Optimizing Treatment Strategies Using Nucleos(t)ide Analogues for Patients with Chronic Hepatitis B

Jurriën Reijnders

Colofon

ISBN: 978-90-8559-095-8

Layout and printing: Optima Grafische Communicatie, Rotterdam, The Netherlands

Illustrated by: Ed Sammelius

The work presented in this thesis was conducted at the Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, the Netherlands.

Financial support for printing this thesis was kindly given by the Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, de Nederlandse Vereniging voor Gastroenterologie, Novartis, Gilead Sciences, Bristol-Myers Squibb and the J.E. Jurriaanse Stichting.

Optimizing Treatment Strategies Using Nucleos(t)ide Analogues for Patients with Chronic Hepatitis B

Antivirale behandeling van chronische hepatitis B: het optimaliseren van behandelstrategieën met nucleos(t)ideanalogen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. H.G. Schmidt
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 24 november 2010 om 15.30 uur

door

Jurriën Gerben Peter Reijnders

geboren te Klundert

PROMOTIECOMMISSIE

Promotor: Prof.dr. H.L.A. Janssen

Overige leden: Prof.dr. C.A.B. Boucher
Prof.dr. J.P. Drenth
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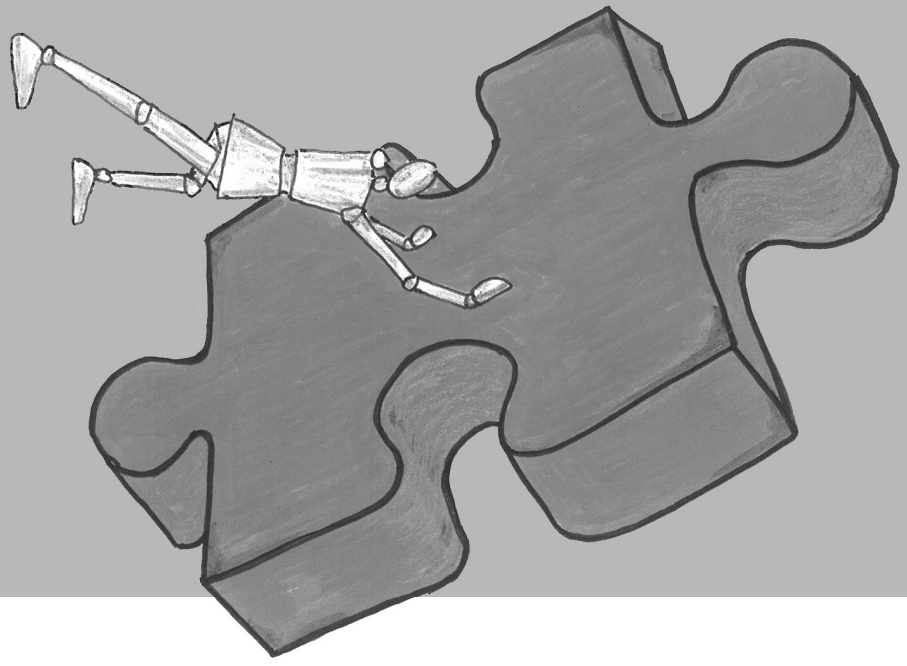

General introduction

Derived from:

Janssen HLA, Reijnders JGP *Treatment with nucleos(t)ide analogues in chronic hepatitis B: Where does the road map lead us?* J Hepatol. 2009 Jul;51(1):1-3

Reijnders JGP, Janssen HLA. *New approaches in the management of chronic hepatitis B: role of tenofovir*. Infection and Drug Resistance 2009; 2: 13-26

Reijnders JGP, Janssen HLA. *How effective is treatment with adefovir plus lamivudine for patients with lamivudine-resistant chronic hepatitis B infection?* Nature Clinical Practice Gastroenterology & Hepatology 2008;5(12):668-9

Reijnders JGP, Janssen HLA. *Potency of tenofovir in chronic hepatitis B: mono- or combination therapy?* J Hepatol 2008;48(3):383-6.

Hepatitis B infection has a complex natural history and causes a wide spectrum of disease.(1) Although effective vaccines are available, universal vaccination has yet not been reached. Currently, an estimated 350 million people are chronically infected, and 0.5-1.2 million subjects die every year due to long-term sequalae of hepatitis B related chronic liver disease, such as liver cirrhosis and hepatocellular carcinoma.(2) Despite evidence-based treatment guidelines areas of disagreement on the management of chronic hepatitis B virus (HBV) infection still exist.

With the currently approved treatment options the ultimate goal is to prevent the development of long-term sequelae of chronic liver disease. Current treatment strategies consist of either therapies with finite duration that aims to achieve sustained off-treatment remission (interferon-based therapy), or long-term therapy that aims to maintain on-treatment response (nucleos(t)ide analogues).(3-4)

Interferon therapy

Interferon (IFN) alfa has been used since the early 1990s for the treatment of chronic HBV infection. It largely acts through enhancement of the immunological response of the host against the virus, although there is also limited direct antiviral effect on HBV replication.(5) This immunomodulatory mode of action is reflected in higher rates of HBeAg- and HBsAg seroconversion, and a more durable response once treatment is discontinued (sustained response) compared to treatment with nucleos(t)ide analogues.(6-10) Among HBeAg-positive patients, subjects with HBV genotype A tend to respond much better than subjects with genotype non-A.(11) Follow-up studies demonstrated that IFN had long-term benefits in that it promotes cumulative HBeAg- and HBsAg seroconversion, prevention of cirrhosis and hepatocellular carcinoma, and prolonged survival.(6, 9) However, IFN-based therapy is associated with a wide spectrum of adverse events, including flu-like symptoms, emotional lability, and bone marrow depression. Still, only few patients require dose modification or discontinuation of treatment, and symptomatic therapy fulfils in most instances.(12) IFN is contraindicated in patients with decompensated liver cirrhosis, but has proven to be safe and effective in patients with advanced fibrosis and compensated liver cirrhosis.(13)

Nucleos(t)ide analogue therapy

The introduction of nucleos(t)ide analogues heralded a new era in the treatment of chronic hepatitis B, and provided a safe, effective, and well-tolerated alternative for IFN. Nucleos(t)ide analogues target the reverse transcriptase of hepatitis B virus and are potent inhibitors of viral replication. Current guidelines recommend that, in HBeAg-positive patients, treatment may be stopped after HBeAg seroconversion and at least six months of consolidation therapy. In HBeAg-negative patients, discontinuation is

only possible after HBsAg loss.(3-4) Therefore, in most patients treated with nucleos(t)ide analogues only a maintained remission seems possible, and nucleos(t)ide analogue therapy has to be administered for extremely long periods of time, if not indefinitely.

Development of antiviral resistance is a major limitation to long-term efficacy of nucleos(t)ide analogue therapy. It leads to reversion of virologic and histological improvement, and enhances the rate of disease progression.(14) One of the first manifestations of antiviral resistance is a virologic breakthrough which is defined as a > 1 $\log_{10}$ increase in serum HBV DNA from nadir during treatment in a patient who had an initial virologic response. It is usually also followed by a biochemical breakthrough.

It is currently recommended to start with monotherapy and to use an add-on strategy in case of development of resistant HBV mutants.(3-4, 15) However, in the last few years management of resistance has evolved, and treatment strategies and clinical guidelines have been developed, which are focused at prevention of antiviral drug resistance.(3, 16) First, only potent nucleos(t)ide analogues with a high genetic barrier, meaning drugs requiring multiple resistance mutations, should be used as monotherapy. Second, treatment should be adapted at an early stage in case of incomplete viral suppression, as several studies have already shown that an initial virologic response is associated with lower rates of antiviral drug resistance in HBV patients in the long term. A third option would be to offer de novo combination of nucleos(t)ide analogue therapy, although the clinical benefit in HBV-monoinfected patients will be difficult to demonstrate in the light of antiviral drugs with excellent resistance profiles.

The general aims of this thesis are (1) to explore the durability of nucleos(t)ide analogue-induced HBeAg seroconversion, (2) to investigate the efficacy of oral antivirals in the treatment-naïve chronic HBV population, and (3) to optimize treatment strategies using nucleos(t)ide analogues to combat antiviral drug resistance.

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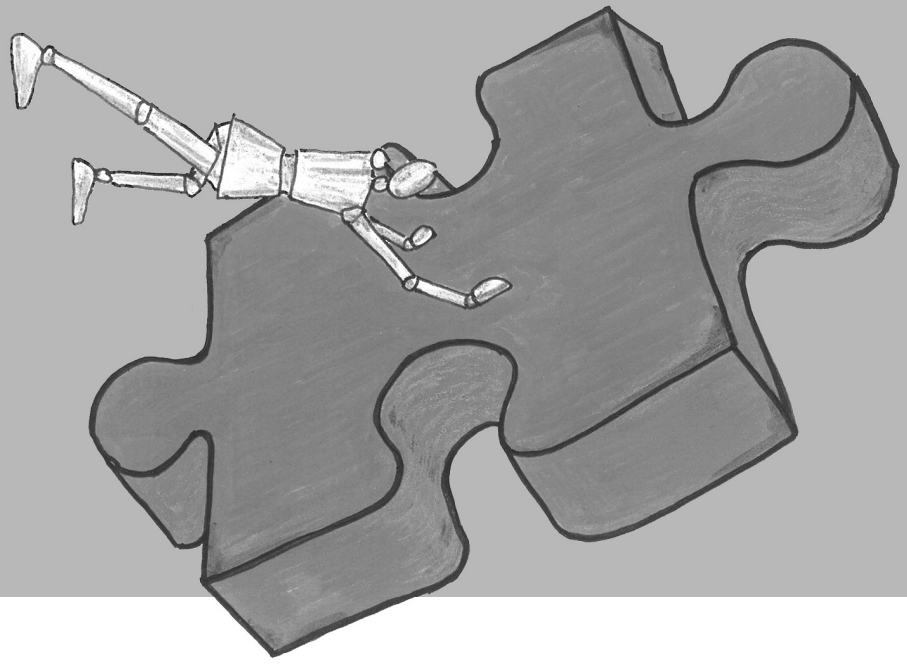

Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B

Jurriën G.P. Reijnders, Moniek J. Perquin, Ningping Zhang, Bettina E. Hansen, Harry L.A. Janssen

Gastroenterology. 2010 Aug;139(2):491-8

ABSTRACT

Background

Inconsistencies in results and guideline recommendations regarding the durability of nucleos(t)ide analogue-induced hepatitis B e antigen (HBeAg) seroconversion require clarification. We studied the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion in patients with chronic hepatitis B virus (HBV) infection.

Methods

We performed a single-center cohort study of 132 HBeAg-positive patients that had received nucleos(t)ide analogue therapy.

Results

During a median treatment duration of 26 months (16–43 months), HBeAg seroconversion occurred in 46 of 132 subjects (35%). Forty-two subjects (91%) had follow-up after HBeAg seroconversion. During a median follow-up of 59 months (28–103 months) after HBeAg seroconversion, 13 of 42 patients (31%) demonstrated a durable remission (defined as HBeAg negative and HBV DNA < 10,000 copies/mL). Overall, 33 of 42 subjects (79%) continued therapy after HBeAg seroconversion; of these, 22 (67%) demonstrated serological and/ or virological recurrence. Nine of 42 subjects (21%) discontinued therapy after HBeAg seroconversion and at least 6 months of consolidation therapy. Only 2 patients demonstrated a durable response in the absence of therapy. Disease recurrence in patients that continued therapy after HBeAg seroconversion was preceded by the development of resistance (80% of these patients); resistance only occurred in subjects given lamivudine monotherapy. In contrast, recurrence after treatment discontinuation or non-compliance was observed in all patients given nucleos(t)ide analogues.

Conclusions

Induction of HBeAg seroconversion by nucleos(t)ide analogues is temporary in most patients with chronic HBV infection. Long-term continuation of nucleos(t)ide analogue treatment, irrespective of the occurrence of HBeAg seroconversion, appears to be necessary.

INTRODUCTION

With approximately 400 million persons infected worldwide, chronic hepatitis B virus (HBV) infection is a major challenge in medical health care. The current treatment aims to prevent development of liver cirrhosis and hepatocellular carcinoma.(1) As these endpoints can only be assessed after decades of infection, short-term surrogate outcomes are used to assess the effect of therapeutic regimens. In HBeAg-positive patients, an important endpoint is HBeAg loss or seroconversion, as it is usually associated with sustained remission and a low risk for the development of cirrhosis and hepatocellular carcinoma.(2-7)

Nucleos(t)ide analogues interfere with the elongation of viral DNA chains through competitive inhibition with the viral polymerase, and are potent inhibitors of viral replication. (8) In general, HBeAg seroconversion rates are approximately 20% after one year of nucleos(t)ide analogue treatment, and these rates increase with prolonged therapy. International guidelines on the therapy of hepatitis B suggest that finite duration of treatment with nucleos(t)ide analogues is a reasonable option, and recommend that treatment may be stopped after HBeAg seroconversion and an additional 6 to 12 months of consolidation therapy.(9-10)

However, the long-term durability of HBeAg seroconversion induced by nucleos(t) ide analogues is controversial, as several studies have reported contradictory results. Whereas some authors reported nucleos(t)ide analogue-induced HBeAg seroconversion to be durable in up to 90%, others reported relapse rates as high as 70%.(11-13) Studies investigating durable response to newer agents as entecavir (ETV) and tenofovir (TDF) are still scarce. Furthermore, off-treatment response data for nucleos(t)ide analogues are available for relatively short periods only, whereas interferon (IFN)-induced response has been documented to be durable for years with increasing numbers of HBsAg seroconversion. (14-16)

Clearly, inconsistencies in results and guideline recommendations regarding the durability of nucleos(t)ide analogue-induced HBeAg seroconversion require clarification. We therefore studied the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion.

METHODS AND MATERIALS

Study population

All adult patients with chronic HBV infection (HBsAg-positivity for at least 6 months), referred to the Erasmus Medical Center between 1996 and 2009, who were treated with nucleos(t)ide analogue therapy for at least 6 months, and were tested HBeAg positive,

anti-HBe negative at the start of treatment, were eligible. Major exclusion criteria were: co-infection with hepatitis C virus, hepatitis D virus or human immunodeficiency virus and treatment with (pegylated) interferon less than 6 months prior to start of nucleos(t)ide analogue treatment.

Follow-up

All subjects were monitored at least every 3-6 months. Biochemical (serum alanine aminotransferase) and virological parameters (quantitative HBV DNA, HBeAg, and anti-HBe) were assessed at every visit. HBV genotype was determined in available serum samples. HBsAg and anti-HBs values were determined in all patients who were HBeAg-negative with HBV DNA levels < 1,000 copies/mL at last follow-up. A genotypic analysis was performed in case of virological breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir after initial virological response. Patients were asked to confirm their adherence to their treatment regimen at all outpatient clinic visits. Patients were defined as non-compliant if (1) unexpected virological rebounds were observed, and (2) the treating physician clearly judged the patient to be non-adherent.

Endpoints

HBeAg seroconversion was defined as loss of HBeAg with concurrent appearance of anti-HBe. Serological recurrence was defined as reappearance of HBeAg confirmed by HBeAg positivity in a consecutive sample. As a viral load of more than 10,000 copies/mL is associated with progression of liver disease(17-18), virological recurrence was defined as a rise of HBV DNA to above 10,000 copies/mL after HBeAg seroconversion having previously been below 10,000 copies/mL, confirmed in a consecutive sample.

Laboratory tests

Serum alanine aminotransferase (ALT) was measured using automated techniques. The upper limit of normal (ULN) was 40 IU/L for male and 30 IU/L for female subjects. Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and anti-body against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. Serum HBV DNA levels were measured using a previously described in house developed quantitative real-time polymerase chain reaction (PCR).(19-20) Currently, this assay is multiplexed without compromising the lower limit of detection (373 copies/mL) with an internal control (pHHV) in order to control the process from DNA isolation through PCR.(21) HBV DNA was extracted from serum samples using the MagnaPureLC (Roche Applied Science, Almere, The Netherlands) as described before.(19) HBV genotypes were assessed by sequence alignment of the overlapping hepatitis B surface antigen with HBV sequences derived from GenBank. Presence of

HBV polymerase gene mutations was determined using InnoLiPA DR2 and DR3 line probe assay (Innogenetics, Gent, Belgium).

Statistical analysis

Continuous variables are expressed as means (± SD) or median (interquartile range) where appropriate. Follow-up times were calculated from the date of nucleos(t)ide analogue treatment initiation or HBeAg seroconversion to the date of event or censorship. Cumulative probabilities of different endpoints were estimated by Kaplan-Meier analysis. Survival analysis with Cox regression model was used to analyze which of the following baseline factors were associated with HBeAg seroconversion and with serological and/or virological recurrence after development of HBeAg seroconversion: Age, gender, race, body mass index (BMI), HBV genotype, time to HBeAg seroconversion, viral load at initiation of nucleos(t)ide analogue therapy and at HBeAg seroconversion, ALT level at initiation of nucleos(t)ide analogue therapy and at HBeAg seroconversion, presence of cirrhosis, and treatment regimen (lamivudine (LAM) monotherapy vs. other treatment regimens).

RESULTS

Baseline characteristics of all patients are shown in table 1. A total of 132 patients with chronic HBV infection treated with nucleos(t)ide analogues in our hospital were included for analysis. Overall, 67 patients were treated with LAM monotherapy, 33 with adefovir (ADV), 22 with ETV, 6 with TDF, 2 with ADV-LAM combination therapy and 2 with TDF-LAM. One hundred seventeen (89%) patients were nucleos(t)ide analogue treatment-naïve, whereas 15 (11%) subjects had previously been treated with nucleos(t)ide analogues. Median duration of therapy was 26 (16-43) months. Of all patients, 100 (76%) subjects were men and the mean age was 38±15 years. Most common HBV genotypes were genotype A (35%) and D (26%).

HBeAg seroconversion

HBeAg loss occurred in 55 of 132 (42%) subjects, of whom 46 (84%) patients seroconverted to anti-HBe during the study period. The cumulative probabilities of achieving HBeAg seroconversion after 1, 2 and 4 years of treatment were 25%, 32% and 44%, respectively (Fig. 1). Median duration of therapy until HBeAg seroconversion was 7 (3-14) months. In multivariate analysis, independent predictors of HBeAg seroconversion were high serum ALT values (HR 1.11 per 1×ULN increase, 95%CI 1.07-1.14, $P<0.001$), and low HBV DNA levels (HR 0.81 per 1 $\log_{10}$ increase, 95%CI 0.68-0.96, $P=0.02$) at baseline.

Table 1 Baseline characteristics (N=132)

Age (years)	38 ± 15
Gender (male %)	100 (76%)
Race	
Caucasian	77 (58%)
Asian	41 (31%)
Other	14 (11%)
BMI	24 ± 4.1
ALT (xULN)*	2.3 (1.4-4.8)
HBV DNA (log_{10} copies/ml)	8.2 ± 1.6
Genotype (N=127)#	
A	45 (35%)
B	25 (20%)
C	19 (15%)
D	33 (26%)
Other	5 (4%)
Cirrhosis	23 (17%)
Treatment course	
Lamivudine	67 (51%)
Adefovir	33 (25%)
Entecavir	22 (17%)
Other	10 (7%)
Nucleos(t)ide analogue treatment naïve	117 (89%)

* Median and interquartile range; # In five patients no serum samples with detectable HBV DNA were available any more.

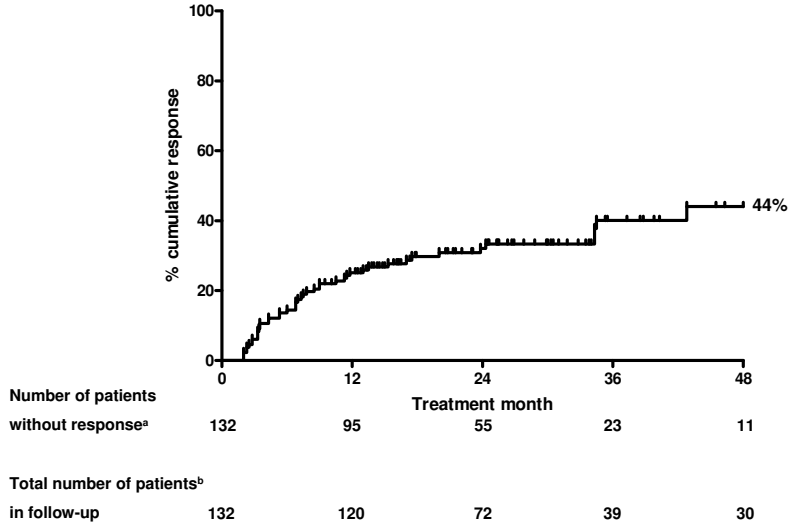

Figure 1 Kaplan-Meier curve for the cumulative probabilities of achieving HBeAg seroconversion during nucleos(t)ide analogue therapy. (a) Number of patients who have not achieved HBeAg seroconversion and are still in follow-up. (b) Number of patients who are still in follow-up, whether or not they have achieved HBeAg seroconversion.

Serological recurrence after HBeAg seroconversion

Follow-up after HBeAg seroconversion was available in 42 of the 46 (91%) subjects, and analysis of recurrence was limited to these 42 patients. Median follow-up time after HBeAg seroconversion for these 42 subjects was 59 (28-103) months. Serological recurrence occurred in 17 of 42 (41%) patients. Cumulative serological recurrence rates at 1, 2 and 4 years after nucleos(t)ide analogue induced HBeAg seroconversion were 17%, 31%, and 44% respectively (Fig. 2). Median duration between HBeAg seroconversion and recurrence was 13 (6-25) months. In 13 (76%) cases serological recurrence occurred more than 6 months after HBeAg seroconversion, in 10 (59%) more than 1 year after HBeAg seroconversion.

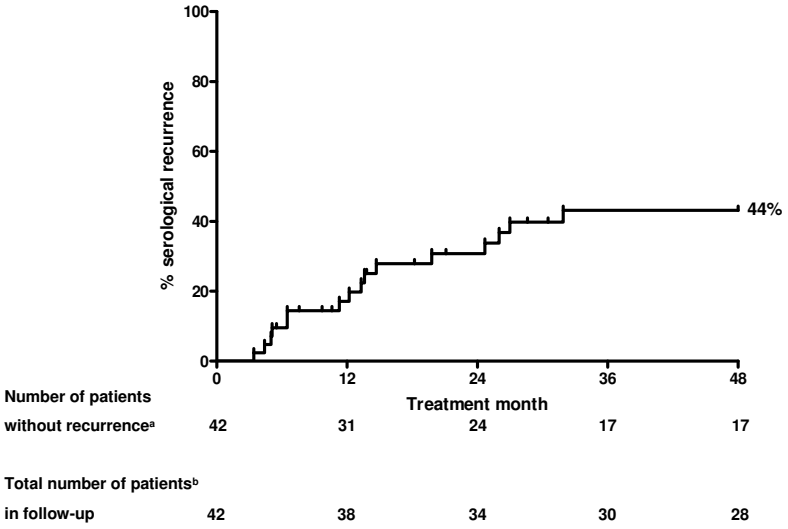

Figure 2 Kaplan-Meier curve for the cumulative probabilities of developing serological recurrence, defined as reappearance of HBeAg confirmed by HBeAg positivity in a consecutive sample, after HBeAg seroconversion. (a) Number of patients who have not developed serological recurrence and are still in follow-up. (b) Number of patients who are still in follow-up, whether or not they have developed serological recurrence.

Virological recurrence after HBeAg seroconversion

Eight of 42 (19%) patients had ongoing viral replication with HBV DNA levels > 10,000 copies/mL despite the achievement of HBeAg seroconversion, and directly progressed to chronic HBeAg-negative HBV infection. In 6 of 8 patients resistance to LAM was detected shortly before the development of HBeAg seroconversion. Two patients (LAM and ADV monotherapy) demonstrated a partial virological response and never achieved HBV DNA levels less than 10,000 copies/mL throughout follow-up. In addition, virological recurrence occurred in 19 of 34 (56%) remaining subjects after the development of HBeAg seroconversion. Cumulated virological recurrence rates at

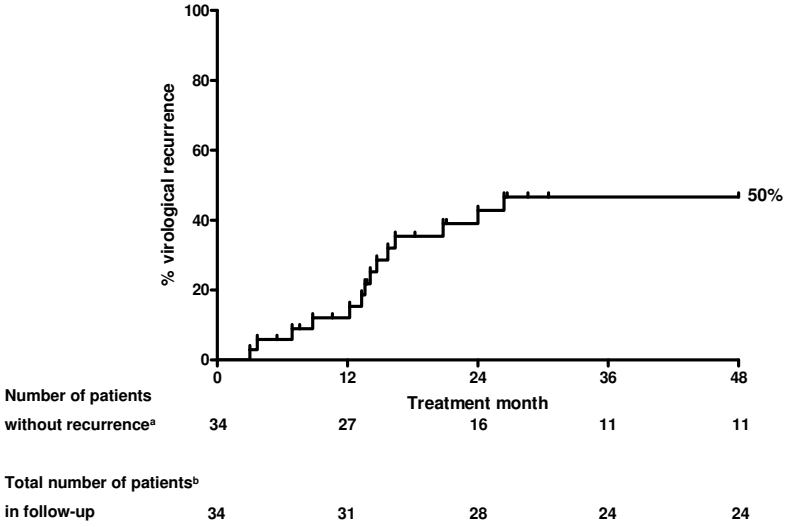

Figure 3 Kaplan-Meier curve for the cumulative probabilities of developing virological recurrence, defined as a rise of HBV DNA to above 10,000 copies/mL after HBeAg seroconversion having previously been below 10,000 copies/mL, confirmed in a consecutive sample. (a) Number of patients who have not developed virological recurrence and are still in follow-up. (b) Number of patients who are still in follow-up, whether or not they have developed virological recurrence. Eight patients had ongoing viral replication with HBV DNA levels > 10,000 copies/mL despite the achievement of HBeAg seroconversion, and were excluded from this analysis.

1, 2 and 4 years after nucleos(t)ide analogue induced HBeAg seroconversion were 12%, 42% and 50%, respectively (Fig. 3). Of these 19 patients, 15 reverted to chronic HBeAg-positive HBV infection (12 on-treatment; 3 off-treatment), and 4 progressed to chronic HBeAg-negative HBV infection (all off-treatment). Median duration between HBeAg seroconversion and virological recurrence was 16 (12-26) months. Seventeen (89%) cases of virological recurrence occurred more than 6 months after HBeAg seroconversion, 15 (79%) cases occurred more than 1 year after HBeAg seroconversion.

Durable response

Among the 42 patients who had HBeAg seroconversion during nucleos(t)ide analogue therapy, only 13 (31%) had a durable response, as defined by continued absence of HBeAg and HBV DNA levels below 10,000 copies/mL, during a median follow up of 59 months (28-103 months) after HBeAg seroconversion. Serological or virological recurrence was significantly more frequent among LAM-treated subjects compared to all others (HR 3.54, 92% CI 1.33-9.42, P = 0.005). No other factors were associated with recurrence. Overall, 33 of 42 subjects (79%) continued nucleos(t)ide analogue therapy after HBeAg seroconversion; of these, 22 (67%) demonstrated serological and/or virological recurrence during follow-up. Disease recurrence in patients that continued

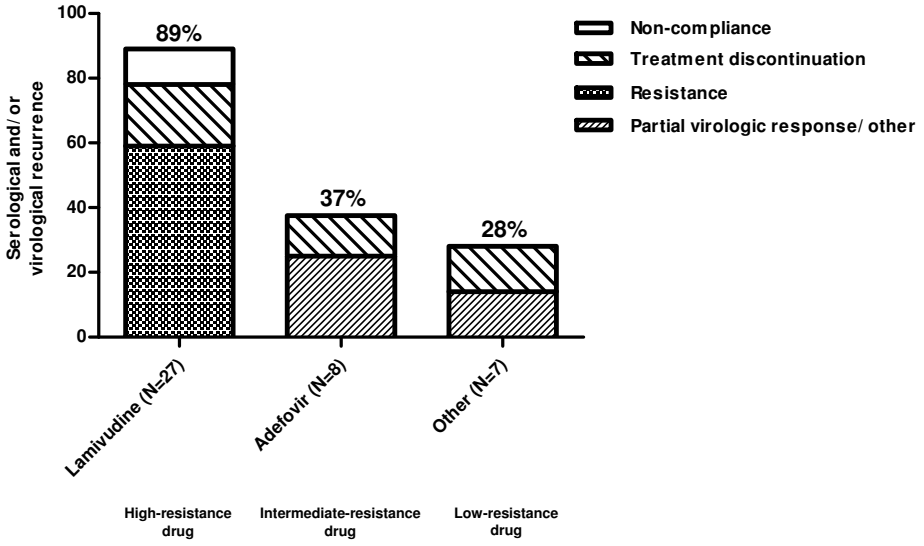

Figure 4 Proportion of patients who developed serological/ and or virological recurrence after nucleos(t)ide analogue induced HBeAg seroconversion, and causes of serological and/ or virological recurrence, specified per treatment regimen.

antiviral therapy after HBeAg seroconversion, was preceded by the development of antiviral drug resistance in 80% of these patients; resistance only occurred in subjects given LAM monotherapy. Nine of 42 subjects (21%) discontinued nucleos(t)ide analogue therapy after HBeAg seroconversion and at least six months of consolidation therapy. Only two patients demonstrated a durable response in the absence of therapy. The clinical features of the nine patients who stopped therapy after HBeAg seroconversion are summarized in Table 2. Among the seven with recurrence after HBeAg seroconversion, five had received LAM, one had received ETV and one ADV. The causes of the recurrence are shown by treatment regimen in Figure 4. Most recurrences in patients treated with LAM were attributable to antiviral resistance, whereas recurrences after HBeAg seroconversion on ADV or other agents were due to discontinuation of treatment or unknown.

HBsAg loss

Four of the 132 treated patients became HBsAg negative during or after treatment. Interestingly, three of these patients had demonstrated recurrence after seroconversion but then were retreated and again had an HBeAg seroconversion followed by loss of HBsAg. All four patients remained HBsAg-negative (and without detectable serum HBV DNA) even after treatment discontinuation (for 3 to 45 months).

Table 2 Summary of patients who discontinued treatment after HBeAg seroconversion

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Therapy	LAM	LAM	LAM	ETV	ADV	LAM	LAM	TDF	LAM
Age	36	45	34	27	59	31	16	29	26
Gender	Male	Male	Male	Male	Male	Male	Male	Female	Female
Genotype	C	B	D	B	A	C	D	B	D
Race	Asian	Caucasian	Caucasian	Asian	Caucasian	Asian	Caucasian	Asian	Caucasian
Cirrhosis	No	Yes	No	No	No	No	No	No	No
HBV DNA(log_{10} c/mL) at baseline	8.3	6.9	8.5	5.6	8.2	9.0	7.7	6.3	8.1
ALT (xULN) at baseline	13.3	1.3	1.9	1.7	2.0	12.4	2.8	2.8	1.6
Total duration of treatment(months)	28	14	19	19	55	21	60	42	72
Duration of consolidation treatment(months)	24	12	8	10	51	17	52	29	19
Months between end of treatment and recurrence/end of follow-up	2	4	7	18	4	1	2	29	51
Serological relapse	Yes	Yes	Yes	No	No	No	No	No	No
Virological relapse	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
HBV DNA(log_{10} c/mL) at relapse	8.8	9.7	11.0	4.1	5.3	6.8	5.3	--	--
ALT (xULN) at relapse	7.8	10.2	28.0	0.8	0.6	12.1	0.6	--	--
HBsAg loss	No	No	No	No	No	Yes*	No	Yes	No

* Reinstitution of lamivudine therapy resulted again in HBeAg seroconversion, and subsequent HBsAg loss during maintained antiviral therapy.

DISCUSSION

Our study explores the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion. During a median follow-up of five years after the development of nucleos(t)ide analogue induced HBeAg seroconversion, only 31% of patients demonstrated a durable remission, defined as HBeAg negativity and HBV DNA levels less than 10,000 copies/mL. Moreover, sustained response was demonstrated in just two of nine patients who discontinued therapy after a consolidation therapy of at least 6 months. On-treatment recurrence was preceded by the development of antiviral drug resistance in 80% of patients, which only occurred in subjects treated with LAM monotherapy. In contrast, recurrence after treatment discontinuation or non-compliance was observed with all nucleos(t)ide analogues.

Previous studies on the durability of nucleos(t)ide analogue induced HBeAg seroconversion reported contradictory results. Studies performed in Western countries reported HBeAg seroconversion achieved during nucleos(t)ide analogue treatment to be durable in 80-90% of cases (11, 13), whereas other studies, mainly performed in Asian countries, showed relapse of viral activity in approximately half of patients with nucleos(t)ide analogue induced HBeAg seroconversion.(12, 22-31) The reasons for these discrepancies are not clear. Explanations may be HBV genotype, different lengths of consolidation therapy and follow-up, as well as the use of different endpoints. Both Dienstag et al. (11) and Poynard et al.(13), who reported high rates of durability after 1-3 years of follow-up, only looked at HBeAg seroconversion itself. Although the first study mentioned that 22 of 30 patients with a durable HBeAg seroconversion had undetectable HBV DNA, it should be noticed that an insensitive HBV DNA assay was used, and that five patients did not demonstrate a biochemical remission at the end of follow-up. Moreover, a potential selection bias exists in this study, as only patients with a durable HBeAg seroconversion for at least 12-16 weeks after cessation of LAM treatment, were included in the analysis. The second study was only published as an abstract.[13] In contrast, studies that reported higher relapse rates also included serum HBV DNA in their definition of relapse, which is an important and reliable marker of viral activity and progression of liver disease.(17-18) In our study, both serological and virological recurrence after HBeAg seroconversion were considered and long follow-up was present. A durable remission after HBeAg seroconversion, whether on- or off-treatment, was only achieved in a minority of patients, as approximately 70% of patients demonstrated either serological or virological recurrence.

Current international guidelines suggest that treatment with nucleos(t)ide analogues can be discontinued after 6-12 months of consolidation therapy following HBeAg seroconversion. (9-10) In agreement with these recommendations, nine of our patients discontinued nucleos(t)ide analogue therapy after HBeAg seroconversion and at least

6 months of consolidation treatment. However, during long-term follow-up only two patients demonstrated a sustained off-treatment response, and remained HBeAg negative with HBV DNA levels < 10,000 copies/mL in absence of therapy.

HBsAg loss results in a more stable remission of chronic HBV infection compared to HBeAg loss or seroconversion. In our study, four of 132 patients developed HBsAg loss during nucleos(t)ide analogue therapy. All four patients demonstrated sustained HBsAg negativity after treatment discontinuation. These results might indicate that HBsAg loss is probably a more suitable end point instead of HBeAg loss or seroconversion, and should be one of our primary treatment goals in patients with HBeAg-positive disease treated with oral antivirals as well.(32)

A limitation of our study is that the majority of patients in our study was treated with LAM monotherapy, and developed recurrence before treatment was discontinued. Only a limited number of patients were treated with either TDF or ETV monotherapy, which are nowadays considered to be our first-choice nucleos(t)ide analogues, and conclusions on these newer compounds should be made with caution. As a significant proportion of on-treatment recurrence was related to the emergence of antiviral drug resistance, which was only observed in patients treated with LAM monotherapy, one might expect that nucleos(t)ide analogues with a good resistance profile will result in lower on-treatment recurrence. Yet, this observation may also point out that when the suppressive effect of an oral antiviral agent is omitted, whether by discontinuation of therapy or by development of resistance, recurrence is likely to occur. Indeed, recurrence was observed with all nucleos(t)ide analogue once treatment was discontinued or the patient was regarded to be non-compliant. Our data, therefore, might suggest that the occurrence of high recurrence rates after HBeAg seroconversion is not limited to LAM-treated patients but applies to treatment with newer agents as well.

In conclusion, the current study showed that induction of HBeAg seroconversion by nucleos(t)ide analogues is temporary in most patients with chronic HBV infection. These findings indicate that HBeAg seroconversion, in contrast to what current guidelines suggest, is an imperfect endpoint in assessing nucleos(t)ide analogue therapy. Therefore, long-term continuation of nucleos(t)ide analogue treatment, irrespective of the occurrence of HBeAg seroconversion, appears to be necessary.

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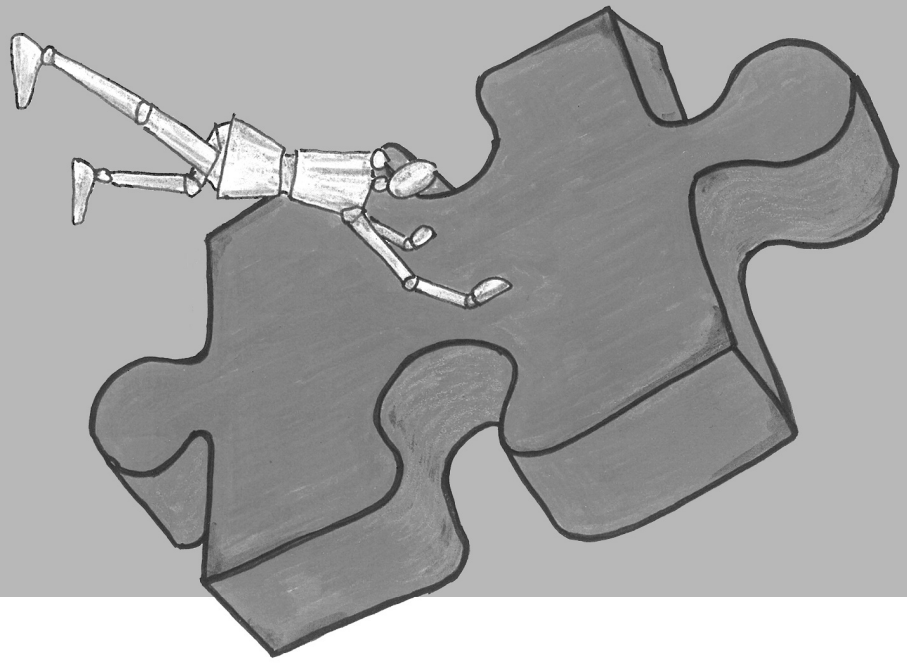
ACKNOWLEDGEMENT

We thank the department of Virology, Erasmus MC University Medical Center Rotterdam, for performing virological testing. We thank Prof. Wang, department of Gastroenterology, Zong Shan Hospital, Fudan University, Shanghai, China, for her contribution to the manuscript.



Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir

Jurriën G.P. Reijnders, Vincent Rijckborst, Milan J. Sonneveld, Sandra M.J. Scherbeijn, Charles A.B. Boucher, Bettina E. Hansen, Harry L.A. Janssen

Journal of Hepatology. 2010 Accepted.

ABSTRACT

Background

We aimed to investigate serum hepatitis B surface antigen (HBsAg) levels in patients with chronic hepatitis B virus (HBV) infection during peginterferon (PEG-IFN) and entecavir (ETV) monotherapy.

Methods

HBsAg was quantified (Abbott ARCHITECT) at baseline and during antiviral therapy (weeks 12, 24, 36, 48) in hepatitis B e antigen (HBeAg-)positive patients treated with ETV (N=33) or PEG-IFN (N=61) and in HBeAg-negative patients treated with ETV (N=37) or PEG-IFN (N=69).

Results

Within the HBeAg-positive population, the degree of HBsAg decline did not differ significantly between patients treated with PEG-IFN and ETV (mean decline 0.94 versus 0.38 log IU/mL at week 48, p=0.15). HBeAg clearance occurred more often in patients treated with PEG-IFN compared to ETV, and HBsAg decline was larger in those who became HBeAg negative, irrespective of treatment regimen. A decline of HBsAg was confined to ETV-treated patients with elevated baseline alanine aminotransferase (ALT) levels, whereas HBsAg decline was not associated with baseline ALT in patients treated with PEG-IFN. Within the HBeAg-negative population, PEG-IFN induced a significant HBsAg decline, while HBsAg did not decrease in ETV-treated patients (0.56 versus -0.10 log IU/mL, p<0.001). Both in HBeAg-positive and HBeAg-negative patients, decline of serum HBV DNA was larger in patients who received ETV compared with PEG-IFN.

Conclusions

In HBeAg-positive patients, decline of serum HBsAg is largely confined to patients who clear HBeAg, either induced by PEG-IFN or ETV. In HBeAg-negative patients, PEG-IFN therapy resulted in a significant reduction in HBsAg levels, whereas HBsAg did not decrease in ETV-treated patients.

INTRODUCTION

Chronic hepatitis B (CHB) can be controlled in most patients with the currently available treatment options, but complete eradication of the hepatitis B virus (HBV) is rarely achieved. HBV covalently closed circular DNA (cccDNA) plays a major role in viral persistence and its clearance is thought to be the limiting factor in eliminating infection (1). Previous studies demonstrated that both (pegylated) interferon (IFN) and nucleos(t)ide analogue (NA) therapy result in a reduction of intrahepatic cccDNA (2-4). In addition, intrahepatic cccDNA was shown to be a strong predictor of sustained off-treatment virological response (5). Serum hepatitis B surface antigen (HBsAg) levels are known to reflect cccDNA in the liver, and reduction of HBsAg levels correlates well with that of cccDNA (2-4).

HBsAg clearance from serum approximates clinical cure and is associated with improved survival (6). The kinetics of HBsAg decline have recently been described in patients treated with standard IFN, PEG-IFN, lamivudine (LAM), and adefovir (ADV) monotherapy (2, 7-8). It was demonstrated that measurement of the serum HBsAg concentration during therapy may allow the identification of sustained responders to PEG-IFN more reliably than serum HBV DNA (9). However, the effect of potent NA such as entecavir (ETV) and tenofovir (TDF) on serum HBsAg levels is unknown. Furthermore, the efficacy of PEG-IFN in terms of HBsAg decline was only compared to inferior oral agents such as LAM and ADV. The aim of our study was (1) to assess on-treatment serum HBsAg kinetics in hepatitis B e antigen (HBeAg-)positive and HBeAg-negative CHB patients treated with PEG-IFN or ETV monotherapy, (2) to compare the efficacy of PEG-IFN and ETV monotherapy in terms of HBsAg decline, and (3) to identify baseline factors associated with HBsAg decline after 48 weeks of antiviral therapy.

METHODS AND MATERIALS

Study population

We studied all consecutive HBV-monoinfected patients treated with ETV monotherapy for at least 48 weeks between January 2005 and May 2008 at the Erasmus MC University Medical Center Rotterdam. Patients treated with PEG-IFN monotherapy were derived from two randomized controlled trials (total treatment duration 48 and 52 weeks) (10-11). The studies conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained from all patients.

Laboratory tests

Patients attended the outpatient clinic at least every 12 weeks for routine examinations and laboratory assessments. Serum alanine aminotransferase (ALT) levels were measured using automated techniques and are expressed as values representing a ratio to the upper limit of the normal range (ULN). Determination of HBeAg and antibody against HBeAg (anti-HBe) status was performed using commercially available enzyme immunoassays. Serum HBsAg was quantified at baseline and during antiviral therapy (weeks 12, 24, 36 and 48) using the ARCHITECT HBsAg assay (Abbott laboratories; range 0.05-250 IU/mL). Serum HBV DNA levels were measured using commercial TaqMan polymerase chain reaction (PCR) assays (Roche Molecular Systems; lower limit of detection 70 copies/mL), except for the HBeAg-positive patients treated with PEG-IFN for whom an in-house-developed TaqMan PCR assay based on the EuroHep standard was used (lower limit of detection 400 copies/mL) (12). It has previously been demonstrated that there is an excellent correlation between these assays (13).

Statistical analysis

Group-matching between the ETV and PEG-IFN groups was performed by their baseline HBV DNA concentration and aimed at a 2:1 ratio in order to increase power, as described by Pocock (14). Baseline HBV DNA was selected, because this factor was previously found to be associated with the degree of HBsAg decline during PEG-IFN therapy (7).

Serum HBsAg and HBV DNA levels were logarithmically transformed for analysis. Continuous variables are presented as mean (standard deviation) or median (interquartile range), where appropriate. The lower limit of detection of 400 copies/mL of the in-house PCR assay was applied to all HBV DNA results to allow comparison between the treatment groups. Continuous variables were compared using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square or Fisher's exact test. The association between baseline factors and the degree of HBsAg decline was assessed by linear regression analyses applying mixed modelling techniques with a random intercept and a random slope per subject, and with a covariance structure depending on the treatment regimen. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the SAS 9.2 program (SAS Institute Inc., Cary, NC, USA). All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

Baseline characteristics

At total of 200 HBV-infected patients were included. The HBeAg-positive population consisted of 61 patients treated with PEG-IFN and 33 patients treated with ETV. The HBeAg-negative population consisted of 69 patients treated with PEG-IFN and 37 ETV-treated patients (Fig.1). Baseline characteristics are presented in Table 1. HBeAg-positive patients treated with PEG-IFN and ETV were comparable at baseline except for serum ALT level, distribution of HBV genotypes and prevalence of liver cirrhosis (Table 1). Within the HBeAg-negative population, the treatment groups were balanced except for ethnicity, distribution of HBV genotypes and prevalence of liver cirrhosis (Table 1). HBeAg-positive patients tended to be younger (38 versus 41 years, p=0.10) and had higher baseline serum HBV DNA and HBsAg levels compared with HBeAg-negative patients (8.4 versus 6.8 log copies/mL for HBV DNA and 4.2 versus 3.8 log IU/mL for HBsAg, p<0.001 for both comparisons), while median ALT levels were similar (2.3 versus 2.5 ULN, p=0.42). Baseline serum HBsAg and HBV DNA levels showed a significant positive correlation in HBeAg-positive patients (R=0.54, p<0.001), while HBsAg and HBV DNA were not correlated in HBeAg-negative patients (R=0.09, p=0.36).

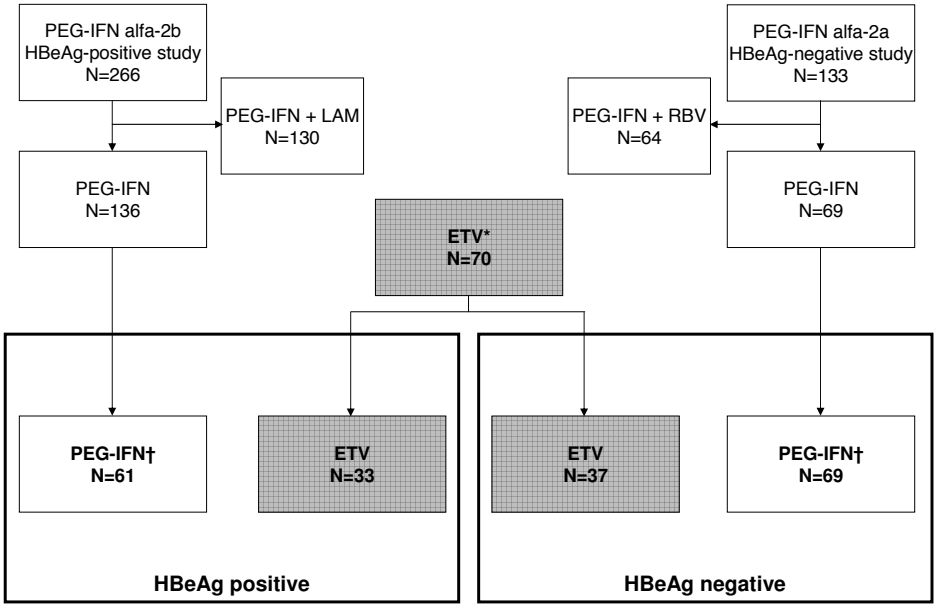


Figure 1 Study profile. *All consecutive patients treated with ETV monotherapy for at least 48 weeks were included; †Patients treated with PEG-IFN monotherapy were randomly selected from two randomized controlled trials [10,11], the PEG-IFN and ETV groups were group-matched according to their baseline HBV DNA level. RBV: ribavirin.

Table 1 Baseline characteristics

Characteristics	HBeAg positive			HBeAg negative		
	PEG-IFN (N=61)	ETV (N=33)	p	PEG-IFN (N=69)	ETV (N=37)	p
Mean (SD) age, years	38 (13)	38 (15)	0.93	42 (10)	40 (12)	0.59
Sex, male (%)	51 (84%)	27 (82%)	0.83	54 (78%)	28 (76%)	0.76
Race (%)			0.12			<0.001
Caucasian	44 (72%)	22 (67%)		66 (96%)	19 (51%)	
Asian	15 (25%)	6 (18%)		1 (1%)	9 (24%)	
Other	2 (3%)	5 (15%)		2 (3%)	9 (24%)	
Median (IQR) ALT*	3.2 (2.3-5.4)	1.5 (1.1-2.4)	<0.001	2.4 (1.7-4.1)	2.2 (1.5-3.3)	0.26
Mean (SD) HBV DNA, log copies/mL	8.6 (1.1)	8.0 (2.1)	0.09	6.9 (1.2)	6.7 (1.7)	0.67
Mean (SD) HBsAg, log IU/mL	4.2 (0.8)	4.3 (0.7)	0.51	3.8 (0.6)	3.7 (0.7)	0.41
HBV Genotype (%)			0.04			<0.001
A	29 (48%)	13 (39%)		7 (10%)	5 (14%)	
B	0	4 (12%)		0	3 (8%)	
C	11 (18%)	2 (6%)		1 (1%)	8 (22%)	
D	14 (23%)	10 (30%)		58 (84%)	20 (54%)	
Other/mixed	7 (12%)	4 (12%)		3 (4%)	1 (3%)	
Presence of cirrhosis (%)	9 (15%)	12 (36%)	0.02	2 (3%)	9 (24%)	0.002

*Multiples of upper limit of the normal range

HBeAg-positive patients

Virological and biochemical response rates

Within the HBeAg-positive population, PEG-IFN therapy resulted in a higher rate of HBeAg clearance at week 48 compared to ETV (21 (34%) versus 3 (9%) patients, p=0.007; Table 2). HBsAg loss (HBsAg <0.05 IU/mL) occurred in 6 (10%) patients in the PEG-IFN group, but was not achieved in patients treated with ETV (p=0.09; Table 2). In contrast, the proportion of patients with HBV DNA <400 copies/mL at week 48 was higher in the ETV group (17 (52%) versus 10 (16%) patients in the ETV and PEG-IFN group, respectively; p<0.001; Table 2).

On-treatment HBsAg and HBV DNA decline

The decline of serum HBsAg during 48 weeks of monotherapy with PEG-IFN and ETV in HBeAg-positive patients is displayed in Figure 2A. HBsAg decreased significantly during PEG-IFN therapy (mean decline 0.94 log IU/mL at week 48, p<0.001) and to a lesser extent in ETV-treated patients (0.38 log IU/mL, p=0.07). The difference in HBsAg decline was not significant between these two groups (p=0.15). Figure 2B shows the decline of serum HBV DNA for the two treatment groups. HBV DNA levels decreased significantly during PEG-IFN and ETV therapy (p<0.001 compared to baseline in both groups). In contrast to HBsAg, suppression of HBV DNA was stronger in the

Table 2 Rates of virological and biochemical response

Response at week 48	HBeAg positive			HBeAg negative		
	PEG-IFN (N=61)	ETV (N=33)	p	PEG-IFN (N=69)	ETV (N=37)	p
HBV DNA <400 copies/mL	10 (16%)	17 (52%)	<0.001	36 (52%)	31 (84%)	0.001
HBeAg clearance	21 (34%)	3 (9%)	0.007	-	-	-
HBeAg seroconversion	16 (26%)	3 (9%)	0.05	-	-	-
HBsAg clearance	6 (10%)	0	0.09	0	0	-
HBsAg seroconversion	4 (7%)	0	0.29	0	0	-
ALT normalization	21 (34%)	16 (48%)	0.18	28 (41%)	27 (73%)	0.001

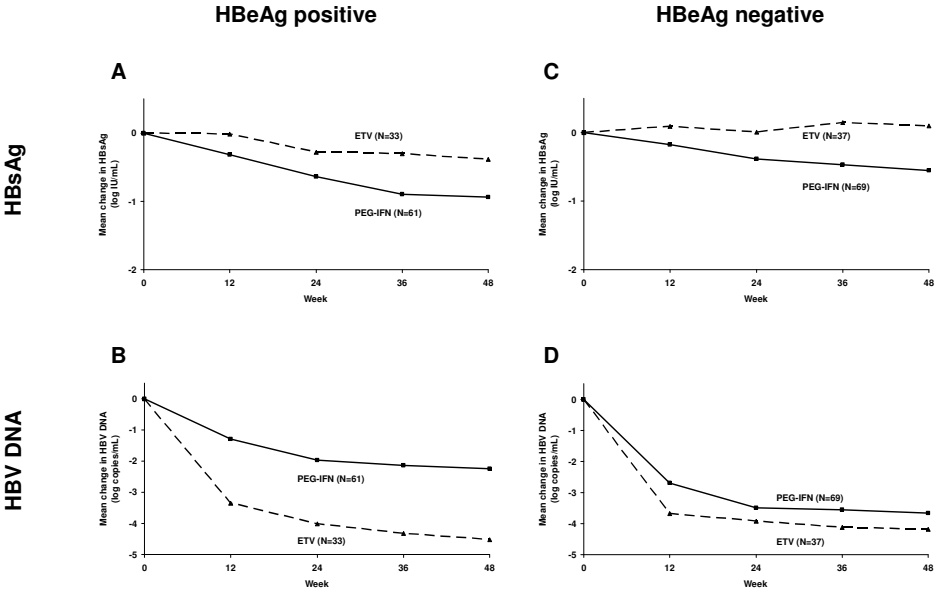


Figure 2 Mean change compared to baseline for (A) HBsAg and (B) HBV DNA levels in HBeAg-positive patients and for (C) HBsAg and (D) HBV DNA levels in HBeAg-negative patients treated with PEG-IFN and ETV.

ETV-treated patients (mean decline 4.5 versus 2.2 log copies/mL at week 48 in the ETV and PEG-IFN group, respectively; p<0.001).

Baseline factors associated with HBsAg decline

By univariate analysis, the baseline factors age, serum ALT level, serum HBV DNA level and HBV genotype (A versus non-A) were significantly associated with HBsAg decline. Patients treated with PEG-IFN tended to have a steeper HBsAg decline compared to patients treated with ETV (p=0.07). After correction of the model for each of these factors in multivariate analysis, a higher HBsAg decline in patients treated with PEG-IFN compared to ETV remained, yet this difference did not reach the level of significance (all p<0.17). Interestingly, HBsAg levels only decreased in ETV-treated patients with

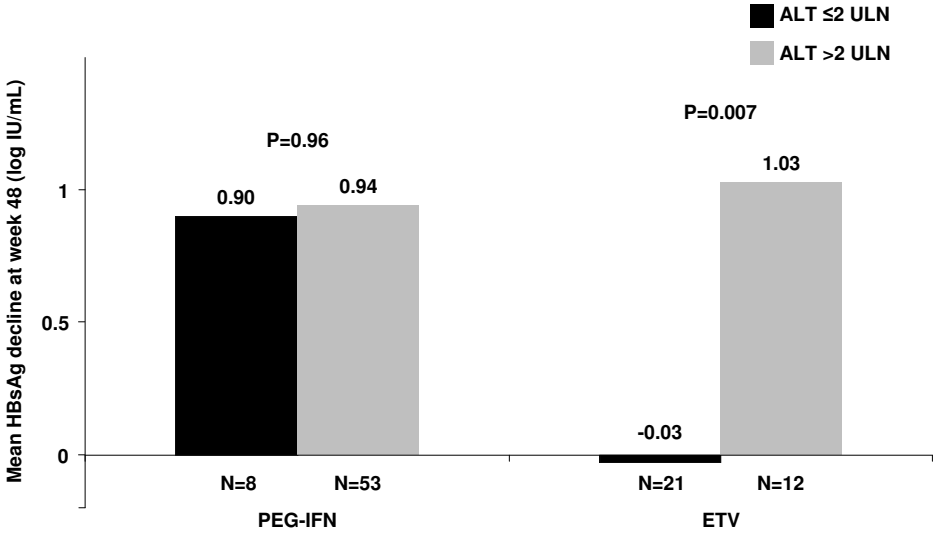


Figure 3 Degree of HBsAg decline after 48 weeks of PEG-IFN and ETV therapy for HBeAg-positive patients according to the categories of baseline ALT ≤2 ULN and >2 ULN. Within the HBeAg-negative population, decline of HBsAg was only related to treatment regimen and not to baseline ALT or any other baseline variable.

elevated ALT levels at baseline (mean decline -0.03 log IU/mL for ALT ≤2 ULN versus 1.03 for ALT >2 ULN at week 48, p=0.007), whereas HBsAg decline was comparable in both ALT categories in HBeAg-positive patients treated with PEG-IFN (Fig. 3). Although the initial decline of HBsAg was more pronounced in the PEG-IFN group with ALT >2 ULN and ETV group with ALT >2 ULN compared with the PEG-IFN group with ALT ≤2 ULN (mean decline 0.67 and 0.73 versus 0.42 log IU/mL at week 24, respectively), this difference was not significant, potentially due to the limited number of patients treated with PEG-IFN who had baseline ALT ≤2 ULN (p=0.61).

Association between HBsAg decline and HBeAg loss at week 48

The magnitude of HBsAg decline was larger in the 24 patients who lost HBeAg compared to patients who remained HBeAg positive after 48 weeks of therapy (mean decline 1.98 versus 0.36 log IU/mL at week 48, p=0.006; Fig. 4). Interestingly, the magnitude of HBsAg decline in the patients who achieved HBeAg loss was not associated with treatment regimen (1.94 and 2.29 log IU/mL for PEG-IFN and ETV, respectively, p=0.85; Fig. 4). The decline of HBV DNA was also associated with HBeAg loss at week 48 (4.6 versus 2.6 log copies/mL for patients who were HBeAg negative at week 48 compared to patients who remained HBeAg positive, p<0.001), irrespective of treatment regimen (4.6 versus 4.9 log copies/mL for PEG-IFN and ETV, respectively, p=0.77).

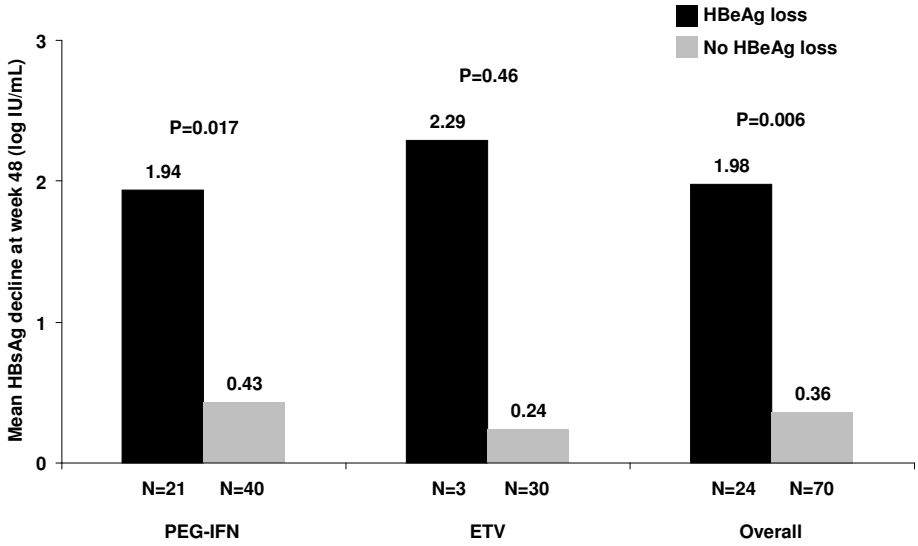


Figure 4 Degree of HBsAg decline at week 48 of PEG-IFN and ETV therapy for HBeAg-positive patients according to the achievement of HBeAg loss after 48 weeks of treatment.

HBeAg-negative patients

Virological and biochemical response rates

Within the HBeAg-negative population, the number of patients with HBV DNA <400 copies/mL at week 48 was higher in the ETV group as well (31 (84%) versus 36 (52%) patients, p=0.001; Table 2). Neither in the PEG-IFN nor in the ETV group HBsAg clearance was observed.

On-treatment HBsAg and HBV DNA decline

The decline of serum HBsAg in HBeAg-negative patients is shown in Figure 2C. Serum HBsAg consistently decreased in the PEG-IFN group (p<0.001 compared to baseline), while HBsAg levels did not change during ETV therapy (mean decline 0.56 versus -0.10 log IU/mL at week 48, p<0.001). Figure 2D shows the decline of serum HBV DNA for the PEG-IFN and ETV group. HBV DNA levels decreased significantly in both treatment groups (p<0.001 compared to baseline), but viral suppression tended to be stronger in the ETV group (mean decline 4.2 for ETV versus 3.7 log copies/mL for PEG-IFN at week 48, p=0.13).

Baseline factors associated with HBsAg decline

Multivariate analysis showed that the decline of HBsAg was only related to treatment regimen (p<0.001) and not to any other baseline variable.

DISCUSSION

This is the first detailed study comparing on-treatment serum HBsAg kinetics in patients with both HBeAg-positive and HBeAg-negative CHB receiving either PEG-IFN or ETV monotherapy. In HBeAg-positive patients, decline of HBsAg was significantly associated with HBeAg loss, and, subsequently, HBsAg decline tended to be higher in PEG-IFN treated subjects compared to ETV-treated subjects (p=0.15). Interestingly, patients who achieved HBeAg loss during either PEG-IFN or ETV therapy demonstrated a similar reduction in HBsAg levels. In contrast, in HBeAg-negative patients, only treatment with PEG-IFN resulted in a significant HBsAg decline, whereas HBeAg-negative patients treated with ETV demonstrated no HBsAg reduction at all.

In HBeAg-positive patients, previous studies suggested that serum HBsAg levels gradually decrease during LAM and ADV therapy (2, 15). Our study indicates that a decline of HBsAg in HBeAg-positive CHB is primarily confined to ETV-treated patients with a baseline serum ALT >2 ULN, and can be attributed to a large extent to patients achieving HBeAg loss. This finding suggests that the presence of an active preexisting immune response against HBV is required to lower HBsAg levels for patients treated with ETV. In contrast to ETV, PEG-IFN is able to modulate immune reactivity itself (16), which is underlined by our observation that PEG-IFN therapy reduced serum HBsAg levels irrespective of baseline ALT level, yet was also mostly confined to patients who demonstrated HBeAg loss after one year of therapy. Even more interesting is that a similar decline of HBsAg was observed in patients who achieved HBeAg loss during either PEG-IFN or ETV therapy.

With regard to our findings in HBeAg-negative patients, it has recently been demonstrated by Brunetto et al. that serum HBsAg levels do not decrease during 48 weeks of LAM therapy (7). Despite the higher antiviral potency of ETV compared with LAM, ETV therapy for 48 weeks did also not result in a reduction of serum HBsAg in HBeAg-negative patients in our study. However, a study from Greece suggested that HBsAg levels decrease in HBeAg-negative patients during long-term LAM monotherapy, although at a significantly slower rate compared with IFN (8). Thus, a longer duration of NA therapy may be required to reduce HBsAg levels in HBeAg-negative CHB. The study from Brunetto et al. also demonstrated a significant on-treatment HBsAg decline of 0.71 log IU/mL in HBeAg-negative patients treated with PEG-IFN monotherapy for 48 weeks, which is somewhat higher compared with our study (0.56 log IU/mL) (7). This difference may be caused by the predominance of HBV genotypes B and C in their study population. HBV genotype D was predominant among the HBeAg-negative patients treated with PEG-IFN in our study, therefore a direct comparison between different genotypes was not possible. However, the degree of HBsAg decline induced by PEG-IFN appears to be less pronounced in genotype D compared with other genotypes (7).

Quantitative HBsAg in serum reflects the cccDNA concentration in the liver, which plays a major role in viral persistence (2-4). It has been shown that ADV monotherapy is able to decrease intrahepatic cccDNA. Yet, when ADV was combined with PEG-IFN, clearance of cccDNA was enhanced, and in contrast to ADV monotherapy, it also resulted in a strong reduction of HBs-antigen- and HBc-antigen-positive hepatocytes (3). Our study, in fact, confirms previous observations that immune modulation is of vital importance to completely eradicate HBV. First, PEG-IFN resulted in higher HBeAg loss rates compared to ETV, and in significant HBsAg decline in both HBeAg-negative and HBeAg-positive patients. Second, although ETV demonstrated to be an potent inhibitor of viral replication, a significant decline of HBsAg was only observed in those patients with preexisting immune activity, reflected by high baseline ALT levels, and in those patients who achieved HBeAg loss after one year of therapy. Moreover, in HBeAg-negative patients, ETV therapy did not even result in HBsAg decline at all.

Our study is limited by the suboptimal study design, which lacked the possibility to randomize at baseline, and therefore led to somewhat different patient characteristics between the two treatment groups at baseline. The higher rate of HBeAg loss among HBeAg-positive patients treated with PEG-IFN compared with ETV may partially be caused by the higher baseline ALT level and the slightly unbalanced distribution of HBV genotypes. However, for the analysis of HBsAg decline in HBeAg-positive patients we corrected for confounders such as HBV genotype, HBV DNA and age using multivariate analysis, and performed a stratified analysis for baseline ALT. Cirrhosis was not associated with HBsAg decline. Furthermore, it should be noted that the predictors of HBsAg decline in our study are exactly the same as the predictors of HBeAg loss identified in previous studies, which only supports our finding that in HBeAg-positive patients reduction in HBsAg levels is largely confined to those patients achieving HBeAg loss during antiviral therapy (17-19). Within the HBeAg-negative population, the only independent factor that was associated with HBsAg decline was treatment regimen. No other baseline factors were related to HBsAg decline, and therefore have not influenced the study results.

In summary, in HBeAg-positive patients decline of serum HBsAg is largely confined to those patients that achieved HBeAg loss during antiviral therapy, either induced by PEG-IFN or ETV therapy. In HBeAg-negative patients PEG-IFN resulted in a significant reduction in HBsAg levels, whereas ETV resulted in no HBsAg decline at all. No other factors were related to HBsAg decline in HBeAg-negative patients.

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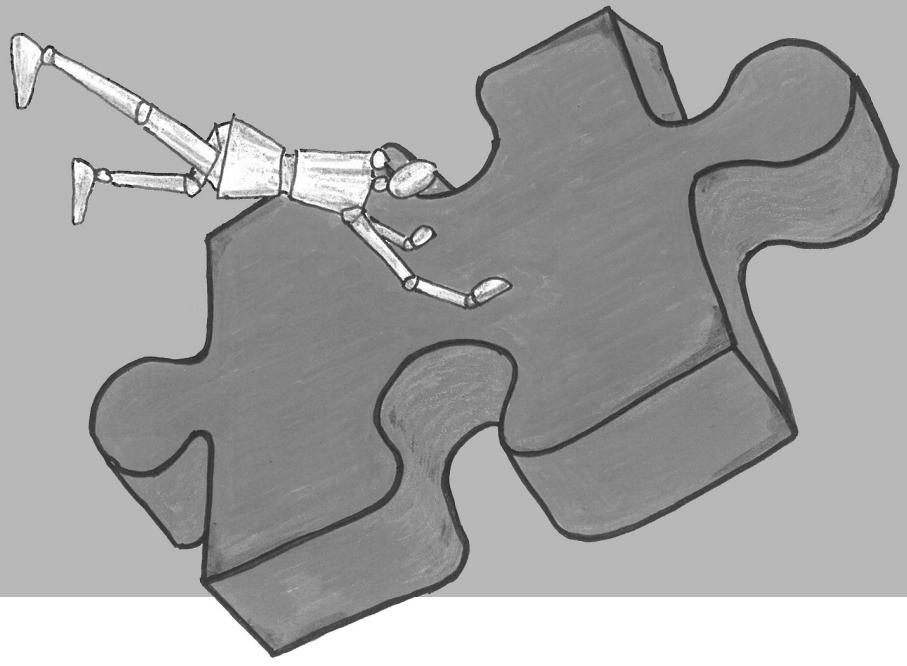

Frequency and clinical outcomes of flares related to nucleos(t)ide analogue therapy in patients with chronic hepatitis B

Ning-Ping Zhang, Jurriën G.P. Reijnders, Moniek J. Perquin, Bettina E. Hansen, Harry L.A. Janssen

Submitted.

ABSTRACT

Background

Flares in chronic hepatitis B are often detrimental but sometimes lead to sustained immune control and disease remission. The aim of this study was to estimate the frequency of hepatitis flares which occur during and/or after cessation of nucleos(t)ide analogue (NA) therapy, and to assess their outcomes.

Methods

In a single center cohort study we investigated 227 patients who received a total of 351 NA treatment courses. NA therapy was discontinued after 149 treatment courses.

Results

In total, 27 flares were observed during 9,779 on-treatment patient-months. The frequency was estimated as 3.2 per 100 person-years (95% CI 2.2-4.7). Lamivudine-treated patients demonstrated the highest frequency (4.9/100 person-years, 95% CI 3.2-7.4). Twenty (74%) of 27 on-therapy flares were associated with development of genotypic resistance, which all occurred during lamivudine therapy. NA withdrawal flares occurred after a median post-treatment follow-up of 3.5 months in seventeen (11%) of 149 treatment discontinuations. No flares were observed in patients who switched to another antiviral agent (n=51). None of the on-therapy and withdrawal flares related to NA therapy were associated with sustained disease remission, and seven flares resulted in decompensated liver disease. Multivariate analysis demonstrated a high baseline viral load to be an independent risk factor of on-treatment flares during NA therapy (RR 1.78; 95% CI 1.22-2.60; p = 0.004). No predictors for withdrawal flares could be indentified.

Conclusions

In this study, flares related to NA therapy never led to immune control and sustained disease remission, and sometimes resulted in decompensated liver disease.

INTRODUCTION

Exacerbation episodes of disease accompanied with abrupt elevation of serum aminotransferase levels are commonly observed during the natural course of chronic hepatitis B (CHB). (1-2) These so-called hepatitis flares, reflecting immune-mediated lysis of infected hepatocytes, may be followed by HBeAg seroconversion.(3-5) However, most flares only result in transient decreases in serum HBV DNA levels without loss of HBeAg. The frequency and severity of these flares are associated with an increased risk of progression to cirrhosis and hepatocellular carcinoma.(4, 6-7)

Similar exacerbation episodes are also observed during anti-HBV therapies, which include (pegylated) interferon (IFN) and nucleos(t)ide analogues (NA). IFN-induced flares affect 25-40% of patients and have been explained by the immunostimulatory properties of this drug, which increases the T cell cytolytic activity and natural killer cell function.(8) Typically, this kind of flare occurs during the second to third month of treatment and may precede HBeAg-seroconversion in HBeAg-positive patients.(9-11) Furthermore, flares related to peginterferon therapy can be divided in host-induced, characterized by decrease in viral load and strongly associated with treatment response, and virus-induced flares, which are preceded by an increase in viral load and less often followed by treatment response.

Flares during NA therapy are primarily reported as results of emergence of therapy-resistant HBV mutants.(12-16) After discontinuation of lamivudine (LAM) monotherapy hepatitis flares are observed in 10-20% of patients,(17-18) which can be associated with decompensated liver disease and fatal outcome.(18-20) However, the knowledge on NA-therapy related flares and their outcome is limited. The aim of this study was to estimate the frequency of hepatitis flares which occur during and/or after cessation of NA treatment, and to assess their clinical outcomes.

MATERIALS AND METHODS

Study population

In this single-centre cohort study, all consecutive adult chronic HBV patients who had received NA therapy for at least three months between 1993 and 2008 were included in this analysis. Patients were excluded if they (1) had received NA therapy combined with (pegylated) IFN, (2) received immunosuppressive medication, (3) had co-infection with hepatitis C, hepatitis D, or human immunodeficiency virus, or (4) had other causes of liver disease.

Follow-up

All subjects were monitored every 3-6 months. At every visit routine examination with biochemical (ALT, bilirubin, albumin, creatinin) and virologic (HBV DNA level, HBeAg, anti-HBe) assessments took place. Genotypic analysis was done in case of virologic breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir after initial virologic response. HBV genotype was determined in all patients.

End points

A flare was defined as an abrupt elevation of serum ALT levels over 10 times the upper limit of normal (ULN) and at least twice the ALT level just prior to the occurrence of the flare.(21) It was categorized as on-therapy flares (occurring during treatment) or withdrawal flares (occurring within 6 months after discontinuation of NA agents). To classify the outcome of a flare, the following end points were considered: HBsAg loss and seroconversion, HBeAg loss and seroconversion for HBeAg-positive patients, ALT normalization, undetectable HBV DNA, and decompensation of liver disease. Decompensated liver disease was defined as any of the following signs newly developed: (1) ascites, (2) persistent jaundice (serum bilirubin > 34 μmol/l, no normalization after serum ALT normalized), (3) hypoalbuminemia (serum albumin < 28 g/l), (4) international normalized ratio (INR) > 2.2, (5) encephalopathy and/or (6) variceal bleeding.(21) An end point was considered to be related to a flare, if it occurred within 6 months after the start of the flare.

Laboratory tests

Serum alanine aminotransferase (ALT) was measured using automated techniques. Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) and anti-body against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays. Serum HBV DNA levels were measured using a previously described in house developed quantitative real-time polymerase chain reaction (PCR).(22-23) Currently, this assay is multiplexed without compromising the lower limit of detection (373 copies/mL) with an internal control (pHHV) in order to control the process from DNA isolation through PCR.(24) HBV DNA was extracted from serum samples using the MagnaPureLC (Roche Applied Science, Almere, The Netherlands) as described before.(22) HBV genotypes were assessed by sequence alignment of the overlapping hepatitis B surface antigen with HBV sequences derived from GenBank. Presence of HBV polymerase gene mutations was determined using InnoLiPA DR2 and DR3 line probe assay (Innogenetics, Gent, Belgium).

Statistical analysis

Continuous variables are expressed as means ± SD or median (range) where appropriate. We calculated the incidence rate of flares per person-year overall to measure the frequency. In calculations of incidence rates, patients could be included in multiple time periods, and could contribute more than one flare in each time period. Poisson regression model was used to estimate and compare the incidence rate(25). All statistical testing was two-tailed at the 5% level. In order to determine independent predictors for the risk of flares, the baseline characteristics such as age, race, sex, pre-existing cirrhosis, ALT, $\log_{10}$ HBV DNA, HBV genotype, treatment regimen were included in the univariate analysis. All tested variables with a p value < 0.15 were entered in the multivariate regression analysis assuming a Poisson distribution. The analysis softwares used were the Statistical Package for Social Science (SPSS, version 14.0, SPSS Inc. Chicago, Ill., USA) and Science Analysis Software (SAS, version 9.13; SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population

Overall, 227 patients received a total of 351 nucleos(t)ide analogues (NA) treatment courses (136 LAM monotherapy; 96 adefovir dipivoxil (ADV); 22 tenofovir disiproxil fumarate (TDF); 77 entecavir (ETV); 4 LAM-ADV and 16 LAM-TDF combination therapy) and were included in this study. Median duration of every single treatment period was 19 months (range 3-113). Of the 227 patients, 173 (76%) were male, and the mean age at baseline was 39±14 years. The most common virus genotypes were A (27%) and D (36%) (table 1). Therapy was discontinued or changed to another type of agent 214 times within 149 patients. Sixty-one percent of them changed to another type of regimen because of development of resistance to the drug. The reason of therapy discontinuation/switch did not show a relation with off-therapy flare occurrence (p = 0.542). In five subjects, there was no six months of follow-up available, two patients stopped treatment because of HBsAg seroconversion, and seven patients died at the end of treatment course. Hence, 200 courses (LAM 126, ADV 60, ETV 3, TDF 11, LAM+ADV 1) after which therapy was discontinued (n=149) or changed to another type of agent (n=51) were available to explore the frequency and outcome of withdrawal flares.

Table 1 Baseline characteristics

Number of patients	227
Mean age (yr)	39±14
Male – no. (%)	173 (76)
Race – no. (%)	
Asian	67 (30)
Caucasian	128 (56)
Other	30 (13)
Viral genotype – no. (%)	
A	61 (27)
B	38 (17)
C	33 (15)
D	81 (36)[a]
Other	13 (6)
Number of treatment courses	351
Courses of NA[b] therapy – no. (%)	
Lamivudine	136 (39)
Adefovir dipivoxil	96 (27)
Tenofovir	22 (6.3)
Entecavir	77 (22)
Lamivudine + adefovir dipivoxil	4 (1.1)
Lamivudine + tenofovir	16 (4.6)

[a] One patient was co-infected with genotype G.
[b] NA, nucleos(t)ide analogues

On-therapy flare

Frequency of flares

In 23 (7%) of 351 treatment courses (9,779 on-treatment patient-months) at least one flare was observed. During three treatment courses multiple flares occurred, resulting in a total of 27 (8%) on-therapy flares. Figure 1 shows the frequency of on-therapy flare, which was 27/9,779 on-treatment patient-months, and was estimated as 3.2 per 100 person-years (95% CI 2.2-4.7). Patients treated with LAM demonstrated the highest frequency (22/5,501 on-treatment patient-months: 4.9/100 person-years, 95% CI 3.2-7.4). The lowest frequency was observed in ADV-treated patients (1/2,913 on-treatment patient-months: 0.4/100 person-years, 95% CI 0.06-3.0). Median peak ALT level was 20 x ULN (range 10-125 x ULN). Twenty (74%) of 27 on-therapy flares were associated with development of genotypic resistance, which all occurred during LAM therapy.

Outcomes of flares

The outcome of flares is presented in table 2. Undetectable HBV DNA and/or HBeAg loss was not achieved after any of the flares. In contrast, three of six patients who

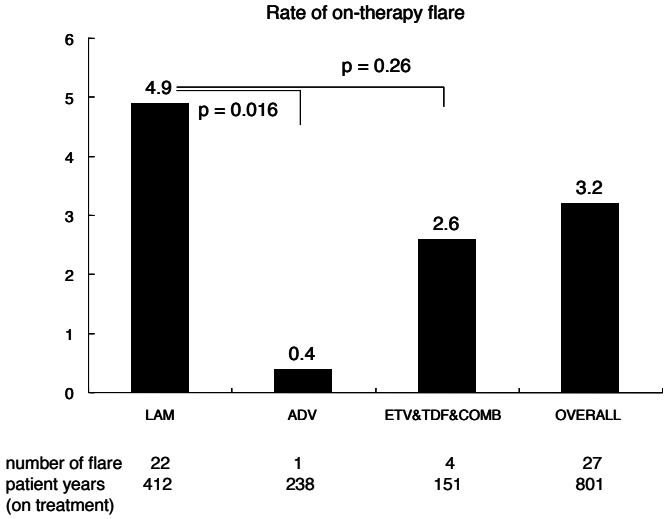

Figure 1 Frequency of on-therapy flare for different type of nucleos(t)ide analogue treatment. pys, person-years. Other, nucleos(t)ide analogue treatments with low resistance profile which consist of ETV, TDF, combination of LAM and ADV and combination of LAM and TDF.

Table 2 Characteristics of flares

	On-therapy	Withdrawal
Number of flares[a]	27	17
Number of patients[a]	23	16
Median peak ALT level (x ULN)	20 (10-125)	17 (11-81)
Genotypic resistance (%)	20 (74.1)	NA
HBeAg loss before flares[b]	6/21	7/16
Outcome of flares		
HBeAg reversion during flares[b]	3/6	5/7
HBeAg loss after flares[b]	0/18	0[c]/14
Undetectable HBV DNA (<373 c/mL)	0/27	0[c]/17
Sustained serum ALT normalization	12/27	4/17
Decompensation of liver disease	4[d]/27	3/17
Death	2[d]/27	0/17

[a] Some patients experienced multiple flares during the same treatment period.
[b] 21 and 16 patients had positive HBeAg at the start of treatment. In some patients HBeAg loss occurred before a flare, but HBeAg reappeared during a flare.
[c] Only patients who restarted antiviral therapy demonstrated HBeAg loss and/or undetectable HBV DNA.
[d] Two patients had an episode of decompensation, and died subsequently of liver failure.
NA= not applicable

achieved HBeAg seroconversion before a flare had HBeAg reversion during the flare. Twelve flares were followed by ALT normalization which was sustained for at least six months. Four flares were associated with an episode of decompensation, and two patients died subsequently of liver failure.

Withdrawal flare

Frequency of flares

After 149 treatment courses in which antiviral therapy was discontinued, seventeen (11%) withdrawal flares (16 after discontinuation of LAM, 1 after ADV) occurred after a median follow-up of 3.5 (range 1- 7.3) months. No flares were observed in patients who switched to another antiviral agent (n=51). The frequency of withdrawal flare was 17/852 follow-up patient-months, which was estimated as 24 per 100 person-years (95% CI 16-38). The highest frequency was observed in patients who discontinued LAM monotherapy (16/610 follow-up patient-months: 32 per 100 person-years, 95% CI 19.5-51.5) (Figure 2).

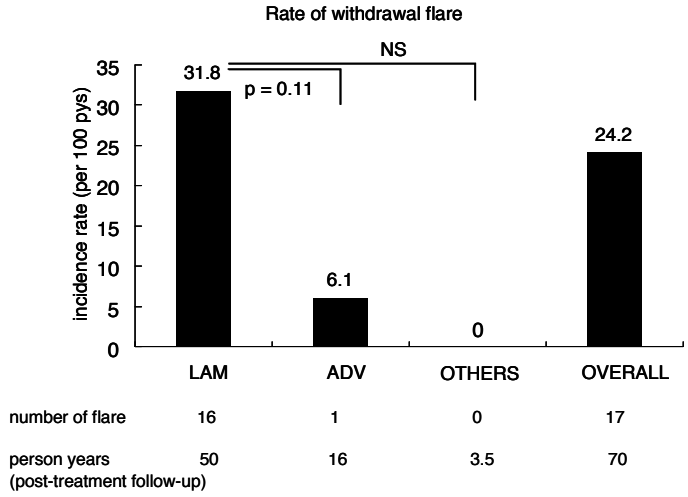

Figure 2 Frequency of withdrawal flares among patients who discontinued nucleos(t)ide analogue therapy.

Outcomes of flares

Rescue treatment was started within ±1 month after 11 (65%) of 17 withdrawal flares. None of the flares resulted in HBeAg loss or undetectable HBV DNA, and only four flares were followed by ALT normalization which was sustained for at least 6 months in the absence of antiviral therapy. HBeAg reversion occurred during a flare among 5/7 (71%) patients who had achieved HBeAg loss before the exacerbation episode. Furthermore, an episode of mild jaundice was observed during three (18%) flares. (table 2)

Risk factor of flares

Most on-therapy flares were associated with the development of genotypic resistance to NA regimens. The relative risk was estimated as 9.05 (resistance vs. non-resistance,

Table 3 Risk factors of on-therapy flares

Parameters	aRR	(95%CI)	*p*-value
HBeAg positivity	1.01	(0.27 - 2.79)	0.98
Baseline HBV DNA (per $\log_{10}$ increase)	1.78	(1.22 - 2.60)	0.003
Baseline ALT (>2×ULN vs. <2×ULN)	1.84	(0.81 - 4.14)	0.14
Treatment regimen			
LAM	1.00	reference	
ADV	0.11	(0.01 - 0.80)	0.03
ETV/TDF/COMB	0.74	(0.25 - 2.22)	0.59

95% CI: 3.65-22.42, p<0.001). Multivariate analysis demonstrated that on-therapy flares occurred more often amongst patients who had a high baseline HBV DNA (RR 1.78; 95% CI 1.22-2.60; p = 0.004) and were treated by LAM compared to ADV (RR 0.09; 95% CI 0.01–0.64; p = 0.016) (table 3). Higher baseline viral load and an inferior genotypic barrier to resistance profile of NA agents were also predictors for risk of genotypic resistance in Poisson regression analysis, which further confirmed them to be predictors of on-therapy flares. No predictors for withdrawal flares could be indentified. Neither an association between on-therapy and withdrawal flares was found.

DISCUSSION

This is the first study which extensively evaluated the characteristics and outcome of hepatitis flares related to NA therapy in chronic hepatitis B. In our study, the frequency of on-therapy flares was 3.2% per year, and 11% of patients who discontinued NA therapy, experienced a withdrawal flare. The highest frequency of on-therapy flares was observed in LAM-treated subjects, and the majority of on-therapy flares were associated with the development of antiviral drug resistance. Similarly, the highest frequency of withdrawal flares was observed in patients who discontinued LAM monotherapy as well. None of the on-therapy and withdrawal flares related to NA therapy were associated with a beneficial outcome. High baseline viral load was an independent risk factor of on-treatment flares. No predictors for withdrawal flares could be identified.

Prospective studies on the natural history estimated the incidence of spontaneous flares for chronic HBV infection 10-27% per year.(26) HBeAg-seroconversion is often preceded by these hepatitis flares; however, most flares only result in transient decreases in serum HBV DNA levels without loss of HBeAg.(3) Previous studies indicated that IFN-induced flares affect 25–40% of HBeAg-positive patients. Furthermore, it was demonstrated that especially host-induced flares, characterized by a decrease in viral load, were associated with treatment response, and can herald HBeAg loss or even HBsAg loss.(9-11) In our study, the frequency of flares during NA therapy was estimated as 3.2% per year, which is lower than the frequency observed in studies on

the natural history and studies in which (pegylated) IFN was used. The majority of these on-therapy flares were associated with the development of antiviral drug resistance and thus also associated with LAM therapy. In contrast to IFN-induced flares, on-treatment flares during NA therapy in our study appeared to be mostly virus-induced and none of them were associated with a beneficial outcome. Four flares resulted in an episode of hepatic decompensation.

Withdrawal flares have been observed after cessation of both peginterferon and LAM monotherapy.(18-20, 27) It occurred in 22% of patients after discontinuation of peginterferon monotherapy, and only few of them resulted in virologic response.(27) After cessation of lamivudine monotherapy, 10-20% of patients experienced withdrawal flares, which have been associated with decompensated liver disease and fatal outcome.(18-20) In our study, 11% of patients developed a flare after cessation of NA therapy. Flares were more often seen after discontinuation of LAM monotherapy compared to other anti-HBV agents, although this is probably related to recent guidelines which indicate when and in which patient it is safe to stop antiviral therapy.(28-29) In contrast, no flares were observed if patients were directly switched to another antiviral agent. None of the flares resulted in sustained disease remission, and three flares were accompanied by an episode of mild jaundice. As all these flares seemed to have a detrimental outcome, close monitoring of patients who for any reason discontinue NA therapy is warranted, and rapid re-initiation of antiviral therapy is recommended. Furthermore, the importance of treatment compliance should be stressed to all HBV patients on oral anti-HBV agents.

A limitation of our study is that most patients were treated with LAM monotherapy. One might hypothesize that the frequency of on-therapy flares will be lower during ETV or TDF monotherapy due to their excellent resistance profiles.(30-31) Furthermore, the frequency of withdrawal flares may also be somewhat overestimated, as the current guidelines recommend, that in HBeAg-positive patients treatment can only be stopped after HBeAg-seroconversion with at least six months of consolidation treatment. In HBeAg-negative patients discontinuation may only be possible after HBsAg clearance.(28-29)

In conclusion, the frequency of flares during NA therapy was approximately 3% per year. As the majority of on-therapy flares was associated with the development of antiviral drug resistance, the frequency will probably decrease when anti-HBV agents with good resistance profiles are used. A withdrawal flare was observed in 11% of patients who discontinued NA therapy. In this study, flares associated with NA therapy appeared to be virus-induced, never led to sustained disease remission, and even resulted sometimes in decompensated liver disease. Therefore, close monitoring of patients who discontinue NA therapy is warranted, and the importance of treatment compliance should be stressed to all HBV patients on oral anti-HBV agents.

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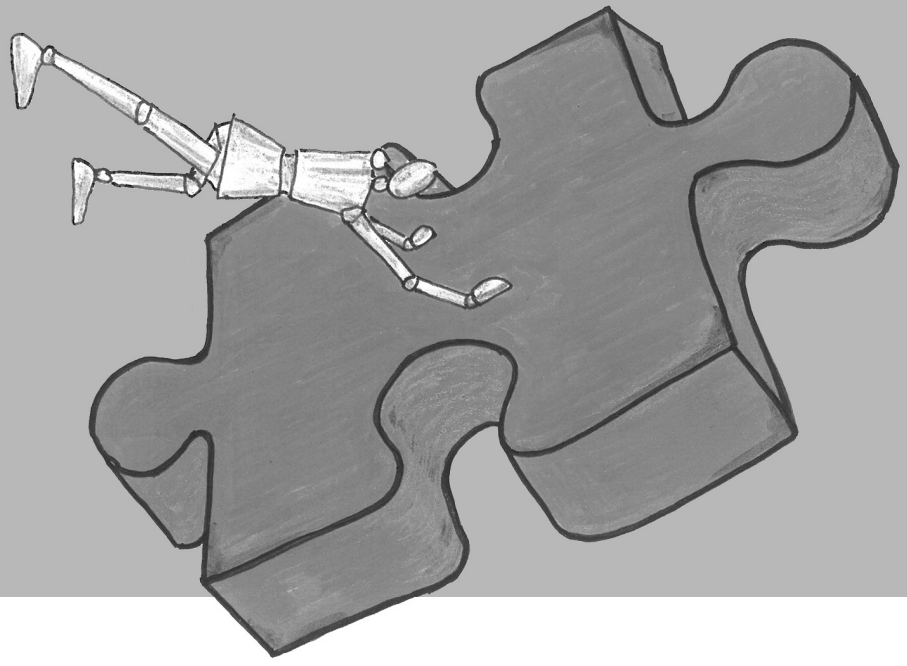

Long-Term Therapy with Tenofovir is Effective for Patients Co-Infected with HIV and HBV

Jurriën G.P. Reijnders*, T.E.M.S. de Vries-Sluijs*, Bettina E. Hansen, Hans L. Zaaijer, Jan M. Prins, Suzan D. Pas, Martin Schutten, Andy I.M. Hoepelman, Clemens Richter, Jan-Willem Mulder, Rob A. de Man, Harry L.A. Janssen, M.E. van der Ende

* These authors contributed equally to this project and should be considered co-first authors

Gastroenterology. 2010 Accepted

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ABSTRACT

Background

We investigated the long-term efficacy and renal safety of tenofovir disoproxil fumarate (TDF), administered to patients co-infected with HIV and hepatitis B virus (HBV) as a part of anti-retroviral therapy.

Methods

We performed a multicenter, prospective cohort study of 102 patients co-infected with HIV and HBV who were treated with TDF.

Results

At baseline, 80% of patients had a detectable viral load (HBV DNA>20 IU/mL). Among patients positive for hepatitis B e antigen (HBeAg, n=67), 92% had a virologic response (HBV DNA < 20 IU/mL) after 5 years of treatment. There was no difference between patients with or without lamivudine-resistance at baseline (P=0.39). Loss rates of HBeAg and hepatitis B s antigen (HBsAg) were 46% and 12%, respectively. Among HBeAg-negative patients (n=15), 100% had a virologic response after 4 years of treatment and 2 (13%) lost HBsAg. Twenty subjects (20%, all HBeAg-negative) had undetectable HBV DNA at baseline; during a median follow-up of 52 months (41–63 months), 19 (95%) maintained a virologic response and 2 (10%) lost HBsAg. Overall, one patient acquired a combination of resistance mutations for anti-HBV drugs and experienced a virologic breakthrough. Three (3%) patients discontinued TDF because of increased serum levels of creatinin. The estimated decrease in renal function after 5 years of TDF therapy was 9.8 mL/min/1.73m^2, which was most pronounced shortly after TDF therapy was initiated.

Conclusions

TDF, administered as part of anti-retroviral therapy, is a potent anti-HBV agent with a good resistance profile throughout 5 years of therapy. Only small, non-progressive decreases in renal function were observed.

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) was licensed for the treatment of human immunodeficiency virus (HIV) infection in 2001, and plays since then a pivotal role in HIV management. Currently, the combination of TDF and emtricitabine is the most widely prescribed nucleos(t)ide analogue reverse transcriptase inhibitor (NRTI) backbone in Europe. Because HIV and hepatitis B virus (HBV) share similar routes of transmission, prevalence of HBsAg-carriership is more than five-fold higher among HIV-infected patients compared to the general population.(1-2) Furthermore, HIV/HBV co-infected patients are at increased risk for development of cirrhosis and hepatocellular carcinoma, and have higher overall mortality rates compared to HIV monoinfected patients.(3-6)

The efficacy of TDF in HBV therapy was first described in studies including mainly patients with HIV-1 co-infection.(7-11) Recent data showed the efficacy of TDF in the treatment of chronically HBV-monoinfected patients as well.(12) TDF was superior to adefovir dipivoxil in both nucleos(t)ide-naïve HBeAg-positive and HBeAg-negative HBV patients, and appeared to be one of the most potent anti-HBV agents so far. Several reports showed that TDF was also effective in the nucleos(t)ide-experienced population, although conflicting results have been presented concerning patients with genotypic resistance to adefovir dipivoxil.(13-16) Moreover, TDF has a good resistance profile, and no convincing proof of HBV-resistant mutants to TDF has been presented so far.(17)

Long-term therapy is indicated for all HIV/HBV co-infected and most HBV monoinfected patients treated with oral nucleos(t)ide analogues, as a sustained response after cessation of therapy is rare.(18-19) However, follow-up in studies investigating the efficacy of TDF in HIV/HBV-coinfected and HBV-monoinfected patients is limited to only two years. In addition, there are concerns about the risk of renal toxicity with TDF.(20-25) We investigated the long-term efficacy and renal safety of TDF administered as a part of anti-retroviral therapy in a large cohort of HIV/HBV-coinfected patients.

MATERIALS AND METHODS

Study population

Six Dutch centers specialised in HIV management participated in this multicenter cohort study. From 2001 to July 2006 all consecutive adult HIV-infected patients positive for hepatitis B surface antigen (HBsAg) for more than six months, and treated with TDF as a part of anti-retroviral therapy for at least six months were included. Patients were excluded if they had hepatitis C or hepatitis *delta* co-infections, or received

concomitant treatment with (pegylated) interferon during the on-treatment follow-up period. Patients were categorized to those with or without the presence of detectable HBV-DNA at baseline

Follow-up

Virologic, haematological and biochemical parameters were recorded at least at 6-month intervals in the first two years of follow-up and at yearly intervals thereafter. At every visit routine examination with measurement of serum alanine aminotransferase (ALT), creatinin, CD4 cell count, serum HIV RNA, serum HBV DNA, HBeAg, and anti-HBe took place. HBsAg status was measured in case of the combined presence of undetectable HBV DNA and negative HBeAg. A mutation analysis was done (a) at baseline in all lamivudine (LAM)-experienced HBV patients, (b) in case of virologic breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir on at least two occasions after initial virologic response, or (c) in case of serum HBV DNA > 200 IU/mL at the end of follow-up. HBV genotype was determined at baseline. At baseline, the diagnosis of cirrhosis was based on the treating physician's judgment. Abdominal ultrasound was performed if there was clinical suspicion of progression to cirrhosis, development of decompensated liver disease or hepatocellular carcinoma.

Endpoints

The primary outcome was virologic response (VR), defined as serum HBV DNA levels < 20 IU/mL during the on-treatment follow-up period. Secondary endpoints were HBsAg loss, HBeAg loss for HBeAg-positive patients, ALT normalization, and emergence of antiviral resistant mutations. Progression to cirrhosis was defined on clinical grounds, that is, albumin level < 3,5 g/dL, platelet count < 100,000 mm^3, clinical decompensation, and ultrasound demonstration of surface nodularity, splenomegaly, and > 15-mm portal vein diameter. Clinical decompensation was defined as development of ascites, encephalopathy, jaundice, or gastro-intestinal bleeding, defined to internationally agreed criteria.(26) Renal function was assessed by monitoring the estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2, which was calculated using the Modification in Diet in Renal Disease (MDRD) equation, based on the serum creatinin, age, sex and race.

Laboratory tests

Serum alanine aminotransferase (ALT), and creatinin levels were measured using automated techniques. Absolute numbers of CD4 T lymphocytes were assessed on whole blood by flowcytometry. Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined using

commercially available enzyme immunoassays. HIV-RNA was quantitatively assessed with the Cobas Ampliprep/Cobas Amplicor version 1.5 (Lower limit of detection: 50 copies/mL; Roche Molecular Systems, Penzberg, Germany). HBV DNA was quantified in serum as previously described.(27-28) The lower limit of this assay was recently determined at 20 IU/mL by probit analysis (M. Schutten, unpublished results). HBV genotype was determined by Sanger sequencing on a 752 basepair fragment in the S gene as previously described.(29) Antiviral resistance associated mutations were determined using the Inno-LIPA HBV DR v2 (Innogenetics NV, Zwijnaarde, Belgium) for highly sensitive detection of mutant species and by Sanger sequencing of the HBV reverse transcriptase gene to detect mutations not present on the Inno-LIPA HBV DR v2 (rtT184, rtA194, rtS202, rtI233, rtM250).

Statistical analysis

Continuous variables are expressed as means ± standard deviation or median (interquartile range) where appropriate. Continuous variables were compared using the t-test or the Mann-Whitney test. Categorical variables were compared using the Chi-square or Fisher's exact test. Follow-up times were calculated from the date of TDF treatment initiation to the date of event or censorship. The cumulative probabilities of virologic response, HBeAg loss and HBsAg loss during treatment were calculated by the Kaplan-Meier method. Survival analysis with Cox regression model was used to analyze which baseline factors were associated with virologic response in patients with a detectable HBV DNA at baseline (n=82). Changes in creatinin during treatment were analyzed with a repeated measurement model estimating an overall smooth quadratic decline while allowing for a random intercept and a decline per patient. All statistical tests are two-sided, and a p-value < 0.05 was considered to be statistically significant. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) were used for all statistical analysis.

RESULTS

Baseline characteristics of the study population are presented in table 1. A total of 102 patients were included in this analysis. Ninety-two (90%) subjects were men and the mean age was 42±8.9 years. The treatment regimens that were used in addition to TDF were for most patients either a NRTI and a non-NRTI regimen (64%) or a NRTI and ritonavir boosted protease inhibitor regimen (24%). During the on-treatment follow-up all patients received concomitant treatment with either LAM or emtricitabine. Median follow-up of the whole study population was 55 (42-64) months.

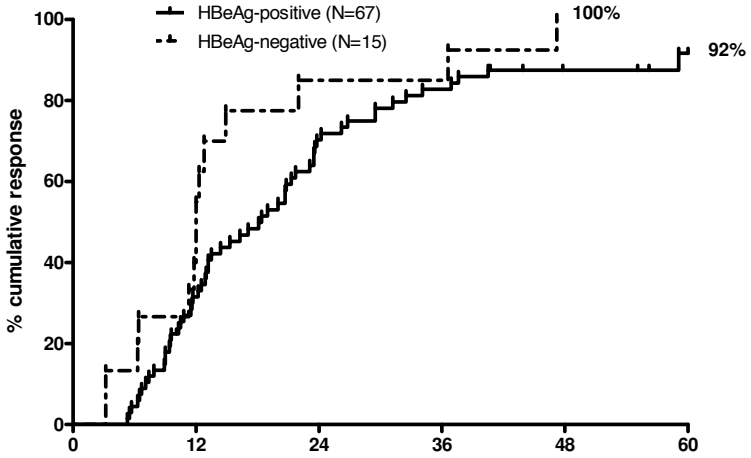
Table 1 Baseline characteristics

	Detectable HBV DNA	Undetectable HBV DNA	*P* - value
	N=82	N=20	
Age	42±8.7	43±10	0.68
Gender (male %)	77 (94%)	15 (75%)	0.02
Race			0.04
Caucasian	54 (66%)	8 (40%)	
Black	18 (22%)	10 (50%)	
Other	10 (12%)	2 (10%)	
BMI	23±5.2	25±3.4	0.31
ALT (xULN)	1.6 (1.0-2.7)	0.7 (0.4-1.0)	< 0.001
HBV DNA (Log_{10} IU/ml)	7.0±2.1	UD*	< 0.001
HBeAg-positive	67 (82%)	0 (0%)	< 0.001
Genotype (N=81)			0.15
A	47 (62%)	5 (100%)	
other	29 (38%)	0 (0%)	
Presence cirrhosis	12 (15%)	2 (10%)	0.66
CD4 count	285 (120-473)	320 (155-460)	0.68
HIV RNA (Log_{10} copies/mL)	3.1±1.6	2.0±1.3	0.002
Creatinin (mg/dL)	0.86±0.17	0.88±0.19	0.66
eGFR (mL/minute)	106±31	102±30	0.62
Treatment regimen			0.41
2 NRTI + 1 NNRTI	50 (61%)	15 (75%)	
2 NRTI + PI/r	20 (24%)	4 (20%)	
Other	12 (16%)	1 (5%)	
Concomitant anti-HBV therapy			0.26
Lamivudine	77 (94%)	20 (100%)	
Emtricitabine	5 (6%)	0 (0%)	
Previous anti-HBV therapy			
LAM-experienced	50 (61%)	18 (90%)	0.02
LAM-resistance at baseline	33 (40%)	0 (0%)	< 0.001
Duration of LAM therapy#	42 (22-74)	45 (24-64)	0.73

*Undetectable; # Months

Virologic response in patients with detectable HBV DNA at baseline

Of 82 patients with detectable HBV DNA at baseline, 67 (82%) subjects were HBeAg-positive at the initiation of TDF, and the mean HBV DNA was 7.0±2.1 log_{10} IU/mL. Fifty (61%) patients were previously treated with LAM for a median duration of 42 (22-74) months. TDF was added to LAM therapy as a second anti-HBV drug in 45 (90%) of 50 patients and in 5 patients LAM was reintroduced in combination with TDF. In 33 (66%) subjects LAM-resistant mutations could be detected at the initiation of TDF. During a median follow-up of 56 (43-64) months, 72 (88%) patients achieved VR. For HBeAg-positive patients (n=67), the cumulative probability of achieving VR at 1, 2, 3, 4 and 5 years of treatment was 31%, 70%, 83%, 88%, and 92%, respectively (figure 1).



Number of HBeAg- patients without response[a]	132	95	55	23	11	11
Number of HBeAg+ patients without response[b]	132	120	72	39	30	30

Figure 1 Kaplan-Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV DNA < 20 IU/mL, for HBeAg-positive (n = 67) and HBeAg-negative (n = 15) HIV/HBV with patients with detectable HBV DNA at baseline (n = 82).

There was no significant difference between patients with or without LAM-resistance at baseline (p = 0.39) (figure 2). In univariate analysis only HBeAg negativity at baseline demonstrated a trend towards a higher chance of achieving undetectable HBV

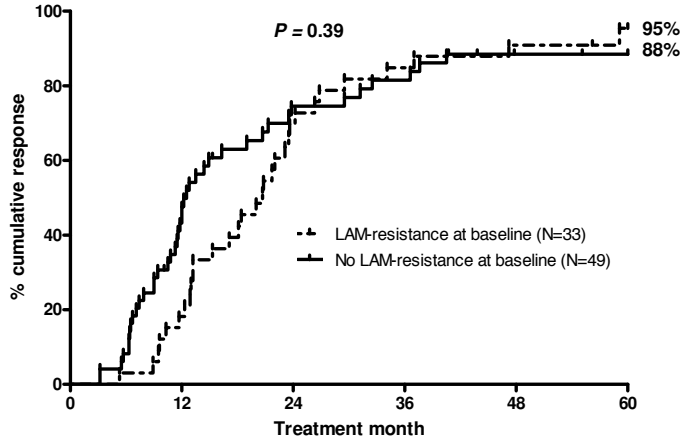
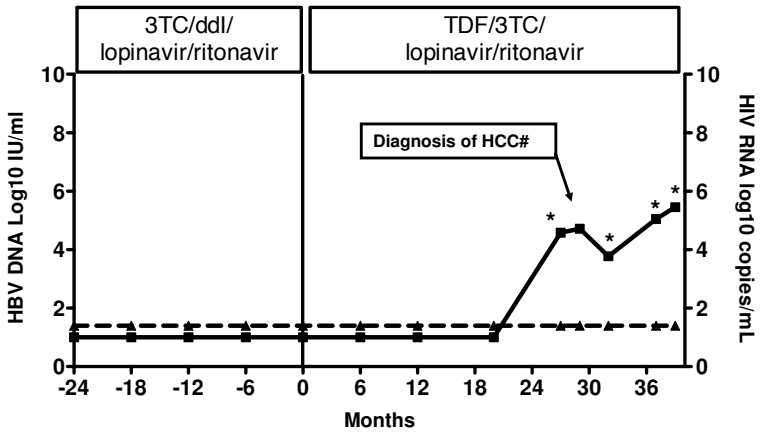
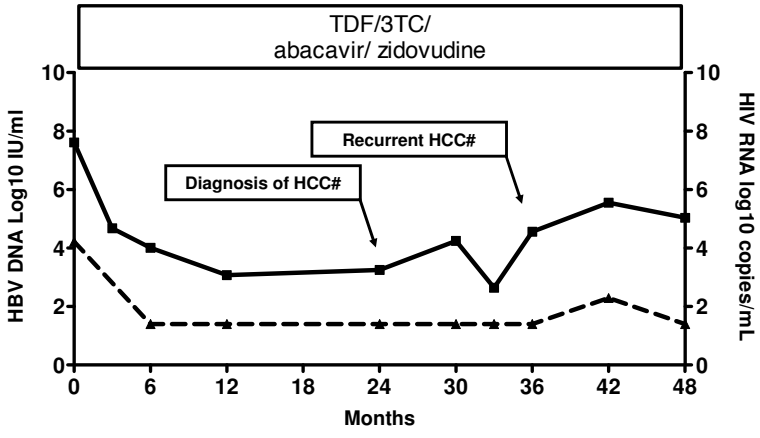


Figure 2 Kaplan-Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV DNA < 20 IU/mL, for HIV/HBV patients with detectable HBV DNA at baseline (n = 82) with lamivudine-resistant (n = 33) or no lamivudine-resistant mutations (n = 49) at the initiation of TDF.





Mutation analysis	Wild-type	Wild-type	Wild-type	rtM204M/I rtL80L/I rtL180M rtA181A/V	rtM204M/I rtL80I rtL180M rtA181A/V

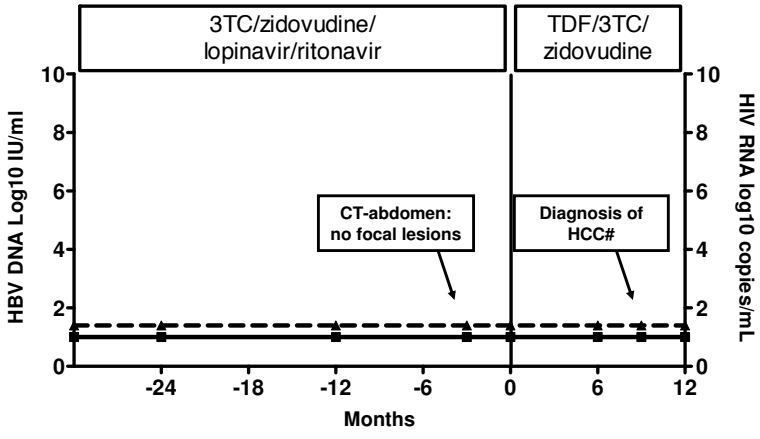


Figure 3 Clinical course of three patients who developed hepatocellular carcinoma throughout follow-up. Two patients demonstrated a virologic breakthrough as well.
A. #: MRI abdomen demonstrated diffuse hepatocellular carcinoma in segment 4-8, with infiltration of the portal vein. In addition, there was a focal lesion in segment 2 with a diameter of 8mm, suspicious for hepatocellular carcinoma. *: Mutation analysis demonstrated wild-type hepatitis B virus
B. #: MRI abdomen demonstrated a focal lesion in segment 4a, suspicious for hepatocellular carcinoma with a diameter of 3.3 cm, for which he received treatment with radio-frequent ablation. After 36 months recurrent hepatocellular carcinoma was diagnosed.
C. #: MRI abdomen demonstrated a focal lesion in segment 8, suspicious for hepatocellular carcinoma, with a diameter of 13 cm. Two other focal lesions, suspicious for hepatocellular carcinoma were observed in segment 2 and 3, with a diameter

DNA (p = 0.09). Loss rates of HBeAg and HBsAg were 46% and 12%, respectively, after 5 years of TDF therapy. For HBeAg-negative patients (n=15), the cumulative probability of achieving VR at 1, 2, 3 and 4 years of treatment was 47%, 85%, 85% and 100%, respectively (figure 1). During follow-up 2 (13%) of 15 HBeAg-negative patients achieved HBsAg loss. Of 59 patients with elevated ALT levels at baseline, 46 (78%) demonstrated ALT normalization at the end of follow-up. Three (4%) patients experienced a virologic breakthrough during the observation period. In two subjects no genotypic resistance could be detected; one patient demonstrated the combined presence of rtM204I, rtL80I, rtL180M, and rtA181V in the HBV polymerase gene (figure 3B).

Virologic response in patients with undetectable HBV DNA at baseline

Twenty patients (100% HBeAg-) had undetectable HBV DNA at baseline. Two patients were treatment-naïve; 18 patients were pretreated with LAM for a median duration of 38 (24-64) months, in whom all TDF was added as a second anti-HBV drug per internal protocol. During a median follow-up of 52 (41-63) months 19 (95%) subjects maintained virologic response, and two (10%) patients showed HBsAg loss. One (5%) subject experienced a virologic breakthrough after which a hepatocellular carcinoma was diagnosed. No genotypic resistance could be detected.

HBV resistance surveillance

During a median follow-up of 55 (42-64) months nine of 67 (13%) HBeAg-positive and one of 15 (7%) HBeAg-negative patients with a detectable HBV DNA at baseline did not achieve virologic response. Of these ten subjects, three experienced a virologic breakthrough as well. One of 20 patients with undetectable HBV DNA at baseline demonstrated a virologic breakthrough. None of the subjects with a virologic breakthrough demonstrated LAM-resistant mutations at baseline. In two patients non-adherence was suspected, as a simultaneous rebound HIV RNA was observed. A hepatocellular carcinoma was diagnosed in the other two patients, of whom one

subject also demonstrated multiple anti-HBV drug-resistant mutations (rtM204I, rtL80I, rtL180M, and rtA181V). Of the patients with a detectable viral load at the end of follow-up without fulfilling the criteria of virologic breakthrough (n=7), four subjects showed LAM-resistance at baseline, and in one patient these substitutions persisted throughout the observation period. No therapy-resistant mutations were observed in the other patients at the end of follow-up.

Progression of hepatitis B and survival

Of the 14 cirrhotic patients at baseline, 3 developed de novo hepatocellular carcinoma after 10-32 months (figure 3), and two subjects decompensated liver disease after 42 and 48 months of follow-up, respectively. In total, four patients died due to hepatocellular carcinoma progression (n=3) or complications related to end-stage liver disease (n=1). Of the 88 non-cirrhotic patients, none progressed clinically to cirrhosis or developed de novo hepatocellular carcinoma. Three patients died because of HBV-unrelated causes.

Entecavir as rescue therapy in patients with persisting HBV replication

In four patients who demonstrated persistent HBV replication during antiviral therapy, entecavir (ETV) (dosed at 1 mg once daily) was added to the treatment regimen as rescue therapy (table 2). Patients were compliant with the treatment regimen, which is supported by the undetectable HIV RNA levels in these four patients at the moment ETV was added. The addition of ETV resulted in undetectable HBV DNA in all subjects after 3-15 months of follow-up; one patient also achieved HBeAg loss.

HIV RNA and CD4 cell count changes

At the initiation of TDF, the mean HIV RNA was 2.9±1.6 $\log_{10}$ copies/mL, and 51 patients (50%) demonstrated serum HIV RNA < 50 copies/mL. At the end of follow up a significantly increased proportion of patients (84%; $p < 0.001$) demonstrated undetectable HIV RNA. The median CD4 cell count increased from 293 (138-470) cells/mm^3 at baseline to 455 (340-643) cells/mm^3 at the end of follow-up ($p < 0.001$).

Renal safety

Two patients (2%) experienced an increase in serum creatinin > 0.5 mg/dL after 5 (peak creatinin level: 1.5 mg/dL; eGFR: 54 mL/min) and 16 (peak creatinin level: 2.2 mg/dL; eGFR: 32 mL/min) months of follow-up, respectively. In both patients TDF was stopped, after which serum creatinin levels stabilized, but did not return to normal in both patients. One additional subject TDF was discontinued after 45 months because of an increase in serum creatinin of 0.38 mg/dL from baseline. The mean eGFR at baseline was 105±30 mL/min/1.73m^2. The estimated decrease after five years of TDF

Table 2 Summary of patients with persistent HBV replication in whom entecavir was added as rescue therapy

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	26	43	41	37
Gender	male	male	male	male
Previous therapy with LAM	no	yes	yes	no
At start of tenofovir				
HBeAg status	positive	positive	positive	positive
HBV DNA (log_{10} IU/mL)	8.9	7.3	7.8	8.2
HIV RNA (log_{10} copies/mL)	4.6	3.5	UD	3.5
HBV Genotype	A	A	A	A
Concomitant anti-HBV therapy	LAM	LAM	LAM	FTC
Virologic breakthrough	no	no	no	no
At time of initiation of entecavir				
Month of follow-up	42	48	48	15
HBV DNA (log_{10} IU/ml)	5.3	3.3	4.2	4.2
HBeAg status	positive	positive	positive	positive
HIV RNA (log_{10} copies/mL)	UD	UD	UD	UD
Mutation analysis	wild-type	wild-type	wild-type	wild-type
Non-compliance	no	no	no	no
Response to salvage therapy				
Salvage therapy	Addition of ETV	Addition of ETV	Addition of ETV	Addition of ETV
Follow-up (months)	15	27	15	12
HBV DNA (log_{10} IU/mL) at last F/U	UD	UD	UD	UD
HBeAg status at last F/U	positive	negative	positive	positive

UD = Undetectable; F/U = Follow-up

therapy was 9.8 (95%CI: 5.4 – 14.2) mL/min/1.73m^2. The major part of decline in renal function occurred shortly after initiation of TDF therapy (p = 0.02), and was observed especially in those subjects with a baseline eGFR > 100 mL/min/1.73m^2 (p < 0.001). The use of ritonavir-boosted protease inhibitors was not related to decline in eGFR. (p = 0.60).

DISCUSSION

This is the first study to assess the long-term efficacy of TDF administered as part of anti-retroviral therapy in a large cohort of HIV/HBV-coinfected patients. Previous studies on the efficacy of TDF in both HIV/HBV-coinfected and HBV-monoinfected patients were limited by a relatively short follow-up period for up to two years.(7-8, 12, 14) In our study, there is a median follow-up of almost five years, and, moreover, it presents the largest cohort of HIV/HBV-coinfected patients treated with TDF so far. It is shown that after five years of follow-up, approximately 90% of patients achieved undetectable

HBV DNA (< 20 IU/mL), almost 50% of HBeAg-positive patients demonstrated HBeAg loss, and HBsAg loss was even observed in approximately 10% of subjects. There was no significant difference between patients with or without LAM-resistance at baseline. More importantly, only one patient demonstrated a combination of known anti-HBV drug-resistant mutations, and experienced a virologic breakthrough thereafter. In three patients TDF was discontinued because of increases of serum creatinin levels. The estimated decrease in renal function after at five years of TDF therapy was approximately 10 mL/min/1.73m2, and was most pronounced directly after initiation of TDF therapy.

The widespread use of highly active anti-retroviral therapy (HAART) has significantly increased the life expectancy of HIV-infected patients, and liver disease has now emerged as a significant cause of non-AIDS-related death.(3, 5) A large prospective cohort study demonstrated active HBV infection to be strongly associated with liver-related mortality.(3) Current guidelines recommend, therefore, inclusion of HBV-active agents within the HAART regimen, and to initiate HAART early if an indication to treat HBV infection exists.(30) However, the benefits of long-term treatment may be negated by the development of anti-HBV drug resistance, which can lead to reversion of virologic and histological improvement. In two recently performed randomized clinical trials in HBV-mono-infected patients, TDF resulted in HBV DNA levels lower than 400 copies/mL in 76% and 93% of HBeAg-positive and HBeAg-negative patients, respectively.(12) Continued therapy produced additional viral suppression, HBeAg- and HBsAg-loss at week 72 and 96.(31-32) Our study now shows TDF, combined with either LAM or emtricitabine, to be an effective anti-HBV agent through five years of therapy with 90% of HIV/HBV co-infected subjects achieving undetectable HBV DNA.

In the phase III trials in HBV-mono-infected patients no evidence of TDF-resistance was shown up to 72 weeks of treatment despite extensive resistance surveillance.(17) Until now TDF resistance has only been described in two HIV-HBV co-infected patients demonstrating the A194T mutation in addition to LAM-resistance (33), yet the association between this mutation and TDF resistance was not confirmed in another study.(34) In our study, four subjects experienced a virologic breakthrough. In two patients this was explained by non-compliance and only one patient demonstrated a combination of LAM- and adefovir (ADV)-resistant mutations in the HBV polymerase gene. The rtA194T mutation was not observed. An interesting phenomenon was that two virologic breakthroughs occurred in association with the development of hepatocellular carcinoma. A satisfactory explanation for this relation could not be found. There are many reports which demonstrate an association between development of resistance and the risk of hepatocellular carcinoma, which is largely explained by the recurrence of viral replication; only one report noted that significantly more hepatocellular carcinomas were observed shortly after development of LAM resistance.(35)

The recently published EASL guidelines on the management of hepatitis B state that "in patients receiving entecavir or tenofovir with a partial virologic response at week 48, some experts would suggest adding the other drug in order to prevent resistance in the long term."(36) In agreement with the follow-up data of the two large phase III trials in HBV-monoinfected patients(12), our study shows that most patients are still able to achieve undetectable HBV DNA in the second year without changing the treatment regimen. Moreover, this is also the first report which demonstrates that adding ETV to existing TDF therapy is still effective after at least 15 months of treatment, and resulted in undetectable HBV DNA in all patients. Our study, therefore, suggests that one can probably wait at least 24 months before adding ETV in patients who are viremic on a TDF-containing treatment regimen.

There have been concerns about the risk of renal toxicity with TDF due to an association between related compounds such as ADV and nephrotoxicity.(37-38) In our study, a small but significant increase in serum creatinin levels was observed after five years of treatment. Yet, only 3% of patients developed serum creatinin elevations which necessitated the discontinuation of TDF. Furthermore, serum creatinin elevations usually occurred early, which suggests that frequent monitoring of renal function is necessary shortly after initiation of TDF treatment, but that thereafter, monitoring can probably decreased.(22) Overall, this study supports the renal safety of TDF as a part of anti-retroviral therapy through five years of treatment.

To date, no confirmed genotypic substitutions in the HBV polymerase gene associated with decreased sensitivity to TDF have been identified. Although direct sequencing does allow for all mutations to be identified, in vitro phenotypic confirmatory assays are mandatory to detect new substitutions. A limitation of our study is therefore, that we were only able to search for known anti-HBV drug-resistant mutations. In addition, no liver biopsies were available during follow-up in all our patients, and abdominal ultrasound was only performed if there was clinical suspicion of progression to cirrhosis, decompensated liver disease, or hepatocellular carcinoma. The frequency of progression of hepatitis B, and more specifically, the development of cirrhosis and hepatocellular carcinoma, may therefore be underestimated in our study.

In conclusion, TDF administered as part of anti-retroviral therapy, demonstrated to be a potent anti-HBV agent with a good resistance profile throughout five years of therapy. The antiviral efficacy of TDF was not influenced by presence of LAM resistance. Furthermore, this study supports the renal safety of TDF through five years of treatment, as only a small, non-progressive decline in renal function was observed. Nevertheless, close monitoring of renal function is still indicated. Adding ETV to the treatment regimen resulted in achievement of undetectable HBV DNA in patients who demonstrate persistent HBV replication during a TDF-containing treatment regimen.

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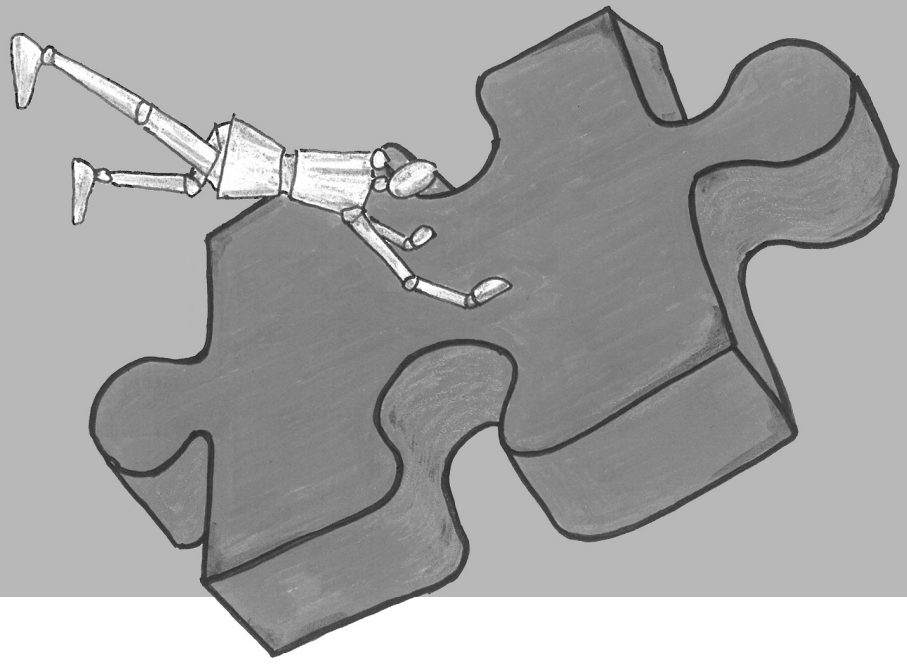
ACKNOWLEDGEMENT

The authors would like to thank F.P. Kroon and N.C. Siegersma for their assistance during data collection.



Antiviral effect of entecavir in chronic hepatitis B: Influence of prior exposure to nucleos(t)ide analogues

Jurriën G.P. Reijnders, Katja Deterding, Jörg Petersen, Fabien Zoulim, Teresa Santantonio, Maria Buti, Florian van Bömmel, Bettina E. Hansen, Heiner Wedemeyer, Harry L.A. Janssen for the VIRGIL Surveillance Study Group

J Hepatol. 2010 Apr;52(4):493-500

ABSTRACT

Background

Entecavir is a potent inhibitor of viral replication in nucleos(t)ide analogue (NA)-naïve chronic hepatitis B patients, but data on the efficacy in NA-experienced subjects is limited.

Methods

In a multi-center cohort study we investigated 161 chronic hepatitis B patients (34% NA-experienced) treated with entecavir monotherapy.

Results

During a median follow-up of 11 (3-23) months, 82 (79%) of 104 NA-naïve patients achieved virologic response (VR), defined as HBV DNA < 80 IU/mL, and none of the patients (0%) developed genotypic entecavir-resistance. VR was demonstrated in 31 (54%) of 57 NA-experienced patients during a median follow-up of 12 (3-31) months. Patients with lamivudine-resistant mutations at the start of entecavir monotherapy had a reduced probability of achieving VR compared to lamivudine-naïve patients (HR 0.14; 95%CI 0.04-0.58; p = 0.007). Antiviral efficacy was not decreased by prior treatment with lamivudine in case lamivudine-resistance had never developed (HR 0.81; 95%CI 0.43-1.52; p = 0.52). Prior adefovir therapy without development of adefovir-resistance (HR 0.84; 95%CI 0.43-1.64; p = 0.61) and presence of adefovir-resistance (HR 0.86; 95%CI 0.27-2.71; p = 0.80) did not influence antiviral response to entecavir. Switching to a tenofovir-containing treatment regimen resulted in viral load decline in patients with entecavir-resistance associated mutations.

Conclusions

Entecavir proved to be efficacious in NA-naïve patients. The antiviral efficacy of entecavir was not influenced by prior treatment with adefovir or presence of adefovir-resistance. Entecavir should not be used in patients with previous lamivudine-resistance, yet it may still be an option in lamivudine-experienced patients in case lamivudine-resistance never developed.

INTRODUCTION

Nucleos(t)ide analogues (NA) target the reverse transcriptase of hepatitis B virus (HBV), and are potent inhibitors of viral replication. In the absence of antiviral drug resistance continued NA therapy is able to suppress viral replication over prolonged periods, and can result in delay or even prevention of clinical progression to liver cirrhosis and hepatocellular carcinoma.(1)

Entecavir (ETV) is a cyclopentyl guanosine analogue, and a potent and selective inhibitor of HBV replication in vitro.(2) In the phase III registration trials it resulted in superior virologic, biochemical and histological efficacy after one year of therapy compared to lamivudine (LAM) in both HBeAg-positive and HBeAg-negative chronic HBV patients.(3, 4) Moreover, ETV proved to have a high genetic barrier to resistance with only 1.2% of NA-naïve HBV patients demonstrating genotypic resistance to ETV after five years of ETV monotherapy.(5) In LAM-refractory chronic HBV patients ETV appeared to be less potent and the frequency of resistance was increased.(6, 7) After five years of treatment 51% of LAM-refractory patients showed genotypic ETV resistance, and in 43% a virologic breakthrough was observed as well.(8) However, study populations of registration trials consist of selected groups of HBV patients, and results can not always be translated to clinical practice. Furthermore, as the increasing number of patients who experienced treatment failure to different NA treatment regimens poses a growing problem for the clinician, data on the efficacy of ETV in these NA-experienced patient groups is warranted.

The aim of this cohort study was to asses the efficacy of ETV in both NA- naïve and –experienced chronic hepatitis B patients, and to explore baseline factors associated with virologic reponse (VR) to ETV.

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 chronic HBV patients treated with ETV monotherapy between 2005 and May 2008 in 7 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 co-infections (HIV, HCV, HDV). In total, 220 HBV-infected patients treated with ETV monotherapy were identified. Fifty-nine 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, 19 patients had

a baseline HBV DNA of less than 2000 IU/mL, six patients were co-infected with HCV, and one patient was co-infected with HDV. Thus, a total of 161 patients were eligible for this analysis.

Follow-up

All subjects were prospectively monitored at least every three months. At every visit routine examination with biochemical (ALT, bilirubin, albumin) and virologic (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 virologic breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir on at least two occasions after initial virologic 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 previously given NA-treatment regimes. HBV genotype was determined at baseline.

Endpoints

The primary outcome was virologic 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 HBsAg loss and seroconversion, HBeAg loss and seroconversion for HBeAg-positive patients, ALT normalization, and emergence of ETV-related mutations.

Laboratory tests

Serum alanine aminotransferase (ALT), bilirubin, albumin levels and international ratio of prothrombin time were measured 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 six of seven 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. Presence of HBV polymerase gene mutations was determined by direct sequencing. HBV genotypes were assessed by sequence alignment of the overlapping hepatitis B surface antigen with HBV sequences derived from GenBank.

Statistical analysis

Continuous variables are expressed as means ± SD or median (range) where appropriate. Follow-up times were calculated from the date of ETV treatment initiation to the date of event or censorship. Cumulative probabilities of different endpoints were estimated by Kaplan-Meier analysis. Survival analysis with Cox regression model was used to analyze which of the following baseline factors were associated with virologic 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 adefovir dipivoxil (ADV), prior history of ADV resistance, prior treatment with (peg)interferon, dosage, and treatment centre. Factors that correlated highly with each other were compared in separate models with each collinear variable by using the Akaike information criterion method. To estimate the influence of prior treatment with LAM and prior treatment with ADV on the virologic response to ETV, hazard ratios were calculated in a multivariate Cox PH model, and adjusted for other confounding effects of other baseline characteristics (HBeAg status, viral load, ALT level, and prior treatment with LAM). Interaction terms in Cox regression model were used to test for the statistical significance of effect modification by baseline HBV DNA on the efficacy of ETV. We applied a dichotomous cut point of HBV DNA > 7 $\log_{10}$ IU/mL based on published data.(6) The estimated curves of probability of achieving VR were calculated according to Cox regression model that adjusted for the influence of significant variables (figure 3 and 4).(9) All statistical tests are 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 presented in table 1. Sixty-eight (42%) patients were HBeAg-positive, median ALT was 1.8 (0.3-75) xULN, and mean HBV DNA was 6.3±1.7 $\log_{10}$ IU/mL. NA-experienced patients were more often HBeAg-positive ($p < 0.001$) at baseline compared to NA-naïve patients. Median follow-up of the whole study population was 12 (3-31) months.

Efficacy of ETV in NA-naïve patients with chronic HBV infection

In total, 104 (66%) patients were NA-naïve, of whom all were treated with ETV 0.5 mg (table 1). Eighty-two (79%) patients achieved VR after a median follow-up of 4 (1-23) months (table 2). For HBeAg-positive patients (n=32), the cumulative probability of achieving VR at 3, 6, and 12 months was 13, 23, and 55%, respectively (figure 1).

Table 1 Baseline characteristics

	All patients	NA-naïve	NA-experienced	
	N=161	N=104	N=57	*p*-value
Age	43±14	44±14	40±14	0.12
Gender (male %)	118 (73%)	74 (71%)	44 (77%)	0.41
Race				
Caucasian	108 (67%)	68 (65%)	40 (70%)	0.83
Asian	31 (19%)	21 (20%)	10 (18%)	
Other	22 (14%)	15 (14%)	7 (12%)	
BMI	25±4.2	25±3.4	25±5.3	0.37
ALT (xULN)	1.8 (0.3-75)	2.1 (0.4-75)	1.6 (0.3-47)	0.18
HBV DNA (Log_{10} IU/ml)	6.3±1.7	6.3±1.6	6.2±1.8	0.86
HBeAg-positive	68 (42%)	32 (31%)	36 (63%)	< 0.001
Genotype (N=148)				
A	30 (20%)	18 (20%)	12 (21%)	0.48
B	10 (7%)	6 (7%)	4 (7%)	
C	19 (13%)	12 (13%)	7 (12%)	
D	80 (54%)	52 (57%)	28 (49%)	
Other	9 (6%)	3 (3%)	6 (11%)	
Dosage entecavir (0.5 mg %)	119 (74%)	104 (100%)	15 (26%)	< 0.001
Presence of cirrhosis	44 (27%)	25 (24%)	19 (33%)	0.21
Previous treatment with (peg)ifn	51 (32%)	30 (29%)	21 (37%)	0.30
Previous treatment with LAM				
LAM-experienced	43 (27%)		43 (75%)	
Prior history of LAM resistance	23 (14%)		23 (40%)	
LAM-resistance at baseline	9 (6%)		9 (16%)	
Previous treatment with ADV				
ADV-experienced	42 (26%)		42 (74%)	
Prior history of ADV resistance	12 (8%)		12 (21%)	
ADV resistance at baseline	11 (7%)		11 (19%)	
Previous treatment with TDF	3 (2%)		3 (5%)	
Previous treatment with LdT	1 (1%)		1 (2%)	

Six (19%) of thirty-two HBeAg-positive patients lost HBeAg after a median treatment duration of 12 (10-22) months, of whom four patients also seroconverted to anti-HBe. For HBeAg-negative patients (n=72), the cumulative probability of achieving VR at 3, 6, and 12 months was 40, 65, and 92%, respectively (figure 1). In none of the patients HBsAg loss was observed. Two (2%) patients experienced a virologic breakthrough during follow-up, but in none of the subjects genotypic resistance to ETV could be detected (table 2).

Efficacy of ETV in NA-experienced chronic HBV patients

VR was demonstrated in 31 (54%) of 57 NA-experienced patients during a median follow-up of 12 (3-31) months. Fifteen (26%) patients were treated with 0.5 mg, of whom ten patients were LAM-experienced and one patient had a prior history of

Table 2 Virologic and biochemical response to entecavir

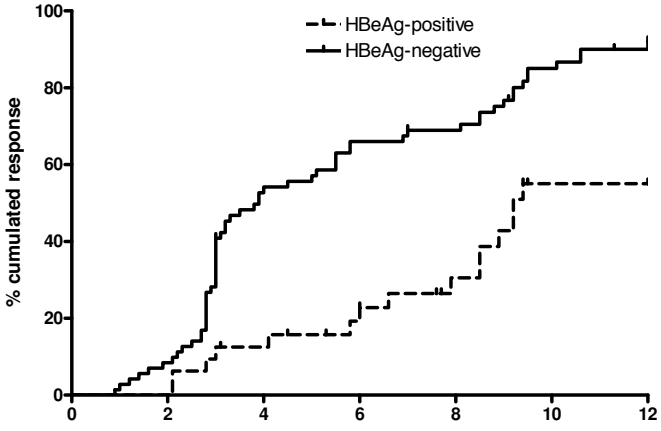
	NA-naïve patients (N=104)	LAM-experienced patients (N=43)			ADV-experienced patients* (N=36)	
		No LAM-resistance (N=20)	Prior history of LAM-resistance (N=14)	LAM-resistance at baseline (N=9)	LAM-experienced (N=24)	LAM-naïve (N=12)
Baseline HBV DNA	6.3±1.7	6.3±2.0	6.6±1.5	6.0±1.9	6.1±1.8	5.7±2.0
Median follow-up (month)	11 (3-23)	9 (4-24)	15 (4-31)	12 (3-26)	12 (3-31)	14 (6-25)
Virologic response	82/104 (79%)	12/20 (60%)	7/14 (50%)	2/9 (22%)	10/24 (42%)	9/12 (75%)
Virologic breakthrough	2/104 (2%)	0/20 (0)%	4/14 (29%)	2/9 (22%)	5/24 (21%)	0/12 (0%)
Genotypic ETV resistance	0/104 (0%)	0/20 (0%)	3/14 (21%)	2/9 (22%)	4/24 (17%)	0/12 (0%)
ALT normalization	58/83 (70%)	6/14 (43%)	7/9 (78%)	3/5 (60%)	6/13 (46%)	10/10 (100%)
HBeAg loss	6/32 (19%)	0/12 (0%)	1/10 (10%)	1/5 (20%)	1/19 (5%)	1/7 (14%)
HBsAg loss	0/104 (0%)	0/20 (0%)	0/14 (0%)	0/9 (0%)	0/24 (0%)	0/12 (0%)

* To explore the role of ETV as rescue therapy for ADV-treated patients, the antiviral effect of ETV is described for those subjects, who were directly switched to ETV monotherapy (n = 36 (86%)).

LAM-resistance. There were no centre differences ($p = 0.23$). Forty-three (75%) subjects were directly switched to ETV monotherapy after failure to preceding NA-therapy. In the remaining 14 patients, the median time between the end of previous NA treatment and the start of ETV monotherapy was 46 (5-132) months.

Lamivudine

Forty-three (27%) patients had received prior treatment with LAM for a median duration of 20 (3-64) months (figure 2). Twenty-three (53%) subjects developed LAM resistance, of whom in 9 (21%) subjects LAM-resistant mutations could still be detected at the initiation of ETV monotherapy. Median duration between the end of LAM treatment and the start of ETV treatment was 23 (0-114) months. Table 2 shows the efficacy of ETV in different subgroups of LAM-experienced patients. Adjusted for baseline viral load, HBeAg status and ALT level, only presence of LAM-resistant mutations at the start of ETV monotherapy was significantly associated with a reduced probability of achieving VR compared to LAM-naïve patients (HR 0.14; 95% CI 0.04-0.58; $p = 0.007$). Previous treatment with LAM without development of LAM resistance (HR 0.81; 95% CI 0.43-1.52; $p = 0.52$) and a prior history of LAM resistance (HR 0.59; 95% CI 0.26-1.34; $p = 0.21$) did not influence the antiviral response to ETV (figure 3). A test for interaction among baseline HBV DNA, LAM-naïve and different subgroups of LAM-experienced patients, and virologic response was not significant ($p = 0.63$), suggesting that the



Number of patients without response[a]							
HBeAg-positive patients	32	32	27	21	17	9	8
HBeAg-negative patients	72	66	33	24	20	9	4
Total number of patients[b]							
in follow-up	104	104	98	89	77	56	42

Figure 1 Kaplan-Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV DNA < 80 IU/mL, for HBeAg-positive and HBeAg-negative NA-naïve hepatitis B patients. (a) Number of patients who have not achieved virologic response and are still in follow-up. (b) Number of patients who are still in follow-up, whether or not they have achieved virologic response.

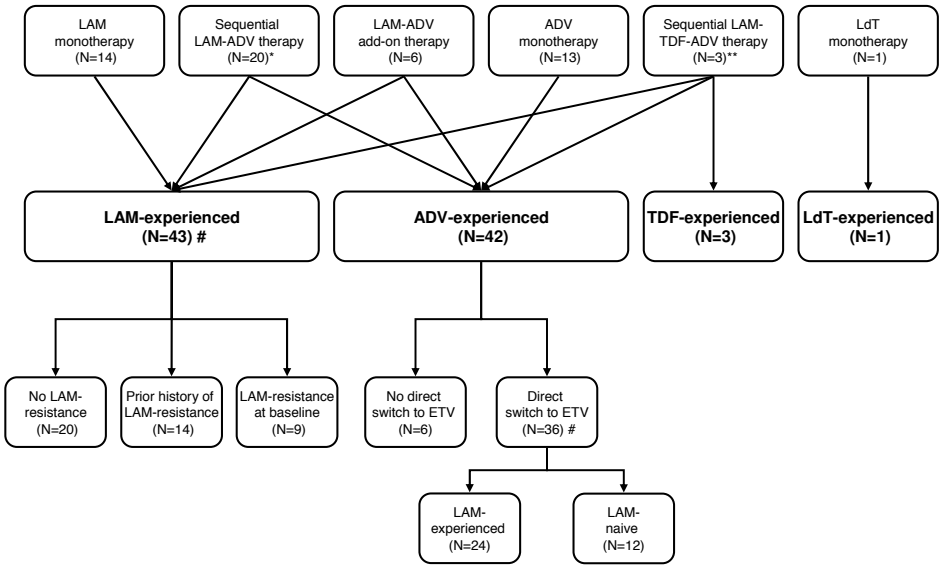


Figure 2 Overview of NA patients (N=57): treatment schedules prior to ETV monotherapy
* Two patients received sequential ADV-LAM therapy; ** One patient received sequential LAM-ADV-TDF therapy; # Patients described in table 2

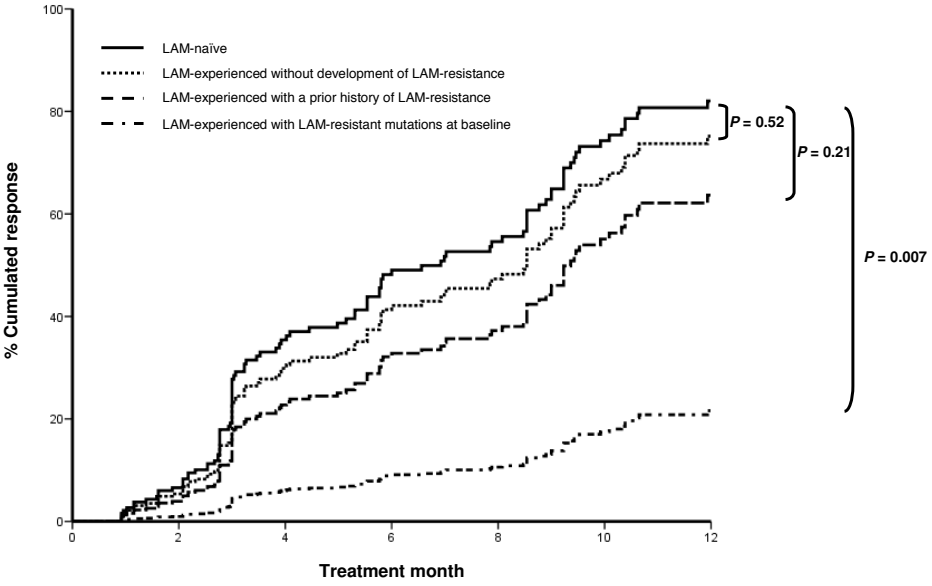


Figure 3 Adjusted estimated survival curve for the cumulative probability of achieving virologic response, defined as HBV DNA < 80 IU/mL, for LAM-naïve and different subsets of LAM-experienced hepatitis B patients, based on the Cox's model for mean values of baseline HBV DNA, ALT level and HBeAg status.

influence of previous treatment with LAM on the antiviral response to ETV, is similar in patients with a high viral load (>7 $\log_{10}$ IU/mL) and patients with a low viral load (≤7 $\log_{10}$ IU/mL) at the institution of ETV (figure 4). Of the fourteen patients with a prior history of LAM-resistance, four (29%) subjects developed a virologic breakthrough during follow-up, and in three (22%) patients genotypic resistance to ETV could be detected (table 2).

Adefovir

Forty-two (26%) patients had received prior treatment with ADV (figure 2). Six (14%) subjects were not directly switched to ETV monotherapy, of whom three patients initially received LAM monotherapy as rescue therapy, one subject was first treated with tenofovir (TDF) monotherapy, and two other patients stopped antiviral therapy after two and twelve months of ADV monotherapy, respectively. To explore the role of ETV as rescue therapy for ADV-treated patients, the antiviral effect of ETV is described in table 2 for those subjects, who were directly switched to ETV monotherapy (n = 36 (86%)). Median ADV treatment duration was 19 (3-55) months. In 10 (28%) of 36 patients genotypic resistance to ADV (rtN236T/ rtA181V/T) could be detected at the initiation of ETV monotherapy, of whom nine subjects were LAM-experienced. Adjusted for previous LAM treatment, baseline viral load, HBeAg status, and ALT level, antiviral

Table 3 Baseline predictors of virological response: time-to-event analysis

Parameters	Multivariate Cox PH model		
	HR	(95%CI)	*p*-value
HBeAg negativity	1.95	(1.25-3.06)	0.004
Baseline HBV DNA (per $\log_{10}$ increase)	0.67	(0.59-0.77)	< 0.001
Baseline ALT (per 1*ULN increase)	1.06	(1.03-1.09)	< 0.001
Lamivudine resistance at baseline	0.16	(0.04-0.64)	0.01

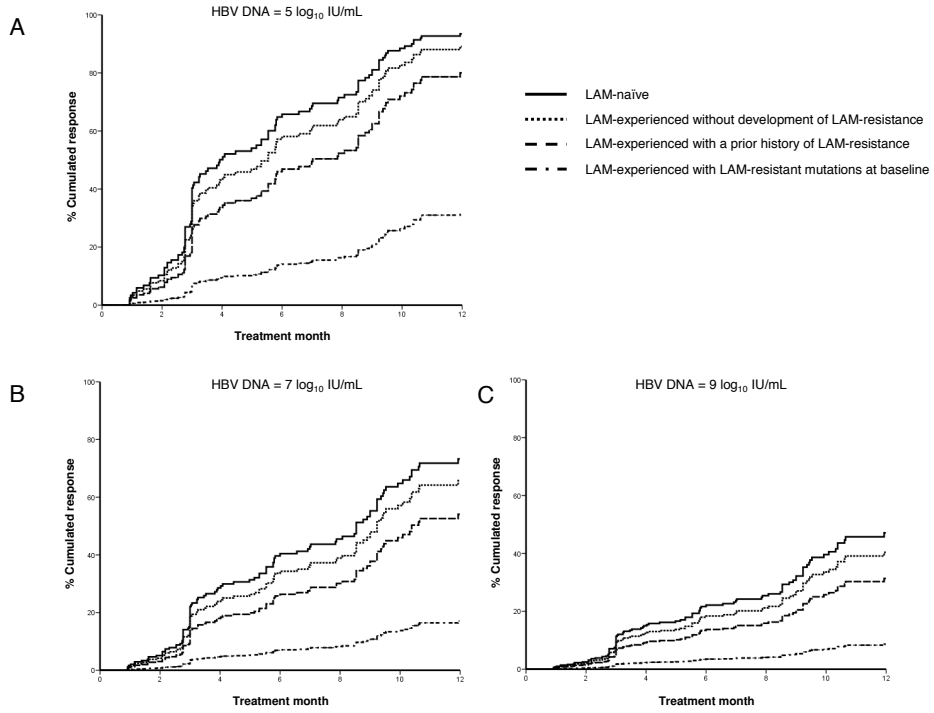


Figure 4 Adjusted estimated survival curves for the cumulative probability of achieving virologic response, defined as HBV DNA < 80 IU/mL, for LAM-naïve and different subsets of LAM-experienced hepatitis B patients, based on the Cox's model for mean values of baseline ALT level, HBeAg status, and HBV DNA = 5 $\log_{10}$ IU/mL (A), HBV DNA = 7 $\log_{10}$ IU/mL (B), and HBV DNA = 9 $\log_{10}$ IU/mL (C).

response to ETV was neither influenced by prior ADV therapy without development of ADV resistance (HR 0.84; 95% CI 0.43-1.64; $p = 0.61$) nor by presence of ADV resistance at the start of ETV monotherapy (HR 0.86; 95% CI 0.27-2.71; $p = 0.80$).

Predictors of virologic response

Table 3 shows predictors of VR. Increased probabilities of VR were seen in HBV patients with HBeAg negativity at baseline (HR 1.95; 95%CI 1.25-3.06; $p = 0.004$), high baseline ALT levels (HR 1.06; 95% CI 1.03-1.09; $p < 0.001$), low baseline HBV DNA levels (HR 0.67; 95% CI 0.59-0.77; $p < 0.001$), and absence of LAM-resistant

mutations at baseline (HR 0.16; 95% CI 0.04-0.64; $p = 0.01$). There was no significant difference in antiviral response between the participating centers ($p = 0.13$). Due to a short follow-up period and a low number of patients developing mutations related to ETV resistance, no predictors of resistance could be identified.

Resistance surveillance

During a median follow-up of 12 (3-31) months eight (5%) patients experienced a virologic breakthrough (table 4). Six patients had a prior history of LAM-resistance, of whom in two subjects LAM-resistant mutations could still be detected at the start of ETV monotherapy. Two NA-naïve patients experienced a virologic breakthrough, but non-adherence was suspected in both subjects. In three patients only LAM-resistant substitutions were seen; two patients demonstrated additional mutations related to ETV-resistance. Only one of five patients who developed genotypic resistance, had achieved virologic response. A TDF containing treatment regimen was initiated in five patients, and a subsequent decline in HBV DNA was observed in four of five patients. No mutations associated with decreased sensitivity to ETV were observed in patients with a viral load > 200 IU/mL at the end of follow-up.

DISCUSSION

This study explores the influence of previous exposure to nucleos(t)ide analogues on the efficacy of ETV, and identifies which factors predict virologic response to ETV. It was shown that in patients with LAM-resistant mutations at baseline ETV is less potent, but that antiviral efficacy was not decreased by prior LAM treatment without development of LAM-resistance. Previous treatment with ADV and presence of ADV-resistant mutations did both not influence the potency of ETV as well. Baseline HBeAg-negativity, high serum ALT, low serum HBV DNA levels, and absence of LAM-resistant mutations were independent predictors of VR for ETV monotherapy. ETV was shown to be a potent antiviral with a favorable resistance profile in NA-naïve chronic HBV patients.
Currently, the increasing number of patients who experienced treatment failure to different NA treatment regimens poses a growing problem in daily clinical practice. Nevertheless, data on the efficacy of different anti-HBV agents in these NA-experienced patient groups are scarce. In accordance with the studies by Sherman et al. (6, 7), we showed that antiviral efficacy of ETV is significantly decreased in patients with LAM-resistant mutations at the start of ETV monotherapy. No difference in potency was seen compared to NA-naïve patients in subjects who were previously treated with LAM without development of resistance to LAM, or patients with a prior history of LAM-resistance but in whom these mutations could not be detected at baseline.

Table 4 Summary of patients with a virologic breakthrough during entecavir monotherapy

	Patiënt 1	Patiënt 2	Patiënt 3	Patiënt 4	Patiënt 5	Patiënt 6	Patiënt 7	Patiënt 8
Age (years)	37	52	45	63	29	29	29	42
Gender	Male	male	male	male	male	male	male	female
At start of entecavir								
HBeAg status	positive	negative	positive	negative	positive	positive	negative	negative
HBV DNA (log_{10} IU/mL)	8.7	5.4	5.1	5.2	5.7	7.9	6.1	6.0
Prior LAM exposure	Yes	yes	yes	yes	yes	yes	no	no
Prior LAM resistance	Yes	yes	yes	yes	yes	yes	no	no
LAM-resistance at baseline	No	no	no	rtM204V, rtL180M	rtM204V, rtL180M	no	no	no
HBV Genotype	B	C	D	D	A	A	D	A
Viral load at max. viral suppression (log_{10} IU/ml)	2.5	< 1.9	< 1.9	5.1	2.3	2.9	< 1.9	< 1.9
Month of virologic breakthrough	15	8	24	6	18	29	9	11
At time of virologic breakthrough								
HBV DNA (log_{10} IU/ml)	4.4	3.0	5.3	6.0	3.5	6.3	3.8	4.2
Mutational pattern	rtM204I, rtL80F	none	rtM204V, rtL180M	rtM204V, rtL180M, rtM250V	rtM204V, rtL180M	rtM204V, rtL180M, rtV173L, rtI169T, rtS202G	none	none
Adverse outcome	None	decompensation, death	none	none	death	none	none	none
Non-compliance	No	no	no	no	no	no	suspected	suspected
Response to salvage therapy								
Salvage therapy	Addition of tenofovir	none	Emtricitabine - Tenofovir	Addition of tenofovir	none	Addition of tenofovir	Addition of adefovir	Tenofovir
Follow-up (months)	24		1	5		5	0	4
HBV DNA at last F/U (log_{10}IU/ml)	< 1.9		3.9	< 1.9		4.0		4.3

However, in the latter group 4 of 14 patients experienced a virologic breakthrough, and in 3 subjects mutations related to ETV-resistance were detected. In the first group no virologic breakthroughs were seen. Furthermore, the effect of previous treatment with LAM on the efficacy of ETV was not influenced by baseline HBV DNA, which suggests that also in patients with LAM-resistant mutations and a viral load less than 7 $\log_{10}$ IU/mL at baseline, the antiviral response to ETV was reduced. This challenges earlier experience in a small number (n=11) of LAM-resistant HBV patients.(6) Based on these results, ETV should not be used as rescue option for patients with a prior history of LAM-resistance. ETV may still be used in LAM-experienced patients in case LAM-resistance never developed, although longer follow-up is required to confirm these initial observations.

Until now, only small case reports have described the in vivo efficacy of ETV in HBV patients previously treated with ADV and/ or patients with ADV-resistant HBV.(10-12) In vitro studies showed that ADV-resistant HBV strains remain completely sensitive to ETV, or only demonstrate a slightly decreased susceptibility.(13-17) Consistent with these in vitro and limited in vivo data, antiviral efficacy of ETV was not influenced by prior treatment with ADV or presence of ADV resistance. A subset of these patients with a poor virologic response to ADV has been described previously by us,(18) in which ETV monotherapy resulted in a limited virologic response. The contrasting outcome might be related to selection of patients who respond poorly to NA in general instead of previous ADV therapy itself. Furthermore, in this study we were able to control for confounding factors such as baseline HBeAg-status, HBV DNA and ALT levels, and prior exposure to LAM.

The antiviral effect of ETV in NA-naïve patients in our study is consistent with results demonstrated in the large phase III registration trials.(3, 4) Our study, therefore, confirms ETV to be a highly potent antiviral agent with a good resistance profile within this population. Furthermore, we demonstrate that patients who are the initiation of ETV HBeAg-negative, have low HBV DNA levels, and high ALT levels, are the most likely to respond. These baseline predictors of VR were also found in patients treated with other NA.(10-12) Due to a short follow-up period and a low number of patients developing ETV-resistance associated mutations, no predictors of resistance could be identified.

In total, eight virologic breakthroughs were observed during the follow-up period, after which in five subjects mutations associated with ETV resistance could be detected. In three LAM-experienced patients, only LAM-resistant substitutions were present at the moment of virologic breakthrough. Minor populations (< 20%) existing within the quasispecies with additional ETV-resistant mutations can not be ruled out, but it confirms that LAM-resistant mutations are maintained or can reappear during ETV monotherapy.(19-21) To our knowledge no patient with a virologic breakthrough during ETV monotherapy with just an rtM204I and an rtL80F mutation has been described

before.(22) In vitro studies suggest TDF and ADV to remain susceptible to LAM- and ETV-resistant HBV mutants.(21, 23) In our study, five patients were switched to a TDF-containing regimen, resulting in decline of HBV DNA levels in four of them.

Limitations of our study include the observational design and the heterogeneous group of patients. Nevertheless, Kaplan-Meier analysis was used to address the variability of treatment duration, and the influence of prior NA therapy on the antiviral response to ETV monotherapy, was estimated in a multivariate model, and adjusted for confounding effects of other baseline characteristics. Indeed, results from our study are consistent with results from previous in vitro and in vivo studies, including phase III randomized clinical trials, and the concordance in results between the different participating centers underlines the reliability of the data.

In conclusion, in NA-experienced patients, ETV should not be used as rescue therapy in patients with a prior history with LAM-resistance, even when LAM-resistant mutations can not be detected at baseline. Also in patients with LAM-resistance and a low viral load at baseline, response to ETV appears suboptimal. ETV may still be used in LAM-experienced patients in case LAM-resistance never developed, yet longer follow-up is required to confirm these initial observations. The antiviral efficacy of ETV was not influenced by prior treatment with ADV or presence of ADV resistance. In NA-naïve HBV patients ETV proved to be highly efficacious. Switching to TDF, whether in mono- or in combination therapy, resulted in decline of viral load in patients with ETV-resistance associated mutations.

ACKNOWLEDGEMENTS

The authors would like to thank D. Tabernero, M. Lütgehetmann, M. Heichen, and P. Pradat for their assistance during data collection.

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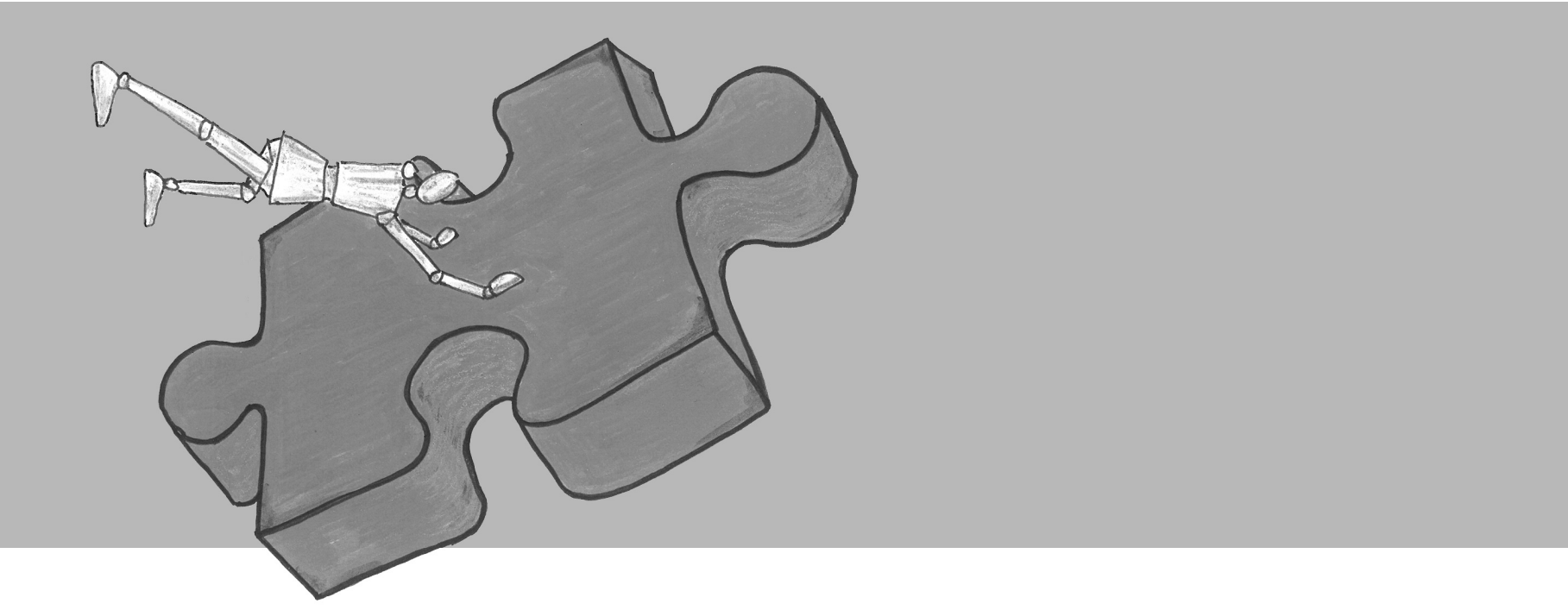


Entecavir shows limited efficacy in HBeAg-positive hepatitis B patients with a partial virologic response to adefovir therapy

Jurriën G.P. Reijnders, Suzan D. Pas, Martin Schutten, Robert A. de Man, Harry L.A. Janssen

J Hepatol. 2009 Apr;50(4):674-83

ABSTRACT

Background

We investigated the efficacy of entecavir in lamivudine-experienced and -naïve patients with persistently high HBV DNA during adefovir treatment.

Methods

Fourteen chronic hepatitis B patients (57% lamivudine-experienced) with a viral load above $5\log_{10}$ copies/mL after twelve months of adefovir therapy and thereafter were treated with entecavir 1 mg daily.

Results

During a median follow-up of 15 months (range: 8-23 months) one of six lamivudine-naïve and none of the eight lamivudine-experienced patients achieved undetectable HBV DNA (< 373 copies/mL). HBeAg loss occurred in none of the subjects. Two lamivudine-experienced patients demonstrated the rtM204I mutation; no other entecavir-resistant substitutions were detected (rtI169, rtT184, rtS202, rtM250). Two of three patients with genotypic adefovir resistance at baseline demonstrated a rapid virologic response to entecavir, but undetectable HBV DNA was not achieved. To achieve a better antiviral response the dosage of entecavir was increased to 2 mg daily in two patients, resulting in further viral load decline for both of them.

Conclusions

Entecavir monotherapy dosed at 1 mg resulted in a slow reduction of viral load in both lamivudine-experienced and -naïve patients with persistently high HBV DNA during adefovir therapy. Increasing the dosage of entecavir led to further HBV DNA decline.

INTRODUCTION

Treatment of patients with chronic hepatitis B virus (HBV) infection remains an important challenge. With the currently approved treatment options the main goal of treatment is complete suppression of viral replication, because persistent HBV viraemia is associated with development of liver cirrhosis and hepatocellular carcinoma.(1, 2) Furthermore, a rapid virologic response after initiation of nucleos(t)ide analogue treatment is associated with lower rates of antiviral drug resistance in the long term.(3-5)
Adefovir dipivoxil (ADV) is an oral prodrug of adefovir, a phosphonate acyclic nucleotide analogue of adenosine monophosphate.(6) Previous studies demonstrated its efficacy in both patients with HBeAg-positive and patients with HBeAg-negative chronic HBV infection, showing significant virologic, biochemical, and histological improvement after 48 weeks of treatment.(7, 8) However, after five years of treatment resistance rates can be up to 29%.(9) Furthermore, ADV demonstrates a relatively high rate of primary nonresponse, probably due to its suboptimal dosage.(10, 11) In the treatment of lamivudine (LAM)-resistant HBV infection, switching to ADV monotherapy resulted in significant HBV DNA decline, but several case series reported increased resistance rates compared to LAM-naïve patients.(12, 13)
Entecavir (ETV) is a cyclopentyl guanosine analogue, and a potent and selective inhibitor of viral replication in vitro.(14) A large phase III study demonstrated its efficacy in nucleoside-naïve patients with HBeAg-positive chronic HBV infection. After 48 weeks of treatment with ETV a greater reduction in viral load was achieved compared to LAM ($6.9\log_{10}$ vs. $5.4\log_{10}$).(15) In LAM-refractory patients ETV is less effective, and results in a $5.1\log_{10}$ HBV DNA decline after 48 weeks of treatment.(16) It has yet not been evaluated in patients with a partial virologic response to ADV therapy. Here we describe 8 LAM-experienced and 6 LAM-naïve patients with chronic HBV infection and a persistently high level of viral replication after twelve months of treatment with ADV, who were switched to ETV monotherapy.

MATERIALS AND METHODS

Study population

All adult patients with chronic hepatitis B, referred to the Erasmus Medical Center Rotterdam from September 2005 till February 2007 who had a persistently high viral load during ADV treatment and subsequently received rescue therapy with ETV 1 mg daily for at least six months were included. A persistently high viral load during ADV treatment was defined as presence of HBV DNA levels greater than $5\log_{10}$ copies/mL after twelve months of treatment and thereafter. All patients had repeatedly

self-reported good compliance to ADV and ETV treatment. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all patients.

Follow-up

All subjects were monitored at least every three months. At every visit routine examination with biochemical (ALT, bilirubin, albumin, creatinin) and virologic (HBV DNA level, HBeAg, anti-HBe) assessments took place. Genotypic resistance was assessed by the InnoLiPA DR2 and DR3 line probe assay (Innogenetics, Gent, Belgium), and was done (a) at baseline (start of ETV treatment) in all subjects, (b) in case of virologic breakthrough, defined as an increase in serum HBV DNA level > $1\log_{10}$ (10-fold) above nadir on at least two occasions after initial virologic response, or (c) in case of serum HBV DNA > $3\log_{10}$ copies/mL at the end of follow-up. If ETV-resistant mutations were detected at the end of follow-up, genotypic analysis was retrospectively performed at six and twelve months of treatment. Genotypic LAM- and ADV resistance was assessed using the InnoLiPA DR2 line probe assay in stored serum samples obtained at the end of LAM and/ or ADV treatment. HBV genotype was determined at baseline in all patients.

Treatment schedules prior to application of entecavir

Lamivudine-naïve patients with high HBV DNA during adefovir therapy

Six patients were LAM-naïve and received ADV treatment for at least twelve months, demonstrating a viral load of more than $5\log_{10}$ copies/mL after 12 months of therapy and thereafter (figure 1a and 2a). The median duration of ADV treatment was 17 (12-31) months. At the start of ADV therapy, all patients were HBeAg-positive, and median HBV DNA was 9.0 (6.5-9.9) $\log_{10}$ copies/mL. After twelve months of treatment the median viral load decreased to 7.1 (6.1-10.1) $\log_{10}$ copies/mL. In none of the subjects HBeAg loss was observed. ALT levels normalized before switching to ETV in one of five patients with initially elevated ALT. No ADV-resistant mutations could be detected at the end of ADV therapy.

Lamivudine-experienced patients with high HBV DNA during adefovir therapy

Eight patients had received prior treatment with LAM for a median duration of 20 (12-70) months. Six (75%) subjects developed LAM resistance. After detection of resistance, LAM was discontinued in five patients. One patient was directly switched to ADV, another patient continued LAM, while ADV was added. All patients received ADV treatment for at least twelve months, demonstrating a viral load of more than $5\log_{10}$ copies/mL after 12 months of therapy and thereafter (figure 1b and 2b). Median

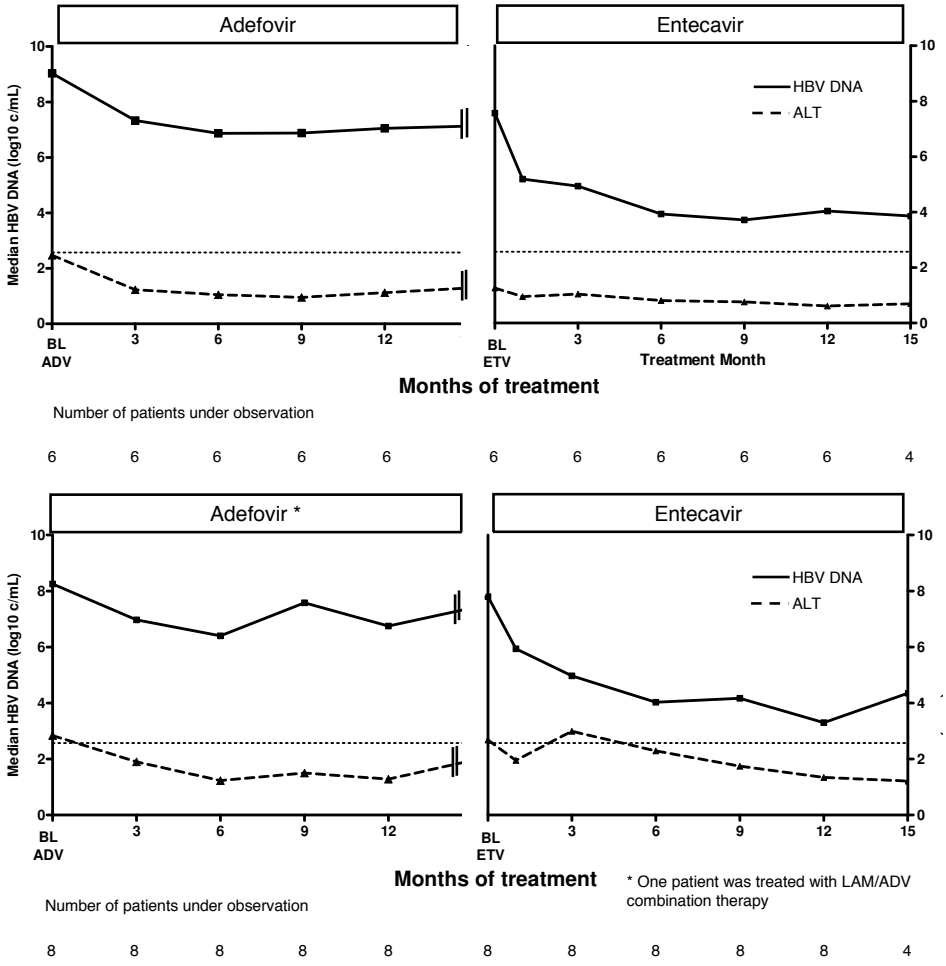


Figure 1 Median serum HBV DNA and ALT levels during adefovir and consecutive entecavir therapy in lamivudine-naïve (A) and lamivudine-experienced patients (B). The dashed line represents the lower limit of detection of the HBV DNA assay.

duration of ADV therapy was 20 (12-30) months. Seven (88%) patients were HBeAg-positive and median HBV DNA was 8.3 (6.3-10.0) $\log_{10}$ copies/mL at the start of ADV treatment. After twelve months of treatment median HBV DNA levels decreased to 6.8 (5.8-9.5) $\log_{10}$ copies/mL. HBeAg loss occurred in none of the subjects. All patients had increased serum ALT levels at the start of ADV therapy; of these, one had ALT normalization prior to switching to ETV monotherapy. At the end of ADV treatment three (38%) patients demonstrated genotypic ADV resistance (rtN236T ± rtA181V/T), and in the one patient in whom LAM was continued after detection of LAM-resistance, the rtM204I mutation could also be detected (table1).

Table 1 Baseline characteristics

Patient No.	Gender	Age (yr)	Race	HBV Genotype	Pretreatment with lamivudine	Prior history of lamivudine resistance	Duration of pretreatment with adefovir (months)	Mutations in polymerase gene at baseline ETV	Presence of cirrhosis	HBeAg status	HBV DNA (log copies/ml)	Serum ALT (*ULN)
1	male	44	Caucasian	D	no	no	12	wild-type	no	positive	10.1	1.2
2	male	25	Caucasian	A	no	no	23	wild-type	no	positive	9.6	1.7
3	male	44	Other	A	no	no	31	wild-type	no	positive	6.7	1.4
4	male	64	Caucasian	A	no	no	18	wild-type	no	positive	8.5	1.0
5	male	32	Caucasian	F	no	no	13	wild-type	no	positive	6.1	0.9
6	female	23	Caucasian	A	no	no	15	wild-type	yes	positive	5.2	1.5
7	male	73	Other	B	yes	yes	12	N236T + M204I	yes	positive	7.8	5.1
8	male	43	Caucasian	D	yes	yes	30	N236T + A181V	yes	positive	9.7	5.8
9	male	45	Caucasian	D	yes	yes	23	N236T + A181V	no	positive	5.8	2.8
10	male	36	Asian	B	yes	yes	13	wild-type	yes	positive	9.4	2.6
11	male	52	Asian	C	yes	yes	19	wild-type	yes	negative	6.1	2.1
12	male	29	Caucasian	D	yes	yes	12	wild-type	no	positive	7.8	1.0
13	female	28	Caucasian	D	yes	no	21	wild-type	no	positive	8.2	4.2
14	female	23	Caucasian	D	yes	no	21	wild-type	no	positive	7.5	1.5

Laboratory tests

Serum alanine aminotransferase (ALT) levels were measured 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 a Microparticle Enzyme Immune Assay (MEIA, Abbott, Chicago, IL). Serum HBV DNA levels were measured using a previously described in house developed quantitative real-time polymerase chain reaction (PCR).(17, 18) Currently, this assay is multiplexed without compromising the lower limit of detection (373 copies/mL or < 69 IU/mL) with an internal control (pHHV) in order to control the process from DNA isolation through PCR.(19) HBV DNA was extracted from serum samples using the MagnaPureLC (Roche Applied Science, Almere, The Netherlands) as described before. (17) Presence of HBV polymerase gene mutations was determined using the InnoLiPA DR2 and DR3 line probe assay (Innogenetics, Gent, Belgium).

Clonal analysis

To determine the kinetics of ADV resistant mutants during ETV treatment a clonal sequence analysis was performed. HBV DNA was isolated using the MagnaPureLC and part of RT (domain B, C and D) was amplified using primers S1 (5'-GTATGTT-GCCCGTTTGTCCTC'-3') and YMDD2tripple (a combination of three primers to ensure the amplification of all genotypes, 5'-ACCCCATCTTTTTGTTTT-3' + 5'-ACCCCAAC-GTTTGGTTTTATTAGG-3' + 5'- ACCCCATCTTTTTGTTTTGTTAGG-3').(17) These primers amplify a PCR product of 403 bp (nt position 461 to 863). If a product of correct size was observed on agarose gel, it was ligated into PCR2.1 vector and transformed into competent E.coli bacteria according to manufacturer's protocol (Invitrogen, Breda, NL). White colonies were picked and colony PCR was performed using the same primers as described above. Sequencing was performed on the PCR products of the colony PCR using the same primers as describes above with Big Dye terminator V3.1 cycle sequencing kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and a ABI3100 instrument (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Subsequently, sequence analysis was performed using Sequence Navigator software sequencer (Applied Biosystems), Lasergene v7 (DNASTAR, Madison, WI) and BioEdit Software (v7.05).

Statistical analysis

HBV DNA levels were logarithmically transformed for analysis. To correct for differences in reference between males and females, ALT levels are expressed as values representing a ratio to the local upper limit of normal (xULN). Continuous variables are expressed as median (range). To compare two continuous variables the Mann-Whitney *U* test was used. Comparisons of categorical endpoints were assessed by the Chi^2

test. SPSS version 14.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of all 14 patients who met inclusion criteria are listed in table 1. Eleven (79%) patients were men and the median age was 40 (range: 23-73) years. Thirteen (93%) patients were HBeAg-positive, median ALT was 1.6 (0.9-5.8) x ULN, and median HBV DNA was 7.8 (5.2-10.1) $\log_{10}$ copies/mL.

Lamivudine-naïve patients with high HBV DNA during adefovir therapy

Serum HBV DNA levels and ALT levels of these six patients throughout the clinical course of ETV treatment are shown in figure 1a and 2a, and table 2. After 6 and 12

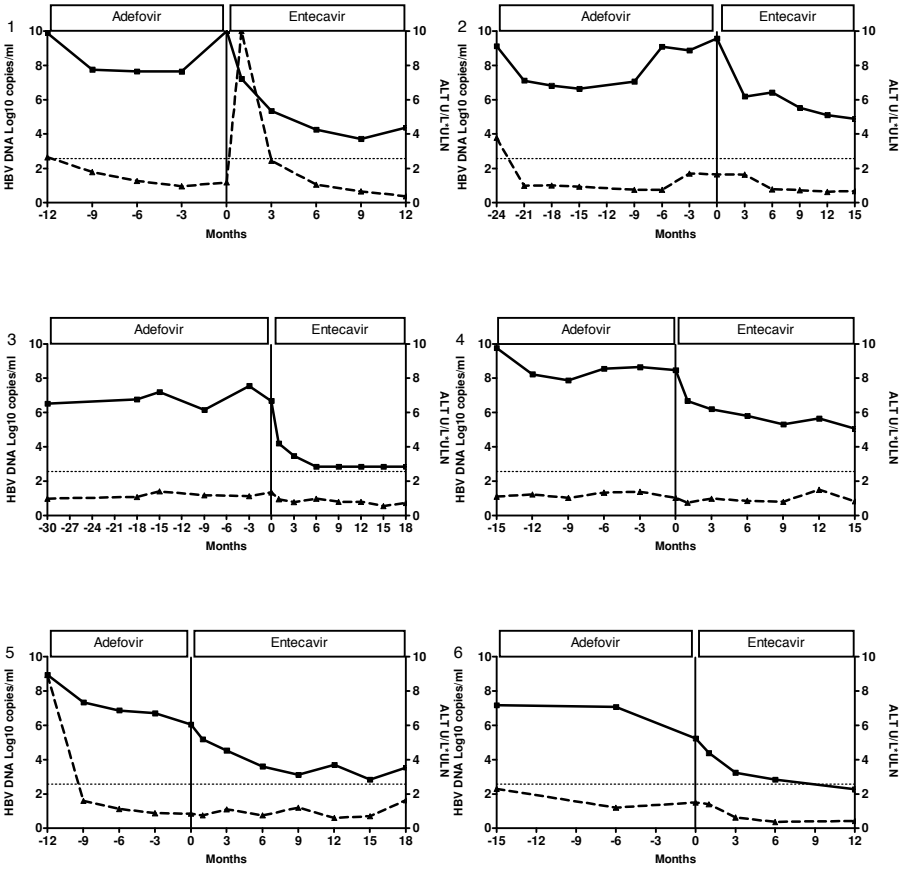


Figure 2A

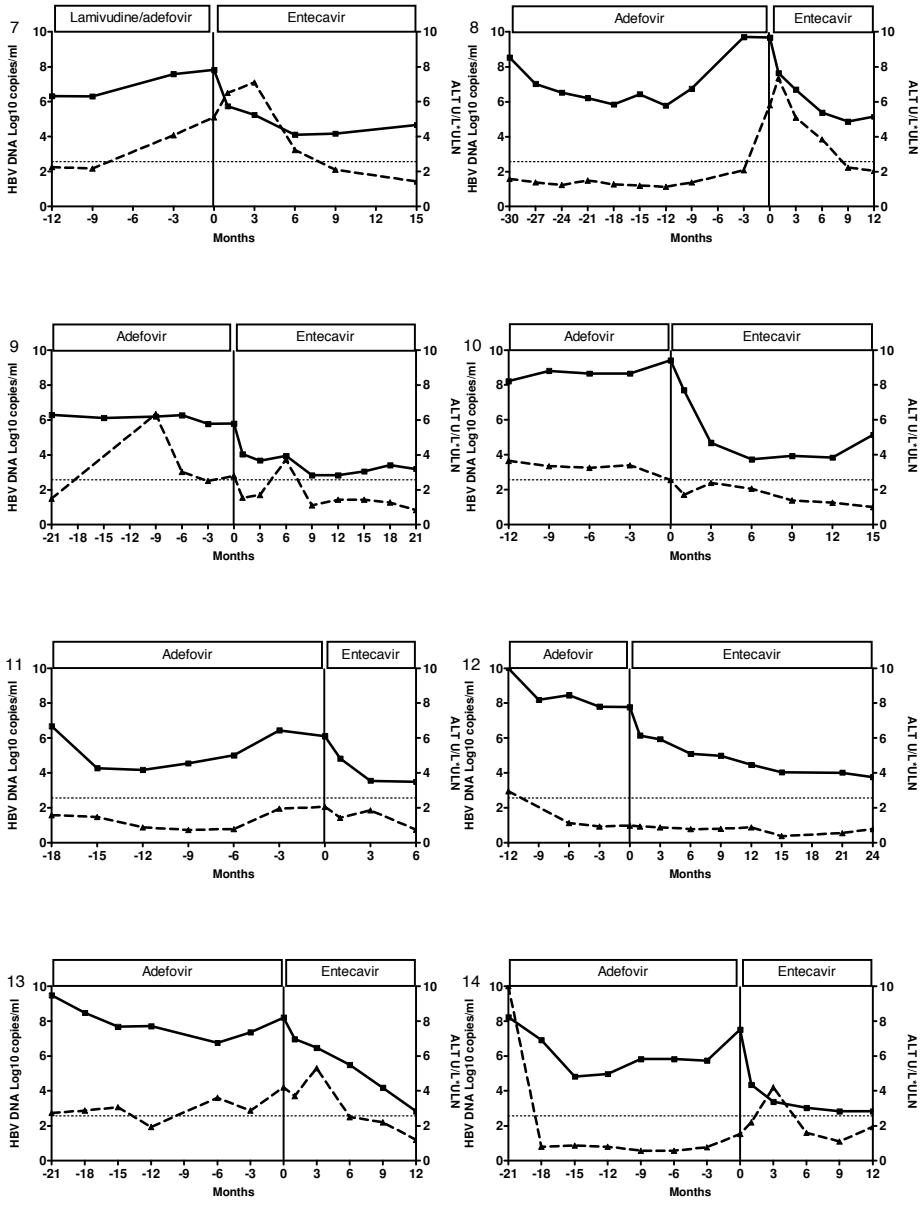


Figure 2B

Figure 2 Individual courses of HBV DNA (■) and ALT (▲) levels during ADV and consecutive ETV treatment in six lamivudine-naïve (A) and eight lamivudine-experienced (B) HBV-infected subjects with a persistently high level of viral replication during ADV treatment. The dashed line represents the lower limit of detection of the HBV DNA assay.

months of treatment the median decline of viral load was 2.9 (2.4-5.9) and 3.4 (2.4-5.8) log_{10} copies/mL, respectively. At the end of the observation period (median 16 months, range 12-20 months) the median decrease of HBV DNA was 3.6 (2.5-5.8) log_{10} copies/mL. Only one patient achieved HBV DNA levels below the detection limit after twelve months of ETV treatment. HBeAg loss occurred in none of the patients. All patients with serum ALT levels above the normal range prior to switching to ETV (n=5) demonstrated ALT normalization at the last visit. No virologic breakthroughs were observed during ETV treatment, and in none of the patients ETV-resistant mutations were detected at the end of follow-up.

Lamivudine-experienced patients with high HBV DNA during adefovir therapy

Eight patients had received prior treatment with LAM for a median duration of 20 (12-70) months. Six (75%) subjects developed LAM resistance. Median duration between the end of LAM treatment and the start of ETV treatment was 40 (0-74) months, and in one patient LAM-resistant mutations (rtM204I) could be detected at the start of ETV treatment. Serum HBV DNA levels and ALT levels of the patients throughout the clinical course of ETV treatment are shown in figure 1b and 2b, and table 2. None of the patients achieved HBV DNA levels below the detection limit during follow-up. Concerning virologic response to ETV, no significant differences were observed between LAM-experienced and LAM-naïve subjects. At the end of the ETV observation period two (25%) patients demonstrated the rtM204I mutation, but no other ETV-resistant substitutions were detected (rtI169, rtT184, rtS202, rtM250). In patient 7 this mutation could already be detected at baseline, and persisted throughout the follow-up period.

Table 2 Virologic and biochemical response to entecavir

		History of LAM therapy		
	All patients (n=14)	Present (n=8)	Absent (n=6)	*p*-value
Follow-up	15 (8-23)	14 (8-23)	16 (12-20)	0.44
HBV DNA				
Baseline	7.8 (5.2-10.1)	7.8 (5.8-9.7)	7.6 (5.2-10.1)	1.0
Month 6	4.0 (2.8-6.4)	4.0 (3.0-5.5)	3.9 (2.8-6.4)	0.90
Month 12	3.7 (2.3-5.7)	3.3 (2.8-5.2)	4.0 (2.3-5.7)	0.79
End of follow-up	3.8 (2.3-5.2)	3.8 (2.8-5.2)	4.0 (2.3-5.1)	0.90
HBV DNA decline				
Month 6	2.9 (1.9-5.9)	3.2 (1.9-5.7)	2.9 (2.4-5.9)	0.70
Month 12	3.6 (2.3-6.6)	3.9 (2.3-6.6)	3.4 (2.4-5.8)	0.52
End of follow-up	3.9 (2.3-5.8)	4.3 (2.3-5.4)	3.6 (2.5-5.8)	1.0
Undetectable HBV DNA	1/14	0/8	1/6	
HBeAg loss	0/14	0/8	0/6	
HBsAg loss	0/14	0/8	0/6	
ALT normalization	8/12	3/7	5/5	0.08

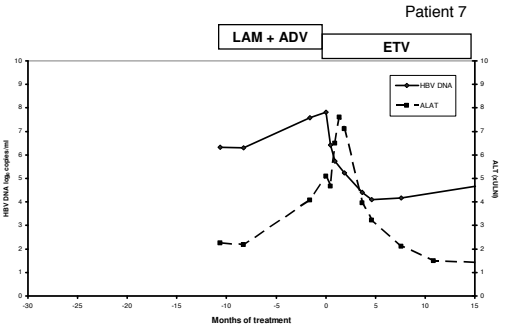
Patient 10 developed a virologic breakthrough subsequently, after which tenofovir (TDF) was added to treatment. The mutation could not be detected at baseline, or after six and twelve months of treatment. Both subjects had a prior history of LAM resistance.

Patients with genotypic adefovir resistance at baseline

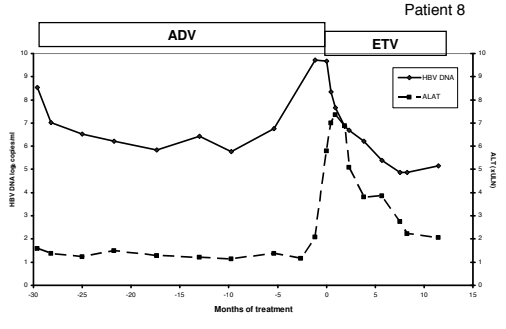
Of eight LAM-experienced subjects, three (38%) patients developed ADV-resistant mutations during ADV treatment, after which treatment was changed to ETV monotherapy. Figure 3 shows the clinical response to ETV of these patients. No significant differences were observed between patients with and without ADV-resistant mutations at baseline (data not shown). Clonal sequence analysis of follow-up samples with serum HBV DNA levels > 1000 copies/mL was performed to examine the evolution of ADV-resistant mutations during ETV treatment. In patient 7 the LiPA assay showed rtM204I and rtN236T substitutions at baseline. Yet, in all 27 clones only wild-type sequence was found. After six months of treatment serum HBV DNA levels decreased to 4.1 $\log_{10}$ copies/mL, and stayed at that level. In follow-up samples at month 3, 6 and 15 the rtM204I substitution was detected in all clones. No other mutations were selected (fig 3a). RtN236T and rtA181V mutations were demonstrated in all clones at baseline in patient 8. Serum HBV DNA levels decreased to only 5.2 $\log_{10}$ copies/mL after 12 months of treatment, and at month 12 all clones still harbored the ADV-resistant substitutions (fig 3b). At baseline patient 9 had rtN236T and rtA181V mutations in all 52 clones. HBV DNA decreased to 3.6 $\log_{10}$ copies/mL after three months of treatment and remained at that level. Due to low viral load clonal analysis was not successful in subsequent blood samples (fig 3c).

Efficacy and safety of entecavir 2 mg daily

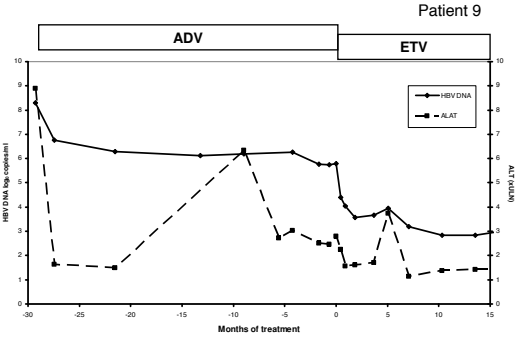
In an attempt to gain an increased antiviral response the dosage of ETV was enhanced to 2 mg daily in two patients. Figure 4 shows HBV DNA and ALT kinetics in these patients during treatment with ADV 10 mg, ETV 1 mg, and ETV 2 mg. Both patients demonstrated a persistently high viral load during treatment with ETV 1 mg, showing a viral load greater than 5 $\log_{10}$ copies/mL after twelve and fifteen months of treatment, respectively. No ETV-resistant mutations could be detected at the moment the dosage of ETV was increased to 2 mg daily. In patient 8 serum HBV DNA levels decreased from 5.2 to 3.4 $\log_{10}$ copies/mL within ten months of treatment. In patient 4 HBV DNA decreased from 5.1 to 3.9 $\log_{10}$ copies/mL after eight months of treatment. ETV 2 mg daily was well tolerated by both patients.



Clonal analysis						
Month	-11	0	3	6		15
Wildtype (rtM204M) (%)		100	0	0		0
rtM204I(%)		0	100	100		100
Number of clones		27	13	17		42
InnoLiPA	M204I	M204I/M N236N/T	M204M/I N236N	M204I N236N		M204I N236N



Clonal analysis						
Month	-30	0	6	9	12	
rtN236T + rtA181V (%)		100	100	95	100	
rtN236N + rtA181V (%)		0	0	5	0	
Number of clones		17	8	48	28	
InnoLiPA	M204I L80V/I	A181V N236N/T	A181V N236N/T	A181V N236N/T	A181V N236N/T	



Clonal analysis			
Month	-30	0	15
rtN236T + rtA181V (%)		100	
Number of clones		52	
InnoLiPA	M204I L80V/I	A181A/V N236N/T	NA

Figure 3 Clinical course and cloning results under entecavir monotherapy of three lamivudine-experienced patients who had genotypic adefovir resistance (InnoLiPA) at the start of entecavir treatment. No significant differences were observed between patients with and without ADV-resistant mutations at baseline.

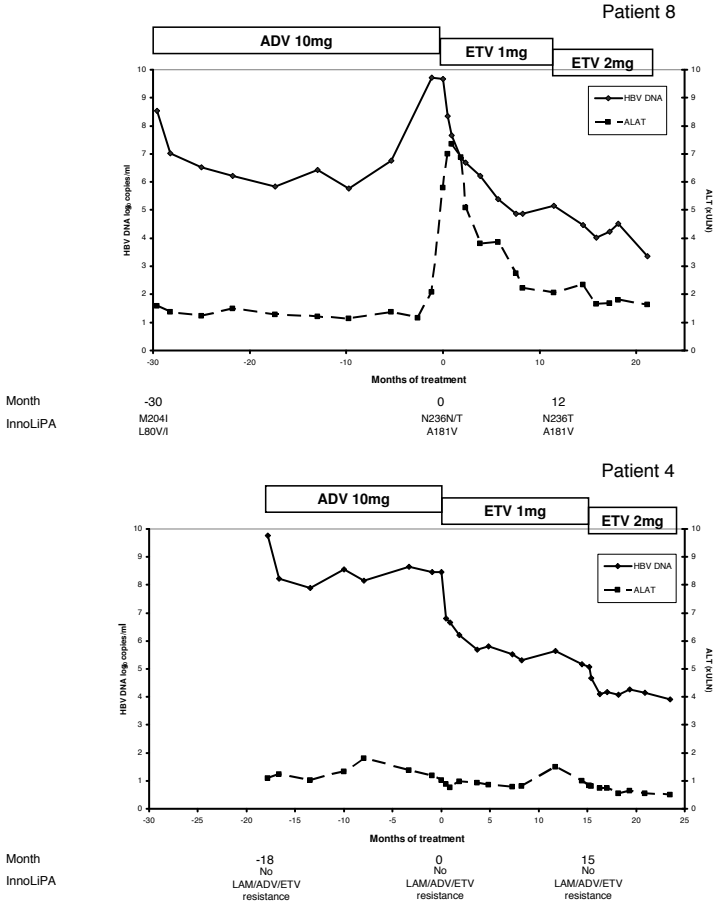


Figure 4 Clinical course of two patients during sequential treatment with adefovir 10 mg, entecavir 1 mg, and entecavir 2 mg.

DISCUSSION

This is the first study to describe the efficacy of ETV as rescue therapy for patients with a persistently high viral load during ADV treatment. We showed that ETV monotherapy resulted in a limited HBV DNA decline in both LAM-experienced and -naïve patients with persistently high HBV DNA during ADV treatment. Only one of fourteen patients achieved undetectable HBV DNA levels (< 373 copies/mL), and none of the patients showed HBeAg loss or seroconversion. Two LAM-experienced patients demonstrated the presence of the rtM204I mutation, but no other ETV-resistant mutations were detected at the end of follow-up. Increasing the dosage of ETV to 2 mg daily resulted in a further decline in viral load, and was tolerated well. Furthermore, ADV-resistant substitutions persisted during ETV monotherapy.

Non-compliance to either ADV or ETV was unlikely for several reasons. First, all patients repeatedly confirmed their adherence in all outpatient clinic visits. Second, all patients demonstrated a continuous HBV DNA decline and no unexpected virologic rebounds were observed. Third, the two patients who were switched to 2 mg demonstrated further viral load reduction. We did not perform random sampling of entecavir plasma levels, because it is still of limited value to assess adherence. Due to the relatively low accumulation index for ETV identification of "white coat" adherence is not feasible. In addition, the long terminal elimination phase makes it difficult to reliably distinguish between a sample taken 24-hours after the last dose and a sample taken 2-4 days after the last dose. Therefore, only a sample with no detectable drug would assure you that a patient had not taken any ETV for a period of at least several weeks.(20)

A general host and/ or pharmacological effect, which results in the inability to achieve sufficient intracellular concentrations of the active moiety of nucleoside analogues, could be an explanation for the poor virologic response to both treatment regimens. One could therefore postulate that the selected group of patients in our study might be in fact "underdosed". Indeed, two patients with a limited virologic response to ETV 1 mg daily demonstrated further HBV DNA decline after increasing the dosage to 2 mg daily. This observation suggests that increasing a drug to its maximum tolerated dose may provide a reasonable option to maximize antiviral activity besides switching to or adding another drug.

Another possible explanation might be the presence of HBV strains with reduced susceptibility to ETV at baseline. In vitro studies demonstrated that LAM-resistance associated mutations induce a decrease in susceptibility to ETV, and it is also suggested that ETV exhibits a positive selective pressure on LAM-resistant mutants in vivo.(21, 22) In our study, no significant differences were observed between LAM-experienced and LAM-naïve patients, which may be related to the large interval period (median 40 months; range 0-74 months) between the end of LAM treatment and the start of ETV monotherapy. Yet, both subjects in whom the rtM204I mutation could be detected at the end of follow-up were in fact previously treated with LAM. In contrast to LAM, ADV-resistant mutations are expected to remain sensitive to ETV. Therefore, an unexpected finding was that rtN236T and rtA181V mutations persisted during ETV therapy, which may indicate a selective advantage for these HBV variants. In vitro studies showed, however, that the rtN236T mutant remains sensitive to ETV with <3-fold change in IC_{50}. (23-25) A recent study reported that rtA181V/T mutations remain completely sensitive to ETV as well (26), although other studies demonstrated a slightly decreased susceptibility. (23-25, 27) Nevertheless, the higher antiviral potency of ETV may overcome this potential weak decrease in susceptibility. In our study, two of three patients with genotypic ADV resistance at baseline demonstrated a rapid virologic response to ETV, although undetectable HBV DNA levels were not achieved.

In accordance with previous studies (16), our study shows that ETV monotherapy may not be the best rescue option for LAM-experienced patients. However, our study also shows that ETV resulted in a limited virologic response in LAM-naïve patients with a persistently high level of viral replication during ADV treatment. In contrast, TDF resulted in undetectable HBV DNA levels in nineteen of twenty LAM-refractory patients with high HBV DNA levels during ADV treatment after a median time of 3.5 months. (28) In another study, 81% of patients (57% LAM-experienced) with an incomplete response to ADV achieved undetectable HBV DNA levels (< 400 copies/mL) after 48 weeks of TDF monotherapy.(29) However, it should be noted that the median baseline viral load was considerably lower in both these studies compared to our study (6.4 $\log_{10}$ and 6.1 $\log_{10}$ vs. 7.8 $\log_{10}$). Furthermore, the median HBV DNA decline after twelve months of treatment (3.8 $\log_{10}$) in the study by Van Bommel et al. was actually comparable to the observed reduction in our study.(28) Therefore, TDF monotherapy proved to be effective in both LAM-experienced and –naïve patients with low viral load at baseline, but its efficacy has still to be demonstrated in highly viraemic HBV patients with a poor response to ADV. As TDF and ETV are the most potent antiviral drugs that are currently approved, our data suggest that combination therapy may be more beneficial in this subset of patients.

In conclusion, in both LAM-experienced and –naïve patients with a persistently high level of viral replication during ADV treatment, switching to ETV monotherapy resulted in a limited virologic response. During treatment only one of fourteen patients achieved undetectable HBV DNA, which indicates the need for close monitoring for development of ETV resistance, especially in the lamivudine-experienced population. Furthermore, ADV-resistant mutations persisted during ETV monotherapy, which may suggest a selective advantage for these HBV variants, and highlights the importance of potential presence of cross-resistance among nucleos(t)ide analogues. Increasing the dosage of ETV to 2 mg daily resulted in a further decline in viral load and was tolerated well.

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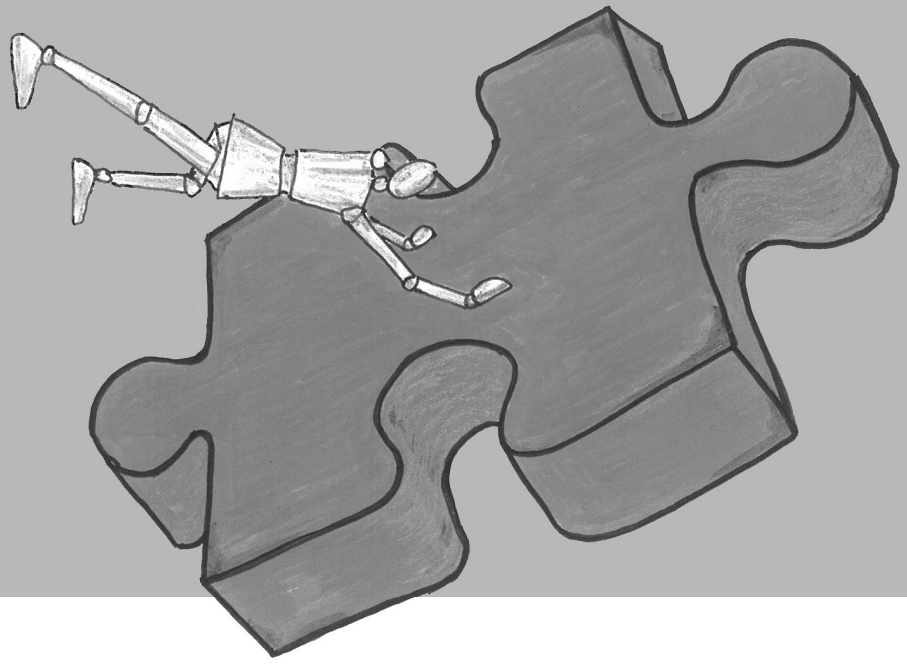

On-treatment monitoring of adefovir therapy in chronic hepatitis B: Virologic response can be assessed at 24 weeks

Jurriën G.P. Reijnders, Wilhelmus F. Leemans, Bettina E. Hansen, Suzan D. Pas, Robert A. de Man, Martin Schutten, Harry L.A. Janssen

J Viral Hepat. 2009 Feb;16(2):113-20

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ABSTRACT

Background

Patients with chronic hepatitis B (CHB) who will and who will not respond to adefovir (ADV) monotherapy need to be identified in an early stage in order to adjust treatment and prevent future development of antiviral resistance.

Methods

In a single center cohort study we investigated seventy-six CHB patients (50% HBeAg-positive) treated with long-term ADV monotherapy.

Results

During a median follow-up of 122 (24-185) weeks 42 (55%) patients achieved virologic response (VR), defined as HBV DNA levels < 10^3 copies/mL, and ten patients (13%) developed genotypic ADV resistance. Independent baseline predictors of VR were HBeAg negativity (HR (hazard ratio) HR 2.98; 95%CI 1.24-7.19; p = 0.02), high ALT levels (HR 1.11; 95% CI 1.05-1.18; p = 0.001), and low HBV DNA levels (HR 0.56; 95% CI 0.41-0.75; p < 0.001). HBV DNA at week 24 demonstrated a higher predictive value for VR than HBV DNA at week 48. Important predictors of genotypic resistance were presence of cirrhosis (HR 6.54; 95% CI 1.39-30.9; p = 0.018), and not achieving VR during treatment (HR 6.60; 95% CI 1.35-32.4; p = 0.008). Patients without VR at week 24 already demonstrated a trend towards the emergence of ADV resistance (p = 0.07)

Conclusion

HBV DNA at week 24 was a better on-treatment predictor of VR than HBV DNA at week 48, and ADV-resistant mutations developed more frequently in patients without VR at week 24. Therefore, our study suggests that virologic response to ADV therapy can already be assessed at 24 weeks, instead of the generally recommended 48 weeks.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is still a serious global health problem with an estimated 350 million people chronically infected, and 0.5-1.2 million deaths a year. (1-2) With the currently approved treatment options the major goal of treatment is HBV DNA suppression in order to prevent development of liver cirrhosis, end-stage liver disease, and hepatocellular carcinoma.

Adefovir dipivoxil (ADV) is an oral prodrug of adefovir, a phosphonate acyclic nucleotide analogue of adenosine monophosphate.(3) Previous studies demonstrated its efficacy in patients with HBeAg-positive and HBeAg-negative chronic HBV infection, showing significant virologic, biochemical, and histological improvement after 48 weeks of treatment.(4-5) However, genotypic resistance rates are up to 29% after five years of treatment with ADV.(6) Two mutations (rtN236T, rtA181V) have been described to confer resistance to ADV.(7-8) Other mutations have also been reported to be associated with reduced susceptibility to ADV, including the rtA181T and rtI233V mutations, but the significance of these mutations remains unclear.(9-13) Furthermore, it is known that a significant proportion of patients have slower and poor primary responses to ADV, probably related to the suboptimal approved dose. In one study, 25% of patients had a less than 2.2 $\log_{10}$ reduction in HBV DNA levels after 48 weeks of treatment. (14) It is currently recommended that in HBeAg-positive patients treatment can be stopped after HBeAg-seroconversion with at least six months of consolidation treatment. In HBeAg-negative patients discontinuation may only be possible after HBsAg clearance, necessitating long-term therapy for a significant proportion of patients.(15-17) However, development of antiviral drug resistance is a major limitation to long-term efficacy of nucleos(t)ide analogues and will thus be an important factor in treatment failures. (18) It is known that resistance only emerges when replication occurs in the presence of drug selection pressure, and complete suppression of viral replication allows little opportunity for resistance to develop.(19) Several studies have already shown that a rapid virologic response is associated with lower rates of antiviral drug resistance in HBV patients in the long term.(20-22) Therefore, antiviral therapy, once initiated, should aim to suppress viral replication as quickly and completely as possible, and patients who will or will not respond to ADV monotherapy need to be identified in an early stage in order to adjust treatment and prevent future development of antiviral drug resistance.

The primary aim of our observational study was to assess virologic response to ADV in patients with chronic hepatitis B virus infection, and to identify baseline and on-treatment factors associated with virologic response in the setting of clinical practice. Secondary aims were to evaluate rates of HBeAg loss, and genotypic resistance rates, and to explore associated baseline and on-treatment parameters.

METHODS AND MATERIALS

Study population

In this retrospective cohort study, all adult HBV patients with compensated liver disease and a viral load of at least 4 $\log_{10}$ copies/ mL referred to the Erasmus Medical Center Rotterdam from August 2003 to March 2006, who had received ADV monotherapy for at least six months, were included in the analysis. Patients were excluded if they had decompensated liver disease or a diagnosis of hepatocellular carcinoma at baseline, received immunosuppressive medication, or if they had co-infections (HIV, HCV, HDV) or other liver diseases.

Follow-up

All subjects were monitored every 3-4 months. At every visit routine examination with biochemical (ALT, bilirubin, albumin, creatinin) and virologic (HBV DNA level, HBeAg, anti-HBe) assessments took place. Genotypic analysis was done in case of virologic breakthrough, defined as an increase in serum HBV DNA level > 1 $\log_{10}$ (10-fold) above nadir on at least two occasions after initial virologic response, or in case of serum HBV DNA > 4 $\log_{10}$ copies/mL at the end of follow-up. HBV genotype was determined at baseline in all patients.

Endpoints

The primary outcome was virologic response (VR), defined as serum HBV DNA levels < 3 $\log_{10}$ copies/mL during the on-treatment follow-up period. Secondary endpoints were loss of HBeAg for HBeAg-positive patients and emergence of ADV-related mutations.

Laboratory tests

Serum alanine aminotransferase (ALT) levels were measured 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 a Microparticle Enzyme Immune Assay (MEIA, Abbott, Chicago, IL). Serum HBV DNA levels were measured using a previously described in house developed quantitative real-time polymerase chain reaction (PCR).(23-24) Currently, this assay is multiplexed without compromising the lower limit of detection (373 copies/mL) with an internal control (pHHV) in order to control the process from DNA isolation through PCR.(25) To investigate resistance-associated mutations related with ADV treatment, HBV DNA was extracted from serum samples using the MagnaPureLC (Roche Applied Science, Almere, The Netherlands) as described before and part of the HBV polymerase reverse transcriptase (domain A, B, C, D and F) was PCR amplified and sequenced directly, using a nested PCR.(23) The outer primers were

HT26-5 (3'-CAGGCCATGCAGTGGAA-5') and YMDD2tripple (a combination of three primers to ensure the amplification of all genotypes, 5'-ACCCCATCTTTTTGTTTT-3' + 5'-ACCCCAACGTTTGGTTTTATTAGG-3' + 5'- ACCCCATCTTTTTGTTTTGTTAGG-3') amplifying a PCR product of 880 bp and in the semi-nested PCR reaction the forward primer was replaced by HT26-2 (5'-CCTGCTGGTGGCTCCAGTTC-3'), amplifying a product of 806 bp. Sequencing was performed using Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and a ABI3100 instrument (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Subsequently, sequence analysis was performed using Sequence Navigator software sequencer (Applied Biosystems), Lasergene v7 (DNASTAR, Madison, WI) and BioEdit Software (v7.05), Ibis Therapeutics, Carlsbad, CA, USA). The same region and procedure were used to determine HBV genotypes. The consensus sequences for genotypes A-H were obtained from the GenBank.

Statistical analysis

HBV DNA levels were logarithmically transformed for analysis. To correct for differences in reference between males and females, ALT levels are expressed as values representing a ratio to the local upper limit of normal (xULN). Continuous variables are expressed as means ± SD or median (range). Follow-up times were calculated from the date of ADV treatment initiation to the date of event or censorship. Cumulative probabilities of different endpoints were estimated by Kaplan-Meier analysis. The relative risk of several baseline and on-treatment parameters was estimated as an hazard ratio (HR) in an univariate and multivariate Cox's proportional hazards model and presented with a 95 percent confidence interval (95%CI). Multivariate analysis was performed with all variables with a p-value < 0.2 in univariate analysis. As the low number of patients achieving both secondary endpoints did not provide enough power to include multiple variables, only univariate analysis was performed to assess baseline and on-treatment predictors. All statistical tests are two-sided, and a p-value < 0.05 was considered to be statistically significant. SPSS version 14.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of the study population are presented in table 1. A total of 76 patients treated with ADV monotherapy in our hospital were included in this analysis. Eighteen patients were excluded. Fourteen patients had a baseline HBV DNA < 4 $\log_{10}$ copies/mL, of whom eight subjects were switched from a tenofovir (TDF)-containing regimen. One patient had coexisting auto-immune hepatitis, two patients were

Table 1 Baseline characteristics (N=76)

Age	46 ± 14
Gender (male %)	57 (75%)
Race	
Caucasian	44 (58%)
Asian	19 (25%)
Other	13 (17%)
BMI	25 ± 4.1
ALT (*ULN)	3.8 ± 4.2
HBV DNA (log_{10} copies/ml)	7.5 ± 1.6
HBeAg-positive	38 (50%)
Genotype	
A	24 (32%)
B	12 (16%)
C	9 (12%)
D	26 (34%)
Other	5 (7%)
Cirrhosis	30 (40%)
Previous treatment with peginterferon	27 (36%)
Previous treatment with lamivudine	42 (55%)
Patients with LAM resistance at baseline	14 (18%)
Patients with a prior history of LAM resistance	25 (33%)

co-infected with HIV, and one patient was co-infected with HDV. Of the 76 patients, fifty-seven (75%) subjects were men and the mean age was 46±14 years. Thirty-eight (50%) patients were HBeAg-positive, mean ALT was 3.8±4.2 xULN, and mean HBV DNA was 7.5±1.6 log_{10} copies/mL. The most common genotypes were A (32%), B (16%), C (12%), and D (34%). Thirty (40%) subjects had a diagnosis of cirrhosis at baseline. Thirty-two (42%) patients received adefovir monotherapy as de novo treatment, while 27 (36%) and 42 (55%) patients were previously treated with (pegylated) interferon or lamivudine (LAM), respectively. Twenty-five (33%) patients had a prior history of LAM resistance. In fourteen (18%) patients LAM-resistant mutations could still be detected at the start of ADV treatment. Median follow-up was 122 (24-185) weeks.

Virologic response to adefovir

Overall, 42 (55%) patients achieved VR after a median follow-up of 23 (4-173) weeks, of whom 30 subjects (71%) maintained it throughout the on-treatment follow-up. In 8 additional patients (19%) serum HBV DNA levels remained below 4 log_{10} copies/mL. The cumulative probability of achieving VR at 12, 24, 48, and 96 weeks was 11, 28, 39, and 49%, respectively (figure 1). Undetectable HBV DNA (<373 copies/mL) was demonstrated by only 19 (25%) patients during follow-up. Table 2 shows predictors of virologic response. Using multivariate analysis increased probabilities of VR were seen in HBV patients with HBeAg negativity at baseline (HR 2.98; 95%CI 1.24-7.19;

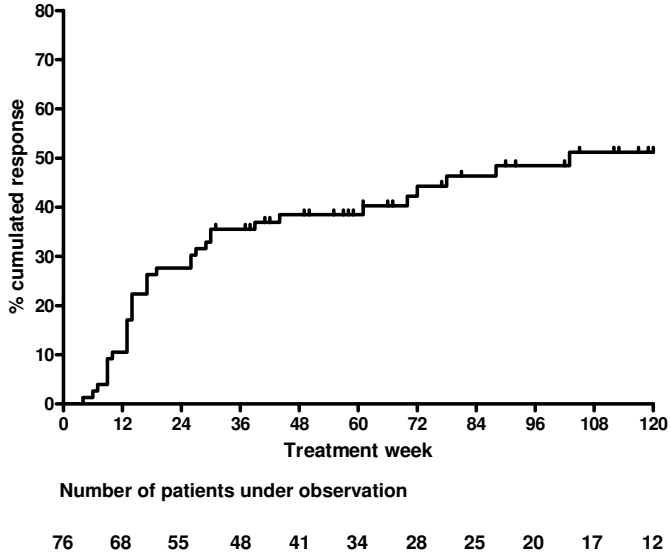

Figure 1 Kaplan-Meier curve for the cumulative probabilities of achieving virologic response, defined as HBV DNA < 10^3 copies/mL.

p = 0.02), high baseline ALT levels (HR 1.11; 95% CI 1.05-1.18; p = 0.001), and low baseline HBV DNA levels (HR 0.56; 95% CI 0.41-0.75; p < 0.001). In univariate analysis HBV DNA levels at week 24 and 48 of ADV treatment were associated with VR as well, but both parameters did not reach statistical significance in multivariate analysis. Nevertheless, HBV DNA at week 24 demonstrated a higher predictive value for VR than HBV DNA at week 48. Clear cut off points for both ALT and HBV DNA levels could not be found. Initial virologic response, defined as serum HBV DNA levels < 4 $\log_{10}$ copies/mL after 24 weeks of treatment (26), was achieved in 35 (46%) patients, of whom 86% reached VR subsequently. Of the 41 patients who did not achieve initial virologic response, VR was found before week 48 and at the end of follow-up in two (5%) and twelve patients (29%), respectively. Seventeen patients (22%) demonstrated primary nonresponse, defined as a decrease in serum HBV DNA of less than 2 $\log_{10}$ after 24 weeks of treatment (16), of whom four subjects (24%) showed VR at the end of follow-up. Of the patients with an HBV DNA decline of more than 2 $\log_{10}$ after 24 weeks (59 subjects), only 64% achieved VR during follow-up.

Serological response to adefovir

In total, eight of thirty-eight HBeAg-positive patients (21%) lost HBeAg during follow-up. Six patients also seroconverted to anti-HBe. The cumulative probability of HBeAg loss during ADV treatment was 10% after 48 weeks and 19% after 96 weeks of treatment. In none of the patients ADV was discontinued after HBeAg loss. One patient demonstrated a seroreversion during follow-up. In the univariate analysis, HBeAg loss

Table 2 Baseline and on-treatment predictors of virological response: time-to-event analysis

Parameters	Univariate Cox PH model			Multivariate Cox PH model		
	HR	(95%CI)	*p*-value	HR	(95%CI)	*p*-value
Gender	0.70	(0.32-1.51)	0.36	-	-	-
Age (per 1 year increase)	1.02	(1.0-1.05)	0.05	-	-	-
Race						
Caucasian	1.00	reference		-	-	-
Asian	1.92	(0.97-3.82)	0.06	-	-	-
Other	1.14	(0.46-2.83)	0.78	-	-	-
BMI (per 1 unit increase)	0.96	(0.89-1.05)	0.38	-	-	-
Genotype						
A	1.00	reference		-	-	-
B	2.78	(1.18-6.58)	0.02	-	-	-
C	0.56	(0.16-1.95)	0.36	-	-	-
D	0.91	(0.43-1.94)	0.80	-	-	-
Other	1.06	(0.24-4.74)	0.94	-	-	-
HBeAg negativity	7.55	(3.39-16.8)	< 0.001	2.98	(1.24-7.19)	0.02
Baseline HBV DNA (per $\log_{10}$ increase)	0.50	(0.40-0.64)	< 0.001	0.56	(0.41-0.75)	< 0.001
Baseline ALT (per 1*ULN increase)	1.06	(1.00-1.13)	0.05	1.11	(1.05-1.18)	0.001
Previous treatment						
None	1.00	reference		-	-	-
(PEG) IFN	0.96	(0.13-7.29)	0.97	-	-	-
Lamivudine	0.76	(0.34-1.71)	0.51	-	-	-
(PEG) IFN/ Lamivudine	0.81	(0.39-1.65)	0.55			
Lamivudine resistance at baseline	1.33	(0.63-2.81)	0.46	-	-	-
Prior history of lamivudine resistance	0.78	(0.40-1.55)	0.49	-	-	-
Viral load during ADV treatment						
HBV DNA (per $\log_{10}$ increase) at week 24	0.48	(0.32-0.72)	< 0.001	0.70	(0.47-1.03)	0.07
HBV DNA (per $\log_{10}$ increase) at week 48	0.33	(0.13-0.82)	0.02	-	-	-
Cirrhosis	0.73	(0.38-1.38)	0.33	-	-	-

was only associated with high baseline serum ALT levels (HR 1.15; 95% CI 1.02-1.31; $p = 0.029$).

Development of genotypic resistance to adefovir

Ten patients (13%) developed genotypic ADV resistance during a median follow-up of 122 (24-185) weeks of treatment. The cumulative probability of developing ADV-resistant mutations was 3% and 8% after 48 and 96 weeks of treatment, respectively. In the univariate analysis, predictors of genotypic resistance were female gender (HR 4.99; 95% CI 1.40-17.8; $p = 0.013$), a higher BMI (HR 1.18; 95% CI 1.02-1.37; $p = 0.026$), presence of cirrhosis (HR 6.54; 95% CI 1.39-30.9; $p = 0.018$), and not achieving VR during treatment (HR 6.60; 95% CI 1.35-32.4; $p = 0.008$). Furthermore, HBeAg positivity at baseline (HR 3.09; 95% 0.79-12.0; $p = 0.10$), and HBV DNA levels above

Table 3 Predictors of development of genotypic adefovir resistance: time-to-event analysis (univariate Cox's proportional hazards model)

Parameters	HR	(95%CI)	*p*-value
Gender (female vs. male)	4.99	(1.40-17.8)	0.013
Age (per 1 year increase)	1.03	(0.98-1.09)	0.24
Race			
Caucasian	1.00	reference	
Other	0.44	(0.09-2.12)	0.30
BMI (per 1 unit increase)	1.18	(1.02-1.37)	0.026
Genotype			
A	1.00	reference	
D	3.51	(0.70-17.6)	0.13
Other	1.53	(0.20-11.5)	0.68
HBeAg positivity	3.09	(0.79-12.0)	0.10
Baseline HBV DNA (per $\log_{10}$ increase)	1.09	(0.71-1.66)	0.70
Baseline ALT (per 1*ULN increase)	0.93	(0.79-1.10)	0.41
Prior exposure to lamivudine	2.55	(0.53-12.3)	0.24
Lamivudine resistance at baseline	0.94	(0.19-4.82)	0.94
Prior history of lamivudine resistance	2.51	(0.66-9.63)	0.18
Cirrhosis	6.54	(1.39-30.9)	0.018
Not achieving VR	6.60	(1.35-32.4)	0.008
Viral load during ADV treatment			
HBV DNA > 3$\log_{10}$ c/mL at week 24	7.15	(0.89-57.6)	0.07
HBV DNA > 3$\log_{10}$ c/mL at week 48	3.69	(0.76-17.8)	0.07

3 $\log_{10}$ copies/mL after 24 and 48 weeks of treatment exhibited a trend towards the emergence of ADV-resistant mutations (table 3). No patients with genotype B or C developed genotypic ADV resistance.

Clinical outcome of patients with genotypic resistance

Table 4 shows a summary of the ten patients developing genotypic resistance during follow-up. Of these ten patients only three patients were initially switched to ADV after LAM breakthrough. At baseline, seven patients were HBeAg positive, median serum HBV DNA level was 8.4 (5.2-9.4) $\log_{10}$ copies/mL, and median serum ALT level was 2.5 (1.2-10.6) xULN. Eight patients had a diagnosis of cirrhosis. Median maximal viral suppression was 3.9 $\log_{10}$ copies/mL. ADV-resistant mutations were detected at a median of 98 (36-177) weeks after treatment was initiated. At time of ADV resistance, median serum HBV DNA level was 5.1 (3.9-8.1) $\log_{10}$ copies/mL, and median serum ALT level was 1.2 (0.6-2.5) xULN. Two patients had an episode of decompensation, and one of these patients died subsequently of liver failure. Specific ADV-resistant mutations included rtN236T and rtA181V (two patients), rtN236T (two patients), rtA181V/T (four patients), and rtV214A, rtQ215S/P (two patients). After detection of resistance, ADV was discontinued in five patients, and four of these subjects were switched to entecavir

Table 4 Summary of patients with genotypic adefovir resistance

	Patiënt 1	Patiënt 2	Patiënt 3	Patiënt 4	Patiënt 5	Patiënt 6	Patiënt 7	Patiënt 8	Patiënt 9	Patiënt 10
Age (years)	70	48	55	53	21	40	52	50	51	65
Gender	female	female	female	female	female	male	male	male	female	male
At start of adefovir										
HBeAg status	positive	positive	negative	positive	positive	positive	negative	negative	positive	positive
HBV DNA (log_{10} c/ml)	6.8	9.4	5.5	9.1	8.2	8.5	5.3	5.2	9.2	9.4
ALT (xULN)	2.7	1.2	1.8	4.9	10.7	1.6	2.8	2.2	4.4	1.3
Cirrhosis	yes	no	yes	yes	no	yes	yes	yes	yes	yes
LAM-resistance	yes	no	no	no	no	no	no	yes	no	yes
Prior LAM resistance	yes	yes	no	yes	no	yes	no	yes	no	yes
Prior LAM exposure	yes	yes	no	yes	yes	yes	no	yes	yes	yes
Genotype	D	E	E	D	D	D	D	A	D	A
ADV-resistant mutation	no	no	no	no	no	no	no	no	no	no
Max. viral suppression (log_{10} c/ml)	4.8	3.8	3.1	3.6	4	5.8	undetect-able	2.3- 3.0	4.7	6.4
Week of ADV mutation detection	36	42	90	118	81	105	165	91	117	177
At time of ADV resistance										
HBV DNA (log_{10} c/ml)	5.4	5	3.9	4.2	5	6.8	4.2	5.1	5.9	8.1
ALT (xULN)	2.5	0.63	1.7	1.8	0.77	1.4	1.1	1.3	0.9	1.1
ADV-resistant mutation	V214A. Q215S. Q215P	A181T, M204V	A181V	A181T	Q215S	N236T. A181V	N236T. A181V	N236T	N236T	A181V
Adverse outcome	decompensa-tion/death	none	none	decom-pensation	none	none	none	none	none	none
Response to salvage therapy										
Salvage therapy	none	none	Addition of lamivudine	Addition of lamivudine	Entecavir	Entecavir	Entecavir	Adefovir*	Entecavir	Tenofovir/ lamivudine
Follow-up (weeks)		36	27	49	48	36	13	70	13	0
HBV DNA at last F/U (log_{10} c/ml)		6.4	undetect-able	3.3	2.3- 3.0	4.9	2.3- 3.0	3.8	3.4	

* Genotypic resistance was retrospectively discovered. ADV was therefore continued and serum HBV DNA levels remained between 3-4 log_{10} c/mL

(ETV). In one patient TDF/ LAM combination treatment was started. Two patients continued ADV and LAM was added. One patient did not receive salvage therapy. In this patient serum HBV DNA levels decreased and stabilized between 3-4 $\log_{10}$ copies/ mL; ALT levels remained normal at continued ADV therapy. At the last follow-up visit ADV-resistant mutations could not be detected.

DISCUSSION

In our study, 55% of patients demonstrated virologic response defined as serum HBV DNA levels < 3 $\log_{10}$ copies/mL, 21% of HBeAg-positive patients lost HBeAg, and 13% of patients developed ADV resistance during a median follow-up of 122 (24-185) weeks. HBeAg-negativity at baseline, high baseline serum ALT, and low baseline serum HBV DNA levels were independent predictors of VR. HBV DNA at week 24 was a better on-treatment predictor of VR than HBV DNA at week 48. A diagnosis of cirrhosis at baseline and not achieving VR were the most important predictors of occurrence of ADV-resistant mutations.

It is known that a significant proportion of patients have slower and poor primary responses to ADV, probably related to the suboptimal approved dose.(14). As demonstrated by our and other studies in ADV- and LAM-treated populations, the most important baseline predictors of achieving VR are HBeAg-negativity, higher ALT, and lower HBV DNA levels.(26-29) Clear cut-off points for both ALT and HBV DNA levels could not be found in this study.

In contrast to the treatment of chronic hepatitis C virus infection, current official guidelines on the management of chronic HBV infection provides only few recommendations on on-treatment evaluation.(16) Recently, a new strategy in the treatment with nucleos(t)ide analogues, the roadmap concept, was proposed. It was recommended that virologic response to ADV should be assessed at week 48 for both HBeAg-positive and HBeAg-negative patients, as ADV has a delayed antiviral effect.(30) Indeed, Locarnini et al. showed that absolute HBV DNA levels above 3 $\log_{10}$ copies/mL after one year of therapy were predictors of ADV resistance at three years.(21) In our study, development of genotypic resistance also occurred more frequently in patients with HBV DNA levels above 3 $\log_{10}$ copies/mL after 48 weeks treatment. Probably due to the low number of patients with ADV resistance this association did not reach statistical significance (P=0.07). However, even more interesting is that this trend could already be found using absolute HBV DNA levels at week 24. In addition, viral load at week 24 demonstrated a higher predictive value for VR than HBV DNA levels at week 48, and only two patients who did not show initial virologic response at week 24, responded before week 48. Yet, it should be mentioned that baseline parameters of VR were far

more important predictors of VR than on-treatment HBV DNA levels, as viral load at week 24 only demonstrated a trend and viral load at week 48 was not associated with VR at all in multivariate analysis. Nevertheless, our study suggests that on-treatment assessment of the efficacy of ADV can be done at an earlier stage than the usually recommended 48 weeks, thereby further optimizing the HBV roadmap concept.

Our study demonstrated that continued viral replication during treatment with an antiviral drug and presence of cirrhosis at baseline significantly increased the risk of antiviral resistance. Therefore, patients with cirrhosis might be a specific population for whom potent antiviral agents with high genetic barriers or even de novo combination therapy should be considered, as viral and biochemical breakthrough can result in severe exacerbations, decompensation, and death.(31) The reason why development of ADV resistance occurred more frequently in patients with cirrhosis remains unclear. Another unexpected finding was that prior lamivudine resistance was not significantly associated with the emergence of ADV-resistant mutations, as previous studies reported increased ADV-resistance rates in lamivudine-resistant HBV patients.(32) However, in our study 6 of 25 (24%) patients with a prior history of LAM-resistance showed ADV-resistant mutations during follow-up, which concurs with previously reported rates.(26, 33) This suggests that the low number of patients with ADV-resistance did not allow demonstrating a significant relation between a prior history of LAM-resistance and development of ADV resistant mutations during ADV monotherapy.

Four patients who developed genotypic ADV resistance received entecavir monotherapy as salvage therapy, of whom three showed a rapid virologic response. In two patients LAM was added, and one patient was switched to TDF/LAM combination therapy. In vitro studies indicate that ADV-resistant HBV strains remain susceptible to ETV and TDF.(8, 34-37) These results were confirmed by anecdotal clinical reports.(26, 37-38) In contrast, substitutions at rtA181 associated with ADV-resistance may also induce a decreased susceptibility to LAM.(36-37) The optimal antiviral salvage strategy for patients with ADV-resistant HBV remains, however, unclear and needs further investigation.

Limitations of our study include the retrospective-observational design and the heterogeneous group of patients. Although this is may be considered a disadvantage, it probably also reflects the real situation of CHB clinical practice in the western world. Undetectable HBV DNA levels (< 300 copies/mL) may be a more precise definition of VR. However, as only few patients achieved undetectable HBV DNA, we decided to set the definition of VR at 10^3 copies/mL to be able to determine baseline and on-treatment predictive factors for achieving VR. In addition, using Kaplan-Meier analysis response rates can be overestimated. A basic assumption of this approach is that a patient will retain the measured outcome whether or not they remain in the study.

However, relapse of HBV DNA levels above 10^3 copies/mL and seroreversion during treatment is known to occur in chronic HBV patients.(39)

In conclusion, after two years of ADV treatment VR is achieved in approximately half of chronic HBV patients. HBV DNA levels at week 24 demonstrated a higher predictive value for VR than HBV DNA levels at week 48. In addition, emergence of ADV-resistant mutations occurred more frequently in patients with a viral load > 3 $\log_{10}$ copies/mL at week 24. Therefore, our study suggests that virologic response to ADV can already be assessed at week 24, instead of the generally recommended 48 weeks. Presence of cirrhosis at baseline and not achieving virologic response were important predictors of the occurrence of ADV-resistance associated mutations. Patients with cirrhosis at baseline might be a specific population for whom more potent antiviral agents with higher genetic barriers or even de novo combination therapy should be considered.

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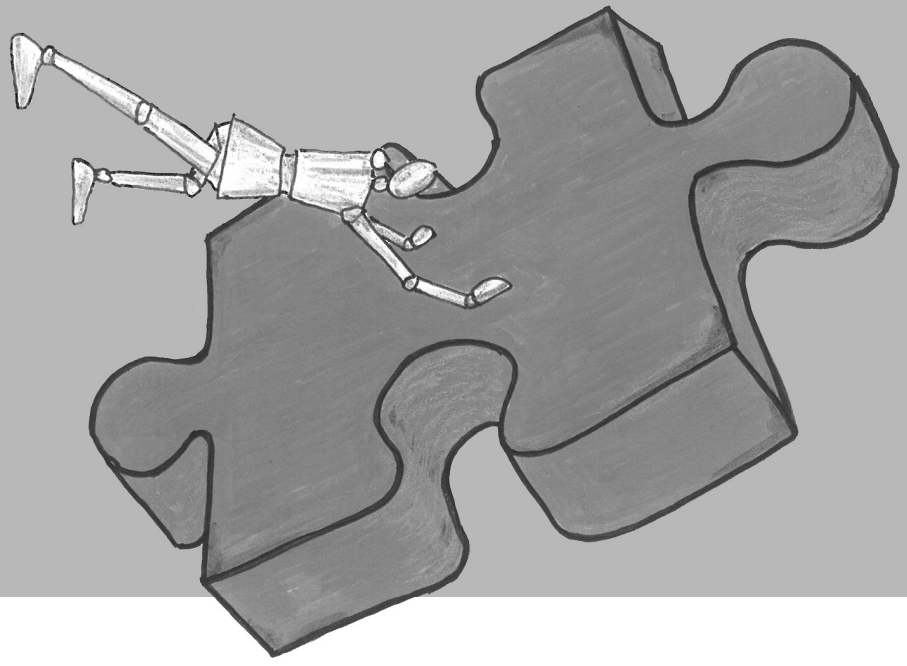

Summary and discussion

Derived from:

Janssen HLA, Reijnders JGP *Treatment with nucleos(t)ide analogues in chronic hepatitis B: Where does the road map lead us?* J Hepatol. 2009 Jul;51(1):1-3

Reijnders JGP, Janssen HLA. *New approaches in the management of chronic hepatitis B: role of tenofovir.* Infection and Drug Resistance 2009; 2: 13-26

Reijnders JGP, Janssen HLA. *How effective is treatment with adefovir plus lamivudine for patients with lamivudine-resistant chronic hepatitis B infection?* Nature Clinical Practice Gastroenterology & Hepatology 2008;5(12):668-9

Reijnders JGP, Janssen HLA. *Potency of tenofovir in chronic hepatitis B: mono- or combination therapy?* J Hepatol 2008;48(3):383-6.

Long-term therapy with oral nucleos(t)ide analogues is nowadays used in most patients with chronic hepatitis B virus (HBV) infection. Three nucleoside analogues, lamivudine (LAM), entecavir (ETV), telbivudine (LdT), and two nucleotide analogues, adefovir dipivoxil (ADV), and tenofovir disiproxil (TDF), are currently approved for the treatment of chronic HBV infection.

All nucleos(t)ide analgues are competitive inhibitors of the viral polymerase by competing with the incorporation of natural intracellular nucleotides in viral DNA. As they lack a hydroxyl group, incorporation prevents the formation of a covalent bond with the adjacent nucleotide, resulting in chain termination. Although all nucleos(t)ide analogues act on HBV polymerase, there are different mechanisms of action. In that perspective, three classes of nucleos(t)ide analogues can be identified.(1) L-nucleosides include LAM and LdT. LAM is mainly an inhibitor of viral minus strand DNA synthesis, that is reverse transcriptase activity, while LdT is supposed to inhibit all the enzymatic activities, including priming of reverse transcription, reverse transcriptase activity, and synthesis and elongation of viral plus strand. Acyclic phosphonates include TDF and ADV, which both are active on the priming of reverse transcription as well as elongation of viral minus strand.(2) ETV is the only compound which belongs to the cyclopentane group, and acts at all three stages of the viral replication cycle.(3)

Treatment end points and durability of response

With the currently approved treatment options the ultimate goal is to prevent the development of long-term sequelae of chronic liver disease. As these clinical outcomes arise only after decades of infection, short-term surrogate endpoints are needed to determine the success of hepatitis B treatment. As a result, permanent and complete suppression of viral replication (at least below 2.000 IU/mL) is our main goal, for persistent HBV viraemia is the most important predictor of progression to liver cirrhosis, hepatic failure, and development of hepatocellular carcinoma.(4-5) HBeAg seroconversion remains another important endpoint in HBeAg-positive HBV infection, because it is usually associated with sustained remission and very low risk for cirrhosis and hepatocellular carcinoma.(6-7)

In general, HBeAg seroconversion rates are approximately 20% after one year of nucleos(t)ide analogue treatment, and these rates increase with prolonged therapy.(8-15) International guidelines on the therapy of hepatitis B suggest that finite duration of treatment with nucleos(t)ide analogues is a reasonable option, and recommend that treatment can be stopped after HBeAg seroconversion and an additional 6 to 12 months of consolidation therapy.(16-17) However, the long-term durability of HBeAg seroconversion induced by nucleos(t)ide analogues is controversial, as several studies

have reported contradictory results. Whereas some authors reported nucleos(t)ide analogue-induced HBeAg seroconversion to be durable in up to 90%, others reported relapse rates as high as 70%.(18-20) In chapter two, the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion is explored. During a median follow-up of five years after the development of nucleos(t)ide analogue induced HBeAg seroconversion, only 31% of patients demonstrated a durable remission, defined as HBeAg negativity and HBV DNA levels less than 10,000 copies/mL (~ 2.000 IU/mL). Moreover, sustained response was demonstrated in just two of nine patients who discontinued therapy after a consolidation therapy of at least 6 months. Disease recurrence in patients that continued therapy after HBeAg seroconversion was preceded by the development of resistance (80% of these patients); resistance only occurred in subjects given lamivudine monotherapy. In contrast, recurrence after treatment discontinuation or non-compliance was observed in all patients given nucleos(t)ide analogues. These findings indicate that HBeAg seroconversion, in contrast to what current guidelines suggest, is an imperfect endpoint in assessing nucleos(t)ide analogue therapy. Therefore, long-term continuation of nucleos(t)ide analogue treatment, irrespective of the occurrence of HBeAg seroconversion, appears to be necessary in most patients.

In contrast to nucleos(t)ide analogue therapy, treatment with (pegylated) interferon results in a more durable response once treatment is discontinued in patients with chronic hepatitis B.(21-22) Intrahepatic covalently closed circular DNA (cccDNA), which plays a major role in viral persistence, is a strong predictor of sustained off-treatment virological response.(23) Serum hepatitis B surface antigen (HBsAg) levels are known to reflect cccDNA in the liver, and reduction of HBsAg levels correlates well with that of cccDNA.(24-26) In chapter 3, on-treatment serum HBsAg kinetics in patients with both HBeAg-positive and HBeAg-negative chronic hepatitis B receiving either pegylated interferon or ETV monotherapy, are compared. In HBeAg-positive patients, decline of HBsAg was significantly associated with HBeAg loss, and, subsequently, HBsAg decline tended to be higher in pegylated interferon treated subjects compared to ETV-treated subjects. Interestingly, patients who achieved HBeAg loss during either pegylated interferon or ETV therapy demonstrated a similar reduction in HBsAg levels. In contrast, in HBeAg-negative patients, only treatment with pegylated interferon resulted in a significant HBsAg decline, whereas HBeAg-negative patients treated with ETV demonstrated no HBsAg reduction at all. This study confirms previous observations that immune modulation is of vital importance to completely eradicate HBV.(25-26) First, pegylated interferon resulted in higher HBeAg loss rates compared to ETV, and in significant HBsAg decline in both HBeAg-negative and HBeAg-positive patients. Second, although ETV demonstrated to be a potent inhibitor

of viral replication, a significant decline of HBsAg was only observed in those patients with preexisting immune activity, reflected by high baseline ALT levels, and in those patients who achieved HBeAg loss after one year of therapy.

That nucleos(t)ide analogues relatively lack any immunomodulatory action is actually confirmed in chapter 4, which explores the role and outcome of nucleos(t)ide analogue treatment-related hepatitis flares. Hepatitis flares are abrupt elevations of serum aminotransferase levels, and reflect immune-mediated lysis of infected hepatocytes.(27-28) It is known that during treatment with (pegylated) interferon approximately 25-40% of patients experience hepatitis flares, which are explained by the immunostimulatory properties of this drug, and are associated with HBeAg seroconversion and sustained response.(29) In our study, flares during (on-therapy flares) and after discontinuation (withdrawal flares) of nucleos(t)ide analogue therapy never led to sustained disease remission, and even sometimes resulted in decompensated liver disease.

Nucleos(t)ide analogue therapy in treatment-naïve patients

As a sustained off-treatment response seems only possible in a minority of patients treated with nucleos(t)ide analogues, nucleos(t)ide analogues probably have to be administered for very long periods, if not indefinitely. Development of antiviral drug resistance is a major limitation to long-term efficacy of nucleos(t)ide analogues, and completely negates the benefits obtained from treatment with oral antiviral agents.(30) It is therefore clear that the main barrier to long-term control in any individual will be drug resistance. The rapidity of emergence of these drug-resistant mutants depends on several factors, including two treatment-related parameters, i.e. genetic barrier and potency. The genetic barrier of a treatment regimen is the number of mutations needed to generate a virus strain with a marked decrease in susceptibility to the drug, yet which still is able to replicate in the presence of these primary resistance mutations. A high antiviral potency of a treatment regimen results in quick and complete suppression of viral replication. As resistance only emerges when replication occurs in the presence of the drug selection, it allows little opportunity for resistance to develop.(31)

TDF was licensed for the treatment of human immunodeficiency virus (HIV) infection in 2001, and plays since that time a pivotal role in HIV management. Recent data showed the efficacy of TDF in the treatment of chronically HBV-monoinfected patients as well.(13) TDF was superior to ADV in both nucleos(t)ide-naïve HBeAg-positive and HBeAg-negative HBV patients, and appeared to be one of the most potent anti-HBV agents so far. In chapter 5, the long-term efficacy and renal safety of TDF administered as a part of anti-retroviral therapy was explored in a largest cohort of HIV/HBV-coinfected patients published so far. It is shown that after five years of follow-up, approximately

90% of patients achieved undetectable HBV DNA (< 20 IU/mL), almost 50% of HBeAg-positive patients demonstrated HBeAg loss, and HBsAg loss was even observed in approximately 10% of subjects. More importantly, only one patient demonstrated a combination of known anti-HBV drug-resistant mutations, and experienced a virologic breakthrough thereafter. Furthermore, this study supports the renal safety of TDF through five years of treatment, as only a small, non-progressive decline in renal function was observed.

ETV is a potent and selective inhibitor of HBV replication in vitro.(3) In the phase III registration trials it resulted in superior virologic, biochemical and histological efficacy after one year of therapy compared to LAM in both HBeAg-positive and HBeAg-negative chronic HBV patients.(8, 32) Moreover, ETV proved to have a high genetic barrier to resistance with only 1.2% of nucleos(t)ide analogue-naïve HBV patients demonstrating genotypic resistance to ETV after five years of ETV monotherapy.(15) In chapter 6 the potent antiviral effect of ETV and its excellent resistance profile in nucleos(t)ide analogue-naïve patients is confirmed. It is demonstrated that 55% of HBeAg-positive and 92% of HBeAg-negative HBV patients achieved undetectable HBV DNA levels after one year of therapy, and none of the patients developed antiviral drug resistance.

In conclusion, the superior potency of TDF and ETV and their excellent resistance profiles in nucleos(t)ide-naïve HBV patients, which were previously demonstrated in large randomized controlled trials, have now been confirmed in large scale investigator-initiated studies with variable follow-up, organised by European research consortia.(33) As recommended in the recent HBV treatment guidelines of EASL, TDF and ETV should, therefore, be prioritised as a first-line treatment. LAM, ADV and LdT should no longer be considered as first-line choice, due to their inferior resistance profile, for HBV-monoinfected patients, even for those with favourable baseline characteristics and even when virological response will be evaluated at week 24.

Nucleos(t)ide analogue therapy in treatment-experienced patients

As mentioned before, we should use only potent nucleos(t)ide analogues with a high genetic barrier as monotherapy to prevent the emergence of antiviral drug resistance. The current guidelines recommend that in case of development of antiviral drug resistance, a second antiviral drug should be added to the treatment regimen, which is not cross-resistant.(16-17, 31)

TDF has been shown to be effective in the nucleos(t)ide analogue-experienced population, although conflicting results have been presented concerning patients with genotypic resistance to ADV.(33) LAM demonstrates significant anti-HIV activity, and

is commonly used as part of an anti-HIV combination treatment regimen. In chapter 5, it is demonstrated that both preceding treatment with LAM and the presence of LAM-resistance did not affect the response to TDF. Furthermore, switching to TDF, whether in mono- or in combination therapy, resulted in decline of viral load in patients with ETV-resistance associated mutations (chapter 6).

Concerning the use of ETV for the nucleos(t)ide analogue-experienced population, it is clearly demonstrated in chapter 6 that ETV should not be used as rescue therapy in patients with a prior history with LAM-resistance, even when LAM-resistant mutations can not be detected at baseline. Also in patients with LAM-resistance and a low viral load at baseline, response to ETV appears suboptimal. ETV may still be used in LAM-experienced patients in case LAM-resistance never developed, yet longer follow-up is required to confirm these initial observations. The antiviral efficacy of ETV was not influenced by prior treatment with ADV or presence of ADV resistance. Furthermore, in a large cohort of HIV/HBV coinfected patients, adding ETV to the treatment regimen resulted in achievement of undetectable HBV DNA in patients who demonstrate persistent HBV replication during a TDF-containing treatment regimen (chapter 5).

In the last few years management of resistance has evolved, and treatment strategies and clinical guidelines have been developed, mostly focusing on prevention of antiviral drug resistance.(16, 34) It is recommended that treatment should be adapted at an early stage in case of incomplete viral suppression, as several studies have already shown that an initial virologic response is associated with lower rates of antiviral drug resistance in HBV patients in the long term. The so-called roadmap concept and the recently published EASL guidelines on the treatment of chronic HBV infection both propose that virologic response should be assessed at week 12 and 24 to identify primary nonresponse and/or partial virologic response, respectively, and to modify treatment accordingly. Although it is questionable whether this concept also applies to the new potent drugs with low resistance rates, some experts would suggest in patients receiving ETV or TDF with a partial virologic response at week 48, adding the other drug in order to prevent resistance in the long term. However, in chapter 5, it is demonstrated that most patients treated with TDF are still able to achieve undetectable HBV DNA in the second year without changing the treatment regimen, and a similar observation was recently made in patients treated with ETV. In contrast, for LAM it was suggested that virologic response could already be assessed at four weeks to predict response at five years of therapy (35), and that on-treatment evaluation of the efficacy of ADV can also be done at an earlier stage than one year (chapter 8).

In chapter 7, the efficacy of ETV as rescue therapy for patients with a persistently high viral load during ADV treatment is described. Surprisingly, ETV monotherapy resulted in a limited HBV DNA decline in both LAM-experienced and -naïve patients with persistently high HBV DNA during ADV treatment. Only one of fourteen patients achieved undetectable HBV DNA levels (< 373 copies/mL), and none of the patients showed HBeAg loss or seroconversion. The contrasting outcome with other studies might be related to selection of patients who respond poorly to nucleos(t)ide analogues in general instead of previous ADV therapy itself. An interesting observation was, therefore, that two patients with a limited virologic response to ETV 1 mg daily demonstrated further HBV DNA decline after increasing the dosage to 2 mg daily, which suggests that increasing a drug to its maximum tolerated dose may provide a reasonable option to maximize antiviral activity besides switching to or adding another drug.

In summary, for patients who developed antiviral drug resistance, it is recommended that a second drug without cross-resistance should be added, meaning TDF or ETV should be added in subjects who developed resistance against a nucleoside or nucleotide analogue, respectively. However, the necessity of the add-on strategy has recently been debated, as the initial results of switching to TDF or ETV monotherapy in LAM- or ADV-resistant HBV patients are promising. Although treatment should be adapted at an early stage in case of incomplete viral suppression to prevent the development of antiviral drug resistance, one can probably wait at least 24 months in patients treated with TDF or ETV monotherapy who are viremic, before switching to an alternative treatment regimen, particularly in case of continued viral load decline.

Conclusion

Long-term continuation of nucleos(t)ide analogue treatment, irrespective of the occurrence of HBeAg seroconversion, appears to be necessary in most patients. The superior potency of TDF and ETV and their excellent resistance profiles in nucleos(t)ide-naïve HBV patients have now been confirmed in large scale investigator-initiated studies. TDF and ETV should, therefore, be prioritised as a first-line nucleos(t)ide analogue treatment. One can probably wait at least 24 months in patients treated with TDF or ETV monotherapy who are viremic, before switching to an alternative treatment regimen. In the nucleos(t)ide analogue-experienced population, TDF or ETV should be initiated if resistance is detected to a nucleoside or nucleotide analogue, respectively. The necessity of the add-on strategy has recently been debated, and additional studies with longer follow-up are needed to confirm these initial observations.

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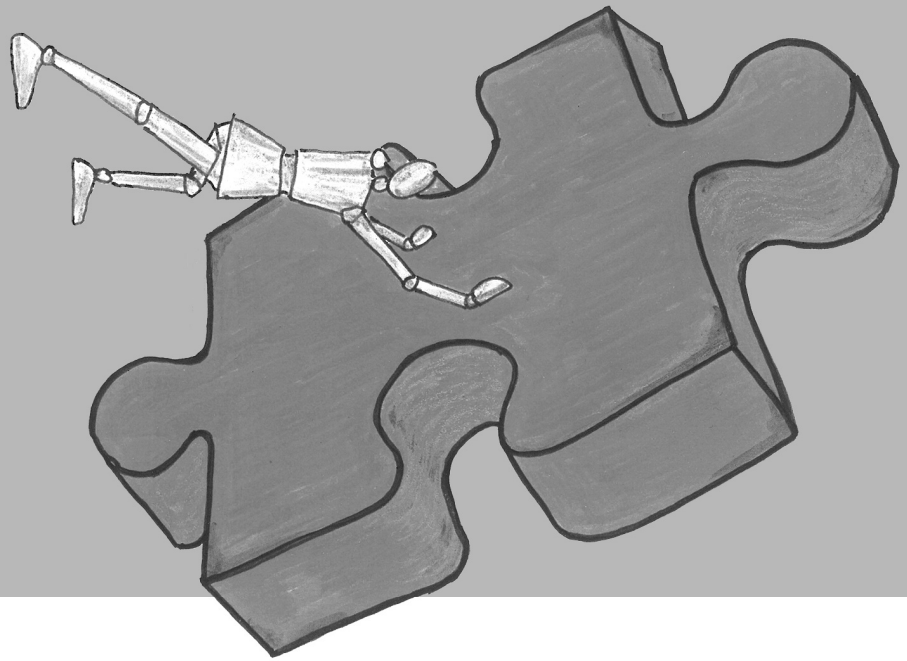

Samenvatting en bespreking

Langdurige behandeling met nucleos(t)ide-analogen is tegenwoordig een veelvuldig gebruikte behandelingsmethode voor een chronische hepatitis B infectie. Op dit moment zijn drie nucleoside-analogen, lamivudine (LAM), telbivudine (LdT), en entecavir (ETV), en twee nucleotide-analogen, adefovir dipivoxil (ADV), en tenofovir disiproxil (TDF), geregistreerd voor de behandeling van chronische hepatitis B.

Alle nucleos(t)ide-analogen zijn krachtige remmers van het virale enzym, DNA- polymerase, door middel van competitie met het natuurlijk substraat, de bouwstenen van het DNA. Door het ontbreken van een hydroxyl-groep, leidt incorporatie van het synthetische nucleoside-analogon tot voortijdige blokkering van de virale DNA-ketenverlenging. Hoewel alle nucleos(t)ide-analogen het HBV-polymerase remmen, zijn er verschillende werkingsmechanismen. In totaal worden er drie klassen van nucleos(t) ide-analogen onderscheiden.(1) LAM en LdT behoren tot de groep van L-nucleosiden. LAM is hoofdzakelijk een inhibitor van reverse-transcriptie van de negatieve streng DNA van het pregenome boodschapper-RNA, terwijl LdT alle drie functionele activiteiten van het virale polymerase remt: priming van het HBV-polymerase, reverse-transcriptie, en synthese van de positieve streng HBV DNA. De acyclische fosfonaten omvatten TDF en ADV, welke beide werken middels remming van synthese van de negatieve streng DNA en priming van het HBV-polymerase. (2) ETV is het enige middel dat behoort tot de groep van cyclopentanen, en remt alle drie de activiteiten van het HBV-polymerase. (3)

Eindpunten van behandeling en duurzaamheid van respons

Met de huidige beschikbare antivirale middelen is het uiteindelijke doel van behandeling het voorkomen van de lange termijnscomplicaties van chronisch leverlijden. Levercirrose en hepatocellulair carcinoom ontstaan echter pas tientallen jaren na besmetting, en derhalve zijn er surrogaat eindpunten op de korte termijn nodig, die geassocieerd zijn met een gunstig effect op de lange termijn. Het belangrijkste surrogaat eindpunt is blijvende en complete remming van virale replicatie, omdat virale load (serum HBV DNA) een uitstekende voorspeller is van progressie naar levercirrose of het ontwikkelen van hepatocellulair carcinoom. Een serum HBV DNA < 2.000 IU/mL lijkt hiervoor voldoende. (4-5) HBeAg seroconversie (verlies van HBeAg met ontwikkeling van anti-HBe) is tevens een belangrijk eindpunt bij HBeAg-positieve chronische hepatitis B, omdat het meestal geassocieerd is met blijvende remissie van ziekte, en een laag risico op het ontwikkelen van levercirrose of hepatocellulair carcinoom.(6-7)

In het algemeen resulteert een jaar behandeling met een nucleos(t)ide-analogon bij ongeveer 20% van de patiënten in HBeAg-seroconversie, en dit percentage neemt toe met doorgaande therapie.(8-15) In de huidige internationale richtlijnen over de

behandeling van hepatitis B wordt aanbevolen dat de behandeling met nucleos(t)ide-analogen ten minste dient te worden gecontinueerd tot het optreden van HBeAg-seroconversie, en pas gestaakt kan worden na zes tot twaalf maanden consolidatietherapie.(16-17) De duurzaamheid van een door nucleos(t)ide analogen geïnduceerde HBeAg-seroconversie is echter onduidelijk, daar verschillende studies tegengestelde resultaten laten zien en het percentage van patiënten die een blijvende remissie van ziekte tonen na HBeAg-seroconversie uiteenloopt van 30-90%.(18-20) In hoofdstuk twee onderzochten wij de duurzaamheid van een door nucleos(t)ide-analogen geïnduceerde HBeAg-seroconversie. Gedurende een mediane follow-up van vijf jaar na het optreden van HBeAg-seroconversie tijdens de behandeling met nucleos(t)ide-analogen, was er slechts bij 31% van de patiënten sprake van een blijvende duurzame respons, hetgeen gedefinieerd werd als een combinatie van HBeAg-negativiteit en een virale load lager dan 10.000 kopieën/ml (~2.000 IU/ml). Daarnaast toonden zeven van de negen patiënten, die de behandeling staakten na een consolidatietherapie van ten minste zes maanden, terugkeer van actieve ziekte. Terugval gedurende behandeling werd voorafgegaan door het ontstaan van antivirale resistentie bij 80% van de patiënten, en alleen bij de patiënten die werden behandeld met LAM monotherapie. Daarentegen trad terugkeer van ziekte na het bewust staken van antivirale behandeling of therapieontrouw op bij alle nucleos(t)ide-analogen. Derhalve lijkt HBeAg-seroconversie een suboptimaal eindpunt te zijn voor de behandeling met nucleos(t)ide-analogen en dient de behandeling langdurig te worden gecontinueerd, ongeacht het optreden van HBeAg-seroconversie bij de meeste patiënten.

Vergeleken met de behandeling met nucleos(t)ide-analogen resulteert (gepegyleerd) interferon in een hoger percentage patiënten met een blijvende, duurzame respons na het staken van therapie.(21-22) Totale eliminatie van het hepatitis B virus is vooralsnog niet mogelijk gebleken met de huidige behandelmogelijkheden, hetgeen samenhangt met het uitermate therapieresistente cccDNA (covalently closed circular DNA) dat zich bevindt in de hepatocyt. Eerder onderzoek toont aan dat de hoeveelheid intrahepatisch cccDNA een belangrijk voorspeller is van een blijvende virologische respons na het staken van antivirale therapie.(23) Bovendien lijken HBsAg concentraties in het serum de hoeveelheid intrahepatisch cccDNA goed te weerspiegelen en is een daling van HBsAg-concentraties in het serum gecorreleerd met de daling van cccDNA in de lever.(24-26) In hoofdstuk 3 vergelijken wij de kinetiek van HBsAg concentraties in het serum van patiënten met chronische HBeAg-positieve en HBeAg-negatieve hepatitis B, die behandeld worden met gepegyleerd interferon of entecavir. In HBeAg-positieve hepatitis B patiënten was de daling in HBsAg concentraties sterk geassocieerd met het optreden van HBeAg verlies gedurende de behandeling. Aangezien behandeling met gepegyleerd interferon leidt tot een hoger percentage patiënten met HBeAg verlies,

resulteerde het in vergelijking met entecavir ook in een grotere daling van serum HBsAg. Indien HBeAg verlies bewerkstelligd werd, was er echter geen verschil te zien in de mate van HBsAg daling tussen deze twee behandelingen. Bij HBeAg-negatieve hepatitis B patiënten leidde alleen gepegyleerd interferon tot een daling van HBsAg concentraties.
Deze uitkomsten bevestigen eerdere bevindingen dat voor totale eliminatie van het hepatitis B virus immuunmodulatie van essentieel belang is.(25-26) Ten eerste resulteert gepegyleerd interferon, een antiviraal middel met immunomodulatoire eigenschappen, in hogere percentages patiënten met HBeAg verlies, en in significante dalingen van HBsAg concentraties in HBeAg-positieve en HBeAg-negatieve hepatitis B patiënten. Daarnaast leidde ETV alleen tot HBsAg daling bij patiënten die of vooraf aan de behandeling immuunactiviteit toonden, hetgeen weerspiegeld werd door hogere baseline ALAT waarden, of HBeAg verlies bereikten gedurende de behandeling.

Dat het nucleos(t)ide-analogen ontbreekt aan immunomodulatoire eigenschappen, wordt in feite gesteund door de bevindingen in hoofdstuk 4, waarin de rol en uitkomst van hepatitis opvlammingen ('hepatitis flare') gedurende behandeling met nucleos(t)ide-analogen wordt onderzocht. Hepatitis flares zijn plotselinge toenames van serum transaminsase concentraties, en geven immuungemedieerde lysis van geïnfecteerde hepatocyten weer.(27-28) Hepatitis flares worden bij 25-40% van de patienten gezien die worden behandeld met interferon. Ze zijn geassocieerd met HBeAg-seroconversie en blijvende virologische respons na het staken van de therapie. In hoofdstuk vier wordt beschreven dat geen enkele nucleos(t)ide-analogen geassocieerde hepatitis flare leidde tot een blijvende remissie van ziekte en soms zelfs resulteerde in gedecompenseerde leverziekte.

Behandeling met nucleos(t)ide-analogen in nucleos(t)ide-naïeve patiënten

Patiënten dienen in het algemeen langdurig met nucleos(t)ide-analogen te worden behandeld, aangezien een blijvende respons na het staken van de behandeling slechts bij een beperkt aantal patiënten kan worden bereikt. Met de duur van de behandeling neemt de kans op het ontstaan van antivirale resistentie toe. Dit manifesteert zich door een stijging van het serum HBV DNA, ondanks het continueren van de behandeling, bij een patiënt met in eerste instantie een goede virologische respons,.(30) Het voorkomen van antivirale resistentie is daarom essentieel om langdurige onderdrukking van virale replicatie te bewerkstelligen door middels van nucleos(t)ide-analogentherapie. De snelheid waarmee antivirale resistentie zich ontwikkelt, wordt onder andere bepaald door twee therapiegerelateerde parameters: de genetische barrière en antivirale potentie. Met de genetische barrière wordt bedoeld het aantal mutaties dat nodig is om een HBV mutant te verkrijgen met verminderde gevoeligheid voor het antivirale middel, maar die

nog steeds in staat is te repliceren ondanks deze mutaties. Een hoge antivirale potentie resulteert in een snelle en complete remming van virale replicatie, hetgeen de kans op het ontstaan van resistentie op de lange termijn vermindert.(31)

TDF werd in 2001 geregistreerd voor de behandeling van een HIV (humaan immunodeficiëntievirus)-infectie en is sindsdien een belangrijk onderdeel van HAART (highly active anti-retroviral therapy). Recente studies tonen tevens de effectiviteit van TDF aan voor de behandeling van een chronische hepatitis B virus monoinfectie.(13) In zowel HBeAg-positieve als HBeAg-negatieve hepatitis B patiënten werden er betere resultaten bereikt met TDF vergeleken met ADV. Daarnaast leek het een van de meeste krachtige anti-HBV middelen te zijn tot nu toe. In hoofdstuk 5 wordt de effectiviteit en veiligheid van TDF, als onderdeel van HAART, onderzocht in het grootste cohort van HIV/HBV gecoinfecteerde patiënten dat tot nog toe gepubliceerd is. Na vijf jaar behandeling bereikte ongeveer 90% van de patiënten een ondetecteerbaar HBV DNA (<20 IU/ml), bijna 50% van de HBeAg-positieve patiënten toonde HBeAg verlies, en bij 10% van de patiënten werd zelfs HBsAg verlies gezien. In slechts een patiënt werd er een virologische doorbraak geobserveerd en detecteerden wij mutaties in het HBV DNA, die geassocieerd zijn met resistentie tegen anti-HBV middelen. Daarnaast bevestigden wij de renale veiligheid van TDF, aangezien er gedurende vijf jaar behandeling slechts een beperkte, niet-progressieve daling in nierfunctie werd geobserveerd.

In vitro studies tonen aan dat ETV een krachtige en selectieve remmer is van HBV replicatie. In grote gerandomiseerde klinische trials werden er na een jaar behandeling met ETV betere virologische, biochemische en histologische resultaten bereikt vergeleken met LAM bij HBeAg-positieve en HBeAg-negatieve hepatitis B patiënten. (8, 32) Daarnaast heeft ETV een goed resistentieprofiel en werd er na vijf jaar ETV monotherapie bij slechts 1.2% van de nucleos(t)ide-naïeve patiënten genotypische resistentie aangetoond.(15) In hoofdstuk 6 wordt het krachtige antivirale effect van ETV en het uitstekende resistentieprofiel bij nucleos(t)ide-naïeve patiënten bevestigd in de klinische praktijk. Na een jaar behandeling bereikten 55% van de HBeAg-positieve en 92% van de HBeAg-negatieve patiënten een ondetecteerbare virale load en ontwikkelde geen van de patiënten resistentie tegen ETV.

De krachtige anti-HBV eigenschappen van TDF en ETV en hun uitstekende resistentieprofielen bij nucleos(t)ide-naïeve hepatitis B patiënten, welke al eerder werden aangetoond in klinische trials, zijn nu ook bevestigd in grootschalige, onafhankelijke cohort studies.(33) TDF en ETV dienen dan ook de middelen van eerste keuze te zijn, indien er wordt gekozen voor een behandeling met nucleos(t)ide-analogen. Dit wordt tevens aanbevolen in de recent verschenen richtlijnen betreffende de behandeling van

chronisch hepatitis B van de EASL. LAM, ADV, en LdT komen hier niet langer voor in aanmerking vanwege hun inferieure resistentieprofielen en dienen ook niet te worden overwogen bij patiënten met voordelige baseline karakteristieken, of bij patienten bij wie op week 24 de virologische respons kan worden geëvalueerd.

Behandeling met nucleos(t)ide-analogen in voorbehandelde patiënten

Zoals hierboven reeds beschreven werd, dienen er alleen krachtige antivirale middelen te worden gebruikt met een hoge genetische barrière teneinde de ontwikkeling van resistentie op de lange termijn te voorkomen. De huidige richtlijnen bevelen aan dat in het geval van het ontstaan van resistentie, er een tweede middel moet worden toegevoegd aan de behandeling die niet kruisresistent is.(16-17, 31)

TDF is een effectief antiviraal middel bij patiënten die al eerder zijn behandeld met nucleos(t)ide-analogen, hoewel studies betreffende voornamelijk ADV-resistente HBV patiënten tegenstrijdige resultaten laten zien.(33) LAM is een goede remmer van HIV replicatie en maakt regelmatig onderdeel uit van HAART. In hoofdstuk 5 wordt aangetoond dat eerdere behandeling met LAM en/of de aanwezigheid van LAM-resistentie geen invloed heeft op het antivirale effect van TDF bij HIV/HBV gecoïnfecteerde patiënten. Daarnaast laten wij zien dat bij patiënten met HBV mutanten die geassocieerd zijn met een verminderde gevoeligheid voor ETV, de omzetting naar TDF, al dan niet in combinatie met een ander middel, leidt tot virusdaling (hoofdstuk 6).

Omzetting naar ETV monotherapie is geen geschikte strategie voor patiënten die in het verleden resistentie tegen LAM hebben ontwikkeld, zelfs niet als op het moment van starten deze specifieke HBV mutanten niet meer gedetecteerd kunnen worden of als er sprake is van een lage virale load. ETV kan daarentegen nog steeds een effectief middel zijn bij patiënten die eerder werden behandeld met LAM, maar bij wie nooit LAM-resistente mutaties zijn gedetecteerd. Er is echter een langere follow-up nodig om deze initiële observaties te bevestigen. Een eerdere behandeling met ADV, of de aanwezigheid van ADV-resistente HBV mutanten, heeft echter geen invloed op het antivirale effect van ETV (hoofdstuk 6). Bovendien leidde het toevoegen van ETV bij HIV/HBV gecoinfecteerde patiënten met persisterende HBV replicatie onder een TDF-bevattend behandelingsschema, tot complete virale suppressie (hoofdstuk 5).

In de laatste jaren is de behandeling van resistentie verder verfijnd, en zijn er behandelingsstrategieën en richtlijnen ontwikkeld die meer gericht zijn op de preventie van antivirale resistentie.(16, 34) Aangezien voortgaande virale replicatie onder antivirale therapie geassocieerd is met een hogere kans op resistentie, wordt in de huidige richtlijnen aanbevolen de behandeling in een vroeg stadium aan te passen indien er

sprake is van incomplete virale suppressie. Het zogenoemde “roadmap” concept en de recent verschenen richtlijnen van de EASL geven beide aan dat de virologische respons op zowel week 12 als week 24 geëvalueerd dient te worden en dat in het geval van een zogenaamde primaire non-respons of partiële virologische respons, de behandeling moet worden aangepast. Hoewel het de vraag is of deze aanbevelingen ook van toepassing zijn op de nieuwere orale middelen met hun uitstekende resistentieprofielen, zijn sommige experts van mening dat bij incomplete virale suppressie na een jaar behandeling met TDF of ETV monotherapie, het andere middel moet worden toegevoegd om resistentie op de lange termijn te voorkomen. Data om deze aanbevelingen te ondersteunen, ontbreken echter op dit moment. In hoofdstuk 5 laten wij juist zien dat een aanzienlijk deel van de patiënten met een detecteerbare virale load na een jaar behandeling met TDF, in het tweede jaar van de behandeling alsnog complete virale suppressie bereiken. Eenzelfde observatie bij ETV monotherapie werd recent ook beschreven. Daarentegen werd met betrekking tot LAM monotherapie in een Aziatische studie aangetoond dat de virologische respons op week 4 al een goede voorspeller is van de uitkomst na vijf jaar behandeling.(35) Verder laten wij in hoofdstuk 8 zien dat de respons op ADV monotherapie waarschijnlijk ook op een eerder tijdstip kan worden geëvalueerd dan de vaak aanbevolen 48 weken.

In hoofdstuk 7 wordt de effectiviteit van ETV beschreven bij een serie HBeAg-positieve HBV patiënten met een persisterend hoge virale load onder ADV monotherapie. Zowel bij patiënten die eerder met LAM werden behandeld als bij LAM-naïeve patiënten, leidde de omzetting naar ETV monotherapie tot een beperkte daling in virale load. Gedurende de follow-up bereikte slechts een van de veertien patiënten een ondetecteerbare virale load (< 373 kopieën/mL) en bij geen van de patiënten werd HBeAg verlies of seroconversie geobserveerd. Deze tegenstrijdige uitkomst vergeleken met andere studies, kan mogelijk verklaard worden door het feit dat er een selectie van patiënten heeft plaatsgevonden die in het algemeen slecht responderen op nucleos(t)ide-analogen. Een interessante bevinding daarbij was dat bij twee patiënten met een partiele respons op ETV monotherapie (1 mg) een verdubbeling van de dosering leidde tot een verdere virusdaling. Dit suggereert dat voor bepaalde patiënten met een suboptimale virologische respons het verhogen van de dosis een alternatief kan zijn naast het omzetten naar of toevoegen van een nucleos(t)ide-analogon.

In het algemeen kan dus worden aanbevolen dat in het geval van antivirale resistentie tegen een nucleoside- of nucleotide-analogon, respectievelijk TDF of ETV moet worden toegevoegd aan de behandeling. Of het toevoegen van een nucleos(t)ide-analogon in plaats van het omzetten naar monotherapie daadwerkelijk nodig is, moeten additionele studies met een langere follow-up uitwijzen. De initiële resultaten van het omzetten

naar TDF of ETV monotherapie bij LAM- of ADV-resistente HBV patiënten zijn echter veelbelovend. Ter preventie van resistentie dient de antivirale behandeling in een vroeg stadium te worden aangepast in het geval van incomplete virale suppressie. Bij TDF of ETV monotherapie lijkt men hiermee echter veel langer meer te kunnen wachten, en waarschijnlijk tot ten minste 24 maanden behandeling.

Conclusie

Langdurige behandeling met nucleos(t)ide-analogen lijkt noodzakelijk bij de meeste patiënten, zelfs indien HBeAg-seroconversie gedurende de therapie optreedt. De krachtige anti-HBV eigenschappen van TDF en ETV en hun uitstekende resistentieprofielen bij nucleos(t)ide-naïeve hepatitis B patiënten zijn nu ook bevestigd in grootschalige, onafhankelijke cohort studies. TDF en ETV dienen dan ook de nucleos(t)ide-analogen van eerste keuze te zijn. Bij patiënten die viremisch blijven onder TDF of ETV monotherapie kan men veel langer wachten dan bij LAM en ADV, voordat de behandeling moet worden aangepast. In het geval van antivirale resistentie tegen een nucleoside- of nucleotide-analogon moet respectievelijk TDF of ETV worden toegevoegd aan de behandeling. Of het toevoegen van een nucleos(t)ide-analogon in plaats van het omzetten naar monotherapie daadwerkelijk nodig is, moeten additionele studies met een langere follow-up uitwijzen.

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Dankwoord

Ik dacht gedurende mijn promotieonderzoek, dat als ik eenmaal was aangekomen bij het dankwoord, ik enorm gelukkig en ontspannen zou zijn. Ik kan echter melden dat in deze fase van afronding zelfs dit een opgave wordt en een deadline heeft, aangezien het drukken van het boekje over twee dagen moet gaan starten. Ik ben echter blij dat ik de afgelopen vier jaar aan dit onderzoek heb kunnen werken, en kijk nog weleens met weemoed terug hoe flexibel je de dagen toen nog kon invullen.

Allereerst gaat mijn dank natuurlijk uit naar mijn promotor, prof. dr. Harry Janssen. Beste Harry, in 2006 kwam ik tijdens mijn oudste coschap tussen de bedrijven door je kamer binnenzetten met de vraag of je niet een promotieplekje voor mij had. Het resulteerde in een soort van sollicitatiegesprek, en binnen een week lag mijn toekomst voor de komende drie jaar vast. Ik denk dat je voor mij *de* ideale begeleider was bij ons onderzoek. Je geeft iemand veel verantwoordelijkheid en vrijheid om het onderzoek naar zijn inzichten in te vullen, en weet de onderzoeksgroep te leiden zonder te veel nutteloos gewauwel eromheen. Daarnaast kijk ik met bewondering hoe je ster wereldwijd rijzende is binnen de hepatologie, waarbij naar mijn mening Rotterdam een unieke, maar nog veel belangrijker, onafhankelijke positie inneemt binnen de commercie van het klinische onderzoek. Als ik een ding van je geleerd heb, dan is het wel dat inhoud op de eerste plaats staat bij een wetenschappelijk artikel, maar dat het “verkopen” van een artikel minstens zo belangrijk is. Alhoewel je agenda drukker en drukker wordt (en er in de toekomst dus maar een ding op zit, en dat is delegeren), wil ik je ontzettend bedanken voor de tijd die je hebt vrijgemaakt in met name het laatste jaar. Ik wens je heel veel succes en plezier toe de komende jaren met het verder uitbouwen van het Rotterdamse hepatologie-imperium. En nog even ter herinnering aan het kleine resistentiecongres dat we hadden in Parijs: wij beide hebben daar in ieder geval geleerd dat het niet verstandig is, dat ik aan het roer van de wijnfles zit, als we de volgende ochtend om 8.00h een breakfast meeting hebben.

Daarnaast wil ik natuurlijk dr. Rob de Man, mijn huidige opleider, bedanken voor zijn hulp bij mijn medische carrière. Bij u kwam ik begin 2006 voor het eerst aankloppen voor het bespreken van mijn oudste coschap. U adviseerde me destijds om er vooral te zorgen dat ik promotieonderzoek ging doen. Nu, in 2010, heb ik mijn promotieonderzoek zo goed als afgerond, en ben ik vorig jaar gestart met de opleiding tot MDL-arts onder uw supervisie. Naast dr. R.A. de Man wil ik verder prof. dr. C.A.B. Boucher en prof. dr. J.P. Drenth bedanken voor het beoordelen van mijn proefschrift. Furthermore, I am honoured to have a committee in which dr. H.W. Reesink, prof. dr. G. Duscheiko,

and prof. dr. F. Zoulim will take place. Daarnaast gaat nog mijn speciale dank uit naar prof. dr. Solko Schalm. Beste Solko, als er iemand een bevlogen wetenschapper is en in feite de belichaming van de Nederlandse hepatologie vormt, dan bent u het. Alhoewel u uiteindelijk te wijs werd bevonden voor mijn kleine commissie, toonde u altijd enorm veel belangstelling voor mijn verrichtingen. Ik wens u veel succes met uw huidige projecten, en ik hoop daar zeker steentje aan bij te dragen.

Promoveren binnen een onderzoeksgroep heeft veel voordelen: een daarvan is dat de meest up-to-date reviewers het manuscript in feite al hebben beoordeeld, voordat het gesubmit gaat worden bij een medisch tijdschrift. Erik, een organisatietalent als jij heb ik nog nooit eerder gezien. Ik zou willen dat ik iets van je mocht lenen, dan krijg jij wat van mijn flexibiliteit ;-) Milan en Roeland, ik wens jullie heel veel succes met jullie verdere promotietraject. Martijn, voor een koffie is altijd tijd. Vincent, al zijn wij twee totaal verschillende personen, ik denk dat onze bijna perfecte samenwerking nu inmiddels al zijn tweede lustrum bereikt heeft, en ik hoop dat er nog een stuk of twintig zullen volgen. Op het moment van de verdediging ben jij ook een van de strijders van het fort in Zuid: het Ikaziaatje. Daarnaast gaat nog speciale dank uit naar de leden van het lab, die mij een korte introductie in de wereld van het pipetteren hebben gegeven: Eric, Andrea, Gertine, en Marjolein (en eigenlijk ook Mark en Andre, ook al doen jullie iets met dat andere virus).

En dan de meest fancy statisticus die er volgens mij ter wereld bestaat, en die, totdat ze vertelt dat ze al twee puberende kinderen heeft, ten minste twintig jaar jonger wordt ingeschat: Bettina Hansen. Bettina, dankzij jou werden de artikelen weer net even naar een hoger plan getild. Veel leuker waren natuurlijk echter de uitjes tijdens de congressen. Heel veel succes met je verdediging van je proefschrift.

Martin en Suzan, mijn steun vanuit de virologie, was ik jullie toch bijna vergeten in mijn dankwoord! Gelukkig kwam ik daar nog net op tijd achter, want jullie bijdrage aan dit boekje is substantieel. Enorm veel dank voor alle hulp bij de aanvullende bepalingen die moesten worden verricht voor de stukken. Mijn start van de week gaat tegenwoordig een stuk trager, er gaat namelijk niets boven een duik in de vriezer op de vroege maandagochtend. Ook voor de andere HBV-promovendi blijkt dit een plezierig uitje te zijn. Milan, Roeland en Vincent zullen dan ook altijd bereid zijn alle samples weer netjes terugzetten.

Moniek en Emily, mijn twee studenten die ik mocht begeleiden bij hun master's thesis. Het leven zou zo veel simpeler zijn als ons project gewoon een rechttoe rechtaan gerandomiseerde klinische trial was. Alhoewel we in het begin niet wisten waar te

moeten beginnen met een eindeloze brij aan heterogene data, die soms terugvoerde tot begin jaren negentig, is er uiteindelijk wel de beloning gekomen met een goede publicatie. Moniek, ik denk dat je een uitstekende keuze hebt gemaakt door met je coschappen te gaan starten in Amsterdam. Heel veel succes met je verdere carrière. Emily, I really enjoyed our cooperation, yet remember that it is important to keep your paper simple and straightforward. Furthermore, I have to thank you for your invaluable contribution to the ARES project.

De directe collega's. Misschien is het goed om te beginnen met onze werkplek, want kippen in de bio-industrie hebben het misschien zwaar, maar ik denk dat ze wel een toontje lager zingen als ze de dakpoli hebben gezien. Het is ongelofelijk dat onder die omstandigheden, er toch nog van die mooie boekjes ontstaan. Allereerst mijn kamergenootjes: Koch, de heerlijke rooklucht die mij tegemoet kwam, als jij lekker je ontbijtje voor de overdracht zat te nuttigen, ik mis het gewoon! Femme, jij bent 24 uur per dag vrolijk en blijft praten, zelfs als er normaal gesproken voor twaalf uur niet veel meer dan zinnen van hooguit een en maximaal twee woorden uit mijn mond komen. Ik vond het ontzettend gaaf om bij je bruiloft te zijn, en succes met je verdere pancreasproject. Paulus van Putten, eigenlijk waren wij ook gewoon kamergenoten. Ik heb ontzettend veel bewondering hoe jij je door je promotietraject weer heen te slaan. Ik hoop dat je het uiteindelijk met succes zal gaan afronden. Nicoline, Desirée, Aaf, Jildou, Leonie, kleine Vincent, Daphne, Jerome, Lieke, Edith, Jilling, Geert, Leonie, Margot, Robert, Joyce, Sarwa, de sfeer was altijd goed, dank voor alle borrels, congressen, skireizen etc.

Marion en Margriet, het onverslaanbare (en enig cynische) secretaresseduo van de Hepatologie, ik zou willen dat jullie bij mij thuis ook eens de administratie op orde kwamen brengen. Marion, jouw hulp bij de gang van zaken rondom de promotie en het submitten van artikelen, met name in het laatste jaar, was onmisbaar.

Meiden van het clinical research bureau, en in het bijzonder Elke en Irene, dank voor jullie hulp bij het schrijven en indienen van protocollen, het doorploegen van de wirwar aan regeltjes, en het omzeilen van de bureaucratie van de METC. Jullie strijd om ervoor te zorgen dat alles verloopt volgens de regels is essentieel (maar…regels zijn toch altijd minder straks dan ze lijken…). Heleen, Cokki, Anneke, Lucille, en Melek, 's ochtends besprak je met jullie het plan, en 's middags was de logistiek rondom inclusie, behandeling en follow-up tot in de puntjes geregeld. Heel veel dank voor jullie hulp.

De hepatitis B-poli op dinsdagmiddag vormde een welkome afwisseling. Alhoewel het enige levensdoel van een medische status is om op die plek te gaan liggen, waar jij niet

verwacht dat hij ligt, wisten Esther, Wilma, Nermin, Lakshmie, Ellen, Esther, en Ronald deze toch altijd weer terug te vinden.

Menno en BP, super dat jullie mijn paranimfen willen zijn. Menno, we kennen elkaar nu al meer dan twintig jaar. Je bent een van mijn beste vrienden, en voormalig ploeg- en huisgenoot. Vanuit Klundert trokken we samen naar Rotterdam om in de gezellige Bas Jungeriusstraat in een van de kansenwijken van Rotterdam te gaan resideren. Jij hebt het daar uiteindelijk zelfs je hele studie volgehouden. Het blijft bijzonder hoe we konden genieten van een ontbijtje onder GTST, Lingo, of Get the Picture, en hoe vol we onze agenda vonden als we een of twee colleges die dag hadden (en die dan nog wisten te missen). Inmiddels woon je al weer een paar jaar met Lil in Breda. Ik wens jullie alle geluk van de wereld. Bas-P, one-of-the-boyz, en voormalig huisgenoot. De mooiste herinneringen blijven toch hoe je wekelijks werd drooggelegd op RS, je de hele dansvloer nodig had om al je moves ten toon te spreiden, om een uurtje of vier aftaaide naar Jaffa voor een knoflooksoepje met hier en der wat shoarma, we samen onze vriend PJ Plakpoep van zijn bedje keerde, en jij vervolgens besloot nog een tweede keer van je broodje shoarma te gaan genieten. BP, (jij) inmiddels de dertig gepasseerd, binnenkort ga je trouwen, ook wij zijn inmiddels “trotse” burgermannen. Ik vind het supergaaf om straks je getuige te zijn, maar eerst ben jij mijn paranimf.

Lieve pap en mam, jullie bijdrage aan wat ik tot nu toe bereikt heb, is enorm. Jullie steun en belangstelling is van onschatbare waarde. Mam, een zoon praat nu eenmaal niet zo veel over emoties en dergelijke als een dochter, zeker ik niet. Het belangrijkste is echter dat ik weet dat ik altijd en overal op jullie terug kan vallen. Pap, als dit laatste drukke jaar met de start van mijn opleiding en het afronden van de promotie voorbij, is moeten onze bijna-wekelijkse uitjes in ere worden hersteld.

Lieve Liek, jij bent verreweg het mooiste wat me tijdens mijn promotietijd is overkomen. Inmiddels zijn we alweer drie jaar bij elkaar, hebben we een schitterend huis, en kan je het zeker niet “pril” meer noemen;-). Het afgelopen jaar heb je er voor gezorgd dat ik werkelijk niets te kort kwam, en heb je er alles aan gedaan om mij zoveel mogelijk te ontlasten. Ik weet niet hoe ik je dit kan terugbetalen, maar ik ga zeker een poging doen. Lieverd, er ligt een wereld van vrije tijd op ons te wachten na november, en bedenk dat we pas een paar procent van onze tijd samen achter de rug hebben.

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Curriculum vitae

Jurriën Gerben Peter werd geboren op 24 september 1982 te Klundert. In 2000 behaalde hij zijn V.W.O. diploma aan het Markland College te Oudenbosch. Aansluitend startte hij met de opleiding Geneeskunde aan de Erasmus Universiteit te Rotterdam. In zijn tweede studiejaar besloot hij naast Geneeskunde, een Master of Science opleiding te volgen op het gebied genaamd Genetic Epidemiology. Een plezierig onderdeel van de Master studie was dat hij een maand aan de Harvard School of Public Health mocht studeren. Deze opleiding werd in 2004 voltooid. Zijn oudste coschap doorliep hij op de afdeling Maag-, Darm-, en Leverziekten van het Erasmus MC te Rotterdam, waarna hij in oktober 2006 zijn artsexamen behaalde. In november 2006 startte hij met promotieonderzoek naar de behandeling van chronische hepatitis B met nucleos(t) ideanalogen onder supervisie van prof. dr. H.L.A. Janssen. Per september 2009 is hij met de opleiding tot Maag-Darm-Leverarts begonnen (opleider: Dr. R.A. de Man). De vooropleiding interne geneeskunde wordt thans verricht in het Ikazia Ziekenhuis Rotterdam (opleider: dr. A. Dees).

PhD Portfolio

Erasmus MC
Universitair Medisch Centrum Rotterdam

Summary of PhD training and teaching

Name PhD student: Jurriën G.P. Reijnders
Erasmus MC Department: Gastroenterology and Hepatology

PhD period: 2006 - 2010
Promotor: Prof. dr. H.L.A. Janssen

1. PhD training

	Year	Workload
Presentations and workshops		
Chronic hepatitis B: a patient-tailored approach. Meeting of the Laboratory for Infectious Diseases, Paterswolde, The Netherlands	2008	12 hours
Liver cirrhosis at baseline is an important predictor of resistance to adefovir. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, The Netherlands	2008	12 hours
Antiviral efficacy of entecavir: results from 153 chronic hepatitis B patients in an international multicenter cohort study. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, The Netherlands	2008	12 hours
Resistance and new anti-HBV drugs. Hepatitis B Masterclass, Utrecht, The Netherlands	2009	18 hours
Clinical decisions in viral hepatitis: using nucleos(t)ide analogue for the treatment of chronic hepatitis B. Cursus Klinische Hepatologie, Lunteren, The Netherlands	2009	8 hours
Five year tenofovir therapy is associated with maintained virologic response, but significant decline in renal function in HIV/HBV coinfected patients. Twice annual meeting of the Netherlands Association of Hepatology, Veldhoven, The Netherlands	2009	12 hours

HBeAg seroconversion during nucleos(t)ide analogue therapy does not lead to durable remission of chronic hepatitis B. 60th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2009	36 hours
Antiviral effect of entecavir: influence of prior exposure to nucleos(t)ide analogues. 44th Annual meeting of the European Association of the Study of the Liver, Copenhagen, Denmark.	2009	36 hours
Treatment of chronic hepatitis B: from clinical trial to clinical practice. BMS satellite symposium. King's College Hospital, University of London, Royal Free Hospital (London), Leeds Hospital (Leeds), United Kingdom.	2009	48 hours
Poster presentations		
Entecavir: a rescue therapy for chronic hepatitis B patients with a limited virologic response to adefovir. 58th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2007	32 hours
Frequency and clinical outcome of flares related to nucleos(t)ide analogues in patients with chronic hepatitis B. 59th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2008	24 hours
HBeAg seroconversion induced by nucleos(t)ide analogues in chronic hepatitis B is not durable in majority of cases. 59th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2008	24 hours
Liver cirrhosis at baseline is an important predictor of resistance to adefovir. 59th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2008	24 hours
Five year tenofovir therapy is associated with maintained virologic response, but significant decline in renal function in HIV/HBV coinfected patients. 60th Annual meeting of the American Association of the Study of Liver Diseases, Boston, MA, United States of America.	2009	32 hours
HBsAg levels during antiviral therapy for chronic hepatitis B: peginterferon vs. entecavir. 45th Annual meeting of the European Association of the Study of the Liver, Vienna, Austria.	2010	32 hours

International conferences

2nd International Conference on the Management of Patients with Viral Hepatitis, Paris, France.	2007	28 hours
42nd Annual Meeting of the European Association for the Study of the Liver (EASL), Barcelona, Spain.	2007	28 hours
The Liver Meeting 2007, 58th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2007	28 hours
EASL-AASLD-APASL-ALEH-IASL Conference Hepatitis B and C virus resistance to antiviral therapies, Paris, France.	2008	28 hours
The Liver Meeting 2008, 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, United States of America.	2008	28 hours
44th Annual Meeting of the European Association for the Study of the Liver (EASL), Copenhagen, Denmark.	2009	28 hours
The Liver Meeting 2009, 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA, United States of America.	2009	28 hours
45th Annual Meeting of the European Association for the Study of the Liver (EASL), Vienna, Austria.	2010	28 hours

Attended seminars and workshops

De Sterkste Schakel hepatitis B en C. September 26, Utrecht, The Netherlands	2007	2 hours
5e Post AASLD symposium. December 6, Rotterdam, The Netherlands	2007	4 hours
Refereeravond Laboratorium voor Infectieziekten: Chronische hepatitis B virusinfectie – Nieuwe richtlijnen voor diagnostiek en behandeling. March 31, Paterswolde, The Netherlands	2008	2 hours
Studiemiddag HIV en hepatitis B: maakt de dokter het verschil? March 19, Rotterdam, The Netherlands.	2008	2 hours
Eerste Lagerhuisdebat Hepatitis B en C. September 25, Amsterdam, The Netherlands	2008	2 hours

2. Teaching

	Year	**Workload**
Lecturing		
Hepatitis B. 2nd year Erasmus MC medical students participating in a 4-week Infectious diseases training program. Rotterdam, The Netherlands.	2009	8 hours

Hepatitis B. 3rd year Erasmus MC medical students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, The Netherlands.	2009	8 hours

Supervising Master's theses

Frequency and clinical outcome of flares related to nucleos(t)ide analogues in patients with chronic hepatitis B – N.P. Zhang	2008 - 2010	60 hours
HBeAg seroconversion during nucleos(t)ide analogue therapy does not lead to durable remission of chronic hepatitis B – M.J. Perquin	2008 - 2010	60 hours