ENTECAVIR TREATMENT FOR CHRONIC HEPATITIS B: ADAPTATION IS NOT NEEDED FOR THE MAJORITY OF NAÏVE PATIENTS WITH A PARTIAL VIROLOGICAL RESPONSE

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List of abbreviations

ETV, entecavir; VR, virological response; PVR, partial virological response; LAM, lamivudine; ADV, adefovir dipivoxil; TDF, tenofovir disoproxil fumarate; LdT, telbivudine; NA, nucleos(t)ide analogues; BMI, body mass index; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HCV, hepatitis C virus; HDV, hepatitis *delta* virus; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; IFN, interferon; ULN, upper limit of normal; CI, confidence interval; HR, hazard ratio

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ABSTRACT

Entecavir (ETV) is a potent inhibitor of viral replication in nucleos(t)ide analogue (NA)-naïve chronic hepatitis B (CHB) patients. The aim of this study was to investigate the long term efficacy and safety of ETV in NA-naïve CHB patients, particularly in those with detectable HBV DNA after 48 weeks, in whom treatment adaptation is suggested by current guidelines.

In a multi-center cohort study we investigated 333 CHB patients treated with entecavir monotherapy. The NA-naïve population consisted of 243 patients, while 90 were NA-experienced. Virological response (VR, HBV DNA <80 IU/mL) was achieved in 48%, 76% and 90% of HBeAg-positive and in 89%, 98% and 99% of HBeAg-negative NA-naïve patients at week 48, 96 and 144, respectively. Thirty-six of 175 (21%) NA-naïve patients with at least 48 weeks follow-up had a detectable load at week 48 (partial virological response, PVR). Twenty-nine (81%) patients with PVR reached VR during prolonged ETV monotherapy and none of them developed ETV-resistance. Among 22 patients with HBV DNA <1000 IU/mL at week 48, VR was achieved in 21 (95%) patients, compared to eight (57%) of 14 patients with HBV DNA ≥1000 IU/mL. Continuous HBV DNA decline was observed in most patients without VR during follow-up and in three patients adherence was suboptimal according to the treating physician. ETV was safe and did not affect renal function or cause lactic acidosis.

Conclusion: ETV monotherapy can be continued in NA-naïve patients with a detectable HBV DNA at week 48, particularly in those with a low viral load at week 48, as long-term ETV leads to a virological response in the vast majority of patients.

INTRODUCTION

Current treatment guidelines consider nucleos(t)ide analogues (NA) and peginterferon (PEG-IFN) as first line treatment of chronic hepatitis B (CHB). The ultimate goal of treatment is prevention of cirrhosis, hepatic decompensation and hepatocellular carcinoma.(1) Entecavir (ETV) is a cyclopentyl guanosine analogue and showed superior biochemical, virological and histological efficacy compared to lamivudine (LAM) in large phase III trials.(2-3) Moreover, genotypic resistance to ETV is rare in NA-naïve patients through five years of continuous therapy.(4) However, the efficacy of ETV is seriously compromised in LAM-refractory chronic HBV patients with increasing rates of genotypic ETV resistance and patients experiencing a virological breakthrough.(5) Recently we translated these previous findings to clinical practice in a large European multicenter study, as ETV was very effective in NA-naïve patients during the first year of therapy, but less effective in patients with LAM resistance at baseline.(6)

Avoiding viral resistance is a cornerstone of CHB treatment, as resistance is associated with a worsened outcome.(7) Moreover, persistent viremia has been identified as a risk factor for a dismal outcome after two years of treatment with telbivudine (LdT).(8) Therefore, current European guidelines have focused on patients with a partial virological response (PVR), defined as >1 log IU/mL decline in HBV DNA from baseline but a detectable load at week 24 (LAM and LdT) or week 48 (adefovir (ADV), ETV and tenofovir (TDF)). It is suggested that these patients could be at risk for developing genotypic resistance and that treatment adaptation in patients with a persisting viral load after 48 weeks should thus be considered.(9) However, evidence supporting these guidelines is scarce and based on data from studies with less potent NA.(8, 10) It is thus unclear whether treatment adaptation is necessary for naïve patients treated with the more potent drug ETV. The aims of this cohort study were therefore (1) to investigate the efficacy of ETV in clinical practice beyond one year for NA-naïve and –experienced chronic hepatitis B patients, (2) to explore baseline

factors associated with a PVR to ETV in NA-naïve patients and (3) to investigate whether a PVR compromises long-term ETV treatment success.

MATERIALS AND METHODS

Study population In this investigator-initiated cohort study within the European network of excellence for Vigilance against Viral Resistance (VIRGIL), all consecutive adult CHB patients treated with ETV monotherapy between 2005 and May 2010 in 10 large European referral centers were included. Further eligibility criteria were: a viral load of at least 2000 IU/mL at the initiation of ETV monotherapy, and duration of ETV monotherapy for at least 3 months. Patients were excluded if they had viral co-infections (HIV, HCV, HDV) and if they had a liver transplantation before start of ETV therapy. All 220 patients from the previously published study cohort were included in this study and 198 new patients were enrolled.(6) In total, 418 chronic HBV patients treated with ETV monotherapy were identified. Eighty-five patients did not fulfill the entry criteria and were excluded from analysis: 33 subjects had been treated with ETV monotherapy for less than three months, 58 patients had a baseline HBV DNA of less than 2000 IU/mL, 7 patients were co-infected with HCV, 1 patient was co-infected with HDV and 1 patient had a liver transplantation before start of treatment. A total of 333 patients were thus eligible for this analysis. Twenty (6%) of these patients could not be traced anymore after several attempts and were therefore considered lost to follow up. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees.

Follow-up of participants All subjects were prospectively monitored every three months. At every visit routine examination with biochemical (ALT, bilirubin, albumin) and virological (HBV DNA level, HBeAg, anti-HBe) assessments took place. Genotypic analysis was done (a) at baseline in all NA-experienced HBV patients, (b) in case of virological breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir on at least two occasions after initial virological response, or (c) in case of serum HBV DNA > 200 IU/mL at the end of follow-up. If ETV-resistant mutations were detected during follow-up,

genotypic analysis was performed at baseline in NA-naïve subjects. In NA-experienced patients, genotypic resistance was also assessed in stored serum samples obtained at the end of all previous NA-treatment regimes. HBV genotype was determined at start of ETV therapy. The diagnosis of cirrhosis was based on histology or ultrasound examinations.

Endpoints The primary outcome was virological response (VR), defined as serum HBV DNA levels < 80 IU/mL (approximately 400 copies/mL) during the on-treatment follow-up period. Secondary endpoints were HBeAg loss and seroconversion (in HBeAg-positive patients), HBsAg loss and seroconversion, emergence of ETV-related mutations and ALT normalization. Renal function was assessed by calculation of the estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2 using the Modification of Diet in Renal Disease equation, based on the serum creatinine level, age, sex, and race.

Laboratory tests Serum alanine aminotransferase (ALT), bilirubin, albumin levels and international ratio of prothrombin time were measured locally using automated techniques. Hepatitis B surface antigen (HBsAg), antibody against HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays in all centers. Serum HBV DNA levels were measured using a quantitative real-time polymerase chain reaction assay, the COBAS AmpliPrep-COBAS TaqMan HBV test (CAP-CTM; Roche Molecular Systems, Inc., Branchburg, NJ, USA), with a lower limit of detection of 12 IU/mL, in nine of ten centers. In one center serum HBV DNA was measured using Roche Amplicor (linear dynamic range, 400 to 200,000 copies/mL; Roche Diagnostic Systems, Branchburg, NJ, USA). A conversion factor of 5.26 copies/IU was used for conversion of copies/mL to IU/mL. HBV genotypes and detection of HBV polymerase gene mutations was determined by direct sequencing or using the INNO-LiPA assay (Innogenetics, Gent, Belgium).

Data analysis HBV DNA levels were logarithmically transformed for analysis. ALT levels are expressed as values representing a ratio to the local upper limit of normal (xULN).

Continuous variables were expressed as means ± SD or median (IQR) where appropriate. Follow-up times were calculated from the date of ETV treatment initiation to the date of event or censorship. The cumulative probability of achieving virological response was estimated by Kaplan-Meier analysis. Cox's regression analysis was used to study which of the following baseline factors were associated with virological response to ETV monotherapy: Age, gender, race, body mass index (BMI), HBV genotype, HBeAg status, viral load, ALT level, presence of cirrhosis, prior treatment with LAM, prior history of LAM resistance, presence of LAM resistance at baseline, duration of LAM therapy, prior treatment with ADV, prior history of ADV resistance, prior treatment with (peg)interferon, ETV dosage, and treatment center. Factors that correlated strongly (that is presence of collinearity), were compared in separate models with each collinear variable by using the Akaike information criterion method. A Cox model was used to estimate the influence of prior treatment with LAM and prior treatment with ADV on the virological response to ETV, adjusted for the confounding effects of HBeAg status, viral load, and prior treatment with LAM. The covariate ETV dosage was not included, as it was not associated with virological response in the univariate proportional hazards analysis and, when included in the model, did not improve model fit. All statistical tests were two-sided, and a P value < 0.05 was considered to be statistically significant. SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of the study population are shown in table 1. One-hundred-forty-three (43%) patients were HBeAg-positive, median ALT was 1.7 (1.0-3.3) x ULN and mean HBV DNA was 6.2±1.7 log IU/ml at baseline. NA-experienced patients were more often HBeAg-positive ($P < 0.001$), had more often cirrhosis ($P = 0.04$), had a higher MELD score ($P = 0.02$) and were more often treated with 1 mg ETV ($P < 0.001$) as compared to NA-naïve patients. In addition, 87% of LAM-resistant patients and 86% of ADV-resistant patients were treated with 1 mg. Overall median follow-up was 20 (IQR 11-32; range 3-51) months.

Efficacy of entecavir in NA-naïve patients In total, 243 (73%) patients were NA-naïve and treated for a median of 19 (IQR 11-32; range 3-45) months (Table 2). For HBeAg-positive patients (n = 86), the cumulative probability of achieving VR at week 48, 96 and 144 was 48% (95% CI 36-60), 76% (66-86) and 90% (81-99), respectively (Figure 1). HBeAg loss rates were 10% at week 48, 21% at week 96 and 34% at week 144. Corresponding rates for HBeAg seroconversion were 8%, 16% and 24%. ETV therapy was not stopped in any patient achieving HBeAg seroconversion. HBsAg loss occurred in one (1%) of the HBeAg-positive patients. For HBeAg-negative patients (n = 157), the cumulative probability of achieving VR at week 48, 96 and 144 was 89% (95% CI 84-93), 98% (95-100) and 99% (97-100), respectively (Figure 1). HBsAg loss occurred in two (1%) HBeAg-negative patients. There were no significant differences in virological response rates per center. Five patients experienced a virological breakthrough, but no genotypic resistance to ETV was detected.

Partial virological response in NA-naïve patients Partial virological response (PVR) at week 48 occurred in 36 (21%) of 175 NA-naïve patients with at least 48 weeks follow-up. High baseline HBV DNA (OR 0.67; 95% CI 0.50-0.89; $P = 0.005$) and HBeAg positivity (OR 0.25; 95% CI 0.10-0.60; $P = 0.002$) were the only independent risk factors for having a PVR.

Overall follow-up of patients with a PVR lasted for 27 (19-35) months. Twenty-nine (81%) of 36 patients achieved a VR beyond week 48 (Table 3). Moreover, 10 patients needed more than 96 weeks of continuous ETV therapy to achieve a VR. Patients achieving a VR had a lower HBV DNA at week 48 than those who did not achieve a VR during prolonged ETV monotherapy. Patients with a PVR were stratified according to their viral load at week 48 (Figure 2). Twenty-one (95%) of 22 patients with HBV DNA <1000 IU/mL and 8 (57%) of 14 patients with HBV DNA ≥1000 IU/mL achieved a VR without treatment adaptation during prolonged treatment beyond week 48. Overall, a continuous HBV DNA decline was observed in six (86%) of seven patients without VR at the end of follow-up. In three (43%) patients non-compliance was suspected by the local physician. Five patients were switched to a TDF containing regimen (in two because of virological breakthrough, one patient first achieved VR); all achieved a VR during follow-up. In two patients ETV dosage was changed from 0.5 mg to 1 mg, both achieved a VR during follow up. However, no ETV-resistance was detected during follow-up, at virological breakthrough or at time point of treatment adaptation.

Efficacy of ETV in NA-experienced patients Fifty-one (57%) NA-experienced patients had received prior treatment with ADV. To investigate the efficacy of ETV as salvage therapy for ADV-treated patients, the antiviral effect of ETV is given in table 2 for 43 (84%) subjects, who were directly switched to ETV monotherapy. Twelve (28%) of these patients had a history of genotypic resistance to ADV (rtN236T/ rtA181V/T). Seventy-two (80%) NA-experienced patients had received prior treatment with LAM. Adjusted for baseline viral load and HBeAg status, antiviral response to ETV was neither influenced by prior ADV therapy (HR 0.92; 95% CI 0.57-1.51; $P = 0.75$) nor by previous ADV-resistance (HR 1.23; 95% CI 0.56-2.70; $P = 0.61$) (Figure 3). In contrast, presence of LAM-resistant mutations at baseline (HR 0.13; 95% CI 0.04-0.42; $P < 0.001$) and a previous history of LAM-resistance (HR 0.40;

95% CI 0.19-0.84; *P* = 0.015) were significantly associated with a reduced probability of achieving VR.

Resistance surveillance Eighteen patients experienced a virological breakthrough after a median follow-up of 20 (11-32) months. Five (2%) NA-naïve patients experienced a virological breakthrough, non-adherence was suspected in two of them and three were switched to a TDF containing regimen (TDF monotherapy in one, TDF add-on in one and TDF+emtricitabine in one). Six NA-experienced patients were switched to a TDF containing regimen (TDF monotherapy in two, TDF add-on in three and TDF+emtricitabine in one) and one patient stopped ETV without starting another NA. In four NA-experienced patients genotypic mutations to ETV were detected, one patient was previously only exposed to ADV and achieved a virological response before developing a virological breakthrough (Table 4). A TDF containing treatment regimen was initiated in all four patients, and a subsequent decline in HBV DNA was observed in three of them. No mutations associated with decreased sensitivity to ETV were observed in any of the NA-naïve patients, including those with a viral load > 200 IU/mL at the end of follow-up.

Safety surveillance Adverse events included: dizziness, headaches and loss of appetite (in three different patients). None of the patients developed clinically evident lactic acidosis, but lactate was not routinely measured. One patient died because of a hepatocellular carcinoma, which was already present at start of ETV, one patient had a recurrence of his hepatocellular carcinoma, and one patient died because of a non-Hodgkin lymphoma. To assess renal safety we analyzed creatinine levels in a subset of 188 patients with an available baseline creatinine. Mean estimated glomerular filtration rate (eGFR) at baseline was 95.8 mL/min/1.73m^2. The mean decrease in eGFR during follow-up was 1.2±18.1 mL/min/1.73m^2 (*P* = 0.38). None of the patients experienced an increase in serum creatinine

> 0.5 mg/dL. In a subset of 9% of patients with an eGFR <70 mL/min/1.73m^2 eGFR increased with 1.4±10.0 mL/min/1.73m^2 (*P* = 0.60*).* Age was the only risk factor significantly associated with developing an eGFR <60 mL/min/1.73m^2.

DISCUSSION

The current multicenter study showed that ETV is effective up to three years in NA-naïve patients, irrespective of having a virological response at week 48. The vast majority of NA-naïve patients with a PVR achieved undetectable HBV DNA through prolonged therapy without treatment adaptation. Genotypic resistance to ETV was not detected in any of the patients with a PVR at week 48. We showed that ETV is a safe antiviral drug with a good renal tolerance and minimal side effects. Persistent viral replication and the development of resistance during treatment with NA for CHB have been associated with an adverse treatment outcome.(8, 11) Therefore, EASL guidelines suggest treatment adaptation in patients with a PVR to prevent treatment failure and the development of resistance.(9) PVR is defined as a decline of >1 log IU/mL in HBV DNA but failure to achieve undetectable HBV DNA levels at week 48 in patients treated with continuous ETV monotherapy.

In our cohort of NA-naïve patients treated with ETV, 36 patients failed to achieve a VR at week 48. Among these patients with a PVR, 81% achieved a VR without treatment adaptation through 15 additional months of therapy. Cumulated probability of achieving VR beyond week 48 was higher for patients with HBV DNA <1000 IU/mL at week 48. Importantly, despite two patients experiencing a virological breakthrough, no resistance was detected in these NA-naïve patients. This is in accordance with the ETV phase III trial in which, albeit with incomplete follow-up, a substantial number of patients achieved a response beyond the first year of treatment, whilst genotypic resistance remained rare through 5 years of continuous monotherapy.(4, 12-13) Our findings are in contrast with previous studies on LdT/LAM and ADV in which persistent viral replication at week 24 and week 48 of therapy was identified as a predictor of the emergence of subsequent viral resistance.(8, 10) This highlights that treatment paradigms based on data from studies investigating agents with a low barrier to resistance cannot be translated to newer and more potent drugs as ETV and TDF.

Nevertheless, not all ETV-treated patients with a PVR achieved VR through prolonged treatment. As we, after thorough examination, determined non-compliance in three (43%) of these seven patients, this explains in our opinion primarily the inability to achieve HBV DNA undetectability. The problem of non-adherence is supported by a previous study suggesting partial response to ADV is most likely due to non-compliance and host pharmacological factors.(14) One of seven patients without a VR experienced a virological breakthrough and treatment was adapted by the treating physician. However, it is important to note that six (86%) of seven patients who failed to achieve a VR during follow-up still had a declining load at end of follow-up, which suggests that achieving a VR can probably be reached in the majority of cases. Patients with a PVR could therefore be considered 'slow responders' instead of partial responders. Taken together, our study shows that continuing ETV appears safe and effective in patients with detectable HBV DNA at week 48, especially in patients with a lower viral load at week 48, of whom 95% achieved VR.

Decreased sensitivity to ETV for LAM-refractory patients was soon known after introduction of this agent.(5, 15) Our study confirms these results as the antiviral efficacy of ETV is seriously diminished in these patients, even after correction for possible confounders as high baseline HBV DNA and HBeAg-positivity. Moreover, our study underlines that even after resistance testing at baseline; the absence of LAM-associated mutations does not guarantee a susceptible virus during ETV treatment. This suggests that if there is a suspected history of LAM-resistance, TDF containing regimens should be preferred instead of ETV monotherapy as LAM-resistance strains remain susceptible to TDF monotherapy.(16)

Consistent with in vitro data, our study showed that antiviral efficacy of ETV treatment was not influenced by prior exposure or resistance to ADV.(17-21) Until now only small studies or studies with a relatively short follow-up have confirmed the in vitro efficacy of ETV in ADV-experienced or ADV-resistant patients in real life practice.(6, 22-24) Our findings are of

particular interest because both ETV and TDF can thus be used as salvage therapy for ADV-experienced patients.(9, 25)

Data from the large phase III trials with a selected population showed that entecavir has few side effects in patients with compensated liver disease.(2-3) However, a recent report indicates that patients with decompensated cirrhosis are at risk for developing lactic acidosis.(26) We showed that ETV is safe during prolonged therapy in this heterogeneous cohort, even in the presence of cirrhosis. Moreover, we proved that ETV does not affect renal function, which might be a concern during TDF therapy.(27)

Limitations of our study are the observational design and the heterogeneous group of patients, yet we used Cox's regression to correct for confounders as treatment duration, HBV DNA, HBeAg status and previous LAM-resistance. Nevertheless, this heterogeneous population is also representative for clinical practice, and makes it possible to compare different groups of (NA-experienced) patients within one study.

In conclusion, in contrast to what is suggested in recently published EASL guidelines on the management of chronic hepatitis B, adjustment of ETV monotherapy in NA-naïve patients with a PVR at week 48 is not necessary. We demonstrated that continuous therapy beyond week 48 is safe and effective, and results in VR in the vast majority of patients, particularly in those with HBV DNA <1000 IU/mL at week 48. Furthermore, genotypic resistance to ETV was not observed in this subset of NA-naïve patients. For both NA-naïve and NA-experienced patients, ETV proved to have a favorable safety profile.

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Figure legends

Figure 1. Kaplan-Meier curve for the probability of achieving virological response for 243 NA-naïve patients according to HBeAg-status at baseline. P-value by Log-Rank testing.

Figure 2. Kaplan-Meier curve for the probability of achieving virological response for NA-naïve patients with a PVR according to HBV DNA at week 48. [a]Three patients were switched to TDF+emtricitabine and one patient received TDF add-on therapy. P-value by Log-Rank testing.

Figure 3. Adjusted hazard ratio (HR) of achieving virological response for both NA-naïve and NA-experienced patients. Based on the Cox's model adjusted for HBeAg status, mean baseline HBV DNA, LAM-experience, history of LAM-resistance, LAM-resistance at baseline, ADV-experience and history of ADV-resistance.

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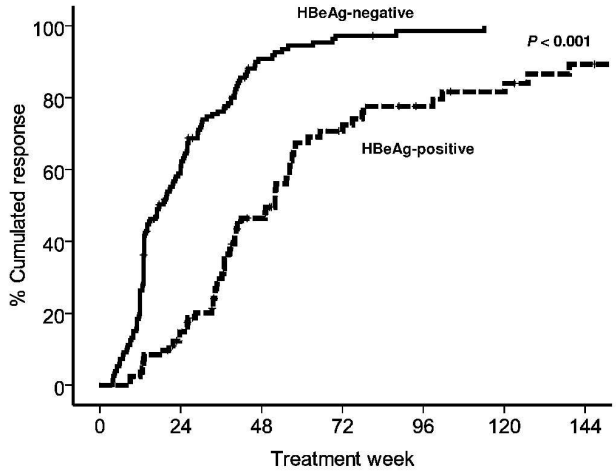
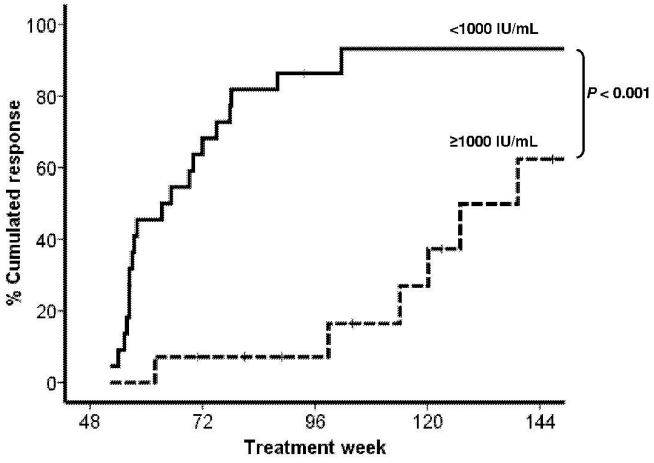


Number of patients without response							
HBeAg-positive	86	70	28	17	11	8	4
HBeAg-negative	157	63	8	3	1	0	0
Total number of patients in follow up	243	226	175	142	106	78	49

Figure 1 v2
279x215mm (300 x 300 DPI)



Number of patients without response					
<1000 IU/mL at week 48	22	8	2	1	1
≥1000 IU/mL at week 48	14[a]	12	10	7	3
Total number of patients in follow up	36	31	23	16	9

Figure 2
279x215mm (300 x 300 DPI)

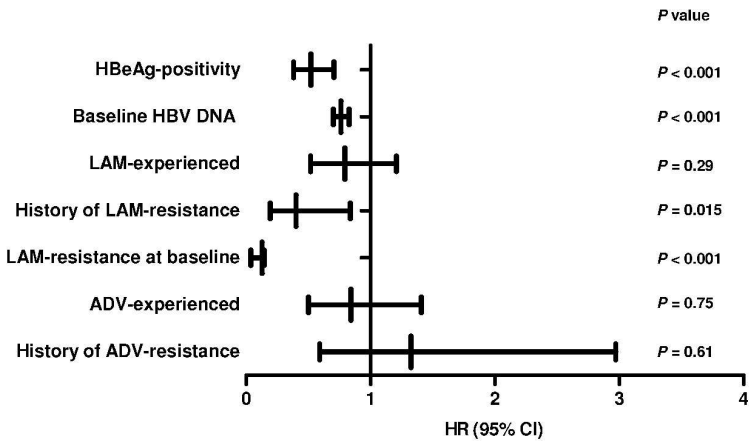


Figure 3
279x215mm (300 x 300 DPI)

Table 1: Baseline characteristics of the study population.

	All patients N=333	NA-naïve N=243	NA-experienced N=90	*P* value
Age	43±14	43±14	41±13	0.12
Gender (male)	248 (75%)	177 (73%)	70 (79%)	0.26
Race				0.57
Caucasian	160 (48%)	114 (47%)	46 (51%)	
Asian	92 (28%)	70 (29%)	22 (24%)	
Other	81 (24%)	59 (24%)	22 (24%)	
BMI	25±4.1	25±3.5	25±5.0	0.21
ALT (xULN)	1.7 (1.0-3.3)	1.7 (1.0-3.5)	1.7 (1.0-3.2)	0.78
HBV DNA (Log_{10} IU/ml)	6.2±1.7	6.2±1.7	6.2±1.8	0.86
HBeAg-positive	143 (43%)	86 (36%)	57 (63%)	< 0.001
Genotype (N=265)				0.80
A	56 (21%)	40 (22%)	16 (20%)	
B	23 (9%)	14 (8%)	9 (11%)	
C	37 (14%)	25 (14%)	12 (15%)	
D	127 (48%)	91 (50%)	36 (44%)	
Other	22 (7%)	14 (8%)	8 (10%)	
Dosage entecavir (0.5 mg)	265 (80%)	238 (98%)	27 (30%)	< 0.001
Presence of cirrhosis	90 (27%)	57 (24%)	33 (37%)	0.04
MELD score	7.8±2.1	7.5±1.9	8.4±2.4	0.02
Previous treatment with (PEG-)IFN	74 (22%)	49 (20%)	25 (28%)	0.14
Previous treatment with LAM				
LAM-experienced	72 (22%)		72 (80%)	
Prior history of LAM resistance	35 (11%)		35 (39%)	
LAM-resistance at baseline	14 (4%)		14 (16%)	
Previous treatment with ADV				
ADV-experienced	51 (15%)		51 (57%)	
Prior history of ADV resistance	14 (4%)		14 (16%)	
ADV resistance at baseline	12 (4%)		12 (13%)	
Previous treatment with TDF	4 (1%)		4 (4%)	
Previous treatment with LdT	2 (1%)		2 (2%)	

Table 2: Virological and biochemical response to entecavir, number of observed events.

	NA-naïve patients (N=243)	**LAM-experienced patients (N=72)**			**ADV-experienced patients[a] (N=43)**	
		No LAM-resistance (N=37)	Prior history of LAM-resistance (N=21)	LAM-resistance at baseline (N=14)	LAM-resistance (N=18)	No LAM-resistance (N=25)
Baseline HBV DNA (Log_{10} IU/ml)	6.2±1.7	6.4±1.8	6.3±1.5	5.6±1.8	5.9±1.8	5.4±1.8
Median follow-up (month, IQR)	19 (11-32)	25 (13-32)	19 (9-31)	10 (6-15)	15 (9-28)	32 (23-39)
Virological response	208/243 (86%)	28/37 (76%)	11/21 (52%)	3/14 (21%)	6/18 (33%)	22/25 (88%)
Virological breakthrough	5/243 (2%)	0/37 (0%)	5/21 (23%)	7/14 (50%)	7/18 (39%)	1/25 (4%)
ETV resistance	0/243 (0%)	0/37 (0%)	3/21 (14%)	0/14 (0%)	3/18 (17%)	1/25 (4%)
ALT normalization	126/171 (74%)	14/24 (56%)	8/11 (62%)	4/7 (57%)	6/9 (67%)	12/16 (75%)
HBeAg loss	18/86 (21%)	6/26 (23%)	4/14 (29%)	0/7 (0%)	3/14 (21%)	4/12 (33%)
HBeAg seroconversion	13/86 (15%)	5/26 (19%)	2/14 (14%)	0/7 (0%)	2/14 (14%)	3/12 (19%)
HBsAg loss	3/243 (1%)	2/37 (5%)	0/21 (0%)	0/14 (0%)	0/18 (0%)	2/25 (8%)

The antiviral effect of entecavir is described for 43 patients who were directly switched to entecavir

Table 3: NA-naïve patients with a partial virological response at week 48. Characteristics of 36 patients with a detectable HBV DNA after 48 weeks of continuous ETV monotherapy.

	All patients N=36	Response N=29	Without response N=7	*P* value
Age	41±14	43±14	33±13	0.13
Gender (male %)	30 (83%)	26 (90%)	4 (57%)	0.07
Race				0.09
Caucasian	17 (47%)	15 (51%)	2 (29%)	
Asian	11 (31%)	8 (28%)	3 (43%)	
Other	8 (22%)	6 (21%)	2 (29%)	
HBeAg-positive	28 (78%)	22 (76%)	6 (86%)	0.58
Presence of cirrhosis	7 (19%)	6 (21%)	1 (14%)	0.70
Follow-up (Months, IQR)	27 (19-35)	28 (19-35)	22 (19-28)	0.23
Baseline HBV DNA (Log_{10} IU/ml)	7.7±1.4	7.5±1.5	8.2±1.1	0.31
HBV DNA week 48 (Log_{10} IU/ml)	2.7±1.3	2.3±1.2	3.8±0.8	0.006
HBV DNA decline at week 48 (Log_{10} IU/ml)	5.0±1.3	5.1±1.3	4.3±0.8	0.13
HBV DNA last visit (Log_{10} IU/ml)			3.0±0.9	
Load >1000 IU/mL at week 48	14 (39%)	8 (28%)	6 (86%)	< 0.001

Table 4: Characteristics of four patients developing genotypic mutations to entecavir.

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Age (years)	26	67	29	26
Gender	Male	male	male	Female
At start of entecavir				
HBeAg status	positive	positive	positive	Positive
HBV DNA (log_{10} IU/mL)	8.9	5.7	7.9	4.2
Prior LAM exposure	No	Yes	Yes	Yes
Prior LAM-resistance	No	Yes	Yes	Yes
Baseline LAM-resistance	No	No	No	No
HBV Genotype	A	D	A	C
Viral load at max. viral suppression (log_{10} IU/ml)	<1.9	5.3	2.9	3.8
Month of resistance	40	9	28	15
At time of resistance				
HBV DNA (log_{10} IU/ml)	5.4	5.3	6.3	5.5
Mutational pattern	rtM204V, rtL180M, rtI169T, L217R	L180M, M204V, V173L, A181T, N236T	rtM204V, rtL180M, V173L	rtM204V, L180M, T184I
Adverse outcome	None	None	None	None
Non-compliance	No	No	No	No
Response to salvage therapy				
Salvage therapy	Addition of tenofovir	Tenofovir monotherapy	Addition of tenofovir	Tenofovir monotherapy
Follow-up (months)	44	18	54	18
HBV DNA at last F/U (log_{10}IU/ml)	< 1.9	< 1.9	< 1.9	4.0