Journal of Viral Hepatitis, 2009, **16,** 279–285 doi:10.1111/j.1365-2893.2009.01074.x

A short course of add-on adefovir dipivoxil treatment in lamivudine-resistant chronic hepatitis B patients

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Received July 2008; accepted for publication September 2008

SUMMARY. The aims of the study were to investigate the efficacy of rescue therapy with lamivudine (LAM) and adefovir (ADV) combination for 6 months followed by ADV monotherapy in lamivudine-resistant chronic hepatitis B (LAM-R CHB) patients, and to analyze the frequency of ADV resistance mutant development in such patients. A total of 170 consecutive LAM-R CHB patients (male/female: 130/40, mean age: 42.9 ± 13.4 years) with viral breakthrough under LAM therapy were analyzed. A total of 68 had HBeAg-positive. Patients received rescue therapy with LAM [100 mg (qd)]+ADV [10 mg (qd)] for 6 months after which LAM was discontinued. HBV-DNA was assessed with the HBV-DNA 3.0 bDNA assay. ADV-resistant mutations were identified by sequencing the reverse transcriptase region. The median duration of rescue therapy was 24 months. Cumulative probability of becoming HBV-DNA undetectable was 33.8%, 59.6% and 68.2% after 24, 48 and 96 weeks of treatment, respectively. These figures were 43.2%, 58.0% and 73.1% for ALT normalization. Among 68 HBeAg-positive CHB patients, 10 patients had an e-antigen seroconversion. Low baseline HBV-DNA level ($<10^7$ copies/mL) was a significant predictor of response to ADV treatment ($P < 0.01$). Cumulative probability of ADV resistance was 1.2%, 15.1% and 37.3% at 12, 24 and 36 months of therapy, respectively. By multivariate analysis, baseline high viral load and primary nonresponse to treatment at week 24 predicted ADV resistance. The data indicate that a time limited add-on strategy does not provide benefit over the switch strategy with respect emergence of ADV resistant mutants in LAM-R CHB patients.

Keywords: adefovir, hepatitis B virus, lamivudine, resistance.

INTRODUCTION

Lamivudine (LAM), adefovir dipivoxil (ADV), entecavir and telbivudine are the current four nucleos(t)ide analogues (NAs) approved for chronic hepatitis B (CHB) treatment, all of which need to be given for prolonged, if not indefinite time, especially in the setting of HBeAg-negative CHB [1,2]. Prolonged use of NAs risks the emergence of HBV strains resistant to the particular NA used. LAM is the first approved NA for the treatment of CHB with potent antiviral efficacy and a very good safety profile [1,2]. The major drawback of LAM treatment is the high rate of LAM resistance, reaching up to 70% after 4 years of treatment [3,4]. LAM resistance is associated with a decrease in its efficacy, may lead to progression of liver disease [5] and requires the introduction of rescue therapy with other NAs. The two available options for treatment of LAM resistant CHB are the use of ADV and entecavir [6,7] although the latter option has been largely abandoned due to an accelerated risk of emergence of entecavir resistance in LAM-R CHB patients [8]. With ADV rescue therapy, two strategies, adding ADV to ongoing LAM treatment and switching to ADV, have been assessed in several recent studies [9–13]. It appears that the 'add-on' strategy is superior to the 'switch to' strategy [10–13]. The latter approach leads to ADV resistance in a substantial number of patients [10–14].

A major concern with prolonged therapy with more than one NA is the development of multiresistant HBV strains. Viral strains resistant to both LAM and ADV have been

Abbreviations: HBV, hepatitis B virus; CHB, chronic hepatitis B; LAM, lamivudine; LAM-R, lamivudine resistant; ADV, adefovir.

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reported to have 59-fold decreased susceptibility to the combination of both drugs [15]. Theoretically, with LAM + ADV combination treatment, ADV would suppress LAM-R mutants and LAM would prevent the extension of potential ADV resistant strains in the viral quasispecies pool. However, ADV is used at a suboptimal dose in the clinical setting because of concern of nephrotoxicity [16]. This is further supported by the finding of persistence of LAM-R strains up to 36 months during rescue treatment with the 'add-on' strategy in LAM-R CHB patients [12]. Likewise, LAM may not be the optimal drug in combination treatment; entecavir and telbivudine may both be more potent antivirals [17–19]. Hence, the 'suboptimal' or less than ideal characteristics of both LAM and ADV may lay a fertile ground for the emergence of multidrug-resistant strains during prolonged LAM + ADV therapy in LAM-R CHB patients which by itself is prone for the emergence of resistant mutants when compared with treatment-naïve patients [15,20]. Thus, a strategy has been recently suggested that assessed a shorter duration of the 'add-on' strategy, where LAM is discontinued after a period of LAM + ADV combination, with the aim of terminating LAM-R strains earlier [21].

In this study, a retrospective analysis of such a strategy is provided in a large cohort of CHB patients with viral breakthrough under LAM therapy. In this cohort, patients had been treated with LAM and ADV combination for 6 months followed by ADV monotherapy. Efficacy of the rescue therapy, frequency of ADV resistance mutant development and potential predictive factors of ADV resistance were analyzed.

MATERIALS AND METHODS

Patients

A total of 170 consecutive LAM-R CHB patients (male/female: 130/40, mean age 42.9 ± 13.4 years), who were seen at two different centers, the Gastroenterology outpatient Clinics of the Istanbul Medical Faculty Hospital of Istanbul University, and the Liver Diseases Outpatient Clinics of the Ankara University Faculty of Medicine, were enrolled in this study between January 2003 and November 2006. All patients had been originally diagnosed with compensated CHB disease, were on treatment with LAM and were discovered to have developed viral breakthrough under LAM treatment. Duration of LAM treatment ranged from 6 to 108 months (median 36 months). All 170 patients subject to this analysis had completed at least 6 months of rescue treatment with LAM and ADV. Data reported here were collected retrospectively from outpatient visit charts from the two university hospital clinics. The median duration of rescue treatment in this cohort was 24 months (range 6–48 months). Liver biopsy was available before starting rescue therapy in 111 patients and had been assessed by the local pathologists according to Knodell *et al.* [22].

Study design

All patients with viral breakthrough under LAM treatment were put on rescue therapy with LAM [100 mg (qd)] + ADV [10 mg (qd)] for 6 months after which LAM was discontinued and ADV was continued as monotherapy. Confirmation of genotypic LAM resistance was seen in all 147 patients (86%), in whom this was explored. Patients were seen at 3 to 6-month intervals and physical examination, biochemical tests and HBV-DNA levels were explored at each visit. HBeAg and anti-HBe was generally tested every 6 months, sometimes at 3-month intervals. Mutation analysis was performed when viral breakthrough was detected. The primary outcome measures were the change in serum HBV-DNA to undetectable and alanine aminotransferase (ALT) normalization in all CHB patients and HBeAg seroconversion to anti-HBe in HBeAg-positive CHB patients. The secondary outcome measure was virological breakthrough and genotypic resistance to ADV. Further investigations include surveillance for hepatocellular carcinoma (HCC) with ultrasound and alpha fetoprotein determinations every 6–12 months as well as clinical assessment for liver decompensation. Possible nephrotoxicity was assessed with blood urea and creatinine determinations.

HBV-DNA quantification

Hepatitis B virus-DNA levels were tested with the Versant HBV DNA 3.0 bDNA assay (Bayer HealthCare LLC, Tarrytown, NY, USA) which has a detection limit of 2000 copies/mL.

Testing for antiviral resistance

Hepatitis B virus-DNA was extracted from 200 μL serum using High Pure Viral Nucleic Acid kit (Roche Diagnostics Corporation, Indianapolis, IN, USA) according to the manufacturer's instructions. HBV pol gene was amplified by nested PCR, the first part of which consists of a 35 cycle of amplification done with denaturation at 94 °C for 5 min, annealing at 45 °C for 50 s and elongation at 72 °C for 80 s with the primers HBVpol1 (5′-CAC CTG CAG CCT CAT TTT GTG GGT CAC CAT A-3′) and HBVpol2 (5′-CAT AAG CTT CAC AAT TCG TTG ACA TAC TTT CCA AT-3′). HBVpol2 and YMDD372 (5′-TCG CTG GAT GTG TCT GCG GCG TTT TAT-3′) primers [23] were used in the second part of nested PCR for 25 cycles with the same conditions. HBV genomes were sequenced by using the 'Big Dye Terminator v3.1 Cycle Sequencing Kit' (Applied Biosystems, Fostercity, CA, USA) according to the manufacturer's instructions in an 'ABI PRISM 310 Genetic Analyzer' (Perkin Elmer, Foster City, CA, USA).

Definitions

Virological response was defined as undetectable serum HBV-DNA with the Versant HBV-DNA assay and biochemical

response as the decline of serum ALT levels to the normal range.

Primary nonresponse was defined as <1 $\log_{10}$ decline of initial serum HBV-DNA after 24 weeks of rescue therapy [24]. A virological breakthrough was defined as >1 $\log_{10}$ increase in serum HBV-DNA level above nadir.

Statistics

Data were expressed as the median and range or mean and SD, were appropriate, for discrete variables and as counts and percentages for qualitative variables. Continuous variables were compared with the student's *t*-test or the Mann–Whitney *U*-test, where appropriate, and categorical variables were assessed using the χ^2 test or Fisher's exact test. The cumulative probability of achieving HBV-DNA undetectability and ALT normalization were assessed with the Kaplan–Meier method. Cox's regression model was used for univariate and multivariate analysis for predictors of emergence of ADV resistance. All *P* values were two-tailed. A value of <0.05 was considered as statistically significant.

RESULTS

Patient characteristics

Baseline demographical, clinical and laboratory characteristics are given in Table 1. In this study, 'baseline' refers to the time point at which therapy for LAM resistance with LAM and ADV combination was started. Of the 170 CHB patients, 68 had HBeAg-positive and 102 had HBeAg-negative CHB. No significant difference existed between the two groups with regard to gender, duration of LAM treatment, the proportion of patients who had used IFN in the past and the proportion of patients with advanced liver disease. However, HBeAg-positive patients were younger ($P < 0.0001$), had higher initial serum HBV-DNA levels ($P < 0.0001$) but lower baseline serum ALT levels ($P < 0.05$) compared with HBeAg-negative CHB patients (Table 1). Seventy-four patients had used standard IFN therapy (6–9 MU, three times a week, for 6–12 months) in the past while 96 patients were treatment-naïve at the time of LAM treatment commencement. In the majority of patients, rescue therapy with LAM and ADV was initiated at the time of biochemical breakthrough (148/170, 87.1%); it was initiated at the time of virological breakthrough in only 22 patients.

Treatment response

The cumulative probability of becoming HBV-DNA undetectable was 33.8% (52/154), 59.6% (84/141) after 24 and 48 weeks of treatment, respectively, and reached 68.2% (51/81) at 96 weeks (Fig. 1). At week 24, 23 patients (23/154, 14.9%) had <1 $\log_{10}$ decline in HBV-DNA, i.e. primary nonresponse. For ALT levels, the cumulative probability of ALT normalization was 43.2% and 58.0% at week 24 and 48, respectively, and reached 73.1% at week 96 (Fig. 2). Mean serum ALT levels declined from 100.3 ± 99.6 IU/mL at baseline to 48.8 ± 31.0 IU/mL, 48.9 ± 47.7 IU/mL and 44.2 ± 33.6 IU/mL at weeks 24, 48 and 96, respectively ($P < 0.0001$). Low baseline HBV-DNA level (<10^7 copies/mL) was a significant predictor of response to ADV treatment at week 48. HBV-DNA negativity at week 48 was more commonly observed in patients with low baseline HBV-DNA levels than in patients with high baseline HBV-DNA levels (more than 10^7 copies/mL) (73.3%, 44/60 *vs* 49.4%, 40/81; $P < 0.01$).

Among 68 HBeAg-positive CHB patients, HBeAg seroconversion occurred in one patient at week 12, in five additional patients at week 24 and in one additional patient at week 48 of rescue therapy. All 68 patients finished 12 months of rescue therapy. Forty-one and 32 patients finished 18 and 24 months of rescue therapy, respectively.

Table 1 Baseline demographical, clinical and laboratory characteristics of all CHB patients

	All CHB patients (n = 170)	E-antigen positive (n = 68)	E-antigen negative (n = 102)	*P*
Age	42.9 ± 13.4	36.4 ± 13.7*	47.2 ± 11.4	<0.0001
Gender (M/F)	130/40	48/20	82/20	>0.05
Previous IFN used	74 (43.5 %)	32 (47.1%)	42 (41.2%)	>0.05
Duration of LAM treatment (months)	36 (6–108)	36 (6–108)	41 (12–84)	>0.05
Baseline serum ALT levels (n: <40 IU/mL)	100.3 ± 99.6	75.0 ± 51.0	117.7 ± 119.9*	<0.05
Baseline serum HBV-DNA levels (log10)	7.0 ± 1.6	7.7 ± 1.3*	6.6 ± 1.6	<0.0001
Cirrhosis	28 (16.5%)	9 (13.3%)	19 (18.6%)	>0.05

HBV, hepatiits B virus; LAM, lamivudine; IFN, interferon; ALT, alanine aminotransferase; CHB, chronic hepatitis B. *HBeAg-positive patients *vs* HBeAg-negative patients $P < 0.0001$.

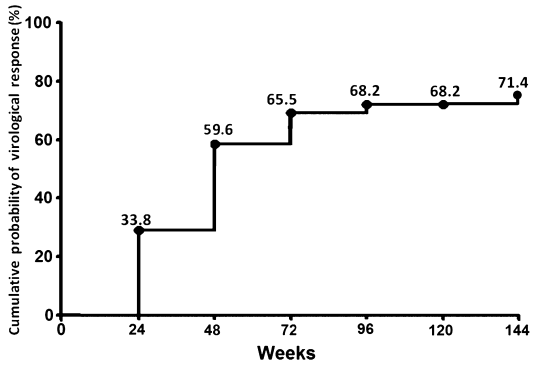

Fig. 1 Cumulative probability of becoming hepatitis B virus-DNA undetectable of a cohort of lamivudine (LAM)-resistant chronic hepatitis B patients treated with 6 months of LAM + adefovir (ADV) combination followed by monotherapy with ADV.

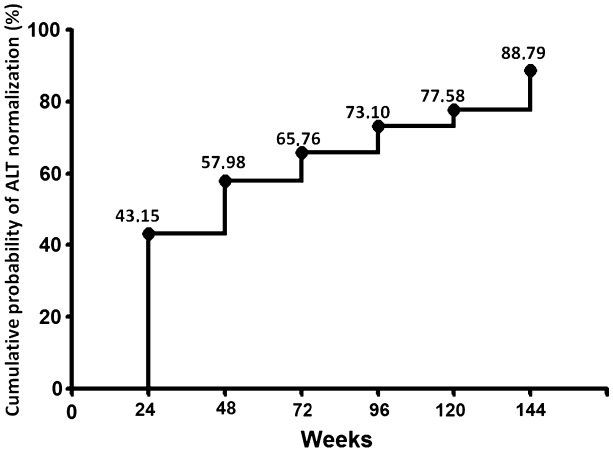

Fig. 2 Cumulative probability of alanine aminotransferase normalization of a cohort of lamivudine (LAM)-resistant chronic hepatitis B patients treated with 6 months of LAM + adefovir (ADV) combination followed by monotherapy with ADV.

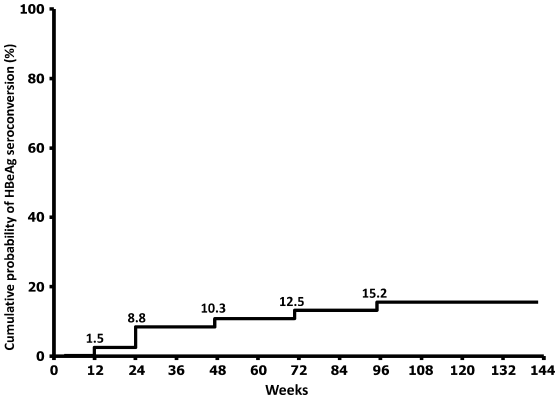

Fig. 3 Cumulative probability of HBeAg seroconversion in the cohort of 68 HBeAg-positive lamivudine (LAM)-resistant chronic hepatitis B patients treated with 6 months of LAM + adefovir (ADV) combination followed by monotherapy with ADV.

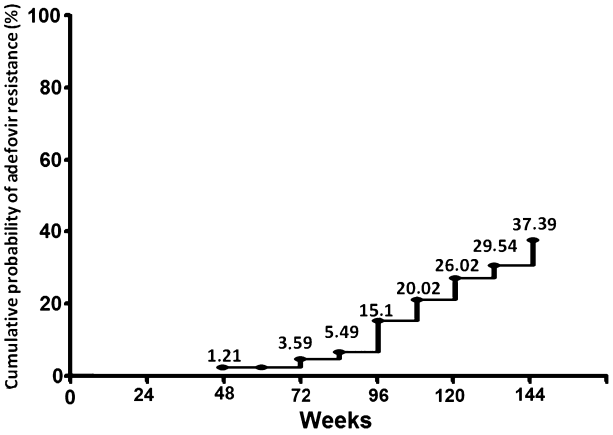

Fig. 4 Cumulative probability of the development of adefovir resistance.

One additional patient seroconverted at week 72 and 2 at week 96. Overall, 10 patients had seroconverted at 96 weeks of rescue therapy (Fig. 3). No seroconversion occurred after week 96.

Resistance to ADV

Adefovir resistance developed in none of the patients at week 24. There were actually 16 patients without HBV-DNA determination at week 24; however, all of these 16 patients continued to have HBV-DNA decrease in the next two determinations until week 48, generally carried out at week 36 and 48. The proportion of patients who developed ADV resistance increased steadily over time and was as follows: 2/159, 5/125, 10/98, 7/33 and 2/16 at months 12, 18, 24, 30 and 36 respectively. The cumulative probability of ADV resistance was 1.2%, 3.6%, 15.1%, 26.0 and 37.3% at months 12, 18, 24, 30 and 36 of rescue therapy, respectively (Fig. 4). In 26 patients with ADV-resistance the following mutations were detected: A181T/V: four patients; N236T: eight patients and A181T/V+N236T: 14 patients.

Predictors of ADV resistance

By univariate as well as multivariate analysis, the only baseline parameter predicting the emergence of ADV resistance was HBV-DNA levels (Table 2). In addition, as on treatment parameter patients with primary nonresponse also predicted ADV resistance. Patients with high baseline HBV-DNA were twice and patients with primary nonresponse were approximately five times more likely to develop resistance to ADV.

Table 2 Predictors of emergence of adefovir resistance

Baseline and on-treatment parameters	Univariate Cox PH model			Multivariate Cox PH model		
	OR	95%Cl	*P*	OR	95%Cl	*P*
Gender	0.66	0.25–1.74	0.41			
Age	1.00	0.97–1.03	0.88			
Duration of LAM treatment	0.99	0.97–1.02	0.90			
Previous IFN used	0.41	0.15–1.09	0.08	0.46	0.15–1.36	0.16
E-antigen status	1.18	0.48–2.86	0.71			
Baseline serum ALT levels	1.00	0.99–1.00	0.16	1.00	0.99–1.00	0.29
Baseline serum HBV-DNA levels	1.52	1.07–2.18	0.02	1.99	1.26–3.14	0.003
Primary nonresponse	4.96	1.51–14.55	0.007	5.25	1.55–17.78	0.008
Fibrosis grade 3–4	1.00	0.29–3.44	1.0			

HBV, hepatiits B virus; LAM, lamivudine; IFN, interferon; ALT, alanine aminotransferase.

Patients who developed ADV resistance appeared to have slightly higher levels of HBV-DNA 24 weeks after commencing rescue therapy than those who did not [5.24 $\log_{10} \pm 1.53$ *vs* 4.44 ± 1.76 ($P = 0.07$)]. Finally, fewer patients who commenced rescue therapy at the time of virological breakthrough developed ADV resistance than those who started rescue treatment at the time of biochemical breakthrough, but again this did not reach statistical significance (1/22 [4.5%] *vs* 28/148 [18.9%] $P = 0.25$).

Follow-up

Among 29 cirrhotics, one patient had HCC at start of rescue therapy and died because of HCC progression; one other patient developed de novo HCC after 12 months of ADV treatment. Hepatic decompensation did not occur in any of the patients including those who developed resistance to ADV. ADV treatment was in general well tolerated. Median initial serum creatinine level was 0.91 ± 0.18 mg/dL. Serum creatinine levels were 0.93 ± 0.19 mg/dL at week 48 and 0.93 ± 0.19 mg/dL at week 96 of treatment. However, serum creatinine level increased in two patients during ADV therapy by more than 0.5 mg/dL. ADV dose was reduced from 10 mg/day to 10 mg every other day. Renal function improved in one of them after dose adjustment.

DISCUSSION

The importance of this study is two-fold: (i) it depicts data on a large cohort of LAM-R CHB patients, most likely genotype D, treated with ADV and represents the largest study in its kind; (ii) it indicates that 6 months of combination treatment with LAM and ADV on long-term follow-up does not provide reasonable benefit with respect emergence of ADV resistant mutants over the 'switch to' ADV strategy. The resistance rate observed is comparable to studies where the 'switch' strategy had been explored; still, the latter depiction could have been strengthened if a comparator arm were available. The shortcomings of the study relate to the fact that it is retrospective. Thus, HBV-DNA was not reassessed with state of the art techniques, since availability of rather few serum samples did not justify re-testing with more sensitive assays. In almost all patients, primary failure to treatment was assessed in the analysis 6 months after rescue treatment and not at 3 months, according to the newer guidelines suggested recently [25]. Despite these shortcomings, this study clearly shows that LAM and ADV combination treatment in LAM-R CHB patients needs to be given for a prolonged if not indefinite period.

An argument may be made about the timing of LAM discontinuation in the context that the LAM arm in the combination treatment would have been better discontinued after achieving HBV-DNA undetectability. However, 6 months of combination treatment is reasonable since HBV-DNA levels at 6 months appear to predict response as well as the development of resistance, nicely shown in treatment-naïve CHB patients treated with LAM or telbivudine [19,26]. This suggests that, even if the combination treatment had been prolonged to the point when HBV-DNA levels became undetectable, at which time LAM would then be discontinued, it most likely would have just postponed the emergence of ADV resistant mutants.

Hepatitis B virus genotype was not assessed in this study. However, previous studies in Turkish HBV patients have shown that the vast majority (almost 100%) are infected with genotype D [27]. The rate of ADV resistance was approximately 1% at 1 year of follow-up and was thus lower than many of previously reported data on ADV monotherapy in LAM-R CHB patients [13,14,28,29]. This benefit was lost when patients were followed beyond 1 year and the cumulative risk of ADV resistance reached 15% at 2 years and 37% at 3 years of follow-up. It is likely that 6 months of combination treatment with LAM + ADV contributed to the low rate ADV resistance at 1 year, but this benefit did not endure beyond the first year of rescue treatment.

Patients with low baseline HBV-DNA levels were more likely to have a virological response at week 48 compared to patients with high baseline HBV-DNA levels in line with Rapti *et al.*'s observation [12]. Patients who started rescue treatment at the time of biochemical breakthrough tended to develop ADV resistance more often than those who started treatment at the time of virological breakthrough. Statistical significance was not reached however which is most likely due to the low number of patients who were put on rescue treatment at the time of virological breakthrough. This observation is in line with the results of Lampertico *et al.* [30] who showed have shown a lower virological response rate in patients with LAM resistance starting rescue treatment at the time of biochemical breakthrough.

Patients with high baseline viral load were more likely to develop ADV resistance. This was in line with some previous studies [12,29] but at variance with others [10,13,28]. The discordant results can be partly explained by the smaller patient numbers [10,13,28] and shorter duration of follow-up [14] in those other studies. Furthermore, patients with primary non response appeared especially sensitive to ADV resistance development. These findings are consistent throughout other studies in which this was explored [14,28,29]. Primary nonresponse may be due to polymorhisms of the enzyme converting ADV to its active moiety or due to patient noncompliance [24].

The outcome of CHB patients who develop ADV resistance is not well known. However, hepatic decompensation after ADV resistance development has been reported with advanced disease [31]. In the present study, ADV resistance was not associated with hepatic decompensation. One cirrhotic patient developed HCC despite being responsive to therapy which underlines the importance of monitoring for HCC in patients with advanced disease.

One concern with a suboptimal combination treatment is the development of resistance mutations to both antiviral agents used and that these multiresistant mutants would coexist on the same viral genome leading to decreased sensitivity to combination treatment [15]. However, the co-existence of multiresistant mutants on the same viral genome has been reported to occur after sequential therapy [32]. The data by Kim *et al.* [32] and the results of the current study emphasizes the importance of long-term combination treatment as the main treatment strategy to combat patients developing LAM resistance. In further support of this, a recent study in LAM-R CHB patients treated with LAM + ADV, no ADV-resistant mutants have been reported during a median 42 months follow-up [11]. Although ADV-resistant mutants have also been reported in patients treated as rescue therapy with LAM + ADV [13] this appears to be the exception and is likely to be due to the suboptimal characteristics of both agents.

Overall, this study underlines once again the importance of long-term combination treatment with LAM and ADV as the main treatment strategy to combat CHB patients developing LAM resistance. It further suggests that 6 months of combination rescue therapy with LAM + ADV to be followed by monotherapy with ADV does not provide benefit with respect to emergence of ADV-resistant mutants in LAM-R CHB patients. High pretreatment HBV-DNA level and primary nonresponse at week 24 predicted resistance to ADV in such patients.

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