potent tenofovir-based regimen in such patients.

Chronic hepatitis B virus (HBV) infection is a serious global public health problem that leads to liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (1–3). The sustained suppression of viral load has been associated with the prevention of liver disease progression and inhibition of the development of long-term complications (4). Therefore, the aim of chronic hepatitis B (CHB) treatment is early and sustained viral suppression (4–6).

Lamivudine (LAM), which was the first nucleoside analogue approved for the treatment of CHB, has been used widely in patients with CHB (7). However, resistance to LAM emerges in approximately 20% of patients after 1 year and in 70% of patients after 5 years of treatment (8, 9). In contrast, entecavir (ETV) has excellent antiviral activity with very low risk of developing resistance (<1.2% during 5 years of treatment) in nucleos(t)ide-naïve patients (10–12). However, the cumulative probabilities of genotypic resistance to ETV and virologic breakthrough increased to 51% and 43%, respectively, in LAM-refractory patients who were switched to ETV monotherapy (11). Therefore, for CHB patients with LAM resistance, current international guidelines recommend switching to tenofovir disoproxil fumarate (TDF), adding on TDF, or adding on adefovir (ADV), but not switching to ETV monotherapy (5, 6). However, earlier international guidelines, which were based on insufficient clinical experiences, recommended switching to 1 mg of ETV per day as one of the treatment options for CHB patients infected with HBV resistant to LAM (13, 14). Unfortunately, as a result of sequential monotherapy, ETV resistance in the form of multidrug resistance has developed in a substantial number of patients.

For patients with both LAM- and ETV-resistant CHB infec-

tion, switching to or adding on TDF or TDF-emtricitabine combination therapy are considered therapeutic options; combination therapy with ADV plus nucleoside analogues can be used in countries where TDF is not yet available (5, 6). It has been demonstrated that both ADV and TDF are active *in vitro* against ETV-resistant HBV, but clinical data on the efficacy of ADV or TDF in patients infected with ETV-resistant HBV strains are limited (15–19).

As rescue therapies for LAM- and ETV-resistant patients, nucleoside analogues, including LAM, telbivudine (LdT), or ETV can be used combined with nucleotide analogues (i.e., ADV or TDF). A previous *in vitro* study reported that ADV had additive activity in combination with pyrimidine analogues (e.g., LAM and LdT), while combinations of ADV with ETV or TDF were synergistic (20). Affecting purine metabolism in a way that enhances the antiviral efficacy may contribute to the synergistic activity of combinations of purine analogues (e.g., ADV, TDF, and ETV) (21).

In this study, we investigated the overall antiviral efficacy and safety of combination therapy with ADV plus one of the nucleo-

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side analogues (LAM, LdT, or ETV) in CHB patients who had developed resistance to both LAM and ETV after sequential monotherapy of LAM and ETV. Additionally, the efficacy of rescue therapy with ADV plus pyrimidine analogues (LAM and LdT) was compared with that of ADV plus purine analogue (ETV).

MATERIALS AND METHODS

Study population. We reviewed the electronic medical records of CHB patients who had developed ETV resistance in addition to prior LAM resistance after sequential monotherapy with LAM and ETV and were treated with LAM-ADV, LdT-ADV, or ETV-ADV combination therapy for at least 6 months. A total of 67 consecutive patients who started treatment with these regimens from February 2009 to August 2012 at a single tertiary care hospital (Seoul National University Hospital in Seoul, Republic of Korea) were included: every day 49 patients received 10 mg ADV with a pyrimidine analogue, either 100 mg LAM (34 patients) or 600 mg LdT (15 patients), and 18 patients received 10 mg ADV with 1 mg ETV as rescue therapy. Patients with the following conditions were excluded: prior exposure to ADV; prior or current ADV resistance; coinfection with hepatitis C, hepatitis D, or human immunodeficiency virus; prior organ transplantation; and a glomerular filtration rate of <50 ml/min, estimated by the Cockcroft-Gault equation.

The study 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.

Study measurements. All patients were evaluated every 2 to 3 months with clinical examination and virologic, biochemical, and serologic evaluations. Hepatitis B e antigen (HBeAg) and antibody (anti-HBe Ab) were determined using radioimmunoassay (RIA ELISA rapid kit; Shin Jin Medics, Seoul, Republic of Korea). Serum HBV DNA levels were quantified in all patients at baseline and at each follow-up visit using the COBAS AmpliPrep/COBAS TaqMan version 2.0 assay (Roche Molecular System, Branchburg, NJ), which has a dynamic range of quantification of 20×10^8 to 1.7×10^8 IU/ml (1.3 to $8 \log_{10}$ IU/ml) (22). Genotypic resistance, defined as the emergence of treatment-associated HBV variants conferring resistance to antiviral drug, was evaluated in all patients at baseline and in patients who developed virologic breakthrough during the treatment period. The amino acid substitutions conferring resistance to LAM (rtV173L, rtL180M, rtL180V, rtM204I, rtM204V, rtM204S and rtV207I), ADV (rtA181V and rtN236T) and ETV (rtL180M, rtL180V, rtM204I, rtM204V, rtM204S, rtI169T, rtV173L, rtT184G, rtT184S, rtT184A, rtT184L, rtT184L, rtS202G, rtS202I, and rtM250V) were analyzed. Genotypic analysis for drug resistance was performed using the direct sequencing method as previously described (23). Briefly, HBV DNA was extracted from serum samples, and the HBV polymerase gene was amplified by nested PCR. The cycle sequencing reaction was performed using 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).

Definitions and study endpoints. The primary endpoint was the proportion of patients with complete virologic suppression, defined as undetectable HBV DNA by a sensitive PCR assay. Secondary endpoints included biochemical response, HBeAg loss, change in serum HBV DNA levels relative to the baseline during the follow-up period, virologic breakthrough, and initial virologic response at 3 months (IVR-3). A biochemical response was defined as normalization of the serum alanine aminotransferase (ALT) level. A virologic breakthrough was defined as an increase in the HBV DNA level of $>1 \log_{10}$ IU/ml above the nadir (lowest value) HBV DNA level during the treatment period. IVR-3 was defined as follows: (i) in the case of baseline HBV DNA of $\geq 4 \log_{10}$ IU/ml, a $\geq 4 \log_{10}$ IU/ml decrease in serum HBV DNA level at month 3 after treatment; or (ii) in the case of baseline HBV DNA of $<4 \log_{10}$ IU/ml, an undetectable HBV DNA level at month 3 (24, 25).

Statistical analysis. For between group comparisons, the chi-squared test or Fisher's exact test was performed for categorical variables, and the

Mann-Whitney U test was performed for continuous variables. Kaplan-Meier methodology was used for time to event calculations, and the between group comparisons of hazard rates were performed using the log rank test. The Cox proportional hazards model was used for multivariate analysis to predict the outcome, complete virologic suppression. All tests were performed as two-sided tests, and a P value of <0.05 was considered significant. All statistical analyses were conducted using SAS software version 9.3 (SAS Inc., Cary, NC) and PASW statistical software version 18.0 (IBM, Chicago, IL).

In this retrospective cohort study, a propensity score analysis was used to select two matched groups of patients and thereby control for potential confounders of the treatment effect (26). Baseline clinical variables, including age, gender, baseline serum HBV DNA level, baseline serum ALT level, baseline serum creatinine level, duration of ETV therapy before rescue therapy, ETV resistance profiles, and time point of rescue therapy, were included in the propensity score generation because these variables were previously found to influence antiviral efficacy (6, 27–31). Logistic regression was applied to generate a continuous propensity score ranging from 0 to 1. Patients who received LAM-ADV or LdT-ADV combination therapy were matched with 2:1 ratio to patients who received ETV-ADV using the nearest neighbor method to select patients for this analysis.

RESULTS

Study population. The baseline characteristics of the 67 patients are summarized in Table 1. Forty-nine patients received a pyrimidine analogue (LAM or LdT) plus ADV therapy (the LAM/LdT-ADV group), 34 patients received LAM plus ADV (LAM-ADV) therapy, and 15 received LdT-ADV therapy). Eighteen patients received ETV, a purine analogue, plus ADV therapy (the ETV-ADV group). The median duration of rescue therapy was 19 (range, 6 to 55) months. All of the patients were confirmed to be infected with HBV with genotypic resistance to LAM and ETV, but not to ADV. The median age of patients in the ETV-ADV group was younger than that of the LAM/LdT-ADV group at baseline ($P = 0.023$). At baseline, the two groups did not differ significantly in gender, serum HBV DNA and ALT levels, HBeAg status, ETV resistance profiles, and time point of rescue therapy.

Virologic responses. Figure 1 shows the changes in the mean HBV DNA level at each time point. The overall mean reduction of serum HBV DNA levels at months 3 and 6 were $-2.03 \log_{10}$ IU/ml and $-2.27 \log_{10}$ IU/ml, respectively, and there was no significant difference between the LAM/LdT-ADV group and the ETV-ADV group (Fig. 1 and Table 2). Complete virologic suppression was achieved in 27 patients (18 patients were in the LAM/LdT-ADV group and 9 in the ETV-ADV group) during the treatment period. The overall mean (\pm standard deviation [SD]) time required for complete virologic suppression was $7.8 (\pm 8.1)$ months; there was no statistically significant difference between the LAM/LdT-ADV group and the ETV-ADV group (8.9 ± 9.2 versus 5.8 ± 4.9 months; $P = 0.328$). The overall cumulative incidence of complete virologic suppression at month 24 was 47.4%: 44.3% in the LAM/LdT-ADV group and 51.4% in the ETV-ADV group (Table 2). There was no significant difference between the two groups (LAM/LdT-ADV group versus ETV-ADV group; hazard ratio [HR], 0.611; 95% confidence interval [95% CI], 0.272 to 1.374; $P = 0.234$) (Fig. 2). The cumulative incidences of complete virologic suppression were comparable between the LAM/LdT-ADV group and the ETV-ADV group among both HBeAg-positive patients (LAM/LdT-ADV group versus ETV-ADV group; HR, 0.495; 95% CI, 0.138 to 1.768; $P = 0.279$) (see Fig. S1A in the supplemental material) and HBeAg-negative patients (LAM/LdT-ADV group versus ETV-ADV group; HR, 0.626; 95% CI, 0.217 to

TABLE 1 Baseline characteristics of all patients by treatment group^a

Characteristic	Value for the following group:		P value
	LAM/LdT-ADV (<i>n</i> = 49)	ETV-ADV (<i>n</i> = 18)	
Age (yr), median (range)	53.0 (28–79)	48.5 (26–62)	0.023
Gender			0.572
Male, <i>n</i> (%)	31 (63.3)	13 (72.2)	
Female, <i>n</i> (%)	18 (36.7)	5 (27.8)	
Serum HBV DNA level (log ₁₀ IU/ml), median (range)	5.02 (2.41–8.23)	5.23 (2.42–8.23)	0.708
Serum ALT level (IU/liter), median (range)	41 (12–809)	26.5 (12–649)	0.172
ULN, <i>n</i> (%)	25 (51.0)	8 (44.4)	0.784
Serum creatinine level (mg/dl), median (range)	0.90 (0.50–1.20)	0.97 (0.63–1.39)	0.357
HBeAg			1.000
Positive, <i>n</i> (%)	28 (57.1)	10 (55.6)	
Negative, <i>n</i> (%)	21 (42.9)	8 (44.4)	
Duration of ETV therapy before rescue therapy (mo), median (range)	28.5 (7.1–61.8)	25.8 (10.3–57.3)	0.490
ETV resistance profiles, <i>n</i> (%)			0.926
L180M + M204V/I + S202G + T184S/A/I/L	6 (12.2)	2 (11.1)	
L180M + M204V/I + S202G	21 (42.9)	10 (55.6)	
L180M + M204V/I + T184S/A/I/L	16 (32.7)	5 (27.8)	
L180M + M204V/I + V173L	5 (10.2)	1 (5.6)	
L180M + M204V/I + M250L	1 (2.0)	0 (0)	
Time point of rescue therapy			0.237
Virologic breakthrough, <i>n</i> (%)	33 (67.3)	15 (83.3)	
Biochemical breakthrough, <i>n</i> (%)	16 (32.7)	3 (16.7)	

^a Data are given as the median (range) or number of patients (*n*). The LAM/LdT-ADV group received lamivudine (LAM) or telbivudine (LdT) and adefovir (ADV), and the ETV-ADV group received entecavir (ETV) and ADV. Abbreviations: HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal; HBeAg, hepatitis B e antigen.

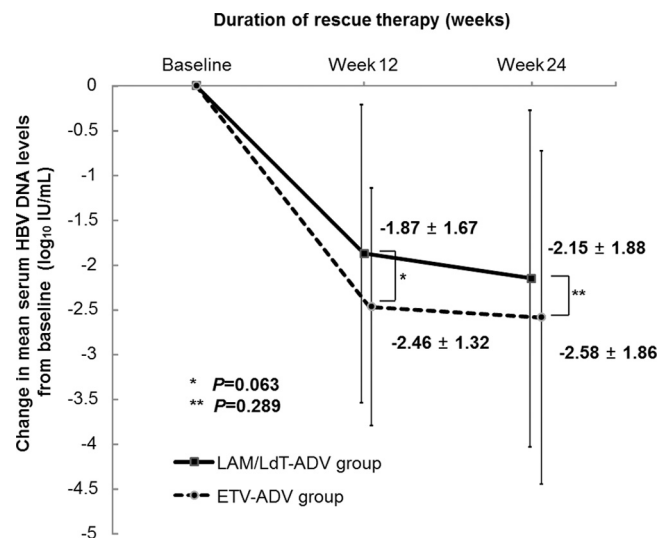


FIG 1 Changes in serum hepatitis B virus (HBV) DNA levels from baseline. The change in serum HBV DNA levels from baseline during rescue therapy, plotted as mean log₁₀ change from baseline values, is shown. The data represent the means for 49 patients in the group given lamivudine (LAM) or telbivudine (LdT) plus adefovir (ADV) therapy and 18 patients in the group given entecavir (ETV) and ADV at weeks 12 and 24. The reduction of serum HBV DNA levels at weeks 12 and 24 was not significantly different between the two groups (*P* = 0.063 and *P* = 0.289, respectively). The error bars (vertical lines) represent the standard deviations.

1.810; *P* = 0.388) (see Fig. S1B in the supplemental material). Among HBeAg-positive patients, the cumulative incidence of complete virologic suppression at month 24 was 32.5% in the LAM/LdT-ADV group and 41.7% in the ETV-ADV group. Among HBeAg-negative patients, the cumulative incidences of complete virologic suppression at month 24 were 60.7% in the LAM/LdT-ADV group and 62.5% in the ETV-ADV group.

Among the pretreatment factors, HBeAg negativity (HR, 2.730; 95% CI, 1.263 to 5.900; *P* = 0.011) and lower baseline HBV DNA level (HR, 0.644; 95% CI, 0.500 to 0.828; *P* = 0.001) were significantly associated with complete virologic suppression in the univariate analysis. In the multivariate analysis, only lower baseline HBV DNA level remained an independent predictor for complete virologic suppression (HR, 0.671; 95% CI, 0.514 to 0.875; *P* = 0.003) (Table 3).

Biochemical and serologic responses. Among the 33 patients with ALT levels above the upper limits of the normal range, 19 patients (57.6%) achieved biochemical response during the treatment period. Cumulative incidences of biochemical response at month 12 were 58.8% in the LAM/LdT-ADV group and 40% in the ETV-ADV group (Table 2). There was no statistically significant difference between the two groups (LAM/LdT-ADV group versus ETV-ADV group; HR, 1.221; 95% CI, 0.404 to 3.688; *P* = 0.723).

Loss of HBeAg occurred in 11 patients (28.9%) among the 38 patients who were positive for HBeAg at baseline. The cumulative

TABLE 2 Treatment responses during rescue therapy by treatment group^a

Response and time point	Cumulative incidence ^b unless specified otherwise for the following group:		P value
	LAM/LdT-ADV group (n = 49)	ETV-ADV group (n = 18)	
Virologic response			
Change in HBV DNA level (log ₁₀ IU/ml), median (range)			
3 mo	-1.58 (-6.21 to 0.98)	-2.35 (-5.91 to 0.46)	0.063
6 mo	-1.67 (-6.93 to 2.31)	-2.14 (-8.23 to 0.52)	0.289
Complete virologic suppression			
3 mo	6.1 (46)	16.7 (15)	0.229
6 mo	22.4 (36)	27.8 (13)	
12 mo	29.2 (24)	44.4 (8)	
24 mo	44.3 (10)	51.4 (3)	
36 mo	52.3 (5)	NA	
Virologic breakthrough			
6 mo	2 (46)	5.6 (17)	0.205
12 mo	4.2 (36)	24.2 (10)	
24 mo	12.4 (20)	24.2 (6)	
36 mo	18.6 (12)	24.2 (5)	
Biochemical response (normalization of serum ALT)			
3 mo	8 (23)	25 (6)	0.722
6 mo	40 (15)	25 (6)	
12 mo	58.8 (7)	40 (3)	
24 mo	64.6 (5)	NA	
36 mo	64.6 (2)	NA	
Serologic response (HBeAg loss)			
3 mo	7.1 (26)	10 (9)	0.381
6 mo	10.7 (23)	10 (9)	
12 mo	23.1 (14)	10 (8)	
24 mo	30.1 (8)	22.9 (4)	
36 mo	40.1 (4)	22.9 (1)	

^a Abbreviations: LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; NA, not applicable.

^b Data are given as cumulative incidence as a percentage with the number of patients at risk within parentheses.

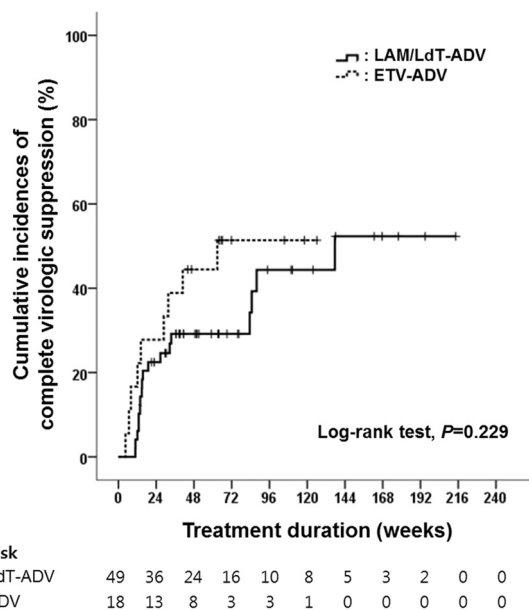


FIG 2 Cumulative incidence of complete virologic suppression during rescue therapy. The cumulative incidences of complete virologic suppression (undetectable levels of HBV DNA according to PCR assays) are shown. One group was given lamivudine (LAM) or telbivudine (LdT) and adefovir (ADV), while one group was given entecavir (ETV) and ADV.

incidences of HBeAg loss at month 24 were 30.1% in the LAM/LdT-ADV group and 22.9% in the ETV-ADV group (Table 2). There was no significant difference between the two groups (LAM/LdT-ADV group versus ETV-ADV group; HR, 1.972; 95% CI, 0.419 to 9.276; $P = 0.390$).

Virologic breakthrough. Figure 3 shows the cumulative incidence of virologic breakthrough analyzed by the Kaplan-Meier method. Virologic breakthrough occurred in 9 patients (5 patients were in the LAM/LdT-ADV group and 4 in the ETV-ADV group) during the treatment period. The mean (\pm SD) time required for virologic breakthrough was 13.3 (\pm 10.0) months, and there was no statistically significant difference between the LAM/LdT-ADV group and the ETV-ADV group (17.3 ± 12.2 versus 8.4 ± 3.5 months; $P = 0.327$). The overall cumulative incidence of virologic breakthrough at month 24 was 15.8%: 12.4% in the LAM/LdT-ADV group and 24.2% in the ETV-ADV group (Table 2). There was no significant difference in the risk of virologic breakthrough between these two groups (LAM/LdT-ADV group versus ETV-ADV group; HR, 0.437; 95% CI, 0.117 to 1.629; $P = 0.217$) (Fig. 3). Virologic breakthrough was accompanied by biochemical breakthrough in 4 patients. Among 9 patients who experienced virologic breakthrough, rtA181V substitution was newly detected in one patient who exhibited rtL180M, rtM204V, and rtT184A substitutions at baseline and received LAM-ADV combination therapy. ETV resis-

TABLE 3 Univariate and multivariate analyses of the clinical factors predictive of complete virologic suppression during rescue therapy^a

Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P value	Adjusted hazard ratio (95% CI)	P value
Age (yr)	1.010 (0.976–1.046)	0.565		
ALT level (IU/liter)	1.000 (0.998–1.003)	0.740		
Rescue therapy regimen (LAM/LdT-ADV vs. ETV-ADV)	0.611 (0.272–1.374)	0.234		
HBeAg (negative vs positive)	2.730 (1.263–5.900)	0.011	2.029 (0.921–4.471)	0.079
Baseline HBV DNA (log ₁₀ IU/ml)	0.644 (0.500–0.828)	0.001	0.671 (0.514–0.875)	0.003
Time point of rescue therapy (VB vs BB)	1.746 (0.702–4.343)	0.231		

^a Abbreviations: ALT, alanine aminotransferase; LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; VB, virologic breakthrough; BB, biochemical breakthrough; 95% CI, 95% confidence interval.

tance profiles were not associated with virologic breakthrough ($P = 0.883$; see Fig. S2 in the supplemental material).

IVR-3 was achieved in 18 patients (26.9%). The patients with an IVR-3 had a significantly higher probability of achieving complete virologic suppression (Fig. 4A, $P < 0.001$) and a significantly lower probability of experiencing virologic breakthrough (Fig. 4B, $P = 0.041$) than did those who did not achieve an IVR-3. None of the patients with an IVR-3 experienced virologic breakthrough during the follow-up period.

Propensity score analysis. A matched study population was constructed to compare the antiviral efficacy of rescue therapy regimens. Clinical variables selected for the propensity score model included age, gender, baseline serum HBV DNA level, baseline serum ALT level, baseline serum creatinine level, duration of ETV therapy before rescue therapy, ETV resistance profiles, and time point of rescue therapy. Twenty-eight patients from the LAM/LdT-ADV group and 14 patients from the ETV-ADV group were selected. The baseline characteristics of the 42 patients

after propensity score matching are summarized in Table S1 in the supplemental material. There were no significant differences in age, gender, serum HBV DNA and ALT levels, HBeAg status, ETV resistance profiles, and time point of rescue therapy. The cumulative incidences of complete virologic suppression and virologic breakthrough were still comparable between the two groups selected and matched using the propensity score model ($P = 0.419$ and $P = 0.337$, respectively; see Fig. S3A and 3B in the supplemental material).

Adverse events. The mean changes in serum creatinine levels at months 3 and 6 were not significantly different between the two groups ($P = 0.574$ and $P = 0.411$, respectively; Table 4). Only one patient experienced deterioration of renal function; the serum creatinine level increased by >0.25 mg/dl during ADV-based rescue therapy. Concurrent diuretic therapy was assumed to be the reason of this event. Myopathy was not observed in any patient during the rescue therapy.

DISCUSSION

In this study, the cumulative incidence of complete virologic suppression at month 24 was approximately 50%, indicating limited antiviral efficacy of combination therapy with ADV plus nucleoside analogues in CHB patients with genotypic resistance to both LAM and ETV. In these patients, combination therapy with ETV-ADV, as well as LAM-ADV and LdT-ADV, failed to achieve sufficient antiviral efficacy. Regarding safety issues, combination therapy with ADV plus nucleoside analogues was tolerable without significant deterioration of renal function or myopathy.

This is the first study that compared the efficacy and safety of ADV-based rescue therapy regimens in LAM- and ETV-resistant CHB patients. Moreover, this is the largest of studies that investigated the efficacy of rescue therapy in patients resistant to both LAM and ETV. In our study, patients with previous or current ADV resistance were excluded, in order to accurately evaluate antiviral efficacy of ADV-containing regimens in patients with dual resistance to LAM and ETV. This is important because *in vitro* studies showed that amino acid substitutions conferring resistance to ADV decrease susceptibility to ADV 3- to 15-fold, and previous clinical studies found that virologic suppression was less profound in patients with triple resistance to LAM, ETV, and ADV than in those with dual resistance to LAM and ETV (18, 32, 33). We acknowledge some limitations resulting from the retrospective nature of the study design and sample size. However, a prospective, randomized trial evaluating the ADV-based therapy in CHB patients resistant to both LAM and ETV cannot be conducted due to ethical considerations in the countries where TDF is

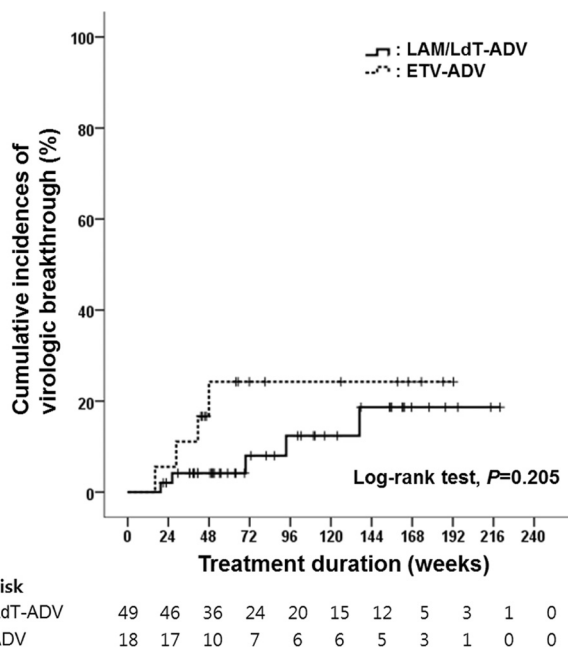


FIG 3 Cumulative incidence of virological breakthrough during rescue therapy. A virologic breakthrough was observed in 9 patients (13.4%): 5 patients of the group given lamivudine (LAM) or telbivudine (LdT) and adefovir (ADV) (10.2%) and 4 patients of the group given entecavir (ETV) and ADV (22.2%) during the treatment period.

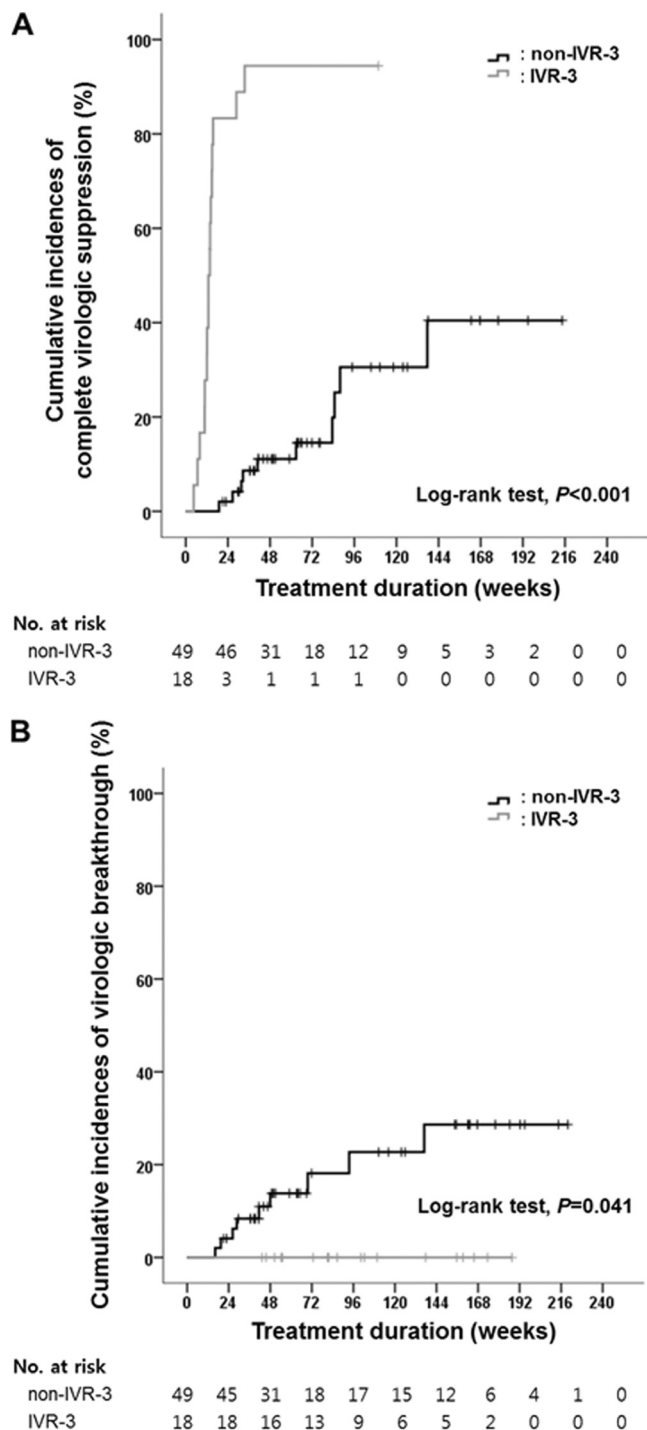


FIG 4 Impact of initial virologic response at 3 months (IVR-3) on long-term efficacy of rescue therapy. (A) The patients with IVR-3 had a significantly higher probability of achieving complete virologic suppression ($P < 0.001$ by log rank test). (B) The patients with IVR-3 had a significantly lower probability of experiencing a virologic breakthrough ($P = 0.041$ by log rank test).

available, because the current international guidelines recommend TDF-based rescue therapy for these patients (5, 6). For the aforementioned reasons, it is difficult to include more patients even in a retrospective study. We introduced the propensity score model to reduce the bias in patient selection.

This analysis demonstrated that antiviral efficacy of combination therapy with ADV plus nucleoside analogues was not effective enough in LAM- and ETV-resistant CHB patients; only half of the patients achieved complete virologic suppression after 2 years. Furthermore, less than 30% of patients who were positive for HBeAg at baseline showed HBeAg loss during this rescue therapy. Previously, two small retrospective studies demonstrated efficacy of ETV-ADV combination therapy in CHB patients infected with HBV resistant to both LAM and ETV (18, 19). However, these two studies (18, 19) included only 17 and 12 patients, respectively, and they also included patients with concurrent ADV resistance. In one of these studies, Yang et al. reported that the cumulative incidences of complete virologic suppression at months 3 and 6 were 57.1% and 100%, respectively, in patients infected with HBV resistant to both LAM and ETV without ADV resistance (18); these responses are much higher than our findings. Important differences from our study are the smaller sample size ($n = 7$) and a larger proportion (71%) of patients that were HBeAg negative, a favorable prognostic factor in our study. The other study, by Jeon et al., reported that serum HBV DNA levels were suppressed effectively during ETV-ADV combination therapy (19). However, cumulative incidence of complete virologic suppression was not shown; hence, cautious interpretation of the results is warranted.

Antiviral monotherapy can promote selection of multidrug-resistant strains of HBV, especially when patients are treated with sequential monotherapies with overlapping resistance profiles, such as monotherapy with LAM followed by ETV (15, 34) or LAM followed by ADV (35–37). As add-on strategies, ETV-TDF or ETV-ADV combination therapy are widely used in patients infected with HBV resistant to both LAM and ETV and recommended by international guidelines (5, 6). In our study, however, ETV-ADV therapy and LAM/LdT-ADV therapy showed limited antiviral efficacy. Furthermore, in patients with triple resistance to LAM, ETV, and ADV, the antiviral efficacy of ADV with combinations of nucleoside analogues could be worse than in patients with dual resistance to LAM and ETV. With the availability of TDF, which has high antiviral potency against multidrug-resistant HBV, TDF monotherapy can be considered a rescue option in LAM- and ETV-resistant CHB patients (5, 29). However, considering the advantage of combination therapy of nucleos(t)ides with complementary cross-resistance profiles, TDF-based combination therapy with a nucleoside analogue should theoretically be a better treatment regimen for multidrug-resistant CHB patients than TDF monotherapy or ADV-based combination therapy. This study demonstrated that a lower baseline HBV DNA level was an independent pretreatment predictor of a favorable virologic response and that IVR-3 was a significant early treatment endpoint that predicted complete virologic suppression. These find-

TABLE 4 Changes in serum creatinine levels according to treatment groups

Time point	Change in serum creatinine (mg/dl) ^a		P value
	LAM/LdT-ADV group ($n = 49$)	ETV-ADV group ($n = 18$)	
3 mo	0.00 (−0.30 to 0.16)	0.00 (−0.77 to 0.09)	0.574
6 mo	−0.02 (−0.30 to 0.19)	−0.01 (−0.77 to 0.21)	0.411

^a Data are given as the median (range). Abbreviations: LAM, lamivudine; LdT, telbivudine; ADV, adefovir; ETV, entecavir.

ings indicate that switching to TDF-based combination therapy should be considered for patients on ADV-based rescue therapy who do not demonstrate these favorable predictors, specifically patients who had high baseline HBV DNA levels and do not achieve an IVR-3 during ADV-based rescue therapy, even though they have not experienced virologic breakthrough yet.

A virologic breakthrough was observed in 9 (13.4%) out of 67 patients. Among those experiencing a virologic breakthrough, the ADV-resistant signature amino acid substitution was newly detected in one patient. Since direct PCR-based DNA sequencing can detect a particular variant only if it is present in $\geq 20\%$ of the total quasispecies pool, the possibility of the emergence in the other patients of additional genotypic resistance to ADV, which was not detected by the direct sequencing method, cannot be excluded (31, 38); the patients on continued ADV-based therapy without complete virologic suppression are at high risk for developing triple resistance to LAM, ETV, and ADV. In addition, ETV resistance profiles were not associated with virologic breakthrough. We could not verify epistatic connectivity of preexisting amino acid substitutions and development of virologic breakthrough.

Meanwhile, ADV-based combination therapy showed acceptable safety profiles. While there have been reports of renal impairment with the use of nucleotide analogues, particularly ADV (30, 39, 40), the changes in serum creatinine levels were minimal. Significant deterioration of renal function related to combination therapy with ADV plus nucleoside analogues was not observed in our study. No patient experienced muscle-related symptoms, including muscle pain and weakness.

In summary, our study found that combination therapy with ADV plus nucleoside analogues, although appearing safe, has limited efficacy as rescue therapy for difficult-to-treat patients infected with LAM- and ETV-resistant HBV strains. In addition, antiviral efficacy of ADV-based combination therapy was predicted to be lower in those patients with higher baseline HBV DNA levels and those who do not achieve an IVR-3 during ADV-based rescue therapy. Combination therapy with TDF, which has more potent antiviral activity and a higher genetic barrier to resistance, may be an optimal treatment option in patients with LAM- and ETV-resistant CHB. Further study to evaluate the antiviral efficacy and safety of TDF-based combination therapy is warranted.

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