

High efficacy of adefovir and entecavir combination therapy in patients with nucleoside-refractory hepatitis B

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Background/Aims: Newly developed and potent antiviral agents suffer from the problem of drug resistance. Multidrug resistance is a major impediment in the treatment of patients with chronic hepatitis B (CHB). In line with American Association for the Study of Liver Diseases guidelines, adefovir dipivoxil (ADV) add-on therapy is recommended in the case of lamivudine resistance, while tenofovir disoproxil fumarate (TDF) is recommended for ADV or entecavir (ETV) resistance. TDF is currently not available in Korea. ADV+ETV combination therapy may be a viable alternative to TDF in patients with either ADV or ETV resistance. However, the efficacy of ADV+ETV combination therapy in patients with CHB and multidrug resistance is unclear. This study investigated the efficacy of ADV+ETV combination therapy in patients with multidrug resistance. **Methods:** Twenty-five patients were enrolled and were administered ADV+ETV combination therapy for at least 6 months. Blood was drawn at baseline and at 3, 6, 9, and 12 months after commencing treatment, and the following blood parameters were analyzed: alanine transaminase, hepatitis B e-antigen (HBeAg), anti-hepatitis B e-antigen, and hepatitis B virus (HBV) DNA levels. The initial virological response (IVR) was defined as an HBV DNA level of $<4 \log_{10}$ copies/mL after 6 months of combination therapy. **Results:** The IVR rate was 76%. The proportion of patients with a high viral load ($\geq 5.0 \log$) dropped from 76% at baseline to only 5% after 6 months of treatment. The biochemical response rate during the first 6 months was 71%. HBeAg was lost in 2 patients (10%). **Conclusions:** ADV+ETV combination therapy induced a good IVR in CHB patients who were refractory to more than 2 antiviral agents. This regimen may be a good alternative to TDF in Korea, where that drug is not available. (*Korean J Hepatol* 2012;18:75-83)

Keywords: Adefovir; Entecavir; Combination drug therapy; Drug resistance; Treatment efficacy

INTRODUCTION

About 350 million people world-wide are chronically infected with hepatitis B (HBV),^{1,2} which can result in severe liver disease that eventually progresses to cirrhosis and hepatocellular carcinoma.^{3,4} To date, interferon alpha and five nucleos(t)ide analogs (NAs), namely, lamivudine (LAM), adefovir (ADV), entecavir (ETV), clevudine and telbivudine have been approved for the treatment of chronic hepatitis B (CHB) in Korea. In South-East Asia, oral

nucleosides are prescribed more frequently than interferon because genotype C is common.⁵ Drug resistance often results from prolonged oral nucleoside treatment. LAM was first released for general use in Korea in 1999. Thereafter, the remaining four antiviral agents were introduced in a sequential manner. ETV resistance occurs frequently in patients with LAM resistance.⁶ Moreover, LAM-resistant patients with CHB sometimes also exhibit ADV resistance, although ADV is thought to be a good rescue therapy for LAM resistance.⁷ In addition, clevudine (CLV) and

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Abbreviations: ADV, adefovir dipivoxil; ALT, alanine aminotransferase; anti-HBe, anti-hepatitis B e-antigen; AST, aspartate aminotransferase; CHB, chronic hepatitis B; CVR, continued virological response; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IVR, initial virological response; LAM, lamivudine; Log, \log_{10} HBV DNA copies/mL; NAs, nucleos(t)ide analogues; ND, not detectable; TDF, tenofovir disoproxil fumarate; VBT, virologic breakthrough

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telbivudine (LdT) are very similar to LAM in terms of their chemical structure and resistance profile. Thus, an alternative treatment for patients with ADV or ETV resistance is needed. One possible alternative is ADV-ETV combination therapy. This approach has the advantage that the drugs may synergize in terms of efficacy while preventing the emergence of mutants. Its disadvantage is its high cost.

The present study investigated the efficacy of ADV-ETV combination therapy in patients with CHB who did not respond to ADV add-on or high dose (1 mg) ETV therapy. In addition, factors that promoted an initial virological response (IVR) to the combination therapy were identified.

MATERIALS AND METHODS

Subjects

In total, 25 patients with nucleoside-refractory CHB were enrolled in the study. All patients exhibited virological breakthrough (VBT) before starting combination therapy, as defined by an increase in HBV DNA levels of at least 1 \log_{10} copies/mL (log) from the nadir during rescue therapy such as ADV add-on and high-dose ETV therapy. Patients were considered to be refractory if they had an inadequate virological response with or without documented genotypic mutations while they were receiving antiviral therapy. An inadequate virological response was defined as an HBV DNA level >4 log at 6 months of rescue therapy. The patients were followed up regularly at 3-month intervals.

The inclusion criteria were as follows: patients were positive for hepatitis B surface antigen (HBsAg) at least 1 year before commencing combination therapy, had HBV DNA level >4 log, and received both ADV and ETV once daily for at least 6 months. Several biochemical parameters and HBV DNA levels were tested at baseline and every 3 months during combination therapy.

None of the patients were co-infected with hepatitis C virus or human immunodeficiency virus or concomitant liver diseases such as alcoholic liver disease or autoimmune liver disease. The IVR was defined as HBV DNA levels below 4 log at 6 months of combination therapy. The continued virological response (CVR) was defined as HBV DNA levels below 4 log after the 6 month time point.

Analysis of virological markers

Routine biochemical tests were performed during therapy by using standard procedures. HBsAg, hepatitis B e-antigen (HBeAg), and anti-hepatitis B e-antigen (anti-HBe) were tested by using a commercial radioimmunoassay kit (Abbott Laboratories, Chicago, IL, USA). HBV DNA levels were determined quantitatively by real time PCR (Roche Molecular Systems, Branchburg, NJ, USA) that had a detection limit of 70 copies/mL. The HBV genotype was not examined because all patients were known to have genotype C.^{8,9}

Genotypic analysis

All baseline patient samples were analyzed for genotypic resistance before starting the combination therapy and at the end of treatment. On-treatment was defined as the time point just before starting the ADV-ETV combination therapy. Off-treatment was defined as the end of combination therapy. Moreover, when VBT was observed, patient samples were analyzed again to identify the mutations that were associated with the genotypic resistance. Genotypic resistance was investigated by restriction fragment mass polymorphism analysis, as described previously by Lee et al (Green-Cross Medical Laboratories, Giheung, Korea).¹⁰

Statistical analysis

Statistical testing was performed by using SPSS version 12 (SPSS Inc., Chicago, IL, USA). The data are reported as median (range). The HBV DNA data were logarithmically transformed prior to analysis. Continuous variables were compared by using the Mann-Whitney *U* test and Student's *t* test. Categorical data were compared by using the Pearson χ^2 test or Fisher's exact test. The cumulative probability of achieving CVR was estimated by using the Kaplan-Meier method. Factors associated with an IVR were analyzed by univariate and multivariate analysis. A *P*-value less than 0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics

The study population comprised 25 nucleoside-refractory patients; with CHB who had previously shown established drug resistance or persistent VBT in at least two measurements. Twenty patients were men and the median age was

39 (25-64) (Table 1). Thirteen patients (52%) had cirrhosis and 20 patients (80%) were positive for HBeAg. The median length of treatment with ADV-ETV combination therapy was 12 (6-24) months.

All patients had previously failed to respond to LAM monotherapy. All patients had taken at least two NAs. Fourteen patients (56%) had failed to respond to two NAs

(LAM and either ADV or ETV) and 11 (54%) had failed to respond to three NAs (LAM, ADV and ETV).

Profiles of genotypic resistance at baseline and after the study time point

On-treatment

At the start of ADV-ETV combination therapy, 12

Table 1. Baseline characteristics of nucleoside-refractory patients with chronic hepatitis B virus (HBV) infection (n=25)

Characteristic	
Median age, years (min-max)	39 (25-64)
No. of males (%)	20 (80)
No. of HBeAg-positive patients (%)	20 (80)
No. with cirrhosis (%)	13 (52)
Median AST, IU/L (min-max)	36 (18-403)
Median ALT, IU/L (min-max)	41 (15-451)
Median albumin, g/dL (min-max)	4.6 (4.0-5.2)
Median total bilirubin, mg/dL (min-max)	0.7 (0.4-10.3)
Median HBV DNA, log ₁₀ IU/mL (min-max)	5.8 (4.1-9.0)
Median duration of ETV plus ADV therapy, months n (min-max)	12 (6-24)
Previous failures to	
2 NAs (n, %)	14 (56)
LAM followed by ADV monotherapy	1 (4)
LAM followed by ETV monotherapy	6 (24)
LAM followed by LAM+ADV combination therapy	7 (28)
3 NAs (n, %)	11 (44)
LAM, ADV and ETV monotherapies sequentially (n, %)	4 (16)
LAM, ETV and LAM+ADV combination sequentially	4 (16)
LAM, LAM+ADV combination and ETV monotherapy sequentially	3 (12)
Resistance mutations to	
LAM (n, %)	12 (48)
M204V/I+L180M	8 (32)
M204I	4 (16)
LAM and ADV (n, %)	4 (16)
M204V/I+L180M+A181V/T	2 (8)
M204I+A181V/T+N236T	1 (4)
A181V/T+N236T*	1 (4)
LAM and ETV	6 (24)
M204V/I+L180M+T184	2 (8)
M204V/I+L180M+S202	4 (16)

*The patient who had been referred due to a high viral load during lamivudine (LAM) plus adefovir dipivoxil (ADV) combination treatment and had previously had a LAM-resistant mutation.

HBeAg, hepatitis B e-antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; ETV, entecavir; ADV, adefovir dipivoxil; LAM, lamivudine; NAs, nucleos(t)ide analogues.

patients (48%) had documented resistance mutations to LAM only, four (16%) to LAM and ADV (rtA181V/T and/or rtN236T), and six (24%) to LAM and ETV (rtT184 or rtS202). Mutations to NAs were not documented in three patients (12%) They had had VBT during the previous LAM treatment period and had a persistent high viral load during ADV add-on or ETV monotherapy. The HBV DNA levels of the three patients at baseline were 6.4, 5.7 and 4.5 log. All three patients had received LAM followed by ETV monotherapy.

Off-treatment

In the off-treatment time point, four samples were not available. Therefore, the data of 21 patients who had both samples at on- and off-treatment time points were analyzed. The HBV DNA was not detectable in three samples at off-treatment time point.

ADV resistance (three patients): Three patients had ADV and LAM resistance at the on-treatment time point. ADV resistance had disappeared at the off-treatment time point in three patients including one patient whose HBV DNA was undetectable.

ETV resistance (five patients): Four of five patients with both ETV resistance on-treatment had the same profile at the off-treatment time point. One patient had a changed resistance profile after the treatment, namely, ETV resistance was lost and only LAM resistance remained.

LAM resistance (ten patients): The LAM resistance was maintained during the treatment. But two patients who had YVDD mutant at the on-treatment time point achieved complete virological response.

Wild type (three patients): The three patients with wild

type at on-treatment did not acquire any genotypic resistance during the treatment period. The HBV DNA levels of these three patients decreased to ≤ 3.3 log (Table 2).

Virological and biochemical responses to combination therapy

From the start to 6 months of treatment, the proportion of patients with fewer than 4 log rose from 0 to 76%. However, the CVR at 9 months of treatment dropped 54%. The median pre-treatment HBV DNA level of all patients was 5.7 log (4.5-9.0). Fourteen patients had elevated alanine aminotransferase (ALT) at baseline (Table 3). After 3 and 6 months of ADV-ETV combination therapy, respectively, three (12%) and four (16%) of the patients had undetectable HBV DNA levels, that is, complete virological response as determined by a PCR assay whose minimum detection limit was <70 copies/mL.

Nineteen of the 25 patients (76%) had an IVR (Table 3) (Fig. 1). Of the 20 patients who were HBeAg-positive at baseline, two (10%) lost HBeAg during ADV-ETV combination therapy at 10.5 months on average (Table 3).

The graph of the cumulative CVR was steep during the early treatment period. After 6 months of treatment, the slope of the curve was gentle (Fig. 2). The patients with and without IVR did not differ significantly in any of the clinical variables, although the patients with IVR to have a lower baseline HBV DNA level ($P=0.052$) (Table 4).

DISCUSSION

Combination therapy was moderately efficacious for those refractory patients with CHB who participated in the

Table 2. Genotypic resistance profiles at the on-treatment and off-treatment time points

Antiviral agent (n)	On-treatment* (n=21)	Off-treatment (n=21)
ADV resistance (3)	M204I+A181+N236 (2)	M204I (1), ND (1)
	L180M+M204V+A181T (1)	L180M+M204V (1)
ETV resistance (5)	L180M+M204V+S202G (3)	L180M+M204V+S202G (2), Wild type (1)
	L180M+M204V+T184I/A (2)	L180M+M204V+T184I/A (2)
LAM resistance (10)	M204V+L180M (7)	M204V+L180M (5), ND (2)
	M204I (3)	M204I (3)
Wild type (3)	Wild type (3)	Wild type (3)

*The number of all patients were 25, but the blood samples of the off-treatment time point in four patients were not available. ND, not detectable; ADV, adefovir dipivoxil; ETV, entecavir; LAM, lamivudine.

Table 3. Virological, serological, and biochemical responses to combination therapy after 3, 6, 9, and 12 months of 25 nucleoside-resistant patients with chronic HBV infection

	Baseline (n=25)	Month 3 (n=25)	Month 6 (n=25)	Month 9 (n=21)	Month 12 (n=13)
No. with HBV DNA, levels that were undetectable	0	3	4	3	3
<3.3 log ₁₀ copies/mL	0	5	6	6	3
3.3-3.9 log ₁₀ copies/mL	0	4	9	7	1
4.0-4.9 log ₁₀ copies/mL	6	9	3	4	5
≥5.0 log ₁₀ copies/mL	19	4	3	1	1
Median ALT, IU/L (min-max)	41 (15-451)	33.5 (23-69)	35 (21-87)	31 (21-57)	24 (12-75)
No. of HBeAg-positive patients who lost expression	2 (10%)				
No. of patients who showed virological breakthrough	2*				

* The virological breakthrough in two patients occurred at 6 and 15 months, respectively.

HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase.

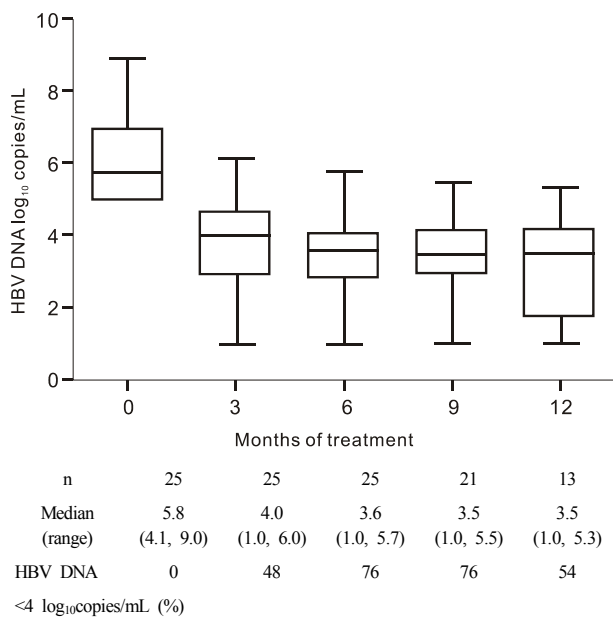


Figure 1. Distribution of nucleoside-resistant patients with chronic hepatitis B virus (HBV) infection relative to their HBV DNA levels. All patients received combination treatment with entecavir (ETV) and adefovir (ADV). The log HBV DNA levels indicated on the y axis reflect all observations within that exponent. HBV DNA levels of <70 copies/mL could not be detected by polymerase chain reaction analysis. The proportion of patients with a high viral load dropped from 76% at baseline to 5% after 9 months of treatment.

study. The IVR rate was 76%. Moreover, the proportion of patients who had high viral loads (≥5.0 log) dropped from 76% at baseline to 5% after 6 months of treatment. Two patients (10%) lost HBeAg, and the biochemical response rate after the first 6 months of treatment was 76%.

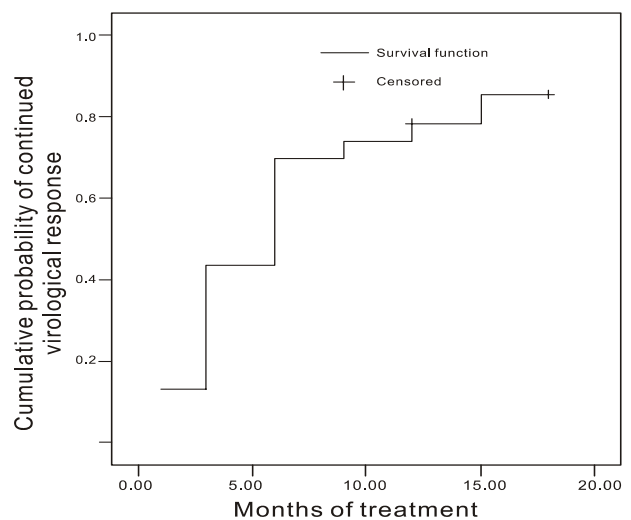


Figure 2. Cumulative probability of achieving a continued virological response (CVR), defined as HBV DNA < 4 log₁₀copies/mL after 6 months of ETV+ADV combination treatment. The cumulative probabilities of CVR at months 1, 3, 6, 9, 12, and 15 were 26%, 43%, 70%, 74%, 78%, and 85%, respectively; there were 22, 17, 13, 7, 6, and 3 patients at these time points.

With regard to the IVR in this study, most of the patients who had high viral loads at baseline developed intermediate or low viral loads after combination therapy. While an IVR of 76% may not appear to be particularly high, it can actually be considered to be a good response when one realizes that these patients are multi-drug resistant patients with CHB who previously responded poorly to monotherapies with all other oral NAs.

CVR was defined as the HBV DNA levels below 4 log after the 6 month time point. The theoretical background of

Table 4. Baseline factors associated with an initial virological response (IVR)

	Patients with IVR (n=19) (Group 1)	Patients without IVR (n=6) (Group 2)	P-value
Median age, years (min-max)	48 (25-64)	57 (41-64)	0.14
No. of males (% of 25)	14 (74)	5 (50)	0.29
Mean serum HBV DNA level			
Baseline, log ₁₀ copies/mL (±SD)	5.7 (1.1)	7.1 (1.3)	0.052
Months 6, log ₁₀ copies/mL (±SD)	2.9 (1.1)	4.9 (0.5)	0.001
Mean change in serum HBV DNA level			
Months 3, log ₁₀ copies/mL (±SD)	2.3 (1.7)	2.6 (1.3)	0.64
Months 6, log ₁₀ copies/mL (±SD)	2.9 (1.6)	2.2 (1.0)	0.22
No. of patients with HBV DNA levels, log ₁₀ copies/mL			
<7.0 log*	16	5	0.13
≥7.0 log	9	1	
Median AST (range)	36 (18-403)	38.5 (29-48)	0.87
Median ALT (range)	49 (15-451)	41 (16-72)	0.50
No. of HBeAg-positive patients (%)	16 (84)	4 (67)	0.56
No. with cirrhosis (%)	8 (42)	5 (83)	0.16
No. with biochemical response (%)	15 (79)	4 (67)	0.61
†Previous failures to <3 NAs	9 (47)	4 (67)	0.65

*This standard was taken from Cho et al¹⁴; † Number of patients who were exposed to less than 3 NAs.

HBV DNA, hepatitis B virus DNA; IVR, initial virological response; NAs, nucleos(t)ide analogues; AST, aspartate transaminase; ALT, alanine transaminase; HBeAg, hepatitis B e-antigen.

this definition was originated from the previous studies. Lim et al defined the virological response as the same level.¹¹ The patients exposed to less NAs showed better treatment response. Lai et al¹² suggested that HBV DNA suppression should continue until the level decreases to less than 10⁴ copies/mL to prevent the development of complication.

The CVR dropped from 76% at 6 months to 54% at 9 months (Fig. 1). This is likely to be due to a blip phenomenon: blips can be defined as small fluctuations in HBV DNA levels that do not represent. Another reason was that the four patients with a good response dropped out of the study after 9 months because of the financial burden of the drugs. Actually, their HBV DNA levels at 6 months were 3.2, 3.0, 2.0 and 1.0 log, respectively.

The cumulative probability of achieving CVR was shown by Figure 2. Kaplan-Meier analysis could be applied only if rebound HBV DNA levels after CVR were not observed in any of the patients during the observation period. Therefore, two patients with breakthroughs at 6 and 15 months,

respectively, were excluded for Kaplan Meier analysis. We strongly suspect that these two patients showed poor compliance at these time points.

The sequential use of NAs monotherapies increases the risk of selection of multi-drug resistant strains.¹³ As a result, the development of multi-drug resistance to LAM, ADV and ETV is becoming a significant problem in Korea.¹⁴ Combination therapy with ETV and tenofovir has been recommended for the treatment of patients who are resistant to LAM and ADV.¹⁵ However, since tenofovir is currently not available in Korea, we speculated that ADV-ETV combination therapy may be a good alternative for patients with multi-drug resistance. However, Korean government insurance only permits the use of one antiviral agent in principle, which means that combination therapy places a considerable financial burden on patients in Korea. Drug adherence should be approached as a social problem rather than as a patient's problem.

Baseline clinical prognostic factors that could predict IVR

or CVR could not be identified. However, if the patients were divided into two groups according to whether they exhibited a decrease in HBV DNA by more than 2 log after 6 months of treatment, only age differed significantly between the two groups upon univariate analysis (data not shown). Multi-variate analysis was performed for four significant factors (P -value <0.1 in univariate analysis). However, none of these factors were proven to be prognostic factors for IVR upon multi-variate analysis (data not shown). Another study showed that patients who were refractory to ADV and who achieved IVR with ETV monotherapy had higher baseline ALT and AST levels than those who did not achieve IVR.¹⁴ A recent study also showed that low pre-treatment HBV DNA levels and previous exposure to fewer NAs were associated with a virological response.¹¹

The loss of HBeAg or a biochemical response during the early treatment period could not be analyzed because too few of the patients had HBeAg ($n=20$) or high ALT levels ($n=16$) at baseline. One patient exhibited a biochemical VBT after 15 months of treatment. This patient confessed that he had sometimes failed to take his medication around 15 months after treatment. A new report about medication compliance that was published recently found that nine of 10 patients who experienced an initial VBT, but did not have confirmed genotypic resistance, had undetectable HBV DNA levels at the most recent follow-up, which was on average 7 months after the initial VBT. These data suggest that medication noncompliance can be a common cause of VBT.¹⁶

With regard to the genotypic resistance data of the present study, the genotypic resistance profile at the on-treatment time point was compared to that at the off-treatment time point. Mutations associated with LAM and ETV resistance were maintained in four of five patients, but ADV resistance had disappeared in three patients by the off-treatment time point. We speculate that ETV can somehow control ADV-resistant viruses, but ADV cannot control the virus with ETV-and LAM-resistant viruses. Indeed, Reijnders et al¹⁷ reported that viruses with ADV-resistant mutations are expected to remain sensitive to ETV. Moreover, in vitro study reported that viruses with the ADV-resistance rtN236T mutation remains sensitive to ETV, albeit with a <3 -fold change in IC_{50} , while viruses with the ADV-resistance rt181V/T mutations remain completely sensitive to ETV.^{18,19}

The limitations of the present study were, first, that the follow-up period was short. Second, drug adherence in the patients was not checked. The three patients who had taken ETV showed an inadequate response to the ADV-ETV combination therapy and we suspect poor drug adherence in these patients. Third, four patients dropped out because of the economic burden of the drug regimen. Two patients completed 6 months of treatment, and the other two patients completed 9 months of treatment. Finally, the present study was retrospective.

In conclusion, patients who experienced ADV or ETV resistance after becoming refractory to LAM showed a good IVR in response to ADV-ETV combination therapy. This combination therapy appeared to be more effective for ADV-resistance mutations than ETV resistance mutations. This combination regimen is thus a good alternative or bridge therapy for patients with multi-drug resistance until tenofovir become available. Further studies with larger number of patients are needed to determine the exact efficacy of this regimen and the factors that favor a good IVR.

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Appendix Table 1. Baseline factors associated with an IVR (defined as a 2 log decrease from the baseline)

	Patients with IVR (n=15) (Group 1)	Patients without IVR (n=10) (Group 2)	P-value
Median age, years (min-max)	48 (25-59)	55 (46-64)	0.045
No. of males (% of 25)	14 (93)	6 (60)	0.121
Mean serum HBV DNA level			
Baseline, log ₁₀ copies/mL (±SD)	6.4 (1.41)	5.5 (0.8)	0.118
Months 6, log ₁₀ copies/mL (±SD)	2.9 (1.5)	4.0 (0.6)	0.041
Mean change in serum HBV DNA level			
Months 6, log ₁₀ copies/mL (±SD)	3.5 (1.4)	1.6 (0.8)	0.001
No. of patients with HBV DNA levels, log ₁₀ copies/mL			
<7.0 log*	10	9	0.345
≥7.0 log	5	1	
Median AST (range)	31 (19-403)	38.5 (18-213)	0.723
Median ALT (range)	49 (15-451)	41 (20-172)	0.97
Median albumin g/dL (range)	4.6 (4.4-5.2)	4.4 (4.0-4.8)	0.097
No. of HBeAg-positive patients (%)	12 (80)	8 (80)	1.0
No. with cirrhosis (%)	7 (47)	6 (60)	0.69
No. with biochemical response (%)	12 (80)	7 (70)	0.65
†Previous failures to <3 NAs	9 (60)	5 (50)	0.70

*This standard was taken from Cho et al¹⁴; † Number of patients who were exposed to less than 3 NAs.

HBV DNA, hepatitis B virus DNA; IVR, initial virological response; NAs, nucleos(t)ide analogues; AST, aspartate transaminase; ALT, alanine transaminase; HBeAg, hepatitis B e-antigen.