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Entecavir plus tenofovir combination as rescue therapy in pre-treated chronic hepatitis B patients: An international multicenter cohort study

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Background & Aims: Long-term viral suppression is a major goal to prevent disease progression in patients with HBV. Aim of this study was to investigate the efficacy and safety of entecavir plus tenofovir combination in 57 CHB partial responders or multidrug resistant patients.

Methods: Investigator-initiated open-label cohort study. Quantitative HBV-DNA measurement and resistance testing (line-probe-assays and direct-sequencing) at baseline and every 3 months.

Results: Fifty seven patients (37 HBeAg+), median age 45 years, previously treated with a median of three lines of antiviral therapy (range 1–6), 24/57 with advanced liver disease, were included. Median ALT at baseline was 1.0 ULN (range 0.3–22) and HBV-DNA 1.5×10^4 IU/ml (range 500–1×10^{11} IU/ml). Median treatment duration of combination therapy was 21 months. HBV-DNA level dropped 3 logs (median, range 0–8 log; p <0.0001), 51/57 patients became HBV-DNA undetectable, median after 6 months (95% CI, 4.6–7). The probability for HBV DNA suppression was not reduced in patients with adefovir or entecavir resistance or in patients with advanced liver disease. Viral suppression led to decline in ALT (median 0.7 ULN; range 0.2–2.4; $p = 0.001$). Five patients lost HBeAg (after 15, 18, 20, 21, and 27 months, respectively), one patient showed HBs-seroconversion. Patients with advanced disease did not show clinical decompensation, two patients with cirrhosis and undetectable HBV DNA developed HCCs. No death, newly induced renal impairment or lactic acidosis were reported.

Conclusions: Rescue therapy with entecavir and tenofovir in CHB patients harboring viral resistance patterns or showing only partial antiviral responses to preceding therapies was efficient, safe, and well tolerated in patients with and without advanced liver disease (249).

Introduction

Treatment of chronic hepatitis B (CHB) has greatly improved with the availability of nucleos(t)ide analogs (NA), which target the viral polymerase [1–3]. The sustained suppression of serum hepatitis B virus (HBV) DNA to very low or undetectable levels by these drugs has been shown to be associated with the prevention of progression of liver disease and inhibition of the development of hepatocellular carcinoma [4–7]. A major shortcoming is the low rate of HBsAg loss and high rate of virological relapse when treatment is discontinued or interrupted due to low adherence [8,9], necessitating long, and in many cases, indefinite treatment. Unfortunately, as the duration of NA treatment is prolonged, the risk of development of drug resistance increases with lesser potent and lower genetic barrier drugs such as lamivudine and adefovir [1–3]. Emergence of drug-resistant HBV usually results in attenuated viral suppression and disease progression, and may lead to significant clinical deterioration [10,11]. With the newer and more potent NAs, especially with entecavir and

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Abbreviations: IFN, interferon; CHB, chronic hepatitis B; HBV, hepatitis B virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate; ADV, adefovir didivoxil; LdT, telbivudine; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; MDR, multidrug resistance.

tenofovir, the risk of resistance in naïve patients is very low as long as patients show a strict adherence to antiviral therapy regimens [9,12]. However, sequential NA monotherapy after the occurrence of resistant mutations with lamivudine and adefovir may result in an increased rate of multidrug-resistant HBV by sequential selection of mutations conferring resistance to both the initial and subsequent therapy. Once antiviral-resistant HBV mutants have been selected, they are persistently archived in cccDNA copies and may therefore be selected rapidly out when using drugs that exhibit cross-resistance [13,14]. Thus, the stability and replenishment of cccDNA is the stumbling block for eradicating CHB infection with current antiviral agents [15–18].

The lower risk of resistance to ETV and TDF (compared with LMV, LdT, and ADV) supports their use as first-line therapy, especially in patients with cirrhosis or decompensated liver disease because development of drug resistance is more likely to precipitate clinical deterioration in these patients [1,19,20].

Combination of drugs without cross-resistance can delay or prevent the emergence of drug-resistant mutants. The ideal combination would target different aspects of the HBV replication cycle, but all of the approved oral drugs against HBV target the viral polymerase. There are some reported differences in the aspects of polymerase function affected by each drug but combining drugs with very similar mechanism of action may lead to drug interference rather than synergy [19]. Although HBV mutants that are resistant to single drugs exist already before therapy starts and can be selected rapidly during antiviral therapy, HBV mutants with multi-drug resistance (MDR) are much less likely to exist before treatment. Combination therapy using NA with a complementary cross-resistance profile may prevent the development of resistance but does not have increased antiviral effects, compared with single-drug therapy [19,21]. Whether specific subgroups of patients may benefit from the combination of a nucleos(t)ide analog with peginterferon has yet to be determined as well [19]. In terms of preventing lamivudine resistance, the use of peginterferon and lamivudine combination was of advantage but present treatment guidelines advocate oral antivirals in patients with advanced liver disease [22–24] and there are no controlled trial data available for patients with polymerase inhibitor resistant strains and subsequent interferon therapy or even combination therapy.

In this real life cohort, we studied the safety and virological, serological, and biochemical responses to the open label combination of entecavir and tenofovir in patients who had already developed resistance to NAs or who responded only partially after sequential mono-therapy. Almost half of these patients showed already advanced liver disease (advanced fibrosis or cirrhosis). For these patients the currently available amount of information is very limited and we tried to explore some treatment options for this difficult to manage patient group.

Patients and methods

Patient recruitment and study design

This retrospective investigator initiated cohort study included patients from 10 European referral centers. The criteria for entering the study were adherence to previous NA treatment, measurable serum HBV DNA before starting combination therapy and presence of resistant HBV, or documented incomplete responses or failure to previous lines of therapy. A minimum period of treatment of 3 months for the combination of ETV (0.5 mg for naive or 1.0 mg for lamivudine experienced patients QD) and TDV (300 mg QD) and age above 18 years were further prerequisites. Informed consent was obtained from all patients and the study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and it was approved by the institution Ethical Committee, the city and state of Hamburg, Germany. Multidrug resistant HBV was defined as presence of rtA181T/V or resistance against ADV (rtN236T) and LAM (rtM204V/I) or LAM + ETV [18]. Incomplete response was defined as persistence and plateauing of HBV DNA during NA treatment (>12 months) and failure of preceding treatment was defined by HBV DNA drop of less than 1 log within 3 months of treatment in line with the EASL clinical practice guidelines [1].

Baseline characteristics and antiviral treatment prior to ETV–TDF combination therapy

In total, 69 HBV infected patients with ETV and TDV combination treatment were identified. Twelve patients did not fulfil the entry criteria and were excluded from this analysis. The remaining 57 patients fulfilled all entry criteria and were included in this analysis (Table 1).

Study objectives and endpoints

The primary study objective was to evaluate the long-term efficacy and safety of ETV and TDF combination therapy in HBV treatment-experienced patients with chronic HBV infection. The primary endpoint was defined as HBV DNA levels by quantitative PCR by <80 IU/ml (lower limit of detection) after 24, 48, and 96 weeks of combination therapy. Secondary endpoints were reduction of aminotransferase (ALT), HBeAg, and HBsAg loss or seroconversion, development of genotypic resistance, safety, and tolerability.

Clinical and laboratory assessments

ALT, albumin, serum creatinine, serum HBV-DNA, HBsAg, HBeAg, antiHBe, and antiHBs were routinely assessed every 3 months by the investigators. Results from laboratory data were collected from patient's records and analyzed retrospectively. Safety and tolerability were assessed from documented clinical side effects and laboratory abnormalities with special focus to kidney function, lactic acidosis, and liver decompensation, HCC development and death. From patients where frozen serum samples before initiation of combination therapy were available (20/57), Inolipa line-probe assay DRV3 and direct sequencing was used to determine resistance status and genotype at baseline. Genotypic resistance testing at baseline was available from another 21 patients from routine work up in different centers (direct sequencing or Inolipa line probe assay).

Pre-treatment prior to ETV–TDF combination therapy

Initiation of the ETV–TDF combination was preceded by 49 months of antiviral treatment (median, range 9–173 months) and median three lines of pre-treatment (range 1–6), see Table 1 for details. Genotypic resistance data were available from 41/57 patients (Table 2). Interestingly, 38/41 patients showed typical resistance patterns to at least one class of nucleos(t)ide analogs, but all 38 patients were genotypically resistant to lamivudine, shown by the presence of the rtM204V and or rtA181V mutations. Furthermore 4/38 patients were genotypically resistant to entecavir and 18/38 to adefovir (rtA181V and or rtN236T), but no tenofovir associated mutations (rtA194T) were detected. Furthermore, besides pre-treatment no entecavir resistance was detected in patients without prior exposure to lamivudine.

Statistical analysis

The Mann Whitney test was used for nonparametric group wise comparisons. Kaplan–Meier methodology was used for time to event calculation and subgroup comparisons were performed using Log-Rank test; all tests were performed two sided and p <0.05 was considered significant.

Results

Antiviral efficacy of ETV–TDF combination therapy

The baseline characteristics of the 57 patients are summarized in Table 1. The median follow up during ETV–TDF combination

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Table 1. Patients characteristics (n = 57).

Age (yr)	45 [median, range 21-79]
Gender (Female/Male)	6/51
ALT (ULN)	1.0 [median; range 0.3-22]
Liver cirrhosis or severe fibrosis	24
HBeAg positive	37/57
HBV DNA (log_{10} IU/ml)	4.1 [median; range 1.9-12]
HBV genotype	4x A, 3x B, 5x C, 26x D, 3x E, 1x D/G, 15x n.a.
Lines of antiviral pre-treatment	3 [median; range 1-6]
Duration of antiviral pre-treatment	49 months [median; range 9-173]
Last treatment before rescue therapy	6x ADV, 14x ETV, 1x LAM, 7x TDF, 1x LdT, 12x ADV + LAM, 10x ADV + ETV, 6x TDF + LAM
Antiviral experience	28% IFN (17/57), 89% LAM (44/57), 77% ADV (44/57), 42% ETV (24/57), 23% TDF (13/57), 2% LdT (1/57)

treatment was 21 months. This treatment induced a median HBV DNA reduction of 3 logs (range, 0–8 log HBV DNA/ml; p <0.0001) and 90% of patients (51/57) became HBV-DNA undetectable (LLoD 80 IU/ml) (Fig. 1). A Kaplan–Meier analysis was used to analyze the probability of reaching HBV DNA undetectability. For the entire cohort, the median time to HBV-DNA undetectability was 6 months (95% CI 4.6–7 months). Interestingly, the presence of genotypic resistance to ADV (n = 17; rtN236T or rtA181V) or to ETV (n = 4; rtT184G, rtS202I, and rtM250V) did not significantly reduce the time to HBV DNA negativity, compared to the remaining cohort (Log rank test; p = 0.8; Fig. 2). Viral suppression was maintained during combination therapy in 51/52 patients (98%).

ALT and serological response

ALT levels decreased by 23% to a median of 0.64 × ULN (p = 0.0002) compared to baseline and normalized in 20 out of 27 patients (74%) with elevated ALT at baseline. HBeAg loss was observed in 5 out of 32 HBeAg positive patients (13, 5%, Fig. 3) after a median treatment duration of 20 months (15, 18, 20, 21, and 27 months, respectively) and HBsAg seroconversion occurred in one patient after 18 months of treatment (genotype D).

Patients with advanced liver disease

Twenty four out of 57 patients (42%) showed advanced liver disease (bridging fibrosis or cirrhosis). Although these patients tended to be older (53.5 years *vs.* 43.5 years; p = 0.35) compared to the other patients in the cohort, there were no significant differences in baseline parameters like pattern of antiviral resistance, HBV DNA and ALT levels, lines of pre-treatment (p = 0.8, p = 0.35, p = 0.45, and p = 0.27) and treatment outcome like time to undetectable HDV-DNA and ALT normalization (p = 0.76 and p = 0.71).

Safety and side effects

In total this ongoing cohort covers currently 1174 months of ETV–TDF combination treatment. As shown in Fig. 4 there were no significant clinical side effects reported, especially no newly induced renal impairment (rise of more than 0.5 mg/dl in serum creatinine level) and no lactic acidosis. In three patients, serum creatinine values were already elevated at baseline and remained stable (1.4, 1.5, and 1.6 mg/dl) although in two of the patients tenofovir dosing was reduced to every second day during therapy. One patient with pre-existing severe renal impairment (serum creatinine 5, 77 mg/dl) showed a further decrease in function to 10.3 mg/dl although antiviral dosing was already initially reduced to 0.1 mg entecavir post dialysis and 245 mg tenofovir/week. Patients with liver cirrhosis did not develop any clinical decompensation, but two patients with cirrhosis and undetectable HBV DNA developed an HCC after 21 and 30 months of combination therapy.

Discussion

With the development of oral antiviral drugs for HBV from less potent first generation drugs such as lamivudine to more potent substances such as entecavir and tenofovir over the last 15 years, a number of patients with chronic hepatitis B have received numerous lines of antiviral therapies, usually as sequential mono-therapies, with insufficient therapeutic responses and the selection of specific antiviral mutations. Of those patients, individuals with advanced liver disease are at highest risk of clinical decompensation due to the development of hepatic flares. We studied a number of these pretreated patients who were finally started on combination therapy with ETV and TDF as rescue therapy. The results of this observational study provide evidence for the efficacy and safety of the combination therapy with ETV and TDF in often heavily pre-treated CHB patients in an open label real life cohort from ten European referral centers.

This analysis revealed that the vast majority of patients showed a clear therapeutic benefit in response to the ETV–TDF combination therapy. Fifty one out of 57 patients became HBV DNA undetectable and ALT levels improved in most patients, suggesting a reduction in liver inflammation. Furthermore, some patients lost HBeAg (5/32). Especially the subgroup of patients with the highest risk of clinical decompensation, i.e. multidrug resistance and advanced liver disease, responded clearly to the combination therapy. Although no patient showed signs of clinical decompensation during the 2 years observation period, two patients with underlying liver cirrhosis already at start of combination therapy developed hepatocellular cancer. This aspect

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Table 2. Patients characteristics prior to combination therapy (n = 41) with genotypic data available.

No.	Adv. fibrosis cirrhosis	Lines of pre-treatment	Last treatment	HBV DNA [baseline, IU/ml]	Genotypic resistance			Time to DNA <LLoD (months)
#1		3	ADV + LAM	1.2×10^8	WT			3
#23		2	ETV	2.5×10^3	WT			(3)
#25	yes	3	TDF	5.5×10^3	WT			(21)
#3	yes	2	ADV + LAM	8.6×10^4	204I			6
#6	yes	1	LAM	1.2×10^5	204I			3
#7		6	TDF + LAM	1.2×10^4	204I			6
#9	yes	3	ADV + LAM	1.0×10^6	204I			3
#10	yes	2	ADV + LAM	1.7×10^5	204V			6
#13		4	ADV + ETV	1.7×10^3	204V			3
#14	yes	4	ADV + ETV	3.4×10^4	204V			6
#15	yes	3	ADV + LAM	3.4×10^6	204V			6
#16	yes	4	ADV + LAM	1.0×10^5	204V			3
#17		4	ADV + ETV	1.5×10^2	204V			6
#18	yes	6	ADV + LAM	5.2×10^5	204V			6
#20		5	ADV + ETV	2.0×10^3	204V			3
#21	yes	3	ETV	1.2×10^4	204I			15
#34		2	TDF + LAM	1.6×10^3	204V			(18)
#42		6	TDF + LAM	9.0×10^4	204V			3
#43		4	ADV + ETV	4.1×10^5	204V			3
#55		4	ADV + ETV	9.1×10^1	204I			3
#5		3	ADV + LAM	3.4×10^6	181V			6
#11		2	ADV + LAM	8.6×10^8	181V			6
#28	yes	2	ADV + LAM	5.4×10^3	181T			6
#30		4	TDF	1.8×10^4	181V			12
#32		2	ADV	1.0×10^8	181V			9
#56	yes	3	ADV	1.1×10^{10}	181T			12
#54		3	ETV	7.6×10^2	181V	204V		3
#50		4	ADV + ETV	3.7×10^5	181V	204V		9
#4		4	ADV + LAM	1.2×10^9	181V	236T		6
#8	yes	1	ADV	8.6×10^5	181V	236T		6
#12	yes	2	TDF	1.2×10^3	181T	236T		3
#26	yes	2	ADV	1.1×10^{12}	181V	236T		(21)
#27	yes	3	ADV + LAM	5.6×10^4	181V	236T		3
#40		3	ADV	1.1×10^4	181V	236T		9
#2	yes	4	ETV	1.7×10^4	181V	204I	236T	9
#31	yes	5	TDF + LAM	3.3×10^2	181V	204V	236T	3
#36		5	TDF	6.9×10^2	181V	204I	236T	3
#22	yes	4	ETV	1.2×10^6	204V	250I		6
#44	yes	4	ETV	9.4×10^1	204V	250I		3
#24		3	ETV	2.5×10^5	184A	204V		6
#33		4	ETV	1.0×10^8	181V	204V	202S	9

again demonstrates the oncogenic potential of chronic HBV infection in advanced liver disease apart from complete viral suppression and calls for stringent HCC surveillance programs for early detection of HCCs in these patients [25].

Only one patient (Supplementary Fig. 1) showed a viral break through to 16, 600 IU/ml after 39 months of ETV–TDF combination treatment and 21 months of HBV-DNA negativity. Non-adherence to treatment was excluded by the medical practitioner and ETV–TDF combination therapy was continued. Without changing therapy, the patient became HBV DNA negative again after 9 months of HBV DNA detectability. Interestingly, the sequence analysis in this patient revealed a new ETV resistance mutation, which could not be detected before the start of combination treatment, suggesting that the combination therapy could

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not entirely prevent further development or spreading of viral resistance, indicating that a stringent detection of genotypic resistance is prudent in patients with viral break through.

Tenofovir is principally eliminated by the kidneys, and there have been reports of renal impairment with the use of TDF [24,26], although with the majority of cases reported in HIV patients. For entecavir, the risk of development of lactic acidosis in decompensated cirrhotic patients with high MELD score of more than 20 have been reported [24,27]. In this cohort, we could not detect significant changes in serum creatinine of more than 0.5 mg/dl during 2 years of observation except in one patient with pre-existing severe renal impairment. A limitation of this real life cohort is that a more thorough analysis of kidney function, i.e. significant changes in filtration rates, serum phosphorus, or calcium and others, was not available. Furthermore, tolerability failures were not systematically observed and in the present study, which measured lactate only if required for toxicity management, no adverse event of lactic acidosis was reported.

This is the first cohort study with ETV plus TDF combination therapy in patients with multidrug resistance HBV. Some short-term studies without known resistance patterns reported already that rescue therapy with combination of entecavir and tenofovir or entecavir and adefovir or tenofovir and lamivudine resulted in a complete virologic response in the majority of CHB patients with multidrug resistance [28–30]. Although further confirmatory long-term studies are definitely required since the number of studied patients so far is very small and the study designs suboptimal, these results suggest the importance of 'adequate' combination therapy in the setting of multidrug-resistant CHB employing the most potent antivirals that have high genetic barriers and potency and do not share cross-resistance profiles. Such therapy should be supported and tailored by genotypic assays to identify the profile of resistance mutations and their virologic evolution.

For mutants resistant to one NA, switching to another NA may exert a selective pressure on the viral quasispecies, allowing additional mutations to occur in preselected mutants, resulting in multidrug resistant HBV [10,31,32]. The mechanism of evolution of multidrug resistance may have implications on the efficacy of rescue therapy. If mutations causing resistance to nucleoside and nucleotide drugs are not on the same viral genome, a combination of two agents that do not show cross-resistance will likely be effective in suppressing mutants resistant to one of the drugs. In contrast, if the antiviral-resistance mutations are present on the same viral genome, combination treatment may not be adequate [13].

Oral therapies in chronic diseases such as arterial hypertension, diabetes and hypercholesterolemia suffer from problems of inconsistent adherence during long-term medications. Counseling of patients with CHB on medication adherence appears to be one

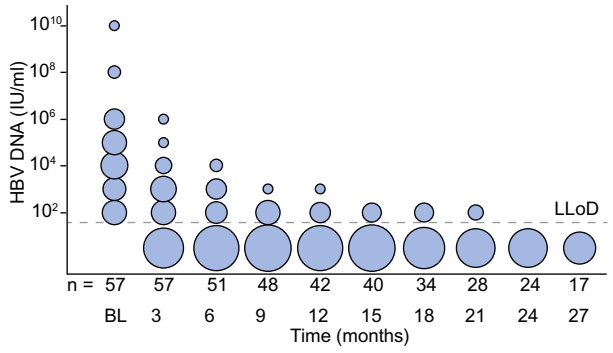

Fig. 1. Viremia during TDF–ETV combination therapy. Displayed are the HBV viremia levels over time. The area of the bubbles is linear, depending on the number of patients with the respective level of viremia. Antiviral treatment induced a median HBV DNA reduction of 3 logs (range, 0–8 log HBV DNA/ml; p <0.0001) with 51/57 (90%) of patients achieving HBV-DNA undetectability (LLoD 80 IU/ml).

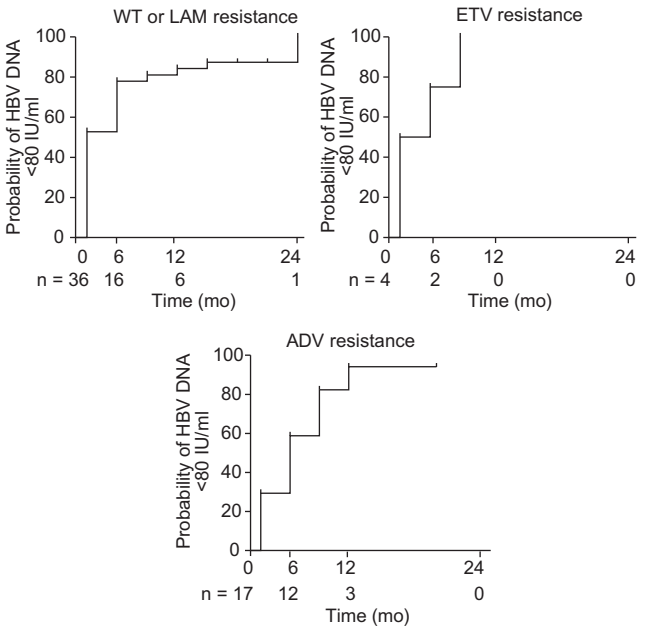

Fig. 2. Probability of HBV DNA below LLoD (80 IU/ml). A Kaplan–Meier analysis was used to analyze the probability of reaching HBV DNA undetectability. For the entire cohort, the median time to HBV-DNA undetectability was 6 months (95% CI 4.6–7 months). Displayed are the analyses for patients with (a) ADV, (b) ETV and (c) the remaining cohort. Interestingly, the presence of genotypic resistance to ADV (n = 17), or to ETV (n = 4), did not significantly reduce the time to HBV DNA negativity, compared to the remaining cohort (Log rank test; p = 0.8).

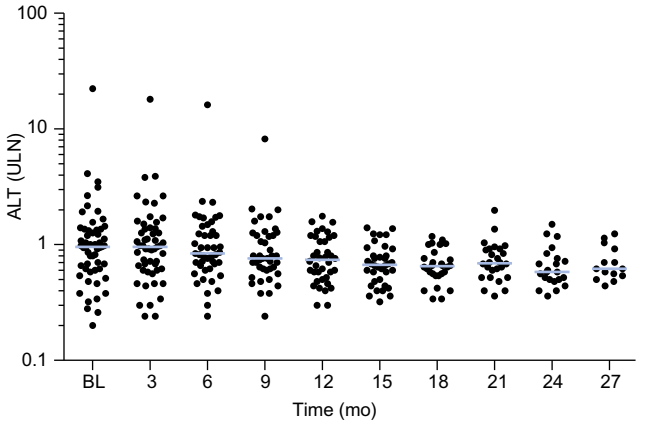

Fig. 3. ALT levels during ETV–TDF combination therapy. Displayed are the ALT levels [ULN] over time with a bar indicating the median value. The decrease of ALT during combination therapy is highly significant (p = 0.0002) and ALT normalization was achieved in 20 out of 27 patients (74%) with elevated ALT (>1 ULN) at baseline.

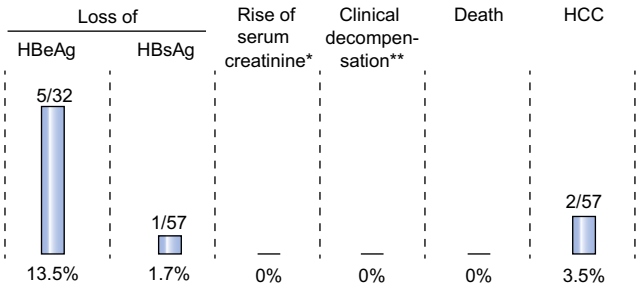

Fig. 4. Clinical outcome. HBeAg loss was observed in 5/32 HBeAg positive patients after a median treatment duration of 20 months (15, 18, 20, 21, and 27 months, respectively) and HBsAg loss occurred in one patient after 18 months of treatment. No significant clinical side effects were reported, especially no deaths, no newly induced renal impairment and no lactic acidosis. Two patients with liver cirrhosis developed an HCC. *Rise of more than 0.5 mg/dl in serum creatinine level. **Clinical decompensation was defined by an episode of jaundice, ascites, bleeding or development of encephalopathy.

of the most important aspects during oral antiviral therapy in CHB as well as sensitive measurements to confirm early on the detection of genotypic resistance. Not all of the patients studied in this cohort developed resistance or multidrug resistance against HBV. Some patients (Table 1) showed only a partial response and viral persistence during mono-therapies and for those patients the question remains if combination therapy is really needed because of true insufficient viral response. Indeed, we cannot rule out that patients showed a better adherence when their treating physicians switched to combination therapy in the sense of a "last chance" to stop the progression of the disease and we have no data other than clinical records on patient adherence. For some patients with possible drug holidays or failures to take the drugs accordingly to the package inserts (for example entecavir without food intake) this could have meant an unnecessary intensification in antiviral medication [9].

Another aspect that has not been investigated thoroughly is the question of duration of combination therapy. In some of the patients it might be possible to switch back from combination therapy to monotherapy at some point after HBV DNA became undetectable, at least in patients without detectable resistance and without advanced liver disease. Those patients should be monitored very closely for continuous viral suppression in a clinical study setting and they should be counseled for adherence.

In summary, we acknowledge that this study has its limitations by its relatively small sample size, the open label design and the lack of systematic data on medical adherence. However, the results of the study show that rescue therapy with the combination of entecavir and tenofovir in a very specific subset of difficult to treat patients with resistant HBV strains or only partial responses to preceding therapies is highly efficient and appears to be safe. Repeated counseling on medication adherence throughout the long-term antiviral CHB therapy and regular HBV DNA monitoring are needed for a fine balance between rapid initiation of rescue therapy versus avoidance of unnecessary changes in antiviral medication in CHB patients.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.09.018.

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