HEPATITIS B VIRUS INFECTION:

IS PATIENT TAILORED TREATMENT FEASIBLE?

WIM LEEMANS

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Hepatitis B Virus Infection:

Is patient tailored treatment feasible?

Hepatitis B virusinfectie:

is op de patiënt toegespitste behandeling haalbaar?

Proefschrift

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Contents

1A Introduction

Success and failure of nucleoside and nucleotide analogues in Chronic Hepatitis B

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INTRODUCTION

The hepatitis B virus (HBV) belongs to the family of hepadna viridae and has a diameter of 42-47 nm.[1] The virus particle encloses a partially double-stranded DNA genome with a length of approximately 3200 base pairs. Within the viral DNA genome four open reading frames (ORFs) can be identified and are termed in analogy of their encoding protein S (surface), C (core), P (polymerase) and X (HBx protein). The ORF S contains 3 regions, the ore S1, pre S2 and S, which encode for the large, middle and small hepatitis B surface glycoproteins depending on the start of the transcription site, respectively. The ORF C is responsible for encoding the hepatitis B e-antigen (HBeAg) and the core antigen (HBcAg). After the binding of the virus particle to the hepatocyte, the HBV viral genome is converted into covalently closed circular DNA (cccDNA) in the hepatocyte nucleus. This cccDNA form the key template for pregenomic RNA in the HBV replication cycle and acts as a reservoir for the HBV. In the hepatocyte cytoplasm, along with the core and polymerase proteins the pregenomic RNA is assembled to virus particles. Sequentially, the RNA is reversed transcribed into a HBV-DNA minus strand, which is finally transcribed by a HBV DNA polymerase into the HBV DNA plus strand. The formed particle can either be excreted via the Golgi apparatus or recycled into the nucleus to form ccc-DNA.[2]

As a result of variety in expression of the viral genome, the HBV is divided into 8 different genotypes, A-H.[3,4] The HBV genotypes are also characterized by different geographical and demographical distribution. Genotype A is predominantly found in North-West Europe and North America, whereas genotypes B and C are mostly seen in Asian countries. Genotype D is most common in the Mediterranean area. Consequently, Caucasians harbor predominantly genotype A and D, while Asians harbor almost exclusively the genotypes B and C.[5,6]

Hepatitis B virus infection is a serious global health problem with more than 350 million people suffering from chronic hepatitis B virus infection. Approximately, 15-40% of these patients will develop cirrhosis, liver failure or hepatocellular carcinoma (HCC).[7] Chronic HBV infection accounts for 500,000 to 1.2 million deaths each year and is the 10th leading cause of death and the 5th most common cause of cancer related deaths.[8-10] Persistent viral replication is associated with liver disease progression; thus, suppression of viral replication is of uttermost importance. Increasing evidence exists suggesting that persistent viral replication is a risk factor for the development of cirrhosis and HCC. Patients with a viral load of 10^4-10^5 copies/ml had an adjusted hazard ratio of 2.5 and 2.3 for the development of cirrhosis or HCC, respectively, increasing to >5 and >6 for patients with over 10^5 copies/ml.[11,12] Treatment of active chronic hepatitis B infection prevents disease progression.[13] Treatment with immunomodulatory agents, interferon-α or its pegylated form, acts mainly by stimulating the immune system and has a modest direct antiviral effect. Interferon, a cytokine that affects many processes in the body, causes a

considerable amount of side effects. Interferon therapy is effective in approximately one third of both HBeAg-positive and HBeAg-negative chronic HBV patients and, in general, responders experience long-term inactivation of their chronic infection.[14-16] Another treatment option for chronic HBV includes the nucleoside and nucleotide analogues, which mainly target the viral polymerase and thereby inhibit viral replication directly.[17] Due to the high replication rate of the virus (up to 10^{10}-10^{12} virions a day) and the lack of proofreading by reverse transcriptase, the de novo mutation rate is high. In combination with selection pressure by antiviral drugs, this may select drug resistant mutants.[18,19] In this review, we discuss the advantages and disadvantages of nucleoside and nucleotide analogues for the treatment of chronic HBV. Also the diagnosis and management of antiviral resistance to nucleoside and nucleotide analogues is discussed.

LAMIVUDINE

Lamivudine (3TC or 2',3'-dideoxy-3'-thiacytidine) is the first registered nucleoside analogue for the treatment of chronic HBV. It is a cytosine analogue, which has to be phosphorylated to its active metabolite. It directly inhibits the viral polymerase by competing with natural thriphosphates for incorporation into the viral DNA by the viral DNA polymerase, thereby terminating chain elongation.[20]

Lamivudine proved to be a very well tolerated drug with an excellent safety profile comparable to placebo. With prolongation of therapy, the safety profile does not change.[21-25]

HBeAg positive disease

Lamivudine is a strong inhibitor of viral replication and a decline of 5.40 $\log_{10}$ copies/ml is feasible after one year of treatment. Due to the generally high viral load in HBeAg positive subjects, 36% of the patients will reach a load below the lower limit of detection (LLD 300 copies/ml). Thus, many patients will still experience considerable viral replication as reflected by a mean HBV DNA of about 10^4 copies/ml. Furthermore, there is a large variation in viral loads greater than 10^4 copies/ml in many patients after 1 year of treatment. Maximum efficacy is reached between weeks 24 and 36 and the viral response weakens thereafter.[21,23]

ALT normalizes in 44-72% of the patients after one year of treatment.[21-23] As a reflection of viral suppression and decline in ALT levels, necro-inflammation improves in 52-64% of patients. No improvement is observed in 34-42% of patients; of these patients, 7-10% experienced worsening inflammation with paired biopsies.[21-23,26] The fibrosis score improves in 35%, remains unchanged in 55% and worsens in 10% of patients.[23] Loss of

HBeAg and the seroconversion to anti-HBe as a sign of partial immunological control occurs in 17-22%.[21-23,26] Loss of HBsAg is only seen in sporadic cases.

With prolonged treatment, some patients have a sustained on-treatment response while in others, lamivudine loses its efficacy due to the emergence of drug resistant mutants. It is hard to estimate from the published mean HBV DNA data whether the viral load declines further if treatment is prolonged after one year due to the rebound in HBV-DNA levels as a consequence of resistance. In a single cohort study, prolongation of treatment showed an increase in the estimated Kaplan-Meier HBeAg seroconversion rates from 27% to 40% to 47% and to 50% at years 2, 3, 4 and 5 respectively.[25,27-29]

Discontinuation of lamivudine resulted in an increase in HBV DNA levels for the entire group.[21] In a long-term follow-up-up study of patients with HBeAg seroconversion during the phase II and III trials, the duration of response was investigated. The median time from the end of the study to enrolment in the follow-up study was 4.3 (0-27) months. At the start of follow-up, 72% had undetectable HBV DNA levels (LLD 7.0 $x10^5$ copies/ml) and this percentage remained 72% at the end of follow-up (median duration 36.6 (4.8-45.6) months). ALT responses diminished as the percentage of patients with normal ALT declined from 73% at baseline to 63% at the end of follow-up. HBeAg seroconversion was sustained in 77% of subjects.[30] The durability of sustained response rates may be affected by the duration of treatment after HBeAg seroconversion. In a Korean study, patients who received up to two months of therapy after HBeAg seroconversion had a higher relapse rate (74%) compared with those receiving treatment for at least 4 months (37%) of lamivudine after HBeAg seroconversion at year 2 of follow-up.[31] In patients in which HBV DNA (hybridization assay, LLD 10^6 copies/ml) and HBeAg were persistently negative for at least 24 months during lamivudine therapy, cumulative reappearance rates of HBV DNA after cessation of therapy were 15%, 21% and 31% at 6, 12 and 24 months of follow-up.[32] Cumulative reappearance rates of HBeAg were 11%, 13% and 16%. These data suggest that long-term administration of lamivudine might enhance the durability of HBeAg seroconversion. Therefore, it is recommended to continue treatment for at least 3-4 months after HBeAg seroconversion. Viral load, at the time of discontinuation, is a predictor for sustained response. Patients with a low viral load (<200 copies/ml) had significantly lower relapse rates (reappearance of HBV DNA (>5.0 $x10^5$ copies/ml and/or HBeAg) (37%) compared to those with a viral load $\geq 10^3$ copies/ml (73%) at 2 year follow-up.[33]

Resistance is caused by mutations in the viral DNA polymerase (YMDD region) and develops as early as six months after treatment; after one year, 14-32% were lamivudine refractory.[21,22] With longer treatment duration, the incidence of YMDD mutants increased to 38%, 53%, 67% and 69% at 2, 3, 4 and 5 years respectively.[25,28,29,34] Over 90% of patients developing resistance experience rebounds in HBV-DNA and ALT levels, although

viral load and ALT level remain significantly lower compared to baseline.[21,28,35] In some patients, the emergence of resistance resulted in reversal of their initial histological improvement.[28,36] A surge of HBV DNA followed by a hepatitis flare with serum ALT >5 upper limit of normal (ULN) occurred in approximately 40% of patients within 1 year after the development of resistance and increased up to >80% by year 5.[37,38] In addition, severe hepatitis with hepatic decompensation or even fatality may occur,[37,39] After the development of resistance, continuation of lamivudine does not seem beneficial. Hepatic flares and decompensation occurred in 67% and 11%, respectively, in patients continuing lamivudine for 12 months. In patients who discontinued lamivudine, hepatic flares and decompensation occurred in 54% and 7%, respectively. In addition, HBV DNA levels increased in 73% of patients in the continuation arm compared to 33% in the discontinued group.. HBeAg seroconverion occurred in 19% of the group continuing lamivudine and 35% of the patients discontinuing lamivudine.[34]

HBeAg negative disease

Treatment of nucleoside naïve HBeAg negative subjects with 100 mg lamivudine once daily resulted in a rapid and strong decline during the first 24 weeks of treatment and a much slower decline until 36 weeks after which there was a slight increase in viral load. After 48 weeks of treatment, the viral load declined by 4.2-4.5 $\log_{10}$ copies/ml.[16,24] In one study, 89% of the subjects had a viral load less than 7.0 $x10^5$ copies/ml and 72% had undetectable HBV DNA levels (LLD 300 copies/ml).[24] In another study, 85% had a viral load below 2.0 $x10^4$ copies/ml and undetectable levels as determined by PCR (LLD 400 copies/ml) in 73%.[16] ALT levels normalized during treatment; at week 48, 71-73% had normal ALT levels.[16,24] Smaller open labeled studies reported ALT normalization in 60-96% of cases after 12 months of treatment.[40-43] Histological improvement, defined as improvement by at least two points in the Knodell necroinflammatory score with no worsening in the Knodell fibrosis score, occurred in 61%. There was no histological improvement in 26% of the patients. Despite improvement and ALT normalization in most patients, the mean Knodell necroinflammatory score was 4.6 at the end of treatment; there was still some inflammation in many patients at microscopic level.[24] The Ishak fibrosis score improved in 38%.[44] In patients with severe fibrosis at baseline, the Ishak fibrosis score improved (≥1 point) in 53%, remained unchanged in 18% and worsened in 5%.[45] Continuation of treatment does not result in significantly improved treatment outcomes as the percentage of PCR negative subjects increased from 72% to 77% and ALT normalization increased from 71% to 84% after 96 weeks of treatment.[44] Other studies found a sustained response with prolonged treatment. HBV DNA negativity (<7.0 $x10^5$ copies/ml) was 89% after 1 year and 75% after 2 years. ALT levels were normal in 81% after 1 year and in 69% after 2 years of continuous treatment.[46] Others experienced loss of efficacy with prolonged treatment with the percentages of HBV DNA negativ-

ity dropping by 21-31% between month 12 and 24.[40,47,48] ALT responses (normal ALT) declined over time being 96% at month 12, 59.5% at month 24 and 42.5% with over 30 months of treatment.[40]

Discontinuation of therapy frequently results in loss of response. After 6 months of follow-up, HBV DNA suppression was lost in most patients as the percentage of PCR negativity (LLD 400 copies/ml) decreased from 73% to 7% and the subjects with HBV DNA levels <2.0 $x10^5$ copies/ml from 85% to 29%. ALT levels increased and 29% lost their biochemical response and after 6 months 44% had normal ALT levels.[16] A study with a longer duration of follow-up after treatment cessation showed an 87% relapse rate in initial responders (HBV DNA <1.4 10^6 copies/ml and normal ALT) and a sustained response in 15%.[49]

Lamivudine resistance emerged in 18-27% after 1 year and increased over time to 44% at year 2 and 60% after 4 years of treatment.[16,40,46,50] The data on the clinical impact of YMDD mutation are controversial. Some studies suggest little impact on therapeutic response.[46,50] Others report increases in serum HBV DNA levels and ALT and loss of histological response in almost all patients.[40,48,51,52] The emergence of resistance can be associated with clinical significant hepatitis.[40,52]

ADEFOVIR

Adefovir dipivoxil (PMEA or 9-(2-(phosphonomethoxyl)ethyl)-adenine) is an oral prodrug of adefovir which already contains a phosphate group and requires only a final phosphorylation step before competing for integration into the forming HBV DNA strand resulting in chain termination. The 10 mg dose used for the treatment proved to be safe acutely and chronically. Treatment with higher doses of adefovir increased the risk of nephrotoxicity.[53,54] Nephrotoxicity is infrequent with the 10 mg dose but dose reduction is still required with declining kidney function.[55]

HBeAg positive disease

Treatment with 10 mg adefovir once daily for 48-52 weeks resulted in a 3.52-4.5 $\log_{10}$ copy decline in viral load and 28-36% reached PCR negativity (LLD 300-400 copies/ml).[53,56] Quantitative viral decline of 4 quartiles ((25%) of patients) could be identified; >4.91 $\log_{10}$ reduction, 3.52-4.91, 2.22-3.51 and <2.22 $\log_{10}$ reduction after 48 weeks of treatment.[57] HBeAg loss occurred in 13-24% of patients and 8-12% had HBeAg seroconversion at 1 year.

ALT levels declined and, at the end of treatment, 48-79% of ALT levels normalized. The decline in viral load and ALT was also reflected in the improvement of histology. Improvement, defined as a reduction of at least two points of the Knodell necroinflam-

matory score with no concurrent worsening of the Knodell fibrosis score, occurred in 53% of the patients. The necroinflammatory score improved in 71%, did not change in 15% and worsened in 13%. Fibrosis improved in 41%, remained unchanged in 45% and worsened in 14%. [53]

A study investigating the efficacy of prolonged treatment was hampered to some extent, as most patients received ≥1 dose of placebo in the second year due to a dose allocation error. As the length of study follow-up varied, Kaplan-Meier estimates were used. With prolonged treatment, the percentage of patients with a viral load below 10^3 copies/ml increased from 28% at year 1 to 45% and 56% at years 2 and 3, respectively. ALT levels became normal in 48%, 71% and 81% after 1, 2 and 3 years of treatment, respectively. Rates of HBeAg-loss increased to 42% and 52% and HBeAg seroconversion rates increased to 29% and 43% at year 2 and 3, respectively. [58] A study continuing treatment up to 2 years showed an increase in viral reduction from -4.5 to -5.0 $\log_{10}$ copies/ml, increase in PCR-negativity (LLD 300 copies/ml) from 28% to 42%, but the percentage of ALT normalization remained unchanged (79% to 78%). The percentage of HBe-loss increased from 13% to 19% and the percentage of patients with HBe-loss and development of antibodies increased to 15%. [56]

In most patients, discontinuation of treatment results in a rapid viral rebound with HBV DNA levels returning to baseline. The increase in viral replication is accompanied by a loss of ALT response and, after 12 weeks of follow-up, 21% had normal ALT levels. [56] However, patients with HBeAg seroconversion had a sustained response in 91% after a median follow-up of 143 months (rang 13-245) with a median HBV DNA of 10^3 copies/ml and a viral load $<10^3$ copies/ml in 93%. Nine percent lost their initial HBeAg response and these patients had a shorter duration of adefovir treatment after HBeAg seroconversion, suggesting adefovir should be continued for several months after the HBeAg seroconversion. [59]

The occurrence of resistance is low. No adefovir related mutations were found after 1 year of treatment and after two years of treatment resistance was found in 1.3-3%. Little research exists investigating the course of liver disease after the development of resistance, but increases in serum HBV DNA and ALT levels occur and there is also the possibility of severe hepatitis and liver decompensation. [60-62]

HBeAg negative disease

Treatment of adefovir for 48 weeks in HBeAg negative subjects resulted in a 3.85-3.91 $\log_{10}$ copies/ml reduction in HBV DNA levels, undetectable HBV DNA levels (LLD 400 copies/ml) in 51% and ALT normalization in 72-80%. [55,63] Histological improvement, defined as improvement by at least two points in the Knodell necroinflammatory score with no worsening in the Knodell fibrosis score, occurred in 64% and the Knodell necroinflammatory score improved from 8.0 points with a median of 3 points. Necroinflammatory scores

improved in 80%, remained unchanged in 17% and worsened in 3% of patients. The Ishak fibrosis score improved in 48%, remained unchanged in 47% and worsened in 4%. [63]

Continuation of treatment up to 2 years, did not lead to much decline in viral load but consolidated the response to adefovir, as 71-75% of the patients had a viral load below 10^3 copies/ml and 73-79% experienced ALT normalization. Long-term treatment up to 5 years resulted in a viral load below 10^3 copies/ml in 78-79% at year 3, 65-68% at year 4 and 67% after 5 years of continuous treatment. ALT levels were normal in 69-78% at 3 years, 70-75% at 4 years and 69% after 5 years. [55]

Improvement in necroinflammatory scores compared to baseline were also sustained with long term treatment with adefovir. Over 80% of patients showed improvement, with a median decline of necroinflammatory scores of 4.5 and 5.0 after 4 and 5 years of treatment, respectively. Fibrosis improved in time during treatment and 55% had improved Ishak fibrosis scores after 4 years of treatment and 71% had improved scores at year 5. The percentage with worsening necroinflammatory scores or fibrosis score was about 5% with long-term therapy. [64]

Discontinuation of therapy after 1 year resulted in a rapid loss of response. HBV DNA levels increased from 10^3 copies/ml to over 10^5 copies/ml, but did not return to baseline values. About 50% lost their ALT response and after a year of follow-up only 30% had normal ALT levels. [55] Even after 4-5 years of treatment, response is not sustained in the majority of patients as only 30% of patients had a viral load below 10^4 copies/ml and 56% had HBV DNA levels of over 10^5 copies/ml after 15 months of follow-up in patients with a complete response during treatment. ALT levels increased transiently followed by sustained normal levels in 36.4%, were elevated in 33.3% at the end of follow-up and 30.3% had normal ALT levels.

Resistance to adefovir was not detected after 1 year of treatment. With continuous treatment, resistance increased to 3%, 11%, 18% and 28% at year 2, 3, 4 and 5, respectively. [65] Little is known about the course of disease after the development of resistance, but increases in serum HBV DNA and elevation of serum ALT occur. Additionally, severe hepatitis and decompensation have been reported. [60-62]

ENTECAVIR

Entecavir (ETV) is a guanosine analogue and has to be metabolized within hepatocytes to its acitive metabolite entecavir-triphosphate. It acts by directly inhibiting three of the four catalytic activities of the viral polymerase: priming, reverse transcription and DNA-dependent DNA synthesis. Toxicology studies, as well as clinical studies, revealed that entecavir is a safe drug with a safety profile comparable to lamivudine. Safety is preserved with extended treatment.

HBeAg positive disease

Treatment of nucleoside/nucleotide naïve HBeAg positive subjects with 0.5 mg once daily resulted in a rapid decline in viral load during the first 24 weeks and a slower but continuous decline to week 48, at which the viral load declined to a median of 6.98 $\log_{10}$ copies/ml. PCR negativity occurred in 67% of patients and 91% experienced viral loads $<7.0x10^5$ copies/ml. ALT levels normalized in 68% of patients. Histological improvement, defined as improvement by at least two points in the Knodell necroinflammatory score with no worsening in the Knodell fibrosis score, occurred in 72%. In 8%, the Ishak fibrosis score worsened and the Knodell necroinflammatory score declined from 8.2 to 4.4.

Continuation of entecavir in patients with detectable HBV DNA beyond 48 weeks resulted in a continuous decline in viral load and PCR-negativity increased to 81% after 96 weeks of treatment.[66,67]

Loss of HBeAg occurred in 22% after 48 weeks of treatment and 21% of patients experienced HBeAg seroconversion. In HBeAg-positive patients without continuing therapy, an additional 10% experienced HBeAg seroconversion resulting in an overall HBeAg seroconversion rate of 31% after two years of therapy.

Discontinuation of therapy in responders to entecavir (HBeAg loss, HBV DNA $<7.0x10^5$ copies/ml and ALT<1.25 upper limit normal) resulted in a sustained response in 73% of patients. HBeAg loss and load below $7.0x10^5$ copies/ml was sustained in 82% of patients.

Resistance to entecavir did not occur during 96 weeks of treatment. A total of 12 viral rebounds (≥ 1 log increase from nadir) were documented during clinical trials, but no entecavir-associated mutations could be detected. In addition, no entecavir mutations were identified in patients failing to PCRachieve negativity. In nucleoside-naïve patients, entecavir proved to have a very high genetic barrier to resistance.[67]

HBeAg negative disease

Treatment of nucleoside treatment naïve, HBeAg negative subjects with 0.5 mg entecavir once daily resulted in a rapid viral decline during the first 24 weeks, after which the majority of patients were PCR negative (LLD 300 copies/ml) and continued to decline to a total of 5.0 $\log_{10}$ copies/ml after 48 weeks of treatment. At this point 90% were PCR negative. ALT levels normalized in 78% of patients. Histological improvement, defined as improvement by at least two points in the Knodell necroinflammatory score, with no worsening in the Knodell fibrosis score occurred in 70% and Ishak fibrosis scores improved in 36%. In 19%, liver histology did not improve. In spite of this improvement, the mean Knodell necroinflammatory score at week 48 was 4.2, indicating some patients still had liver inflammation at a microscopic level.

Treatment discontinuation in patients classified as responders (HBV DNA $<7.0x10^5$ copies/ml and ALT <1.25 ULN) resulted in a sustained response in 48% of patients after

24 weeks of follow-up. Twelve percent experienced an increase in ALT to over 5 times the ULN and 8% experienced a severe flare (ALT >10 times ULN) after discontinuation.[24]

In nucleoside-naïve patients, resistance did not occur during the 96-week study period. Six patients experienced viral rebound (>1 $\log_{10}$ copies/ml increase from nadir), but resistance testing revealed no entecavir-associated mutations.

MANAGEMENT OF TREATMENT FAILURES

A distinction has to be made in patients failing therapy due to the emergence of drug resistant mutants, or those failing treatment for other reasons.

Definitions have been formulated in order to distinguish the cause of failure. Primary treatment failure is defined as < 1 $\log_{10}$ IU/l (=1.78 $\log_{10}$ copies/ml) decrease in HBV DNA after 12 weeks of treatment. Several factors may contribute; non-compliance, inefficient conversion from the prodrug to its active metabolite, inadequate phosphorylation within the hepatocytes or under dosing of the drug. Under-dosing is particularly an issue with 10 mg adefovir which was selected for safety reasons to prevent nephrotoxicity as documented with the 30 mg dose.[53] However, resistance cannot be ruled out completely as some nucleoside or nucleotide patients are already resistant to the drug.[68,69]

Secondary treatment failure is defined as an increase of >1 $\log_{10}$ IU/l after an initial decrease of 1 $\log_{10}$ IU/l in HBV DNA as confirmed by two consecutive measurements at a 1-month interval. Factors to be considered are non-compliance and resistance.

Little is known about the causes of treatment failure in non-drug resistant patients. It is not known why some patients experience excellent viral suppression and others suboptimal suppression. There are predictors of treatment response at baseline: high serum aminotransferase (>5x ULN) levels, high degree of necroinflammatory activity and low serum HBV DNA.[70,71] A high viral load is probably one of the reasons why treatment outcomes on viral suppression are less effective in HBeAg-positive subjects compared to HBeAg-negative subjects.

Because viral factors, as well as individual factors, play a role in treatment outcomes, it is difficult to assess the best treatment option for non-responders. In theory, all other nucleoside or nucleotide analogues should be effective. Very little clinical data are available to prove this. Drug sensitivity testing in cell cultures is of little value as it takes only viral factors into account. The sensitivity of wild-type virus is measured and clinical data about the potency is already known from clinical trials. Presuming randomization leads to an equal distribution of individual factors, more potent drugs are expected to suppress viral replication in subjects failing treatment. Entecavir is a more potent drug than lamivudine in the laboratory setting and proved its higher potency in clinical trial where more entecavir treated subjects had response.[23,24] In adefovir failures, the more potent

drug tenofovir proved effective in treating this patients.[72] Also, the addition of another drug could be feasible. In vitro testing demonstrated the combination of adefovir with an L-nucleoside (lamivudine, telbuvidine, emtricitabine) and exerted additive antiviral effects.[73] Large studies are needed to determine treatment efficacies of antiviral agents or their combination in non-drug resistant subjects failing antiviral treatment.

RISK FACTORS FOR DRUG RESISTANCE

Risk factors for the development of lamivudine resistance are: prior course of lamivudine, duration of lamivudine therapy, high body weight and body mass index, male sex, high baseline HBV DNA, insufficient HBV DNA suppression and elevated ALT levels during treatment.[35,74,75] Risk factors for the development of adefovir resistance are: lamivudine resistance at start of treatment, high baseline viral load, overlap <1 month of lamivudine + adefovir in case of lamivudine resistance, insufficient HBV DNA suppression during treatment.[76-78] Predictors for entecavir resistance are: lamivudine resistance and suboptimal suppression of HBV DNA on treatment.[79] Persistent on-treatment viral replication is a major factor for the development of resistance and this has been shown best in lamivudine treated subjects. A study showed that patients with serum HBV DNA of $>10^3$ copies/ml after 6 months had a 63% chance of developing resistance.[75] In another study in 24 lamivudine treated patients for > 1 year, none of the patients with a nadir HBV DNA <50 copies/ml developed resistance, 2 out of 5 patients had a nadir viral load between 50-300 copies/ml and all 11 patients with a nadir viral load >300 copies/ml developed resistance.[74] A load of 10^5 copies/ml after 48 weeks of treatment is predictive for the development of adefovir resistance. However, as resistance is rare, larger groups of adefovir resistant patients have to be studied in order to identify risk factors.[78] About entcavir, even less is known. Viral load during treatment seems to play a role as 81% of the patients developing resistance had a nadir viral load above 10^4 copies/ml.[79]

DETECTION OF DRUG RESISTANCE

Clinically, antiviral resistance has to be suspected when a virological or biochemical breakthrough occurs. A virological breakthrough is not strictly defined, but it is generally agreed to be a 1 $\log_{10}$ increase in viral load either in copies/ml or as IU/l after an initial response in compliant patients.[80-82] The use of sensitive PCR techniques is advised to monitor the viral load during treatment. The response can be assessed more accurately and an increase in viral load is detected earlier because of the lower level of detection (see fig. 1).

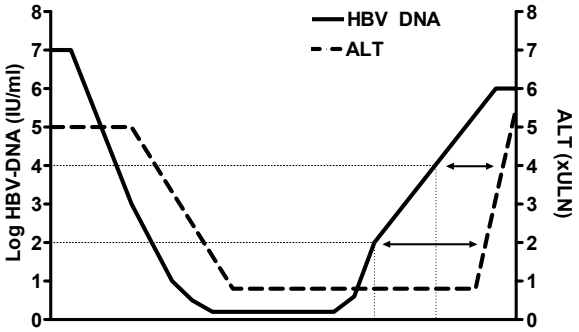

Figure 1. HBV DNA and ALT levels after the initiation of antiviral therapy with nucleos(t)ide analogues in chronic hepatitis B.
After starting therapy, there is a decline in viral load with a concurrent drop in ALT levels afterwards. When resistance develops, there is a rise in HBV DNA. Subsequently, there is an increase in ALT levels. With sensitive HBV DNA assays, a rise in viral load can be observed soon after the development of resistance. When less sensitive tests are used or only ALT levels are measured to detect antiviral resistance, there is less time to switch to other nucleos(t)ide analogues to manage the developed resistance and to prevent an ALT flare.

Biochemical breakthrough is usually defined as an ALT level >1.5 ULN during continuing treatment in patients who initially had normal ALT levels.[81,82] In the majority of cases, a virological breakthrough precedes a biochemical breakthrough and the time lapse may vary from weeks to months.[83] Compliance should be confirmed, as it may be a more common cause of treatment failure as expected. In case of non-compliance, this subject has to be reinforced and follow-up is necessary to determine whether the patient is again responsive to therapy. By using the sensitive tests, the chance of detecting viral breakthrough during the time laps to biochemical breakthrough is increased.

Genotypic testing provides information such as which mutations have arisen during treatment and whether these mutations may correlate with the clinical phenotype. This information is useful when determining the treatment strategy, as there might be cross-resistance to other antiviral agents. Resistance testing can be done by direct sequencing of the HBV polymerase/reverse transcriptase gene or reverse hybridization-based assays, such as the line probe assay. Direct sequencing is able to detect new mutations while the line probe assay is designed to interrogate for a selected known mutation associated with drug resistance. The major advantage of the line probe assay is that it is able to detect selected mutations earlier than sequencing techniques, and in patients with low levels of serum HBV DNA.[84,85]

MANAGEMENT OF DRUG RESISTANCE

Lamivudine resistance

Switching to adefovir dipivoxil is an effective treatment for lamivudine resistant chronic hepatitis B and is able to suppress viral load by 4 $\log_{10}$ copies/ml after 48 weeks of treatment. 26% of patients had a viral load below 10^3 copies/ml (see table 1).[86] There is controversial data on the addition of adefovir to continuing lamivudine. It seems that there is no benefit in terms of viral suppression for adefovir monotherapy compared to adefovir + lamivudine combination therapy as declines in viral loads are similar.[86,87] Combination therapy of lamivudine and adefovir resulted in negative HBV DNA by PCR assay in 20% (LLD 200 copies/ml), below 10^3 copies/ml in 35% of HBeAg positive patients, PCR negativity in 57% (LLD 400 copies/ml) and a viral load below 2.0 x10^3 copies/ml in 78% of HBeAg negative patients at week 48.[86-89] Lamivudine should at least be continued for 2-3 months after initiation of adefovir as this overlap may prevent the emergence of adefovir resistance.[76] The time of initiation of therapy influences treatment outcomes. Fewer patients with phenotypic resistance (HBV DNA >10^6 copies/ml and elevated ALT) responded to treatment compared to the patients with genotypic resistance (HBV-DNA 10^4-10^6 copies/ml) at the time of addition of adefovir to lamivudine. Response in the former was also slower.[88] This emphasizes the importance of regular HBV DNA testing, with the use of sensitive tests.

Although adefovir is effective, in lamivudine refractory patients, there is some degree of cross-resistance. Patients who switched to adefovir monotherapy had a higher rate of resistance to adefovir (up to 19% after one year of treatment).[76,90]

Lamivudine and adefovir combination therapy may result in lower resistance rates to adefovir. As lamivudine suppresses the adefovir resistant mutants, adefovir resistance is lower in patients receiving combination treatment compared to those discontinuing lamivudine.[76,91]

Lamivudine resistance confers a dose-dependent cross-resistance to entecavir in a cell culture model; however, lamivudine refractory strains remain sensitive to entecavir. Treatment outcomes of lamivudine resistant patients with 1 mg entecavir once daily were less compared to the nucleoside naïve patients and resulted in a decline of 5.1

	Lamividine	Adefovir	Entecavir	Tenofovir
Lamivudine-resistant	R	S	S#	S
Adefovir-resistant	S	R	S	S
Entecavir-resistant	R	S	R	S

Table 1. Management of resistance and summary of cross-resistance profiles.
R= resistant, S= sensitive.
#Entecavir remains sensitive to lamivudine resistant strains, but the sensitivity is decreased.

log_{10} copies/ml and PCR negativity (LLD) in 21% of patients at week 48. ALT normalized in 75% of patients and histological necroinflammatory scores improved in 55% of patients. In addition, 34% of patients experienced improvement of fibrosis scores.[92] With prolonged treatment in HBeAg negative lamivudine refractory patients up to 96 weeks, 30% of patients were PCR-negative (LLD 300 copies/ml), 85% had normal ALT levels and HBeAg seroconversion was observed in 16% of patients.[93] Entecavir requires multiple mutations and therefore has a high barrier to resistance. Entecavir resistance emerges after additional mutations in lamivudine resistant strains.[94] After 1 year of entecavir, 1% of lamivudine-resistant patients were resistant to entecavir which increased to 9% after two years.[79]

Tenofovir disoproxil fumaraat, an oral prodrug of tenofovir, is licensed for the treatment of HIV and possesses potent activity against lamivudine resistant HBV and has no cross-resistance with lamivudine resistance.[95-97] Results look very promising but all studies were very small and the drug has to be studied in larger groups.

Adefovir resistance

In vitro, adefovir resistant mutants are susceptible to lamivudine and might therefore be used to treat adefovir resistance (see table 1).[98] However, little data are available on this treatment option.[62,99] The effect of adefovir associated mutations on long-term lamivudine treatment is unknown and no data is available on lamivudine + adefovir combination therapy. Entecavir and tenofovir have been proven to be effective for adefovir resistant mutants, both in vitro and in vivo, as detailed in a number of published case reports.[62,72,98,100] Larger studies are needed to study different treatment options for patients with adefovir resistance, the same counts for patients resistant to both adefovir and lamivudine.

Entecavir resistance

Entecavir resistance is highly cross-resistant with lamivudine as entecavir resistance requires lamivudine resistance (see table 1).[94] These mutant strains are sensitive to adefovir in vitro and clinical treatment with adefovir resulted in viral load declines.[94,101]

DISCUSSION

Over the years, immense progress has been made in the understanding, as well as the treatment of chronic hepatitis B. Most patients with chronic hepatitis B can be treated adequately and as the morbidity and mortality are fairly high especially, in patients with cirrhosis, treatment should be initiated when appropriate. As the field of hepatitis B quickly evolves, the recommendations for the initiation and type of treatment

changes. In the coming years, more antiviral agents will be registered for the treatment of chronic hepatitis B. This will extend the arsenal of agents. This will probably result in better treatment options, but on the other hand, treatment may become more complex. Specifically, resistance issues will be challenging. Fortunately, the resistance rate of some antivirals is relatively low, but due the low incidence, it is hard to study resistance because enrolling large groups of patients will be necessary. In vitro testing provides information on the level of cross-resistance and this work should ideally be done for every new mutation found. Also, this work has to be extended to multi-resistant strains, which will emerge with the use of multiple drugs either as sequential monotherapy and combination therapy. Clinical treatment options for resistant strains will have to be evaluated. Although adefovir has been used clinically for several years and large trials with extended treatment durations have been performed in which numerous people developed resistance, very little data are available about the management of adefovir resistance. The same counts for entecavir, but very few resistant cases have been identified thus far. More studies are needed to investigate the basic mechanisms of treatment of non-responders, especially those caused by means other than drug resistance. Some patients will not respond to treatment. The factors involved should be more closely studied, as well as, the response to other agents. We may be on the verge of a change in the treatment of chronic hepatitis B.

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1B Rationale and aims of the study

In the early days of chronic hepatitis B treatment prospects of patients were grim. No effective therapy was available and liver transplantation was the only option for patients who progressed to end-stage liver disease. To worsen matters, HBV infection soon became a contraindication for transplantation as survival was poor due to re-infection of the transplant liver, followed by an aggressive course leading to graft failure in a short time. With the introduction of HBIg therapy post-transplantation recurrence rates dropped and survival increased. This resulted in acceptation of HBV infected patients in the transplant programs again. In the nineties the only treatment available for chronic HBV was interferon. Although many different interferon regimens were explored, treatment results were limited with a response in about one third of patients. In addition the therapy was associated with multiple side effects and required thrice weekly injection. Relapse after discontinuation was frequent. Retreatment with interferon yielded even lower success rates. A new era started with the introduction of the oral nucleoside analogues.

With Lamivudine treatment it was possible to control HBV disease in the majority of patients, probably even increasing survival. But optimism was short lived as it soon turned out the resistance barrier of lamivudine was low, resulting in resistance in up to two third to three quarter of patients. Resistance was often accompanied by relapse of viral replication and liver inflammation and thus to progression of disease. Sometimes the hepatic flare after relapse leads to decompensation or even death. New drugs for the treatment of HBV came on the market with increasingly shorter intervals. Treatment with PEG-interferon-α improved sustainability of response with much lower side effects during treatment. New nucleosides and nucleotides are more potent and have a higher barrier to resistance. However, treatment is far from perfect. Only a minority of patients is cured by interferon treatment, which is not likely to happen with nucleoside/nucleotide treatment. Thus many patients need life-long monitoring and/or treatment. With the availability of several treatment options it became harder to determine the best treatment for the individual patients. In the early days the choice was simple, as little was known about the prognostic factors for treatment outcome and options were limited. Another challenge has emerged in the form of treatment experienced patients, who failed prior therapy as a result of non-response to treatment or development of resistance. The best for the patient is patient tailored treatment taking in account variables known to influence treatment outcome.

The question is: "Is patient tailored treatment feasible"?

In this thesis we explore the possibility of patient tailored treatment by reviewing the literature and by research on treatment outcomes in patients with hepatitis B virus infection to add and understand some of the variables influencing treatment outcome.

AIMS OF THE STUDY:

1. To examine the response to PEG-interferon-α in lamivudine experienced patients harbouring mutations in the YMDD-motif associated with resistance to lamivudine.
2. To describe the development of entecavir resistance in a treatment experienced patient
3. To determine treatment outcomes in patients switched from tenofovir to adefovir.
4. To explore the response to adefovir in an open population, reflecting daily practice. Outcomes, predictors for response and resistance.
5. To investigate the feasibility of discontinuing HBIg treatment in patients transplanted for acute fulminant hepatitis B virus infection.

2

The effect of pegylated interferon-α on the treatment of lamivudine resistant chronic HBeAg positive Hepatitis B virus infection

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ABSTRACT

Objective: To determine the response to pegylated interferon-α treatment of HBeAg-positive hepatitis B patients with proven lamivudine resistance.

Methods: Sixteen HBeAg-positive HBV patients with YMDD mutations were treated with pegylated interferon. Median treatment duration was 52 weeks (range 20–53), with a 26-week follow up.

Results: Two of 16 (12.5%) patients seroconverted to HBeAg negative and achieved sustained virological (HBV-DNA levels below ^{10}log 5 copies/ml) together with biochemical (normalization of serum ALT levels) responses. Compared with the strong signal in all other patients, only these two patients had a faint signal in the lamivudine resistance assay. For all patients, the median viral load decreased from ^{10}log 9.4 to 7.9 copies/ml (p=0.001) during treatment but rebounded to a median of ^{10}log 8.7 copies/ml after treatment cessation. Similarly, elevated median ALT levels at baseline decreased with treatment but rebounded after the end of treatment.

Conclusion: In the largest cohort study to date, pegylated interferon-α therapy showed marginal efficacy in the presence of lamivudine resistance but such therapy may be beneficial in patients with only small amounts of mutant virus. In our opinion, an analysis of the patient subgroup harbouring an YMDD-mutation should be included in all future studies of pegylated interferon-α in chronic hepatitis B.

INTRODUCTION

Although 350–400 million people worldwide are affected by hepatitis B, to date, treatment for patients is frequently unsuccessful. Chronic hepatitis B is an immunological-based liver disorder, and there is increasing evidence that only a complete and vigorous HBV-specific immune response can achieve control and elimination of the virus, preventing disease progression.[1] The subgroup of HBeAg-positive hepatitis B patients with proven lamivudine resistance are little studied, and existing studies are often hampered by their extent and design, which makes it difficult to draw definitive conclusions about the optimal treatment for this group.[2-5] In the study reported here, which is the largest cohort study to date, we investigate the response of HBeAg-positive hepatitis B patients with proven lamivudine resistance to pegylated interferon-α.

The nucleoside analogue lamivudine is an effective inhibitor of viral DNA polymerase. It suppresses replication of HBV, improving transaminase levels and liver histology and enhancing the loss of hepatitis B e antigen (HBeAg).[6,7] However, sustained response after discontinuation of treatment occurs in only 10–15% of lamivudine-treated patients.[8] Another drawback is the emergence of mutations, in the tyrosine-methionine-aspartate-aspartate (YMDD-motif) of the viral polymerase, which are associated with resistance.[9] Mutations sometimes arise as little as six months after initiation of treatment with a resistance rate of 15–30% after one year, increasing to approximately 60–70% after four years of continuous treatment.[5, 6, 7, 10-13] Some patients who experience virological breakthrough may develop acute exacerbation, leading to liver decompensation and death.[9]

European guidelines recommend pegylated interferon-α as first-line treatment for both HBeAg-positive and -negative patients[13] but there remain several unanswered questions related to its uptake as a panacea treatment for hepatitis B. There is conflicting evidence on the effect lamivudine resistance, caused by mutations in the YMDD-motif, on the outcome of pegylated interferon-α therapy. Recently, our Department co-ordinated a large, independent, randomised, double-blind multicentre trial to determine the effects of pegylated interferon-α treatment in HBeAg-positive patients either alone or in combination with lamivudine.[14] The presence of the complete data set and patient samples from this trial enabled us to devise this retrospective cohort study of the outcome of pegylated interferon-α treatment in HBeAg-positive patients carrying the YMDD-motif mutated virus, which is reported here.

PATIENTS AND METHODS

Study design

In this retrospective, comparative, cohort study, data were compiled from the patient files and virological records of a large multicentre trial, previously conducted in our department in which the efficacy of pegylated interferon alpha-2b, either alone or in combination with lamivudine, was compared in a randomised trial of chronic hepatitis B patients.[14] In addition, patients treated by the same protocol outside this study were also included.

Inclusion and exclusion criteria

Eligible patients were HBeAg-positive with resistance to lamivudine as a result of lamivudine treatment before the start of interferon therapy. Resistance was confirmed by detection of a mutation in the YMDD motif of the RNA-dependent DNA polymerase gene of the virus. All patients with lamivudine-resistant virus were included in this analysis, regardless of differences in the subsequent interferon therapy (mono- or combination therapy). If mutational data were not available on record, retrospective analysis was carried out on the corresponding stored serum samples. Where a time point was missing from the records, results from the nearest date of sampling were taken, within an interval of four weeks.

Patients were excluded from the study if they were receiving antiviral treatment at the time of enrolment in the original study.

Treatment and outcome measures

All patients were treated with pegylated interferon alpha-2b either in monotherapy (100 µg/week) or in combination with lamivudine (lamivudine 100 mg/day) for more than 20 weeks and were followed-up for at least a further 16 weeks post-therapy.

During treatment and follow-up, patients attended outpatient clinics every 4 weeks for routine examination and laboratory tests. Assessments were made at baseline, Weeks 16, 32 and 52, and after 26 weeks of follow-up, as appropriate.

Outcome measures were assessed at the end of treatment (Week 52) and at the end of follow-up (Week 26). The primary outcome measure was loss of HBeAg from serum. Secondary outcomes were return to normal of serum ALT levels, concentrations of HBV DNA below 200,000 copies/ml and concentrations of HBV DNA below the level of detection of the assay (Taqman® assay; 400 copies/ml).

Biochemical and virological assessments

Viral load was determined by HBV DNA serum levels and seroconversion by the presence of HBeAg or anti-HBe. HBeAg and anti-HBe concentrations were determined using a

Microparticle Enzyme Immune Assay (MEIA, Abbott, Chigaco, IL). During treatment with interferon, HBV-DNA serum levels were determined by an in-house qPCR, (Taqman® assay) calibrated using Eurohep HBV DNA standards.[15] Used quantitatively, the Taqman assay enables accurate determination to levels of 1,000 copies/ml.[16] HBV genotypes and mutation analysis of the YMDD motif at the rtM204M side of the viral polymerase gene were determined using the Inno-Lipa assay (Innogenetics Ghent, Belgium).

The extent of liver inflammation was determined by measuring serum alanine aminotransferase (ALT) levels. To correct for the heterogeneity of local assays, ALT levels are expressed as values representing a ratio to local upper limit of normal (x ULN) and shown as medians with their range.

Statistical analysis

Continuous variables are expressed as median with their range. Median scores were compared by Wilcoxon Signed-Rank Test. The Mann-Whitney test was used for the comparison of groups. A two-tailed *p* value of <0.05 was considered statistically significant. All analyses used SPSS (version 12.0.1; Chicago, IL, USA).

Subgroup analyses were performed to determine whether known predictors of response to therapy accounted for response to pegylated interferon treatment in this study. The most frequently cited predictors of response include; previous interferon-alpha treatment, genotype, high ALT levels and low viral load.[13,14]

RESULTS

Sixteen HBeAg-positive patients fulfilled the study criteria. The baseline characteristics of the patients are shown in Table 1.

Of the patients in this analysis, the majority (12/16) received pegylated interferon alpha treatment monotherapy and the remaining four were treated with the same weekly dose of pegylated interferon together with 100 mg of lamivudine per day (combination therapy). Fifteen of the 16 patients received treatment for 52 weeks and, for 13 patients, all data (ALT, HBV-DNA and e-status) were available at 26 weeks of follow-up. In viral samples from two patients only faint mutation bands were visible by the Inno-Lipa Assay.

HBeAg-status

Two of 16 patients (12.5%; 95% ci -6.0% to 31%) seroconverted to HBeAg negative, and these two patients also had a sustained virological and biochemical response (HBV-DNA levels less than 10^5 copies/ml and normal ALT at 26 weeks follow up).

	total	PEG-interferon monotherapy	**Peg-Interferon + lamivudine combination therapy**
Male/ female gender (*)	14 / 2	10 / 2	4 / 0
Median age (range)	41.5 (25-72)	41.5 (27-72)	41.5 (25-48)
Race (caucasian / asian)(*)	14 / 2	11 / 1	3 / 1
Previous IFN treatment (yes/ no;*)	7 / 9	5 / 7	2 / 2
Median time start PEG-IFN after lamivudine (weeks) (range)	42.3 (5.3-176.1)	39.9 (5.3-176.1)	64.6 (31.0-100.6)
Fibrosis according Ishak no. 0 / 1 / 2 / 3 / 4 / 5 / 6 // missing (*)	0 / 3 / 1 / 6 / 0 / 1 / 1 // 4	0 / 2 / 1 / 5 / 0 / 0 / 1 // 3	0 / 1 / 0 / 1 / 0 / 1 / 0 // 1
Median ALT x ULN	3.5 (1.5-11.0)	3.6 (1.5-11.0)	3.5 (1.7-7.2)
Median ^{10}log HBV-DNA	9.4 (8.7-10.4)	9.3 (8.7-10.4)	9.8 (9.1-10.2)
Genotype A/D/other (*)	6 / 6 / 4	5 / 5 / 2	1 / 1 / 2
YMDD mutants V / M+I / M+V / M+V+I (*)	1 / 4 / 6 / 5	1 / 2 / 4 / 5	0 / 2 / 2 / 0

(*) number of patients

Table 1. Baseline characteristics for the total group and the subgroups peg-interferon monotherapy and peg-interferon + lamivudine combination therapy.

Viral load

The median viral load for the whole patient group decreased by 1.5 ^{10}log copies/ml to 7.9 (range 1.7–10.0) during the treatment period (p=0.001). However, this reduction was not sustained and by 26 weeks after treatment cessation the viral load rebounded to a median of 8.7 ^{10}log copies/ml (range 2.6–10.3) (Table 2).

Response assessed by ALT measurements

At baseline all patients had elevated ALT levels and a high viral load (Table 1). By Week 52, ALT levels decreased to a median of 1.3 xULN (range 0.9–11.6), which is significant compared with baseline (p=0.047) (Table 2).

After discontinuation of treatment, ALT levels increased to a median of 2.3 xULN (range 0.9–5.1) by 26-weeks of follow-up. Only 3 (19%) patients had sustainable normal ALT levels.

Sustained response

By 26 weeks of follow up only 2 of 16 (12.5%;95% ci -6.0% to 31%) patients could be considered as sustained responders to treatment with pegylated interferon alpha by our primary outcome measure, and only 2/13 (15.4%) patients had viral load below 10^5 copies/ml. Only one responder had a HBV DNA less than 400 copies/ml at the end of follow-up. It is of note that the two responders were the patients where only very faint

	baseline		Week 52		Week 26 follow-up	
	ALT	HBV DNA	ALT	HBV DNA	ALT	HBV DNA
Total n=16	3.5 (1.5-11.0)	9.4 (8.7-10.4)	1.3 (0.9-11.6)[1]	7.9 (1.7-10.0)[1]	2.3 (0.9-5.1)[1]	8.7 (2.6-10.3)[1]
Monotherapy n=12	3.4 (1.5-11.0)	9.3 (8.7-10.4)	1.4 (0.9-11.6)	7.9 (4.3-9.5)[1]	2.5 (1.5-5.1)	8.7 (8.1-10.3)[1]
Combination therapy n=4	3.5 (1.7-7.2)	9.8 (9.1-10.2)	1.3 (1.1-2.5)	4.2 (1.7-10.0)	1.0 (0.9-3.1)[2]	6.7 (2.6-10.1)

Table 2. The course of liver inflammation as measured as ratio of ALT as upper limit of normal and viral load (^{10}log copies/ml) at baseline, end of treatment (week 52) and 26 week of follow-up for the total group and the subgroups receiving PEG-interferon-α monotherapy or PEG-interferon-α + lamivudine combination therapy. Number in superscript (1) indicate a significant difference ($p<0.05$) compared to the baseline value (1). Number in superscript (2) indicate a significant difference ($p=0.05$) between two groups. No significant differences at any time point were found between mono or combination therapy.

mutation bands were visible in the initial Inno-Lipa assay. Both responders were 48 years of age, an Asian male, interferon naive and a Caucasian male who had received prior interferon-α therapy. Time elapsed after discontinuation of lamivudine was 64.5 and 31.0 weeks and fibrosis according Ishak was 5 and 1 respectively. One patient had genotype B and the other genotype A. Both harboured the M552M + M552I mutation. ALT levels were 7.2 and 4.2 times elevated and HBV DNA levels were 9.1 and 9.7 ^{10}log copies/ml respectively. None of the baseline characteristics was found to be significantly different compared to the nonresponders. One other patient had a normal ALT level after 26 weeks of follow up, but no HBe-seroconversion and HBV DNA above 10^5 copies/ml.

Monotherapy or combination therapy

The majority of the patients 12/16 (75%) were treated with pegylated interferon alpha-2b monotherapy. The baseline characteristics of these patients did not differ significantly from those receiving combination therapy (Table 1).

After 52 weeks of treatment the ALT levels had decreased equally in both groups (p=0.791). In the combination therapy group, ALT levels continued to decrease during follow up and the median ALT level was normal at end of follow up which was significantly lower (p=0.05) (Table 2). For the monotherapy group, despite achieving similar ALT levels to the combination group at week 52, at the end of follow up the ALT levels increased to 2.5 x ULN (Table 2.)

Although the decrease in viral replication was more marked in the group receiving lamivudine in addition to interferon (5.6 ^{10}log compared with 1.4 $\log_{10}$ copies/ml) during treatment, by 26-weeks after therapy overall viral levels for both groups were not significantly different (p=0.643) (Table 2).

None of the 12 (0%) patients receiving pegylated interferon alpha-2b monotherapy had e-loss, normal ALT level or HBV DNA level below 10^5 copies/ml at the end of follow-up. Both responders were in the group that received combination therapy.

DISCUSSION

This is the largest study to date on the response to pegylated interferon-α treatment of HBeAg-positive hepatitis B patients who have YMDD-mutated virus after previous lamivudine treatment. Previous studies with this patient group used non-pegylated interferon-α and were limited in the number of patients studied,[2-4] the length of treatment (six months) and treatment schedules. The small number of responders to treatment prevented us from drawing definitive conclusions about the benefits of interferon therapy in this group. In contrast to the previous studies, in our larger study, all patients were treated with pegylated interferon-α for 52 weeks, in accordance with present guidelines and recommendations.[13,17]

In our group of 16 patients, two (12.5%) responded positively to pegylated interferon-α treatment with HBe-seroconversion, a drop in viral load and normalization of ALT levels. This response is lower than that observed in other trials of pegylated interferon-α treatment for HBeAg-positive patients, where the percentage of e-loss was over 30%.[14,18] However, the response rate was in line with that observed in the earlier trials with lamivudine-resistant patients, where between 16% and 22% of patients responded to pegylated interferon-α.[2-5] If we limit the analyis to the patients receiving monotherapy PEG-interferon-α none of the twelve (0%) responded to therapy.

There are several proposed predictors of response to treatment with interferon, including; previous interferon-α treatment, genotype, high ALT levels and low viral load.[13,14] In this small study we performed a sub-group analysis to determine which, if any, could explain the observed marked lack of response to pegylated interferon-α. None of these predictors were found to influence treatment outcome.

We previously found that rate of e-loss was 25% in patients with genotype D virus, compared with 47% in those with genotype A.[19] Other studies concur that genotypes C and D are less responsive to interferon-α treatment compared with genotypes A and B.[20-23] A large randomised trial with pegylated interferon alpha-2a found no significant difference for response according to genotype, however a trend for higher responses in patients with genotype A was observed.[18] In our study of patients carrying YMDD-mutated virus, the relationship between genotype and treatment response was unclear.

Both responders had genotypes that respond more favourably to treatment (genotypes A and B).

In our study it was striking that all patients with clear lamivudine resistance were unresponsive to interferon therapy. Notwithstanding the possibility that other unidentified factors may have influenced outcome, and although immunomodulatory therapy has not previously been linked to therapy failure with nucleoside analogues,[24] the findings in our study may suggest that YMDD-mutation impairs the immune response to HBV and reduces the efficacy of pegylated interferon-α treatment. In support of our putative explanation, both responders had, in contrast to the other subjects, very faint bands in the Inno-LiPA assay, which has a detection limit of about 5%. This assay was not formally quantified but this qualitative assay suggests that these patients had only a low quantity of mutant virus.

Our results might suggest that, for subjects with emerging lamivudine resistance, early pegylated interferon-α therapy may be beneficial. Alternatively, other nucleoside analogues, such as adefovir, tenofovir and entecavir, which have been shown to be effective against lamivudine-resistant virus, may present a treatment option for this patient group.[25-27]

Although suggestive of the negative impact of the presence of the YMDD-mutation on pegylated interferon-α treatment, this study is too small to yield definitive results. More data are needed to further determine the effect of the YMDD-mutation on the efficacy of pegylated interferon-α treatment. Whether PEG-interferon-α combination therapy with other nucleoside/nucleotide analogues is benificial for patients with lamivudine resistance has to be determined. This study is too small to make definite conclusions. The timing of the therapy may be of importance as both responders receiving combination therapy had only little amounts of mutant virus present in a sensitive assay. In our opinion, an analysis of the patient subgroup harbouring an YMDD-mutation should be included in all future studies of PEG-IFN in chronic hepatitis B.

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3

Switching patients with lamivudine resistant chronic Hepatitis B virus from tenofovir to adefovir results in less potent HBV DNA suppression

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ABSTRACT

Background: The nucleotide analogues, tenofovir disoproxil fumarate and adefovir dipivoxil, inhibit viral replication and are both effective against the hepatitis B virus.
Methods: In our department, tenofovir was prescribed in addition to lamivudine for the treatment of lamivudine resistant chronic hepatitis B. After administration of adefovir, 10 patients were switched to adefovir monotherapy. We studied changes in HBV DNA and ALT in these patients.
Results: The median treatment duration with tenofovir was 78 weeks resulting in a median viral load reduction of 5.4 (range 6.8 to 2.3) $\log_{10}$ copies/ml compared to baseline (p=0.005). Two patients had an increase >1 $\log_{10}$ copies/ml during tenofovir treatment. After the switch to adefovir, 6 out of 10 patients had an HBV DNA >4 $\log_{10}$ copies/ml and the median HBV DNA increased from 2.8 to 4.5 $\log_{10}$ copies/ml (p=0.017). The factors associated with relapse were HBV DNA PCR positivity at the time of switch and genotype B or D. ALT levels at the beginning of tenofovir treatment also might be a factor. Retreatment with tenofovir (n=3) resulted in a rapid decline in HBV DNA.
Conclusion: Tenofovir is a potent antiviral drug. Switching to adefovir resulted in viral relapse in 60% of patients and retreatment with tenofovir resulted again in viral decline, which suggests that tenofovir is a more potent antiviral agent.

INTRODUCTION

Although 350-400 million people worldwide are infected with hepatitis B, to this date, treatment is frequently unsuccessful. Interferon-α (IFN) treatment or pegylated interferon-α (PEG-IFN) results in a sustained response in 30-40% of treated patients.[1,2] Many rely on long-term viral suppression with nucleoside or nucleotide analogues to prevent disease progression. Nucleosides or nucleotides act by inhibiting the viral polymerase, thereby suppressing the viral replication of HBV and improving serum transaminase levels and liver histology. In HBeAg-positive patients, the loss of hepatitis B antigen (HBeAg) is enhanced.[3-6] The long-term treatment may select for mutations within the viral polymerase that promote antiviral resistance resulting in an increase in liver inflammation and worsening of liver histology. Lamivudine has a high resistance rate of 15-30% after one year of treatment, which increases to 60-70% after continuous treatment.[7]

The nucleotides, tenofovir disoproxil fumarate and adefovir dipivoxil, exhibit both activity against wild-type virus and lamivudine resistant mutants in vitro and in vivo.[8-10] Tenofovir disopoproxil fumarate is an oral prodrug of tenofovir, a nucleotide (nucleoside monophosphate) analogue with activity against retroviruses, including HIV-1, HIV-2 and hepadnaviruses. Following absorption, tenofovir DF is rapidly converted to tenofovir, which is metabolised intracellularly to the active tenofovir diphosphate. The active form of tenofovir is a competitive inhibitor of HBV transcriptase and terminates the growing DNA chain.[11-13]

Several small studies suggest a more potent antiviral effect of tenofovir compared to adefovir.[9] It is not known whether viral suppression is maintained after switching to adefovir or whether it is effective in case of viral breakthrough on tenofovir treatment, however several cases suggest the loss of efficacy in some patients.[14,15] In our Department, tenofovir was added to lamivudine to combat viral breakthrough due to lamivudine resistance during a period in which adefovir was not yet available. After adefovir, dipivoxil became available as a registered product and the tenofovir containing regimen was switched to adefovir monotherapy. Its availability enabled us to study the ability of adefovir treatment to sustain tenofovir induced disease remission.

PATIENTS AND METHODS

Study design

In this retrospective cohort study, data were compiled from patient files and virological records from patients treated for chronic hepatitis B virus infection at our Department.

Eligible patients were treated with tenofovir disoproxil fumarate after the emergence of genotypic lamivudine resistance and in whom therapy was switched to adefovir monotherapy. All patients were included in this analysis regardless of HBeAg status or duration of tenofovir therapy. HBV DNA $>10^4$ copies/ml at the start of tenofovir treatment was required.

Treatment and outcome measures

All patients were lamivudine resistant and subsequently treated with tenofovir disoproxil fumarate either in monotherapy (245 mg/day) or in combination with lamivudine (100mg/day) prior to switching to adefovir monotherapy (10 mg/day). There was no overlap with tenofovir and adefovir treatment. Tenofovir (245 mg/day) monotherapy or combination therapy of tenofovir with lamivudine (100mg/day) was started in some subjects with proven failure to adefovir therapy (relapse after the switch). During treatment, patients attended our outpatient clinic on a regular basis for routine examination and laboratory testing.

Outcome assessments were made at the initiation of lamivudine, start of tenofovir treatment, at weeks 12 and 24 of tenofovir therapy, the end of tenofovir therapy/ start of adefovir therapy and last measurement the subject was on adefovir treatment. The primary outcome measure was the change in viral load. Secondary outcomes were concentrations of HBV-DNA below the limit of detection of the assay (Taqman® assay; 373 copies/ml), HBV-DNA below 10^3 copies/ml and serum alanine aminotransferase (ALT) levels <1 times the upper limit of normal (ULN).

Biochemical and virological assessments

HBV-DNA serum levels were measured with an in-house quantitative PCR (Taqman® assay) calibrated using Euroheb HBV-DNA standards.[16] This Taqman assay enables accurate quantitative determination of HBV to levels of 1000 copies/ml and has a lower limit of detection of 373 copies/ml.[17] The extent of liver inflammation was determined by measuring ALT levels using automated techniques and values are expressed as a ratio to the upper limit of normal (xULN).

For sequence analysis, the total HBV genome was sequenced sequentially with primers as described before.[18] These sequence products provided information on mutations related to lamivudine, adefovir and tenofovir resistance. The analysis was performed using an ABI3100 instrument (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and analysed using BioEdit Software (v7.05), (Ibis Therapeutics, Carlsbad, CA, USA).

Statistical analysis

Continuous variables are expressed as medians with their range. Dependent variables were compared using the Wilcoxon's Signed Ranks-test and independent variable by Mann-Whitney U test, Chi-square or Fisher exact test as appropriate.

A two tailed p-value of <0.05 was considered statistically significant. For all analyses, SPSS (version 14.0.0; Chicago, IL, USA) was used.

RESULTS

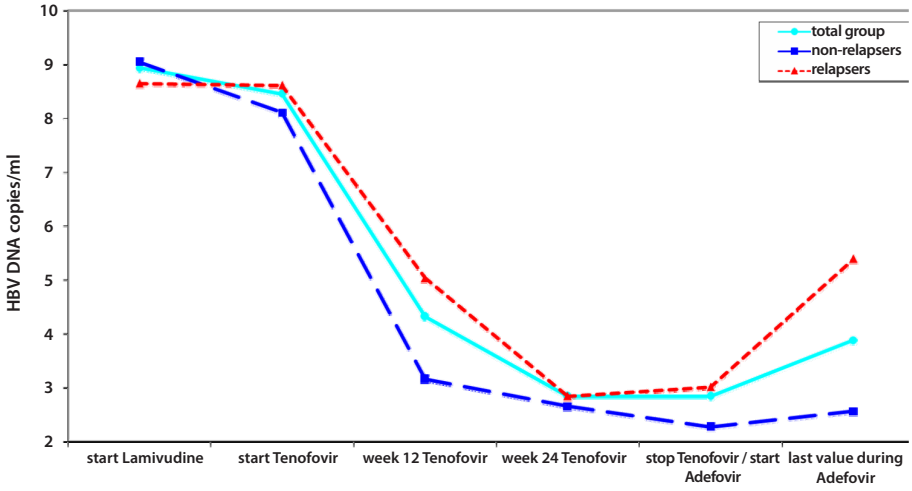
Ten patients fulfilled the study criteria. The baseline characteristics are shown in Table 1. Median treatment duration with tenofovir until the switch to adefovir was 78 weeks and resulted in a rapid and significant decline of 5.4 (6.8 to 2.3) $\log_{10}$ copies/ml in viral load (p=0.005) compared to baseline (Fig. 1 and 2). Maximum HBV DNA decline during tenofovir therapy was 6.1 (7.4 to 2.4) $\log_{10}$ copies/ml, but HBV DNA increased slightly and at the end of tenofovir therapy median serum HBV DNA was 2.8 $\log_{10}$ copies/ml, with 40% (4/10) patients being PCR negative. At the end of tenofovir treatment 30% (3/10) patients had a viral load above 10^3 copies/ml. Two of those patients (patients 2 and 9) clearly showed phenotypic resistance to tenofovir as both had over 1 $\log_{10}$ increase of HBV-DNA, the other patient (patient 7) had an increase in HBV DNA from PCR negativity to 3.2 $\log_{10}$ copies/ml just before the switch to adefovir. No genotypic resistance associated with tenofovir could be documented. None of the patients experienced a hepatic flare. ALT levels declined significantly from 2.4 xULN at start of therapy to 0.74 xULN (p=0.013) at the end of tenofovir therapy, resulting in ALT levels <1 ULN in 80% (8/10) of the patients and all had ALT level <1.5 times the ULN.

Switching tenofovir to adefovir had a negative effect in 60% (6/10) of patients. The viral load for the total group increased significantly to 4.5 $\log_{10}$ copies/ml during adefovir therapy (p=0.046). In the relapse group, the increase in HBV DNA was 2.2 $\log_{10}$ copies/ml and all patients had a viral load of $>10^4$ copies/ml. The increase in HBV DNA was not accompanied by an hepatic flare as ALT levels remained 0.74 times the upper limit of normal and only one patient in the relapse group had an ALT level >2 times ULN. Resistance testing revealed only the rtL80I and rtM204I mutation in the patient responding poorly to tenofovir (patient 9). Genotype related polymorphisms were not detected, especially not those published in the literature. [19]

To study the factors influencing relapse the group of non-relapsers was compared to the relapsers. Patient characteristics were comparable for both groups, as was the duration of lamivudine and tenofovir treatment (Table 1). HBV DNA levels did not differ between the two groups during lamivudine therapy, up to 24 weeks of treatment with tenofovir.

Baseline factors	Total group (n=10)	Non-relapsers (n=4)	Relapsers (n=6)	P-value
Age (years)	33 (18-45)	33 (18-42)	36 (26-45)	0.831
Race (Caucasian / Asian / Other) (%)	40/40/20	25 /50 / 25	50 / 33 / 17	0.732
Sex- male (%)	70	100	50	0.200
HBeAg positive (%)	80	75	83	1.0
Cirrhosis (%)	20	0	33	0.467
Previous IFN (%)	50	25	68	0.524
Genotype (A / B / C / D (%)	20/40/10/30	50/25/25/0	0/50/0/50	
Geno B+D vs rest (% geno B+D)	70	25	100	0.033
HBV DNA start TEN	8.45 (4.62-9.66)	8.10 (4.62-8.84)	8.61 (7.96-9.66)	0.240
ALT start TEN (xULN)	2.43 (0.36-18.50)	5.74 (3.33-18.50)	1.29 (0.36-11.83)	0.055
Duration of LAM prior to TEN (weeks)	92.14 (52.14-160.0)	78.43 (64.0-105.43)	127.5 (52.14-160)	0.286
LAM resistant before initiation TEN (weeks)	47.43 (5.0-120.86)	47.43 (23-0-62.0)	54.90 (5.0-120.86)	0.831
Factors associated with relapse				
Duration TEN therapy (weeks)	77.57 (51.0-105.86)	83.5 (77.14-94.0)	62.57 (51.0-105.86)	0.831
Duration TEN + LAM combination therapy (weeks)	53.57 (2.86-92.0)	71.57 (14.0-89.0)	48.50 (2.86-92.0)	0.336
HBV DNA start LAM	8.93 (7.28-10.20)	9.04 (7.28-9.11)	8.64 (7.33-10.20)	0.796
ALT start LAM	12.03 (0.64-23.75)	12.18 (12.03-13.13)	1.01 (0.64-23.75)	0.439
Lowest HBV DNA during LAM#	3.88 (2.84-7.73)	3.33 (2.84-4.59)	3.96 (2.84-7.73)	0.224
Lowest HBV DNA during TEN	2.27 (2.27-4.42)	2.27 (2.27)	2.27 (2.27-4.42)	0.517
Time HBV DNA $<10^3$ stop TEN (weeks)	25.0 (8.86-62.86)	22.5 (8.86-62.86)	25.0 (11.71-47.0)	0.317
HBV DNA stop TEN	2.84 (2.27-6.43)	2.27 (2.27)	3.01 (2.84-6.43)	0.007
HBV DNA neg stop TEN (%)	40	100	0	0.05
HBV DNA $<10^3$ stop TEN (%)	70	100	50	0.200
ALT stop TEN	0.74 (0.53-1.30)	0.83 (0.53-1.30)	0.70 (0.58-1.08)	0.522
HBeAg positive at switch (%)	70	50	83	0.500

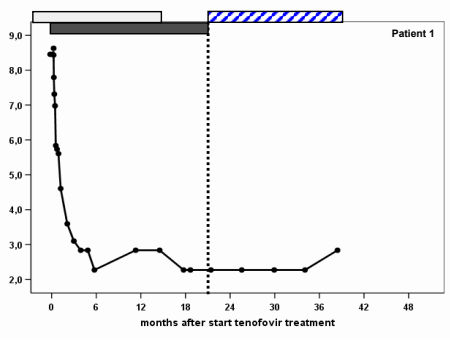
Table 1. Baseline characteristics for the three different groups (total group, non-relapsers, relapsers). P-values are outcome of comparison between non-relapsers and relapsers. Factors associated with relapse at baseline (start of tenofovir therapy) and before, during and after tenofovir treatment. LAM = lamivudine, TEN = tenofovir, HBV DNA neg = undetectable by PCR (lower limit of detection 373 copies/ml), HBV DNA in copies/ml, # = median viral load of the lowest HBV DNA level reached during tenofovir at any time point for each individual.

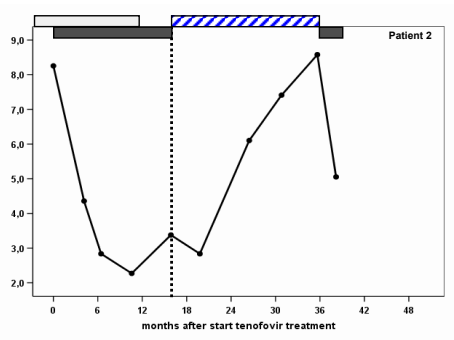


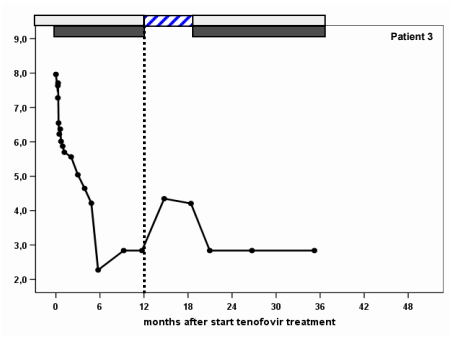
HBV DNA						
Total Group	8.93 (7.28-10.20)	8.45 (4.62-9.66)	4.32 (2.84-6.59)	2.84 (2.27-4.82)	2.84 (2.27-6.43)	4.50 (2.27-8.58)
Non-relapsers	9.04 (7.28-9.11)	8.10 (4.62-8.84)	3.16 (2.84-4.39)	2.65 (2.27-3.73)	2.27 (2.27)	2.56 (2.27-2.84)
relapsers	8.64 (7.33-10.20)	8.61 (7.96-9.66)	5.03 (2.84-6.59)	2.84 (2.27-4.82)	3.01 (2.84-6.43)	5.39 (4.21-8.58)
p-value	0.796	0.240	0.176	0.659	0.007	0.01
ALT (xULN)						
Total Group	12.03 (0.64-23.75)	2.43 (0.36-18.50)	1.30 (0.83-2.65)	0.86 (0.58-1.60)	0.74 (0.53-1.30)	0.74 (0.40-2.92)
Non-relapsers	12.18 (12.03-13.13)	5.74 (3.33-18.50)	2.38 (0.83-2.65)	0.97 (0.60-1.05)	0.83 (0.53-1.30)	0.74 (0.40-0.95)
relapsers	1.01 (0.64-23.75)	1.29 (0.36-11.83)	1.10 (1.10-1.30)	0.81 (0.58-1.60)	0.70 (0.58-1.08)	0.73 (0.44-2.92)
p-value	0.439	0.055	0.285	0.521	0.522	0.670

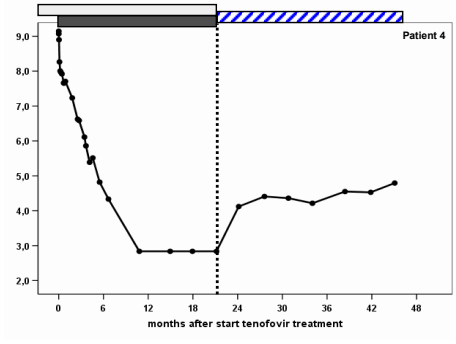
Figure 2. Course of HBV DNA during treatment for the total group, non-relapsers and relapsers at several time points during lamivudine, tenofovir and adefovir therapy. The median HBV DNA (log_{10} copies/ml) and ALT levels as a ratio to the upper limit of normal (xULN) and their range at these time points are provided in the table. P-values are provided for the comparison between non-relapsers and relapsers.

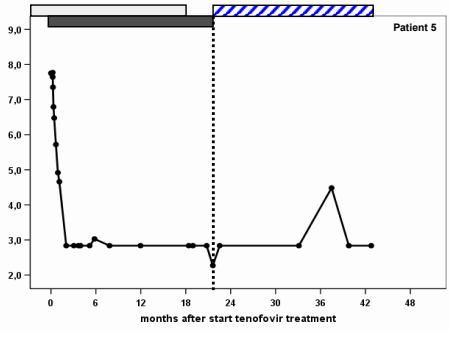
However at the end of tenofovir treatment the viral load was significantly higher in the relapse group (3.0 vs. 2.3 log_{10} copies/ml, p=0.007) and 0% vs. 100% had undetectable HBV DNA (p=0.05). After the switch the viral load remained low for the non-relapsers and increased in the relapse group with 2.2 log_{10} copies/ml to 5.4 log_{10} copies/ml at the end of follow-up. Despite ALT levels at start of lamivudine treatment and HBV DNA at start of lamivudine and tenofovir treatment were comparable, ALT levels at start of tenofovir therapy were higher in non-relapsers (5.7 vs. 1.3 times ULN) and showed a

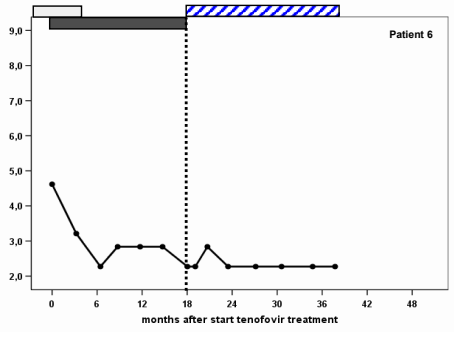

Patient 1
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment

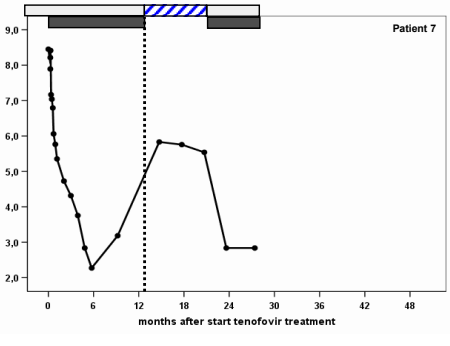

Patient 2
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 3
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 4
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 5
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 6
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 7
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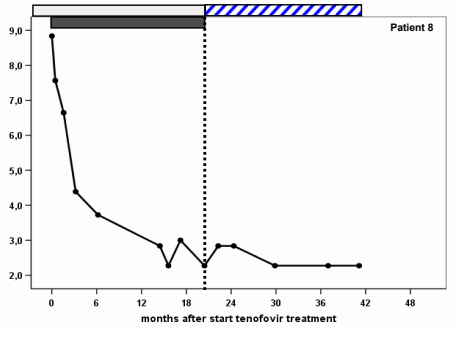
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment


Patient 8
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
0 6 12 18 24 30 36 42 48
months after start tenofovir treatment

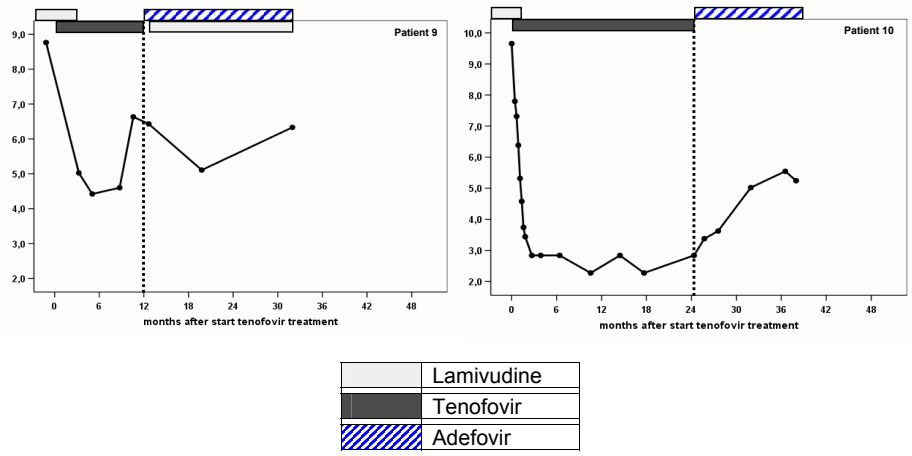


Figure 1. Course of HBV DNA during Tenofovir treatment and after the switch to adefovir therapy in 10 patients. The dashed vertical line indicates the time of the switch to adefovir.

trend to significance (p=0.055). ALT levels normalised in both groups, but ALT decline was higher for non-relapsers (5.1 vs. 0.5 times ULN ALT, p=0.055). HBeAg loss occurred more often in non-relapsers. One patient in the relapse group had HBeAg reversion. After the switch HBeAg status differed between the two groups (25% vs. 100% HBeAg positivity, p=0.033). Genotype B or D were significantly more common in patients with relapse during adefovir treatment (p=0.033).

Salvage therapy

In three patients tenofovir was restarted resulting in a rapid decline of HBV DNA levels. Two subjects treated (patients 3 and 7) with tenofovir + lamivudine combination therapy reached a viral load below 103 copies/ml within 12 weeks. The other subject (patient 2) had a 3.5 log10 copies/ml decline after 18 weeks of treatment with tenofovir mono therapy. In the patient (patient 9) with poor response to tenofovir treatment and no decline in viral load addition of lamivudine resulted in a 1.3 log10 copies/ml decline in viral load, but HBV DNA levels rebounded to the same level as before the addition of lamivudine.

DISCUSSION

The majority of patients infected with hepatitis B, irrespective of HIV infection, showed a good response to tenofovir, with an overall decrease in viral load of at least 4 $\log_{10}$ copies/ml.[9,20-23] Breakthrough during tenofovir therapy has not been reported in HBV

mono-infected patients. Although others have not confirmed a reduction in sensitivity, a mutation (rtA194T) that results in a reduction in in-vitro sensitivity to tenofovir has been reported.[11,24] Its frequency and clinical impact remains to be seen. Recently, the variants rtV214 and rtQ215S in combination with lamivudine associated results have been reported to be associated with a reduced sensitivity to tenofovir.

We studied 10 patients infected with lamivudine resistant chronic HBV treated with tenofovir. The median decline during treatment was 5.4 $\log_{10}$ copies/m. HBV DNA was $<10^3$ copies/ml in 70% and PCR–negativity was reached in 40% of patients. Two patients experienced increases in viral load of over 1 log and increases in PCR negativity to over 10^3 copies/ml was observed in an additional patient. Resistance analysis did not reveal mutations within the polymerase gene except lamivudine associated mutations in one patient.

Six of ten patients in this study failed on adefovir therapy. Three patient already had suboptimal viral suppression (HBV DNA $>10^3$ copies/ml), but the other 3 patients had low viral loads. No viral variants were detected, except the lamivudine associated mutations, rtL80I + M204I, in one subject. The known mutations associated with adefovir resistance (rtV84M, rtA181V/T, rtQ214A, rtQ215S, rtN236D/T) were ruled out by genotypic testing. Therefore, resistance to adefovir does not seem to play a role in the relapse after switching therapy. These findings are in concordance with the patients described before in which viral relapse was observed after switching tenofovir to adefovir.[14,15] A polymorphic form of the HBV genotype A2 rtL217R is sensitive to tenofovir but associated with non-response to adefovir has been described. A similar polymorphic form was described for the genotype D3 rtI233V, which was sensitive to tenofovir or entecavir but displayed a 6-10 fold decreased susceptibility to adefovir.[19,25] In our population, no genotype specific polymorphisms were found. However, genotypic influences have not been ruled out, as there was an association between genotype B or D and relapse. However, in large randomised studies of patients treated with adefovir or entecavir, HBV DNA suppression was comparable across HBV genotypes A-D.[26,27] Genotypes might influence treatment outcomes. Some studies reported different rates for genotypes in patients treated with lamivudine.[28-30] In adefovir treated patients, resistance was associated with HBV genotype D infection.[31] Further studies involving a larger number of patients with various genotypes are required to explore the possible association between genotype and relapse or the development of resistance.

If relapse cannot be explained by the development of resistance, there must be varying pharmacodynamic and pharmacokinetic properties in the host and different responses (viral dynamics and viral kinetics) to antiviral therapy. In vitro data suggest that adefovir and tenofovir are equipotent molecularly.[8,32] The 10 mg dose for adefovir was chosen for safety reasons (i.e. nephrotoxicity) and the higher dose of 30 mg proved to be more

potent in a head to head comparison indicating that the 10 mg dose is marginal for HBV DNA suppression.[5] Thus, a 245 mg dose of tenofovir is likely to be more effective.

A head to head comparison showed a significantly greater reduction in HBV DNA and higher rates of PCR negativity in patients receiving tenofovir, illustrating viral dynamics are in favour of tenofovir.[9] The dose of tenofovir could have been sufficient for potent viral suppression by tenofovir and the relapse of HBV DNA after the switch could have been the result of adefovir under dosing. In the group of relapsers, the viral load, at the time of switch, was significantly higher suggesting suboptimal viral suppression. The difference in potency is further underlined by the potent inhibition of viral replication with the reintroduction of tenofovir. Two patients reached a viral load below 10^3 copies within 12 weeks and the third patient had a 3.5 $\log_{10}$ decline in viral load over a period of 18 weeks, which is similar in magnitude to the other two patients. In one patient, lamivudine was added to adefovir resulting in a 1.3 $\log_{10}$ decline in HBV DNA, but the viral load quickly rebounded.

In conclusion, our data demonstrate that tenofovir is a potent inhibitor of viral replication, but efficacy is partially lost in some patients with prolonged treatment. Switching tenofovir to adefovir monotherapy results in a relapse of viral load in 60% of patients. Specifically, factors such as viral load at the time of switch and genotype B or D were associated with an increased risk of relapse. ALT levels at the initiation of tenofovir treatment could also be a factor influencing treatment outcome. Relapse did not seem to be a result of treatment-resistance and the reintroduction of tenofovir resulted in a strong and rapid decline in viral load. Patients on tenofovir therapy should not be switched to adefovir monotherapy.

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4 Selection of an entecavir resistant mutant, despite prolonged HBV DNA suppression, in a chronic hepatitis B patient with pre-existent lamivudine resistance: successful rescue therapy with tenofovir

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ABSTRACT

Entecavir has potent activity against hepatitis B virus. Drug resistance has not been reported in nucleoside naïve patients and is low in lamivudine-refractory patients.

A 43 year-old man was treated with lamivudine for HBeAg-positive chronic hepatitis B. A viral breakthrough due to a drug resistant mutant was observed and entecavir 1 mg daily was added. After the viral load had been near the lower detection range of the PCR assay for 30 weeks, lamivudine was discontinued. The serum HBV DNA remained low until a second viral breakthrough was observed after 45 weeks of entecavir monotherapy. Entecavir was switched to tenofovir disoproxil 245 mg daily, which resulted in a decline below 1000 copies/ml. Sequence analysis revealed the presence of rtL180M and rtM204V lamivudine resistant associated mutations at the start of entecavir treatment. During entecavir treatment, the rtS202G mutation was selected. Retrospective analysis revealed that during lamivudine treatment three other mutations had been selected as well, namely rtE1D, rtV207L and rtI220L.

Conclusions: We describe the first case of entecavir resistance in a lamivudine resistant patient with good initial suppression of viral replication for 70 weeks. Based on the data from cross-resistance and sensitivity testing in vitro and treatment outcomes, tenofovir proves to be a good treatment option for entecavir-resistant patients.

INTRODUCTION

Hepatitis B infection remains a global health problem despite the availability of effective vaccines. It is estimated that 350-400 million people worldwide are chronically infected. If left untreated, about a third will develop progressive and possibly fatal liver disease.[1] Drugs inhibiting viral replication have a higher on-treatment response compared to interferon-alpha or PEG-interferon-alpha, but relapse is frequent after treatment discontinuation. Treatment with the first registered small-molecule inhibitor of the viral polymerase, lamivudine, results in a rapid 4-5-log decline in viral load and has been shown to improve liver histology after one year of treatment. In addition a reduction of the incidence of hepatic decompensation and hepatocellular carcinoma compared to placebo treatment in patients with cirrhosis was observed.[2-4] A major drawback is the emergence of mutations within the viral polymerase gene rendering resistance resulting in a resistance rate of approximately 20% per year.[5] Adefovir dipivoxil on the other hand has a slower rate of resistance development relative to lamivudine and both drugs have different resistance patterns.[6,7]

A new promising drug, a carboxylic 2'-deoxyguanosine analogue entecavir, is rapidly metabolized to its active triphosphate metabolite.[8] This drug has recently been registered and a dose of 0.5 mg results in an approximately 7-log decline in HBeAg-positive and 5-log decline in HBeAg-negative, nucleoside naive patients. Entecavir (1 mg QD) is also effective against lamivudine resistant strains, however the reduction in viral load is less compared to wildtype.[9-11] The resistance rates in entecavir are very favorable. In large phase III studies, only 18 HBV DNA rebounds in over 900 person years of treatment were observed in nucleoside naïve patients treated with entecavir for up to two years of treatment. Analysis of these patients revealed no genotypic and phenotypic resistance to entecavir.[12] A phase III study for lamivudine refractory patients, revealed 5 out of 141 patients with viral rebound, two of them with entecavir resistance associated substitutions in the polymerase gene, treated with entecavir for 1 year. In the second year of entecavir treatment 17 HBV rebounds were observed in 119 treated patients and in 11 of them entecavir resistance was detected.[13]

Tenofovir disoproxil fumarate is another promising drug and is an acyclic nucleotide analogue analogue reverse transcriptase inhibitor, with showed potent activity both in in vitro and in vivo studies in wild type and lamivudine resistant HBV in HIV-HBV co-infected and HBV mono infected patients.[14-16]

In this report we describe a case of entecavir resistance after initial successful and prolonged viral suppression during entecavir treatment in a lamivudine-refractory patient.

Based on available resistance and drug susceptibility data and the results of our treatment, we discuss the management strategy in case of entecavir-resistance.

CASE

A 43-year old male (weight 87 kg, length 175 cm, BMI 28.4) with chronic active HBeAg positive (HBeAg and anti-HBe concentrations were determined using a Microparticle Enzyme Immune Assay, MEIA, Abbott, Chigaco, IL) hepatitis B and liver cirrhosis was treated in 1994 with IFN-α 5 MU thrice weekly for 30 weeks, but relapsed (fig.1). Lamivudine 100 mg once a day was initiated and serum HBV DNA decreased to undetectable levels as detected by an in-house qPCR, calibrated using Eurohep HBV DNA standards.[17] Used quantitatively, the Taqman assay enables accurate determination to levels of 1,000 copies/ml and a lower detection limit of 373 copies per ml.[18] This was, accompanied by subsidence of liver inflammation (measured by serum alanine aminotransferase levels (ALT)). After discontinuation HBV-DNA levels rebounded, accompanied by a post-treatment flare. Reintroduction of lamivudine 100 mg once daily resulted again in a fast decline in HBV-DNA levels below the detection limit and reduction in liver inflammation. HBeAg remained positive during treatment. After 230 weeks of continuous lamivudine treatment, a hepatic flare was documented as a result of viral breakthrough. The viral load rebounded to over 10^8 copies/ml. Twenty weeks later entecavir 1 mg was added to

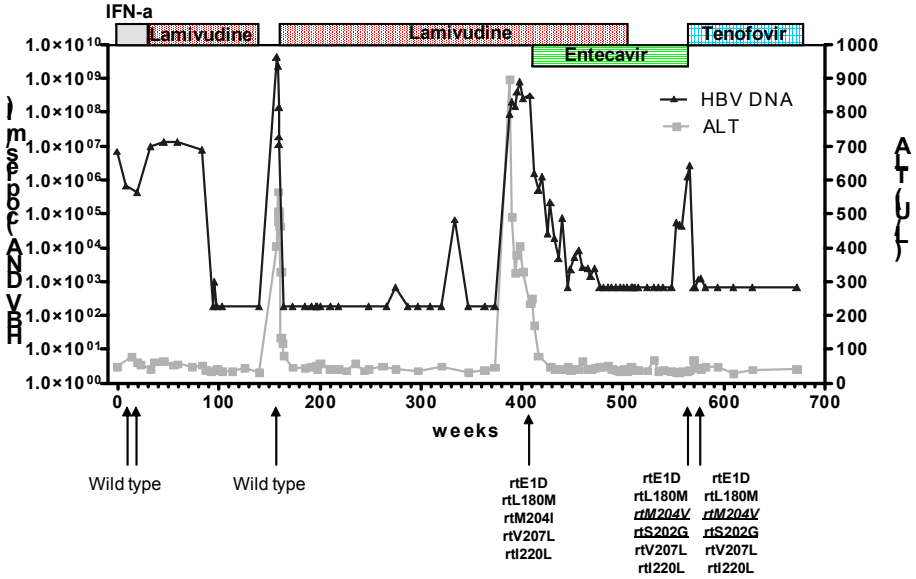

Figure 1. Viral load dynamics and course of ALT during treatment. Samples negative by PCR were calculated as 1.87 x10^2, half of the range from 0 to the lower level of undetectability of 373 copies/ml. Samples <1000 copies/ml but positive by PCR were noted as 6.87 x10^2, which is the value between 373 and 1000 copies/ml. The upper limit of normal for ALT is 40 U/l. The time points, at which sequence analysis was performed, are indicated by arrows and accompanied by the mutations found. Changes in the mutation pattern are indicated in bold, underlined or italic.

the lamivudine regimen, which resulted in a suboptimal decline in viral load of 2.35 $\log_{10}$ copies/ml. Viral load continued to decline to levels below 10^4 copies/ml and ALT levels around the ULN (40 U/l) after 48 weeks of treatment. However, it took over a year (69 weeks) of combination therapy to reduce viral levels below 10^3 copies/ml. The viral load was near the lower detection limit by PCR at the long term and HBeAg loss occurred. After 96 weeks of combination therapy, lamivudine was discontinued with no effect on viral load or extent of liver inflammation. At week 144 of entecavir treatment, after the HBV DNA had been below 10^3 copies/ml for over 70 weeks, a viral load of 5.69 x10^4 copies/ml was documented and HBeAg became positive again. Gradually the viral replication increased to 1.27 x10^6 copies/ml and remained at this level. The ALT levels remained low. Entecavir 1mg once daily was switched, without overlap, to tenofovir disoproxil (Viread) 245 mg once daily. After 25 days of treatment, the viral load was again below 10^3 copies/ml and the patient underwent HBeAg seroconversion after 6 weeks of therapy. A small increase to just above 10^3 copies/ml was observed. After 63 days of treatment, the viral levels were again below 10^3 copies/ml and remained just above the lower limit of detection, but below 10^3 copies/ml, for over 106 weeks. ALT levels were slightly elevated to a maximum of 1.7 times the upper limit of normal and somewhat variable. This might be a consequence of his steatosis hepatis.

SEQUENCE ANALYSIS

For sequence analysis, the total HBV genome was sequenced sequentially with primers as described before.[19] These sequence products gave information on mutations related to lamivudine and entecavir resistance. The analysis was performed using an ABI3100 instrument (Applied Biosystems, Niewerkerk a/d IJssel, The Netherlands) and analysed using BioEdit Software (v7.05), Ibis Therapeutics, Carlsbad, CA, USA).

The genome was sequenced at different time points during antiviral therapy (see fig. 1). The sequence of the viral polymerase at the beginning of lamivudine treatment was wild type. At the time of lamivudine resistance, the lamivudine-resistance associated mutations rtL180M and rtM204I were present. During entecavir treatment, the rtL180M mutation remained

present and the rtM204I mutation changed to the rtM204V mutation. The sequence of the viral polymerase at the time of breakthrough during entecavir therapy revealed the novel rtS202G variant. In addition, three other variants in the reverse transcriptase domain were detected at the time of lamivudine resistance. These rtE1D, rtV207L and rtI220L variants remained present during entecavir therapy as they were also detected in the sequenced samples after breakthrough during entecavir therapy.

DISCUSSION

In this report, we describe the results of sequential monotherapy with lamivudine, entecavir and tenofovir in a chronic HBV infected patient. In this patient, selection of the rtS202G mutation on a background of lamivudine resistance induced entecavir resistance although HBV-DNA initial viral suppression was excellent. This patient differs from previous reports on entecavir resistance by the prolonged period of vigorous viral suppression nearly to the lowest level of detection of the PCR assay before clinical resistance emerged, after an initial slow decline in viral load. In general, persistent replication is a major determinant for the emergence of genomic mutations. [7,20]

Entecavir is a 100-300 fold more potent inhibitor of the wild type viral polymerase compared to lamivudine. Cell culture data demonstrated entacavir still exhibits potent inhibitory capacities of the lamivudine resistant polymerase, although sensitivity is decreased 20-150 fold. [21,22] In the clinical setting, entecavir 1 mg QD proved effective in the treatment of lamivudine resistant patients. [9,11] In the presence of the rtL180M and rtM204V mutations and entecavir-associated mutations, the susceptibility to entecavir decreased dramatically as seen by an increase in IC_{50} values by 280 to over 1500 fold. [23]

Sequencing of the viral polymerase in our patient showed the lamivudine-associated mutations, rtL180M and rtM204V. In addition, the rtS202G mutation was found. This mutation has recently been described in a patient with similar previous treatments including lamivudine therapy leading to both rtL180M and rtM204V mutations. This patient only had a modest decline of $\log_{10}$ 2 copies/ml with a nadir value of 10^5 copies/ml. [24] The error rate of the HBV reverse transcriptase has been estimated to occur at a rate of 10^{-4} base per replication cycle. [25] Because of the high viral load the emergence of the rtS202G mutation conferring resistance to entecavir was a matter of time. [24]

Persistent viral replication is a major factor for the development of resistance. Our patient, however, had excellent viral suppression for over a year, but the decline in viral load was slow and variants could have appeared in the background. Other factors for treatment failure such as poor adherence or an inadequate metabolism (absorption, bioavailability, metabolism of the prodrug to its active metabolites or phosphorylation) does not seem to play an important role in this case. He was adherent as the drug count was satisfying. The decline of viral load was excellent on the long term, for which an adequate metabolism is obligatory.

Lamivudine treatment was continued for 96 weeks after the start of entecavir. The presence of lamivudine in the regimen could put a continuous selection pressure on the lamivudine resistant mutants, preventing reversion to wild-type virus. The rtS202G mutant could already have emerged during lamivudine. It can take quite a while for a drug resistant mutant to become the dominant species and lead to a rise in HBV DNA

as kinetics of emerging drug resistant mutants is usually slow. Free replication space is necessary for the spread of mutant virus and its availability is linked to the time required to observe the mutant (i.e. the increase of viral load).[26] Intrinsic resistance of the mutant and its replicative fitness also influences the time to the clinical emergence. These factors may in part explain the difference in resistance rates between entecavir and lamivudine. Replication fitness of entecavir resistant mutants is diminished to about half compared to the wild type virus. The combination of rtL180M + rt202G + rtM204V harbored by our patient results in a replicative fitness of about 50% of the wild type virus.[23,24] Additional mutations rtV173L and rtP177S are able to restore replicative fitness to similar levels of the wild type. These factors may, in part, explain the difference in resistance rates between entecavir and lamivudine as more genetic changes are necessary for a mutant to become the dominant strain.

The role of the rtE1D, rtV207L and rtI220L mutations, found during lamivudine therapy is not clear. Their impact on sensitivity and replication fitness is unknown, nevertheless they remained present during entecavir therapy, which may imply a certain influence of these mutations. Patients with lamivudine resistance treated with adefovir frequently loose the lamivudine-associated mutations suggesting the mutations rtE1D, rtV207L and rtI220L confers an advantage for the virus.[27] Future in vitro research has to clarify their impact on drug sensitivity and replication fitness.

Almost no data about the clinical management of entecavir resistance are available. In vitro cross-resistance testing gives some insight into the probable outcomes of different treatments. All L-nucleoside analogues lamivudine, emricitabine (FTC), telbuvidine (L-dT), L-dC , L-dA, torcitabine and clemuvidine (L-FMAU) show >100 fold cross resistance to different patterns of lamivudine resistance and are therefore not a treatment option.[14,22,28-32] Lamivudine resistant mutants remain susceptible to acyclic phosphonate nucleotides like adefovir, tenofovir and alamifovir.[14,28,33-35] Adefovir and tenofovir also proved to be clinically effective against lamivudine resistant species.[16,36,37] Although adefovir and tenofovir remain active against lamivudine resistant mutants, their activity is less. For adefovir, increase of 2.8-16 fold in IC_{50} values have been reported, while tenofovir exhibited only a maximum 3.3 fold increase in IC_{50} values.[14,22,33,35] It appears lamivudine mutations have an impact on the treatment efficacy of adefovir in the clinical setting.[6,38] Mutations at the rtL80 site that occur during lamivudine therapy, might negatively influence the effectiveness of adefovir.[39,40] Furthermore, earlier and higher rates of emergence of rtA181V/T and rtN236T mutations associated with adefovir resistance have been reported in lamivudine resistant patients during adefovir therapy.[38,41] The mutation at codon rt181 mutation has also been found in patients refractory to lamivudine. Selection of a mutation at codon rtA181 has been detected during entecavir therapy.[24] As A was substituted to G, its impact on the susceptibility to adefovir is

unknown. However molecular modeling showed that the rtA181V/T mutations alter the position of codon rtM204 resulting in indirect steric hindrance.[42] Therefore, the rtA181G mutation could also effect this site and susceptibility to antivirals, which appears to be the case considering the addition of this mutation to the rtL180M + rt202G + rtM204 mutations reduces IC_{50} values and replication fitness.[24] Due to the many possible interactions of lamivudine and entecavir associated mutations with adefovir, this compound might not be the most logical choice.

Tenofovir disoproxil (tenofovir) has potent antiviral activity against both the wild type and mutant hepatitis B virus. Recently, the mutation rtA194T has been described leading to over 10-fold decreased sensitivity, although clinically this did not lead to resistance.[43] This mutation has no cross-resistance to any other antiviral used for the treatment of hepatitis B.

We switched our patient to tenofovir 245 mg once daily, resulting in a rapid decline of viral load to below 1000 copies/ml and almost to undetectable levels. In addition, HBeAg seroconversion was documented after 1.5 months of treatment. Based on our findings and available resistance data on hepatitis B, tenofovir seems to be a good treatment option for entecavir-resistant patients. Longer treatment duration is necessary to determine the durability of the viral and biochemical response.

In summary, entecavir resistance also arises in lamivudine-refractory patients with prolonged suppression of viral replication. Based on theoretical assumptions and our findings, tenofovir seems to be a good treatment option for entecavir-resistant hepatitis B.

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5

Early treatment modification of adefovir therapy: optimizing the role of on-treatment monitoring in chronic hepatitis B

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ABSTRACT

Introduction: 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. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from all patients.

Follow-up of participants

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.

Data 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 co-infected with HIV, and one patient was co-infected with HDV. Of the 76 patients, fifty-

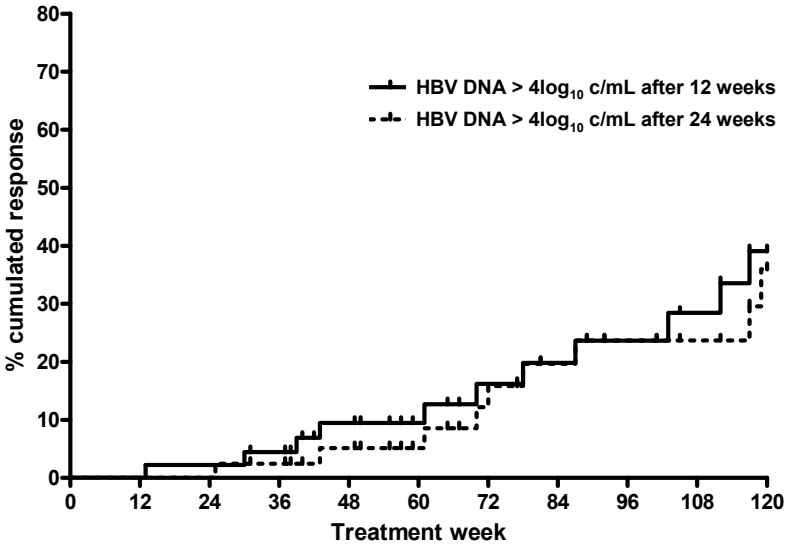
Table 1. Baseline characteristics of the study population (N = 76)

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%)

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 demon-



Number of patients under observation											
HBV DNA > 4log$_{10}$ after 12 weeks	45	45	44	42	35	28	24	21	17	14	10
HBV DNA > 4log$_{10}$ after 24 weeks	41	41	41	39	35	28	23	20	16	14	10

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

strated 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; $p = 0.02$), 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

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	-	-	-

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 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 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

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

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	undetectable	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	decompen-sation/ death	none	none	decompen-sation	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	undetectable	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

LAM breakthrough. At baseline, seven patients were HBeAg positive, median serum HBV DNA level was 8.4 (5.2-9.4) log10 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 log10 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) log10 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. 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 log10 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 rt181 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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6

Discontinuation of prophylactic Hepatitis B immunoglobulin therapy is feasible in patients transplanted for acute fulminant Hepatitis B virus infection

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ABSTRACT

The introduction of hepatitis B immunoglobulin (HBIg) prophylaxis has dramatically reduced the recurrence of hepatitis B viral infection after liver transplantation. However, lifelong HBIg therapy is inconvenient for the patient and very expensive. We studied the safety of discontinuation of HBIg therapy in the subset of liver transplant patients with fulminant acute hepatitis B.

Methods: The study included all patients with fulminant acute hepatitis B who were transplanted between 1991 and 2006 at the Erasmus Medical Center Rotterdam, received HBIg according to protocol, and completed at least one year of follow-up. Immunosuppression consisted of induction with monoclonal antibodies, steroids, and calcineurin inhibitors. Steroids were discontinued after three months; monotherapy with calcineurin inhibitors was continued after 3 months. HBIg was given for at least 12 months before discontinuation.

Results: Nine patients (3 males/6 females) met the inclusion and exclusion criteria. Median follow-up was 86 months (range 23-199). Three patients received lamivudine in addition to HBIg. One and three year HBsAg recurrence rates were 0% and 11%, respectively. The one patient with HBsAg recurrence had a self-limiting course, became HBsAg negative again, and developed high levels of endogenous anti-HBs after reduction of immunosuppression in combination with lamivudine treatment. One patient died 23 months post-transplantation of a non-hepatic cause without any signs of HBV recurrence.

Conclusion: In patients transplanted for acute fulminant HBV infection, withdrawal of HBIg and lamivudine after one year appears to be safe with a low chance of HBV recurrence.

INTRODUCTION

Orthotopic liver transplantation (OLT) is the only effective therapeutic option in patients with decompensated end-stage liver disease secondary to hepatitis B virus (HBV) infection. The management of patients after orthotopic liver transplantation for hepatitis B virus related hepatic failure has evolved tremendously during the past decades. Due to rapid and frequent recurrence of the disease, often with an aggressive course leading to graft failure and death, the overall survival of transplanted patients was initially low.[1,2] Based on this high rate of failure, hepatitis B infection became a contraindication for transplantation in many programs. The poor outcome was primarily due to re-infection: patients with HBV recurrence had a 1 and 3 year survival rate of 73% and 58%, respectively, compared to 98% and 89%, respectively, for those without HBV recurrence.[3] Post-transplant recurrence was drastically reduced by the introduction of passive intravenous (i.v.) immunoprophylaxis using hepatitis B immunoglobulin (HBIg), which is capable of preventing recurrence.[4,5] Discontinuation of HBIg often results in HBsAg relapse and rapid graft failure.[6] Long-term treatment with HBIg, preferably combined with a nucleoside analogue, has therefore become the standard of care for HBV positive transplant recipients.[7]

In patients transplanted for acute liver failure, recurrence rates were considerably lower than that seen in chronically infected patients. With long-term HBIg therapy recurrence rates in these patients are very low.[4,8]

Despite the advantages associated with HBIg treatment, long term HBIg therapy is both expensive and inconvenient for the patient. It has the theoretical disadvantage of being a plasma product and although it appears safe, toxicity over the long-term remains unknown. The high costs and the inconvenience of administration of intravenous HBIg prompted the development of intramuscularly administered HBIg, which reduced side effects and costs.[9,10] Intramuscular therapy is as effective as intravenous administration and has become the most commonly used route of administration.[8] Additionally, studies showed the combination of HBIg with an oral nucleoside or nucleotide analogue to be more effective than either agent alone in the prevention of recurrence.[7,11,12]

Patients with acute fulminant hepatitis receive the same prophylaxis as cirrhotic patients transplanted for decompensated chronic hepatitis B infection. However, patients with acute infection have a different, more vigorous immune response to the virus compared to the immune response in cirrhotic patients. There is almost no data on the efficacy and safety of HBIg discontinuation in patients transplanted for acute fulminant hepatitis B.

In our center, patients who receive hepatic transplant and have chronic hepatitis B virus infection receive life-long HBIg therapy. However, patients receiving orthotopic liver transplantation for an acute fulminant HBV infection discontinue HBIg therapy after

1 year. The HBV recurrence rate, graft survival, and patient survival for this group was investigated to determine the effect of HBIg cessation.

PATIENTS AND METHODS

Patients

Between the start of the program in 1985 and December 31, 2007, 559 liver transplantations were performed at the Erasmus Medical Center Rotterdam. The Erasmus Liver Transplant Research database was used to identify eligible cases. Missing data were completed from medical records. Male and female patients, at least 16 years of age, who were transplanted for liver failure due to fulminant acute hepatitis B infection were eligible. The use of nucleoside analogues in the pre-transplant and post-transplant period was allowed.

Ninety-eight transplants (18%) were performed in 90 patients with HBV infection. All patients with fulminant hepatic failure due to acute hepatitis B were grouped together as acute hepatitis B. The diagnosis of acute hepatitis B required the presence of hepatitis B surface antigen (HBsAg in the serum) at the time of evaluation just prior to transplantation or in the case of negative serum HBsAg, a viral load above 10^4 copies/ml in combination with the presence of IgM-antiHBc antibodies. Exclusion criteria included a follow-up period less than 12 months, no treatment with HBIg prophylaxis, concomitant use of interferon or other immune- or cytokine-based therapies directed against HBV, and organ or bone marrow transplantation other than the liver. Other exclusion criteria were liver disease not caused by hepatitis B, co-infection with hepatitis C, hepatitis Delta or human immunodeficiency virus (HIV), and flare of chronic hepatitis B presenting as "acute on chronic" disease. To exclude the latter diagnosis the explanted liver was examined by a pathologist.

The primary outcome measure was HBsAg recurrence in serum. Secondary end point was post-transplantation survival.

HBIg and lamivudine therapy

Patients transplanted after 1990 received HBIg (Hepatect, Biotest Pharma) 10.000 IU anti-HBs intravenously in the anhepatic phase and daily thereafter. Therapy was stopped if HBsAg was negative and anti-HBs >500 IU/l in HBeAg-positive patients and antiHBs >150 IU/l in HBeAg-negative patients. In patients transplanted after 2000 this was combined with oral lamivudine 100 mg once daily with dose adjustment for kidney function. HBIg and lamivudine were stopped one year after transplantation per protocol.

Biochemical and virological assessments

HBsAg, anti-HBs, HBeAg, and anti-HBe were determined using a Microparticle Enzyme Immune Assay (MEIA, Abbott, Chicago, Ill). Serum HBV-DNA levels were determined by an in-house quantitative polymerase chain reaction, calibrated using Eurohep HBV DNA standards.[13] Used quantitatively, the Taqman assay enables accurate determination to levels of 1,000 copies/ml and has a lower detection limit of 400 copies per ml.[14]

Statistical analysis

Continuous variables were expressed as the median plus range. For all analyses, SPSS (Version 14.0.1; Chicago, IL, USA) was used.

RESULTS

Fourteen patients underwent orthotopic liver transplantation for acute liver failure due to hepatitis B infection. Two of 14 patients (14%) were excluded, as they did not receive HBIg because this was not standard of care at the time of transplantation. Two patients were excluded because they had a follow-up of less than 2 months. One died as the result of graft failure, the other died due to intrapulmonary hemorrhage. One patient was excluded because he did not discontinue HBIg therapy per protocol. A total of 9 patients met the inclusion and exclusion criteria, including three males and six females with a median age of 39 years (range 19-62) (see Table 1). One patient was HBsAg negative at the time of transplant; this patient had an HBV-DNA of 10^4 copies/ml. In addition he had a serological profile and histology of the explanted liver compatible with fulminant HBV infection. Two of the nine patients were HBeAg positive. Median viral load was 2.596×10^4 copies/ml (range 3.75×10^3 to 5.52×10^7). One of 9 patients received lamivudine in the pre-transplant period. After transplantation 3 out of 9 patients received a combination of HBIg and lamivudine.

Two patients (22%) had graft failure and were re-transplanted. One had acute graft failure immediately post-transplantation, and the second patient developed chronic rejection. Neither of these patients showed HBV recurrence in the period before or after re-transplantation. One patient (case 1) died after 23 months as a consequence of metastatic ovarian carcinoma. She did not have recurrence of HBV during follow-up. Overall, the one and three year patient survival rates were 100% and 89%, respectively.

Recurrence rates were 0% and 11% after 1 and 3 years of follow-up, respectively. One patient had recurrence of HBsAg and viral replication 21 months post-transplant. HBeAg was negative and anti-HBe was positive; viral load was 8.51×10^5 copies/ml. The immune suppression at that time consisted of Cyclosporin 175 mg twice daily. Cyclosporin dose was temporarily reduced and lamivudine 100 mg once daily was added. Thereafter the

Case No.	Gender	Age (years)	race	HBsAg	HBeAg	Anti-HBe	HBV-DNA	Nucleoside Pre-trans-plant	Nucleoside post-trans-plant	Retrans-plant (days)	Death	Follow-up/ survival (months)	recur-rence	Time to rec-curence (months)
1	F	55	Cauc	+	+	-	$4.41x10^4$	-	-	-	+	23	-	
2	F	39	Cauc	+	-	+	n.a.	-	-	-		199	-	
3	F	21	Black	+	no data	no data	$5.85x10^5$	-	Lam	165		86	-	
4	M	62	Cauc	-	-	-	$2.26x10^4$	-	Lam	-		41	-	
5	F	23	Cuac	+	-	+	1.27x10e4	-	-	3		189	-	
6	M	24	Asian	+	+	-	$5.52x10^7$	-	-	-		46	-	
7	F	56	Asian	+	-	+	2.59x 10e4	-	-	-		133	+	21
8	F	19	cauc	+	-	+	$3.75x10^3$	Lam	Lam	-		31	-	
9	M	51	Black	+	-	+	n.a.	-	-	-		173	-	
Total	Male/ Female	Age (years)		HsAg(+)/ HBsAg(-)	HsAg(+)/ HBsAg(-)	Anti-HB(+) /anti-HBe(-)	HBV-DNA	Nucleo-side(+)/ nucleoside (-)	Nucleo-side(+)/ nucleoside (-)	retrans-plants	deaths	Follow-up /survival (months)	recur-rence	
	3/6	39 (18-62)		8/1	2/8	5/3	$2.59x10^4$ ($3.75x10^3$-$5.52x10^7$)	1/8	3/6	2	1	86 (23 – 199)	1	

Table 1. Baseline characteristics and outcomes in the patients transplanted for acute fulminant hepatitis B. Cauc=Caucasian, Lam= lamivudine.

viral load declined rapidly below 10^3 copies/ml within 2 months and below the detection limit within 5 months; HBsAg disappeared after 5 months. No HBIg was administered during this period and after 6 months lamivudine was discontinued. HBsAg and HBV DNA remained negative thereafter. Nine months after the recurrence low levels of endogenous anti-HBs were detected which increased to over 1000 IU/l in the months thereafter.

DISCUSSION

Prophylaxis to prevent recurrent HBV infection following liver transplantation has dramatically improved the outcome for acute and particularly chronic hepatitis B patients. With the current prophylaxis regimes, combining HBIg with a nucleoside or nucleotide analogue, survival rates have increased dramatically. Survival rates are nowadays the same or even better in patients transplanted for HBV than in patients transplanted with a non-HBV diagnosis.[5] The dramatic increase in survival is mainly due to a diminished risk for HBV recurrence, which often ran an aggressive course leading to graft failure in a relative short period.[1-4] Our results confirm the excellent survival in patients transplanted for acute HBV, as the three-year survival rate was 89%. One patient died from a non-hepatic cause.

Despite the relatively good results of transplantation for HBV disease, questions remain regarding the optimal dose and duration of HBIg and lamivudine. Most transplant centers continue HBIg therapy indefinitely. Others report the same clinical outcomes with much lower doses of HBIg or administration for shorter periods of time.[15,16]

Our study differs from other studies as we specifically studied the discontinuation of immunoprophylaxis in a small group of well-characterized patients transplanted for acute fulminant hepatitis B. All patients had at least 1.5 years of follow-up and only one patient had a self-limiting recurrence of HBV.

Patients with acute fulminant hepatitis B differ from chronically infected patients in several ways. One of the most important differences is the immune response to the virus. Patients with chronic hepatitis B mount an inadequate immune response, resulting in a situation in which the immune system tries to eliminate the virus but does not succeed in eradicating the infection, leading to ongoing liver inflammation. Patients with acute fulminant HBV infection mount a much more vigorous immune response, resulting in hepatic inflammation that is not adequately controlled and that ultimately leads to liver failure. As patients with acute hepatitis have an adequate immune response to HBV, the immune response may prevent recurrence in the majority of cases. Even in the event of post-transplant recurrence an adequate anti-HBV response is mounted, which was the

case in our patient with HBV recurrence. In this patient, treatment with lamivudine was started and the patient cleared the HBV infection and developed high levels of anti-HBs.

Early discontinuation of HBIg can result in recurrence, as shown in chronic HBV patients receiving a course of less than 6 months HBIg.[4] The minimum duration of HBIg dosing, however, is not known. The majority of recurrences are seen in the first 2 years post- transplantation.[4,8]

In conclusion, our study suggests that in selected patients transplanted for acute fulminant HBV, HBIg prophylaxis can safely be discontinued after one year.

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Discussion

Future perspectives for the management of chronic hepatitis B

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ABSTRACT

Chronic hepatitis B virus infection affects about 400 million people around the globe and causes approximately a million deaths a year. Since the discovery of interferon-α as therapeutic option the treatment of hepatitis B has evolved fast and the management has become increasingly complicated. The amount of viral replication reflected in the viral load (HBV-DNA) plays an important role in the development of cirrhosis and hepatocellular carcinoma. The current treatment modalities for chronic hepatitis B are immunomodulatory (interferons) and antiviral suppressants (nucleoside and nucleotide analogues), which come with their own advantages and limitations. An overview of the treatment efficacy for both immunomodulatory as antiviral compounds is provided in order to provide the clinician insight in the factors influencing treatment outcome. With nucleoside or nucleotide analogues suppression of viral replication by 5-7 $\log_{10}$ is feasible, but not all patients respond to therapy. Known factors influencing treatment outcome are viral load, ALT levels and compliance. Many other factors which might influence treatment are scarcely investigated. Identifying the factors associated with response might result in stopping rules, so treatment can be adapted in an early stage to provide adequate treatment and avoid the development of resistance. The efficacy of compounds for the treatment of mutant virus and the cross-resistance is largely unknown. However genotypic and phenotypic testing as well as small clinical trials provided some data on efficacy in this population. Discontinuation of nucleoside or nucleotide analogues frequently results in viral relapse, however some patients have a sustained response. Data on the risk factors for relapse are necessary in order to determine when treatment can be discontinued safely.

In conclusion: chronic hepatitis B has become a treatable disease, however much research is needed to tailor therapy to each individual patient, to predict the sustainability of response and determine the best treatment for those failing treatment.

INTRODUCTION

Treatment of chronic hepatitis B remains an important clinical objective. Estimates are that 2 billion people have been infected worldwide and chronic hepatitis B currently affects about 400 million people, particularly in developing countries. [1] Chronic hepatitis B is responsible 500,000 to 1.2 million deaths annually from liver cirrhosis and hepatocellular carcinoma (HCC). [2] It is one of the most common infectious diseases and among the world's leading causes of death. There are two strategies to decrease these numbers, prevention of new infections and treatment of those already chronically infected. Treatment options consist of immunomodulatory and viral suppressant drugs. In this review the current standard of care and the future developments in the field of chronic hepatitis B are discussed.

WHAT IS THE OPTIMAL TREATMENT

Naïve patients

The ideal treatment for hepatitis B is an effective cheap treatment, resulting in HBsAg loss and formation of anti-HBs, with finite treatment duration and little side effects. Currently none of the HBV treatments fulfil these conditions. With interferon based therapy HBsAg loss occurs in 3%-10% of the patients within one year of the start of therapy and increases in sustained responders to 11%-32%. [3-9] HBsAg loss is rare (<2% after one year of treatment) in patients treated with nucleos(t)ide analogues, which is about the rate observed in the natural history of the disease. [10-16] However in a small cohort treated with tenofovir, HBsAg loss was observed in 14% of 35 patients. [17] More large size trials have to be conducted to investigate the rate of HBsAg loss for the newer nucleos(t)ide analogues or their combinations. Treatment with Interferon-α or PEG-interferon-α treatment is of finite duration and response is often durable off-treatment. However this treatment has side effects and only a minority responds to it. Nucleosides/nucleotides are well tolerated and most patients respond to therapy but treatment is hampered by the selection of drug resistant mutants leading to loss of efficacy and frequent relapse after discontinuation. As none of the current registered therapies for chronic HBV is ideal, none of the drugs is regarded as the standard first-line therapy for HBV. Strategies have particularly aimed at selecting host and virus characteristics either before or during therapy to increase treatment efficacy and also withdraw ineffective treatments. The argument about the poor tolerability of interferon has been weakened by the introduction of PEG-interferon-α, which only has to be administered once a week. Furthermore it is believed PEG-interferon-α is more potent than the conventional interferons, however good comparative studies have not been performed. [18] Based on treatment outcomes,

Treatment	Increased likelihood of response	Decreased likelihood of response
(PEG)interferon	Baseline ALT >2 ULN [28,148,149]	Baseline ALAT <2 ULN [28,148,149]
	Baseline HBV DNA $<10^9$ c/ml [4,28]	Baseline HBV DNA $>10^9$ c/ml [4,28]
	Genotype A or B	Genotype C or D
	Longer treatment duration [34-36,150]	
Nucleoside/ nucleotide analogues	Baseline serum aminotransferases >2 ULN [151,152]	Baseline serum aminotransferases <2 [151,152]
	Baseline HBV DNA $<10^9$ c/ml [151]	Baseline HBV DNA $>10^9$ c/ml [151]

Table 1. Baseline factors influencing likelihood of response to antiviral therapy. ULN = upper limit of normal; c/p = copies/ml.

the preferable treatment shifts to interferon based therapy or nucleoside/nucleotide analogue therapy for different patient groups. In Table 1 the known predictors for response are given for both interferon-α based therapies or nucleos(t)ide analogues.

Recent studies found a strong correlation between HBV DNA level and the development of liver cirrhosis and HCC. However, as none of the studies concerning natural history separately analysed the outcome in patients with prolonged normal aminotransferases the exact influence of the viral load is not known.[19-21] Interferon based therapies are generally ineffective in patients with low pre-treatment serum aminotransferases levels, and for these patients nucleoside/nucleotide treatment would be indicated. Low pre-treatment serum aminotransferases and high HBV DNA levels also decrease the likelihood of response for these agents, however this confers to HBeAg loss/seroconversion. In theory nucleoside/nucleotide analogues are effective in lowering the viral load and thereby decreasing the risk for the development of cirrhosis and HCC.[22]

However the benefit of this approach on survival is not supported by clinical trials.

For therapy of treatment naïve patients with elevated ALT levels, consensus guidelines have no preference for interferon or nucleoside/nucleotide therapy. Treatment outcomes for different therapies are provided in Table 2. Genotype proved to be an important predictor for the response to interferon-α or PEG-interferon-α therapy, especially in HBeAg positive patients. Genotype A and B show superior end off-treatment responses as well as off-treatment responses compared to genotypes C and D.[3,4,18,23-25] HBeAg loss occurred in 34-36% of patients. In addition HBsAg seroconversion was observed in 13-22% of patients with genotype A.[18,26,27] Therefore a 48 week course of PEG-interferon-α should be considered as first-line therapy for HBeAg positive patients with genotype A or B.

For HBeAg negative patients the distinctions are less clear. Genotype D responds less to PEG-interferon-α compared to genotypes A, B or C. Sustained ALT normalisation and

a viral load <20,000 copies/ml was observed in 27%, 44%, 52% and 16%, for genotype A, B, C and D respectively. Sustained response occurred significantly more frequent in genotype B and C compared to genotype D. The difference in sustained response between genotype A and D was not significant, probably due to the small number of genotype A infected patients.[28] However only patients with genotype A, treated with PEG-interferon-α for 48 weeks, had a considerable chance (18%) to develop HBsAg seroconversion.[27] The long-term follow-up is not known for PEG-interferon-α. Two years of follow-up showed a decrease in response (HBV DNA $<2.0x10^4$ copies/ml) from 43% after 24 weeks of follow-up to 29%.[29] However long-term follow-up studies with conventional interferon showed high relapse rates.[2,5,30] Nucleos(t)ide analogues are effective across all genotypes in both HBeAg positive and HBeAg negative patients and have proven to be a good treatment option for chronic active hepatitis B.[31,32] However recent data suggest a role of genotype in treatment outcome, not related to resistance. Patients with genotype B or D more frequently relapsed when switched from tenofovir to adefovir. The numbers are small, but warrant further investigation.[33]

Retreatment of non-responders

Treatment of non-responders to previous treatment is little studied and most studies are of small size. Studies show that retreatment with conventional interferon can induce HBV DNA loss and HBeAg seroconversion, however the overall results are not conclusive (Table 3) Most of the results are difficult to interpret as the initial schedules of interferon therapy differs as well as the time to retreatment. The retreatment schedules often differ from the initial schedule, and treatment duration is often prolonged influencing treatment outcome positively.[34-36] The real benefit of interferon retreatment, especially with pegylated interferon is unclear. Nucleos(t)ide analogues appear to be effective in interferon failures, although efficacy may be different and data are limited.[37] A clinical trial using adefovir dipivoxil included 123 HBeAg positive and 48 HBeAg negative patients failing prior interferon therapy. HBeAg seroconversion rates were similar for interferon naïve and experienced patients.[38] The efficacy of the use of interferon for lamivudine failures is unclear. (Table 3 and 4) It appears that interferon is effective in patients who received lamivudine, but did not develop resistance. Interferon-α therapy probably has a low efficacy in lamivudine resistant patients, though the numbers published are small.[39,40]

Cirrhotic patients

The life expectancy of patients with cirrhosis, if untreated, is greatly diminished with a 5-year survival of 84% for compensated and 14-35% for decompensated cirrhosis.[41-43] Interferon has to be used with caution in cirrhotic patients and its use is limited to Child A cirrhosis. Interferon may be effective in compensated cirrhotics in which treatment

	Author; Journal; Year	HBeAg loss (%)	HBeAg seroconversion (%)	HBsAg loss (%)	Decline viral load (log10 copies/ml)	HBV DNA negativity (%)	ALT normalisation (%)	Histological improvement (%)	Resistance (%)
HBeAg pos									
PEG-IFN-α 2a	Cooksley; J. Viral Hepatitis; 2003 18 $	35	33			39 9	35		
PEG-IFN-α 2a	Lau: NEJM; 2005 4	34	32	3@	2.4	14 4	41	49	
PEG-IFN-α 2b	Janssen; Lancet; 2005 3	36	29	7	2.3	7 4	32	53*	
lamivudine	Chang; NEJM; 2006 13	20	18	1	5.4	36 2	60	62	13
lamivudine	Alexander; BMC Gastroenter; 2005 153	42	28				56		10
lamivudine	Chan; Ann Intern Med; 2005 154	28	28	0	2.74	10 1	78	59 **	40
lamivudine	Yao; Hepatobil Pancr Dis Int; 2004 155	10	8			36	72		12
lamivudine	Jonas; NEJM; 2002 156	26		2		61 6	55		19
lamivudine	Mazur; Med Sci Monit; 2002 157	49	44	5		37 8	56		
lamivudine	Barbaro; J Hepatol: 2001 158		19	0			23	27*	16
lamivudine	Dienstag; NEJM 1999 48	32	17	2		44 7	41	52*	32
lamivudine	Gane; J Hepatol; 2006 64	23	21		5.5	40 2	75	56	8
adefovir	Marcelin; NEJM; 2003 16	24	12		3.6	21 4	48	53	0
adefovir	Lee; Hepatology; 2006 104	14			4.0	29 5	79		0
adefovir †	Zheng; Hepatology; 2006 127	13	8	0	4.5	28 2	79		0
adefovir	Bzowej; Hepatology; 2006 66	20	18		5.7	39 2	81		2
entecavir	Chang; NEJM; 2006 13	22	21	2	6.9	67 2	68	72	0
telbivudine	Gane; J Hepatol; 2006 64	26	22		6.5	60 2	77	65	3
telbivudine	Bzowej; Hepatology; 2006 66	31	27		6.6	58 2	77		4
HBeAg neg									
PEG-IFN-α 2a	Marcellin; NEJM; 2004 5			4	2.3	19 4	59	59	
lamivudine	Marcellin; NEJM; 2004 5			0	4.2	73 4	73	58^	41
lamivudine	Lai; NEJM; 2006 15			0	4.5	72 2	71	61	6
lamivudine	Lai; NEJM; 1999		16	0			72	56*	14
adefovir	Hadzyannis; NEJM; 2003 14				3.9	51 4	72	64	0
entecavir	Lai; NEJM; 2006 15			0	5.0	90 2	78	70	0
Mixed									
lamivudine	Ooga; J Gastroenterology; 2004 159					78 5	78		16
lamivudine	Suzuki; Intervirology; 2003 111	42	28	0		88 6	86		
lamivudine	Yao; J Hepatology; 2006 65		18		4.3	43 2	78		
entecavir	Yao; J Hepatology; 2006 65		15		5.9	76 2	90		

Table 2. Treatment outcomes after 1 year of treatment for different antiviral drugs for the management of chronic hepatitis B. Although conventional interferon-α is widely used for the treatment of HBV it is not listed as this treatment will be replaced by PEG-interferon-α in the near future. Efficacy measures for PEG-interferon presented are off-treatment responses after 24 weeks of follow-up. Studies are organised by HBeAg status and the mixed studies included both HBeAg positive as HBeAg negative patients.
* only improvement of ≥2 points in necroinflammation according to Ishak
** only improvement of ≥2 points in necroinflammation according to Knodell
@ HBsAg seroconversion
$ treatment duration 24 weeks
^ After 24 weeks of follow-up
Lamivudine study performed in children
† Including some Lamivudine resistant patients

[1] LLD 200 copies/ml
[2] LLD 300 copies/ml
[3] LLD 366 copies/ml
[4] 400 copies/ml
[5] $5.0x10^3$ copies/ml
[6] $7.0x10^5$ copies/ml
[7] $1.0x10^6$ copies/ml
[8] $1.4x10^6$ copies/ml
[9] $5.0x10^6$ copies/ml

outcomes do not differ from those without cirrhosis.[6,34,36,44,45] Adverse events, dose reductions and early discontinuation occur more frequently in cirrhotic patients.[46,47] Nucleos(t)ide analogues appear to be as effective in cirrhotics as in those without cirrhosis regardless of HBeAg status.[48-50] Lamivudine treatment in patients with advanced fibrosis or compensated cirrhosis reduced the progression of liver disease. Loss of efficacy due to resistance resulted in an increase of disease progression.[51] Entecavir treatment resulted in undetectable HBV DNA loss (LLD 300 copies/ml) in >90%, ALT normalisation in over 60% and histological improvement in >70% of patients with compensated cirrhosis.[52]

Decompensated cirrhotic patients should be treated with nucleoside analogues as interferon-α is contra-indicated.[53,54] The timing of the initiation of therapy is essential. If the bilirubin level rises above 3.5 mg/dl the 3 month survival is poor and is not likely to be influenced by nucleos(t)ide analogue therapy. Several studies confirmed the efficacy of lamivudine therapy in patients with HBV related decompensated cirrhosis. Therapy resulted in a significant improvement in virological, biochemical and markers of disease status.[55-59]

Treatment of lamivudine refractory patients, wait-listed for liver transplantation, with adefovir for 48 weeks in resulted in a 4.1 $\log_{10}$ copies decline in viral load. Liver functions improved significantly and the Child Pugh-Turcotte score (CPT) improved or remained stable in 92% of the patients.[60] Adefovir therapy initiated in pre-transplant patients resulted in undetectable serum HBV DNA in 76% and normal ALT in 84% of the patients after 96 weeks of treatment. Markers of synthetic liver function improved in most patients, the Child-Pugh scores improved, or remained the same and survival was over 80% after two years.[61] Another study in lamivudine refractory patients with decompensated cirrhosis showed a HBV DNA response ($\leq 10^5$ copies/ml or ≥2 log decrease from baseline) in 92%, over half of the patients had ALT normalisation and there was improvement of

Author; Journal: Year	Treatment regimen	Pretreatment HBeAg status	HBeAg loss	HBeAg seroconversion (%)	HBV DNA loss (%)	ALT normalisation (%)
Janssen; J Hepatology; 1993 160	IFN 1.5 MU daily for 4 weeks followed by 3 MU daily for 8 weeks and then 5 MU daily for 4 weeks	HBeAg pos (n=18)		11	17	
Carreno; Hepatology; 1999 161	IFN 9 MU thrice weekly for 24 weeks	HBeAg pos (n=27)	41	22	44 5	22
Munoz; J Hepatology; 2002 162	IFN 6 MU 5 times weekly for 24 weeks	HBeAg pos (n=11)		18	18 1	18
Munoz; J Hepatology; 2002 162	IFN 6 MU 5 times weekly for 24 weeks	HBeAg neg (n=18)			22 1	44
Ballauff; Eur J Pediatr; 163	IFN 5-9MU/m2 thrice weekly for 16-24 weeks	HBeAg pos (15)		33	33 4	
Teuber; Z Gastroenterol; 1995 164		HBeAg pos (27)		30	59	
Flink; Hepatology; 2004 165	PEG-IFN-α 2b 100 µg/week for 32 weeks followed by 50 µg/week for 20 weeks	HBeAg pos (18)	28		0 2	22
Lau; J Hepatology; 2005 166	PEG-IFN-α 2a for 48 weeks	HBeAg pos (30)		43		
Prev lamivudine						
Flink; Hepatology; 2004 165	PEG-IFN-α 2b 100 µg/week for 32 weeks followed by 50 µg/week for 20 weeks	HBeAg pos (8)	50		0 2	47
Lau; J Hepatology; 2005 166	PEG-IFN-α 2a 180 µg/week for 48 weeks	HBeAg pos (31)		32		
Marcellin; J Hepatology; 2006 167	PEG-IFN-α 2a 90-180 µg/week for median 48 weeks	HBeAg pos (71)	37$	32$	47 3 $	51
Marcellin; J Hepatology; 2006 167	PEG-IFN-α 2a 90-180 µg/week for median 48 weeks	HBeAg neg (36)			67 3 $	52

Table 3. Response to interferon-α or PEG-interferon-α after having failed a prior course of interferon or response to interferon-α or PEG-interferon-α after having failed a prior course of lamivudine.
$ on-treatment responses

[1] LLD 200 copies/ml
[2] LLD 400 copies/ml
[3] LLD 1.0x10^4 copies/ml
[4] LLD 4.25x10^5 copies/ml
[5] LLD 4.81x10^5 copies/ml

liver synthesis function and Child-Pugh scores after one year of treatment.[62] Although it is possible to inhibit viral replication and prevent clinical decompensation, the occurrence of HCC is not prevented. After 5 years of continuous lamivudine therapy or add-on therapy with adefovir in lamivudine resistant cases in HBeAg negative cirrhotic patients, 16% of patients died, or underwent liver transplantation. However 24% was diagnosed with HCC.[63] The moment of initiation of nucleos(t)ide analogues to prevent the occurrence of HCC has yet to be determined. In cirrhotic patients there seems no benefit, but a study including patients with advanced liver fibrosis or cirrhosis showed a reduction in HCC in lamivudine treated patients compared to placebo. Patients with lower fibrosis and Child-Pugh scores were less prone to disease progression.[51] The data suggest nucleos(t)ide analogue therapy has to be initiated before the cirrhosis develops to prevent HCC and has to be continued indefinitely.[51,63]

TREATMENT WITH NUCLEOSIDE/NUCLEOTIDE ANALOGUES

Four oral nucleoside or nucleotide analogues, lamivudine, adefovir, entecavir and telbivudine, are currently marketed and approved as first line therapy for the treatment of chronic hepatitis B. All therapies result in reduction of viral load, ALT levels and improvement of liver histology. (Table 2) It is difficult to point out one compound which should be the first nucleoside or nucleotide used for the treatment of chronic hepatitis B. At least two major points at least have to be taken into account: (i) efficacy of the treatment on the short and long term, including the development of resistance and (ii) the costs. Comparing the efficacy of treatment is difficult, however some comparative studies have been performed. Both entecavir and telbivudine, proved superior efficacy over lamivudine after 1 year of treatment.[13,15,64,65] Direct comparison of telbivudine or adefovir for 52 weeks showed superior efficacy on viral and biochemical parameters for telbivudine, but resistance was not assessed.[66] Adefovir was never directly compared to lamivudine. Direct comparison of entecavir and adefovir for a duration of 24 weeks showed a decline of HBV DNA of 6.97 $\log_{10}$ copies/ml for entecavir and 4.84 $\log_{10}$ copies/ml for adefovir. PCR undetectability (HBV <300 copies/ml) was reached in 45% of entecavir treated patients vs. 13% of those receiving adefovir.[67] Tenofovir seems to be a promising new drug, but it has only been used in small series in lamivudine or adefovir treatment failures. The long term outcomes are only known for lamivudine and adefovir. Another problem with interpretation and positioning of the outcomes of clinical studies is the lack of standardisation of outcome measures.

The development of resistance is the most important factor for loss of efficacy. Lamivudine has a high rate of resistance of 18-27% after 1 year and this increases over time, being 44% at year 2, 60% at year 3, and after 4 years of treatment almost 70% has devel-

oped resistance.[5,68-74] Adefovir showed no resistance after 1 year, but rates increased to 1-3%, 11%, 18% and 28% at year 2, 3, 4 and 5.[14,16,75] Entecavir showed no resistance up to 2 years of treatment, however complete non-responders did not receive treatment in year 2.[76] Telbivudine had a resistance rate of 2-4% after 1 year of treatment.[64,66]

With long-term lamivudine treatment HBeAg seroconversion increases to 27%, 40%, 47% and 50% at years 2, 3, 4 and 5 respectively, despite the development of resistance.[68,72,74,77] Prolonged therapy with adefovir in HBeAg positive subjects resulted in viral load below 10^3 copies/ml in 28% at year one, 45% and 56% at years 2 and 3. ALT levels became normal in 48%, 71% and 81% after 1, 2 and 3 years of treatment. Rates of HBeAg-loss increased to 42% and 52% and HBeAg seroconversion rates increased to 29% and 43% at year 2 and 3.[78] A study with continued treatment up to 2 years showed an increase in viral reduction from -4.5 to -5.0 $\log_{10}$ copies/ml, increased PCR-negativity (lower limit of detection 300 copies/ml) from 28% to 42%, but the percentage of ALT normalisation remained unchanged (79% to 78%). The percentage HBeAg-loss increased from 13% to 19% and the percentage of patients with HBeAg seroconversion increased to 15%.[79] In HBeAg negative subjects prolonged adefovir therapy of 2 years, showed little additional decline in viral load, but consolidated the response to adefovir, as 71-75% of the patients had a viral load below 10^3 copies/ml and ALT normalisation in 73-79%. Long term treatment up to 5 years resulted in a viral load below 10^3 copies/ml in 78-79% at year 3, 65-68% at year 4 and 67% after 5 years of continuous treatment. ALT levels were normal in 69-78% at 3 years, 70-75% at 4 years and 69% after 5 years.[75] Treatment with adefovir in an open population resulted in less favourable results, as during a mean follow-up of 115 weeks only 55% had a viral load below 10^3 copies/ml. Twenty one percent of HBeAg positive patients lost HBeAg.[80] Entecavir also showed a continuous viral decline in patients with detectable HBV DNA beyond week 48 and HBeAg seroconversion rates increased.[81,82] Another aspect which is little studied is the sustainability of response after discontinuation of therapy. In HBeAg positive subjects who seroconverted during therapy, response is durable in over half of the subjects.[13,83-86] In HBeAg positive patients treated with lamivudine who discontinued after achieving a complete response (HBeAg loss, undetectable HBV DNA and normal ALT) had a sustained response of 78%, 72%, 70%, 67% and 64% after 1, 2, 3, 4 and 5 years of follow-up respectively.[87] In HBeAg positive subjects with HBeAg seroconversion during adefovir therapy the response was sustained in 91%.[86] In entecavir treated HBeAg positive subjects for 48 weeks the sustained response (HBeAg loss and HBV DNA $<7.0 \times 10^5$ copies/ml) was 82% after 24 weeks follow-up.[88] The durability can be increased by continuing treatment for several months after HBeAg seroconversion. Therefore it is recommended to continue treatment for at least 3-4 months.[84,85] As many clinical trials had a predetermined endpoint, sustainability migt be a bit higher if treatment was continued for a longer period in those

patients who underwent HBeAg seroconversion within 3 months before dicontinuation. In HBeAg negative subjects the durability of response is often poor. Patients treated for two years with lamivudine who had undetectable HBV DNA levels (LLD 200 copies/ml) discontinued treatment. After 12 months of follow-up the virological relapse rate was 50%.[89] The viral load at discontinuation and duration of treatment do not accurately predict sustainability of response in HBeAg negative patients.

Other parameters such as intrahepatic total HBV DNA and intrahepatic cccDNA and HBcore expression and the level of hepatitis B virus core related antigen appear to be superior in prediction of sustained response compared to viral load at the end of therapy. The studies where however small and the results have not been confirmed by others.[90,91]

Management of treatment failures to nucleos(t)ide analogues

A distinction can be made for patients failing therapy: due to resistance or other reasons. Many patients do not achieve complete suppression of HBV DNA during treatment. Several factors may contribute such as non-compliance, inefficient conversion from the prodrug to its active metabolite, inadequate phosphorylation within the hepatocytes or underdosing of the drug. Some patients failing to respond initially to treatment may already harbour a resistant mutant prior to the start of therapy.[92,93] Underdosing is particularly an issue with adefovir treatment as the 10 mg dose was chosen for safety reasons. The 30 mg dose was more effective, but also more nephrotoxic.[16]

Little is known why some patients have suboptimal viral suppression. The known baseline predictors for response provide information on the likelihood of response, but the outcome cannot be predicted. (Table 1) A high baseline viral load is probably one of the reasons why more HBeAg-positive patients have a suboptimal response compared to HBeAg-negative patients.[13-16] Recently genotypic dependent polymorfisms have been described associated with primary treatment failure and more might be detected.[93,94] As viral factors, as well as host factors play an import role in response, it is difficult to assess the optimal treatment for sub-optimal responders. Presuming study randomisation led to an equal distribution of both viral and host factors, it is to be expected that more potent drugs are able to suppress viral replication in subjects with suboptimal suppression. Entecavir and telbivudine proved their superior potency over lamivudine in a head to head comparison and for telbivudine this observation was also been made in comparison with adefovir.[13,15,64-66] In adefovir treatment failures the more potent drug tenofovir showed good viral suppression.[95] Patients responding to tenofovir and switched to adefovir showed viral relapse, while no mutants could be detected.[33,96] Another strategy could be adding a second drug to the failing compound. In vitro testing demonstrated that combining adefovir with an L-nucleoside (lamivudine, telbivudine,

emtricitabine) exerted additive antiviral effects.[97] Clinically the combination of adefovir and emtricitabine resulted in stronger viral suppression.[98] In patients failing adefovir switching therapy to tenofovir and either emtricitabine or lamivudine resulted in decrease in viral load in most patients.[99]

Prevention of resistance

For nucleoside/nucleotide analogue treatment, a number of risk factors for resistance have been identified. For lamivudine this includes: prior course of lamivudine, duration of lamivudine therapy, high body weight and body mass index, male sex and high baseline HBV DNA, insufficient HBV DNA suppression at month three, and elevated ALT levels during treatment.[100-102] For adefovir the following risk factors for resistance have been reported: lamivudine resistance at start of treatment, high baseline viral load, <1 month continuation of lamivudine after the start of adefovir therapy in case of lamivudine resistance, insufficient HBV DNA suppression during treatment.[103-105] For entecavir, lamivudine resistance and suboptimal suppression of HBV DNA on treatment were found as risk factors.[76] A key factor in the development of resistance is the persistence of viral replication. Several studies found a relation between ongoing viral replication and the development of resistance. Patients with a serum HBV DNA $>10^3$ copies/ml after 6 months of lamivudine treatment had a 63% chance for developing resistance.[102] Another study in 24 patients, found that none of the subjects with excellent viral suppression (nadir HBV DNA <50 copies/ml), two out of 5 patients with a nadir viral load between 50-300 copies/ml and all 11 patients with a nadir viral load >300 copies/ml developed resistance.[101] For adefovir, a load of over 10^5 copies/ml after 48 weeks of treatment is a risk factor for resistance.[105] Patients treated with adefovir not having a viral load below 10^4 copies/ml at week 12 or 24 had a low probability of reaching virological response and had an increased risk for the development of resistance. These patients should be switched to alternative treatment.[80] In a study in which patients were treated with either telbivudine, or lamivudine, a viral load $>10^3$ copies/ml after 24 weeks of treatment was associated with an increase risk for resistance. Although entecavir has a high genetic barrier slowing down the development of resistance, ongoing viral replication is a major risk factor for its occurrence. Even in patients with good viral suppression mutants can appear in the background resulting in viral relapse.[106]

The role of genotypes is controversial as some have reported influence of the genotype on the development of resistance, while others do not find this association.[103, 107-114] For adefovir genotype D appears to be associated with an increased risk for resistance.[103] Genotype might influence the mutational pattern. When genotype A and D in lamivudine resistant patients were compared, the rate of M204V mutants and rates of mutations at position rtL180 was higher in genotype A. The rate of M204I mutations was higher in

genotype D. The median time of shift from M204I to M204V was shorter in genotype A. Additional resistance associated mutations were only detected in patients infected with genotype D.[115] In genotype C patients HBV DNA was significantly higher compared to genotype B after the development of YMDD mutants.[107] Studies are often hampered by their small size. For compounds with a low rate of resistance it is hard to determine the role of the genotype as large numbers have to be treated often for a prolonged period.

The current strategy of continuous monotherapy is insufficient to completely suppress viral replication in a large number of patients . In vitro testing has to be done in order to find promising combinations of drugs. These combinations of drugs then have be to investigated in long-term large scale trials with clinical response and resistance as outcome measures.[97]

It is important to detect resistance as early as possible during treatment with nucleoside or nucleotide analogues. In case of virological breakthrough, which is generally agreed to be a 1 $\log_{10}$ increase in viral load in either in copies/ml or IU/l after an initial response in compliant patients.[116-118] Sensitive quantitative HBV DNA assays are therefore advised for monitoring, as a viral rebound can be detected earlier. Virological breakthrough mostly precedes biochemical breakthrough and the time lapse may vary from weeks to months.[119] Genotypic testing provides information on the type of mutation which arises during treatment and if there might be decreased drug sensitivity. Knowledge of the specific mutation will be increasingly important in the future as different mutations may have a distinct influence on treatment efficacy of other compounds. Newly detected mutations should be investigated by phenotypic assays to determine their replication fitness and susceptibility to other compounds.[120]

MANAGEMENT OF RESISTANCE

Lamivudine resistance

Adefovir has proven to be effective against lamivudine resistant mutants. Adefovir monotherapy is able to suppress viral load by 2.4-4.0 $\log_{10}$ copies/ml.[104,121] The data of adefovir and lamivudine combination therapy by adding adefovir to ongoing lamivudine is controversial. A randomised study found no difference in viral decline.[121] Another study did not find a difference in viral suppression after one year of treatment, but at month 18 adefovir and lamivudine showed a stronger viral decline (4.3 $\log_{10}$ copies/ml) vs. adefovir monotherapy (3.4 $\log_{10}$ copies/ml).[122] A study comparing combination therapy to monotherapy in lamivudine resistant patients showed significantly higher

	n	Author; Journal; Year	HBeAg loss (%)	HBeAg sero-conversion (%)	HBsAg loss (%)	Decline viral load (log10 copies/ml)	HBV DNA negativity (%)	ALT normalisation (%)	Histological improvement (%)	Resistance (%)
HBeAg pos										
PEG-IFN-α 2b	16	Leemans; J Hepatology; 2006 39	13	13	6	0.6	6 4	19		
adefovir	45	Buti; Hepatology; 2004 168		13	0		33 7	51		
adefovir	19	Peters; Gastroenterology; 2004 121	16	11		4.0	26 5	47		0
HBeAg neg										
PEG-IFN-α 2b	20	Vassiliadis; WJG; 2006 40					5 4	10		
adefovir	75	Buti; Hepatology; 2004 168			0		51 7	63		
adefovir	20	Manilakopoulos; Hepatology; 2005 122				3.3		72		5
adefovir + lamivudine	44	Manilakopoulos; Hepatol; 2005 122				3.3		87		0
adefovir	26	Koskinas; J Hepatology; 2005 169				2.5		92		4
adefovir+ lamivudine	74	Lampertico; Hepatology; 2005 170					78 6	82		0
adefovir + lamivudine	23	Koskinas; J Hepatology; 2006 169				2.8		87		0
adefovir + lamivudine	49	Vassiliadis; AP&T; 2005 171			0	6.5	57 4	75		
Mixed										
adefovir	18	van Bömmel; Hepatology; 2004 17	19		0	2.8	44 4			
adefovir + lamivudine	20	Peters; Gastroenterology; 2004 121	17	6		3.6	35 5	53		0
adefovir	57	Lee; Hepatology; 2006 104	20			2.4	19 3	60		18
adefovir + lamivudine	46	Perrillo; Gastroenterology; 2004 62	15	8	0	4.6	20 1	30		
adefovir ± lamivudine ‡	126	Schiff; Hepatology; 2003 60				4.1	81 4	76		0
adefovir + lamivudine	34	Moriconi; J Hepatology; 2006 172					68 1			
adefovirr ± lamivudine	65	Hann; J Hepatology; 2006 135	7 ††			2.4	21 5			
entecavir	42	Chang; Gastroenterology; 2005 173	11	4		5.6	26 4	68		0
entecavir	141	Sherman; Gastroenterology; 2006 132	10	11		5.1	27 2	61	55	7
entecavir	42	Karino; J Hepatology; 2006 174		15		3.8	60 4	78	60	0
entecavir	116	Yao; J Hepatology; 2006 175	8	6		5.8	27 2	85		

tenofovir ± lamivudine @	35	van Bömmel; Hepatology; 2004 17				5.5	100 4		0
tenofovir ± lamivudine	44	Hann; J Hepatology; 2006 135	4 ††			5.0	86 5		
tenofovir + lam @	11	Van der Eijk; J Viral Hepatitis; 2005 176 $	10	0	0	5.0		91	
tenofovir @	10	Dore; J Infect Dis; 2004 177	20	10		4.9		25	
tenofovir @	12	Núñez; Aids; 2002 178 $		11	8	3.8	58 1		
tenofovir @	20	Nelson; Aids; 2003 179		25		4.0			
tenofovir @	12	Benhamou; NEJM; 2003 180	0	0	0	3.8			

Table 4. Treatment outcomes after 1 year of treatment for different antiviral drugs for the management of lamivudine resistant chronic hepatitis B for both HBeAg positive and HBeAg negative patients. Efficacy measures presented are off-treatment responses for PEG-interferon and on-treatment responses for nucleos(t)ide analogues.
$ results after 24 weeks of treatment
@ including HIV/HBV co-infected patients
± some patients with, some patients without combination therapy
†† after 24 months of treatment
‡ Patients with decompensated cirrhosis

[1] LLD 200 copies/ml
[2] LLD 300 copies/ml
[3] LLD 366 copies/ml
[4] LLD 400 copies/ml
[5] LLD $1.0x10^3$ copies/ml
[6] LLD $2.0x10^3$ copies/ml
[7] LLD $1.0x10^5$ copies/ml

rates of PCR-negativity (81% vs 40%) in patients with a baseline viral load ≥5 $\log_{10}$ copies/ml. [123]

Although adefovir is effective for the treatment of lamivudine refractory patients there seems to be some degree of cross-resistance as in vitro testing shows a 2.8-16 fold increase in IC_{50} values for adefovir for lamivudine resistant strains. [124-126] Clinically mutations also associated with lamivudine resistance appear to influence treatment outcomes. Viral decline and ALT normalisation might be less in lamivudine resistant patients compared to treatment naïve patients, but other studies do no report such difference. [104, 127, 128] The rate of resistance is increased in patients with lamivudine resistance switched to adefovir monotherapy compared to the large phase III trials and to patients switched to adefovir and lamivudine combination therapy. [14, 16, 103, 104, 123] Considering the mounting evidence of more potent antiviral effect and a lower rate of resistance adding adefovir to the ongoing lamivudine therapy is to be preferred above switchting to adefovir monotherapy. If chosen to switch to adefovir monotherapy lamivudine has to be continued for at least 2-3 months as this overlap may prevent the emergence of adefovir resistance. [103, 129]

Lamivudine resistance shows some cross-resistance with entecavir in cell culture, but lamivudine resistant strains remain sensitive to entecavir. [130, 131] Although very effective,

treatment outcomes with 1 mg entecavir in lamivudine resistant patients were less compared to 0.5 mg entecavir in treatment naïve patients as viral decline (6.9 vs. 5.1 $\log_{10}$ copies/ml) and rates of PCR-negativity (67 vs. 19%) were lower after 48 weeks of treatment (Table 4).[13,132] Entecavir has a high barrier to resistance as multiple mutations are necessary for the virus to be resistant. Lamivudine refractory patients already harbour some of these mutations and entecavir resistance occurs therefore more frequent in lamivudine resistant patients. After 1 year 1.4% of patients became resistant increasing up to 9% after two years and 15-19% after 3 years of treatment.[76,132,133]

Tenofovir disoproxil fumarate possesses potent activity against lamivudine resistant hepatitis B (Table 4).[17,95,134,135] Lamivudine resistant mutants lead to a slight 1.8-5.7 fold increase in IC_{50} values. The known mutants however remain sensitive to tenofovir and the mutation pattern of tenofovir has no overlap with the mutational pattern of lamivudine.[124,126,136] Most studies add tenofovir to lamivudine, though tenofovir monotherapy seems to be equally effective.[137] Tenofovir is thought to be a more potent viral suppressing agent for lamivudine resistant HBV compared to adefovir, but its efficacy is only investigated in relatively small groups of patients. Many of them including HIV-HBV co-infected patients.[17,135] Being a very promising drug, more studies have to be conducted to determine the exact role or the combination with other compounds for the treatment of lamivudine resistant hepatitis B.

Adefovir resistance

Adefovir resistant strains are susceptible to lamivudine and lamivudine can thus be used for rescue therapy.[138] Indeed clinically lamivudine is able to reduce the viral load in adefovir resistant patients.[139,140] The effect of adefovir associated mutations on long-term treatment is unknown. It is likely that lamivudine resistant strains severely limit the use of lamivudine. In vitro a strain conferring resistance to both adefovir and lamivudine is viable and has reduced sensitivity to all common drugs used for hepatitis B, although tenofovir and entecavir are likely to be able to suppress HBV DNA.[138] Adefovir resistant strains are susceptible to entecavir and tenofovir in vitro.[138,141] In very small series tenofovir and entecavir proved effective against adefovir resistant HBV.[103,142]

Entecavir resistance

Entecavir resistance is highly cross-resistant with lamivudine as entecavir resistance requires lamivudine resistance.[130] This mutant strains are in vitro sensitive to adefovir and clinically treatment with adefovir resulted in decline of the viral load.[130,143] Tenofovir seems also be effective in case of entecavir resistance.[106]

Future directions for the management of resistance

The development of resistance is the largest limiting factor for long-term treatment with nucleoside or nucleotide analogues and should therefore be studied in detail. Lamivudine as well as many other L-nucleosides have high rates of resistance caused by a single mutation. Due to the high resistance rate and being the only oral drug available for a long time, it gave the opportunity to study the mechanisms and outcomes of resistance. The large number of patients treated with lamivudine with subsequent development of resistance made it possible to study the effect of salvage therapy. Entecavir and adefovir proved their efficacy in large populations. But despite all these opportunities we still do not know the exact incidence of adefovir resistance in lamivudine resistant patients. Although the balance tips to lamivudine and adefovir combination therapy over adefovir monotherapy, the definite answers has not been provided, especially the question whether monotherapy comes with higher rates of resistance. Tenofovir seems to be a very promising drug, although studies are small and little is known on the effect of tenofovir monotherapy on lamivudine resistant strains. Very little data is available on the occurrence and management of adefovir and entecavir as well as newer drugs. Studying resistance for compounds with low rates is difficult as large numbers have to be treated. Large scale initiatives are necessary to study the effectiveness and resistance. Understanding of mutational patterns is very important as each pattern has its own influence on replication fitness and cross-resistance. In vitro studies and molecular modelling have to provide these answers to design optimal treatment regimens. This approach is needed as many drugs have been developed and it is not feasible to test all drugs or combinations for all mutational patterns.

DISCUSSION

The knowledge and therapeutic options came a long way since the discovery of the hepatitis B virus. Nowadays chronic hepatitis B virus infection is a treatable disease. However much remains unknown and treatment options are far from perfect. The natural history is only partially understood and only recently the importance of viral load has been revealed.[19-21] Further studies have to identify the factors involved in the progression of disease in order to be able to identify those patients in need of treatment. Treatment options are diverse and have limitations in tolerability and efficacy. More data are needed to be able to predict treatment outcome in patients. This is especially important for the treatment with interferon, which is costly and is associated with considerable side effects and an overall success rate between 30-40%. However this treatment proved to be able to inactivate the disease for long periods in responders, which might result in HBsAg-seroconversion. Research to identify those patients likely to respond

to treatment before the start of therapy or within a few weeks after start of treatment is urgently needed. Nucleoside or nucleotide analogue therapy is the alternative for interferon based treatments and the response rates on treatment are higher compared to interferon. However relapse is frequent after discontinuation, while identifying those relapsing is not possible. This has resulted in long-term treatment, although it is known that response can be sustained off-treatment. By identifying the factors responsible for sustained response, it might be possible to accurately predict sustainability. In theory this could result in nucleos(t)ide analogue therapy of limited duration. This is especially important in young adults who often have a desire for pregnancy, whilst the antiviral drugs have not been investigated on safety for the unborn child, or long-term in patients themselves.

Data on treatment efficacy in treatment experienced patients is limited. Therefore large cohorts of patients have to be studied. Especially the rate of resistance and the mutational patterns are hard to assess. For some therapies resistance rates are low or mutational patterns are diverse. Genotypic and phenotypic testing and molecular modelling are helpful to determine the level of cross-resistance with other compounds. Promising rescue therapies should be studied clinically in order to determine their efficacy. The data on resistance, (mutational patterns, replication fitness, molecular modelling and cross resistance) is scattered and therefore it is almost impossible to look up the implications of a specific mutational pattern. A large central database combining all the data on resistance could provide this information and would be of great value for everyone interpretating mutational patterns. This database could also provide clinicians advice on treatment for an individual resistant patient. More specific knowledge on resistance calls for the development of new techniques that are sensitive, able to detect new variants, able to determine whether multiple variants are located on the same genome, easy to perform and interpreted, cheap and suitable for mass screening.

As none of the current treatments for chronic hepatitis B is optimal, prevention of infection should be one of the cornerstones of management of chronic hepatitis B. Safe and well tolerated vaccines for hepatitis B have been developed and their effectiveness have been proved. There have been some concerns about the luxation of autoimmune phenomena's.[144] Three WHO large scale evaluations revealed no increased risk for the development of autoimmune diseases.[145-147]

In conclusion: The management of chronic hepatitis B evolved fast and nowadays hepatitis B is a treatable disease, More research on the factors involved in response to treatment or treatment failure is needed to better tailor treatment to the individual patient. Much attention should be paid to universal worldwide vaccination as this may significantly change the burden of disease.

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8 Summary and conclusions

Treatment options and insights have come a long way since the discovery of the hepatitis B virus in 1967. Numerous treatments options emerged and we have gained deeper insight into its epidemiology, natural history, genetics, interaction with the immune system and response to treatment. As a result treatment responses have improved. Nowadays the disease can be controlled in the majority of patients. Treatment however, is still far from ideal, as cure is achieved in only a minority of patients treated with interferons which, have a lot of side effects. The viral replication inhibitors have higher response rates and few side effects, but ideal treatment duration is not known and the drugs at present are continued for long periods. The safety in the long term is not known and the emergence of resistant strains limit their effectivity. Hepatitis B is highly adapted to the human body deluding the immune system in ways only partly understood. In addition it has the capability to maintain a very stable genetic reservoir in the form of covalently closed circular DNA (cccDNA) protecting the virus against eradication from a vigorous immune response or strong inhibition of the viral replication. This cccDNA can be detected even in patients with HBsAg seroconversion and forms the basis for viral relapse in immune compromised subjects. The replication error of the virus is high, wich means it can adapt to new conditions and become resistant to therapy. This combination of diversity of the human host and virus makes each infected individual unique. Initially treatment options were limited, but in recent years options have been expanded. New dilemmas arise with increasing understanding of the disease. In this thesis several of these dilemmas are discussed. PEG-interferon-α therapy seems far less effective in patients who are lamivudine resistant. Only 12% of patients sercoconverted to HBeAg negativity. In addition these patients had only faint bands in the resistance assay suggesting only small quantities of mutant virus were present. Although we studied the largest cohort described to this date, numbers are still too small to draw definite conclusions. However these findings support the concept that genotypic alterations in the viral genome and therefore viral structure which are associated with resistance influence the host immune response system.

Patients becoming resistant to antivirals form a new group, which is rapidly becoming more diverse as new drugs are marketed and new mutations develop. A complicating factor is cross-resistance between many of these drug, what limits the efficacy of other drugs. Resistance rates for nucleoside-naïve patients are very low in patients treated with entecavir. Resistance rates are much higher in lamivudine-resistant patients due to partial cross-resistance. Even long-term vigorous suppression with entecavir in lamivudine resistant patients does not provide complete protection against the development of entecavir resistance. This underlines the importance of viral replication for the development of resistance. Tenofovir therapy proved to be highly effective in case of entecavir resistance.

Changing the treatment regimen from tenofovir to adefovir in lamivudine-resistant patients responding to tenofovir resulted in viral relapse and 6 of 10 patients had a viral load above 10^4 copies/ml. All patients with over 10^3 copies/ml at the time of switch relapsed. This provides additional evidence for tenofovir being a stronger antiviral than adefovir. It also indicates that patients should not be switched from tenofovir to adefovir.

Outcomes of adefovir treatment in an open unselected population are poorer than the outcomes of randomised trials. Many patients do not achieve virological response and resistance rates are much higher. Patients with cirrhosis turned out to have lower response rates and higher rates of resistance. This group is at high risk for the development of hepatocellular carcinoma or decompensation and viral control is therefore extremely important. Cirrhotic patients treated with adefovir require frequent monitoring. HBV genotype has proven to be a predictor of response to therapy in PEG-interferon-α treatment, but also appear to play a role in nucleoside or nucleotide treatment.

Liver transplantation is the ultimate salvage therapy for HBV infected patients. However post-transplant patients need life-long immunosuppressants and HBIg therapy to prevent rejection and recurrence of HBV. Patients with acute fulminant liver failure due to acute hepatitis B are subjected to the same schedule of immunoprohylaxis as patients with chronic hepatitis B. They differ however in their immune response. It seems safe to discontinue HBIg prophylaxis in patients transplanted for acute fulminant hepatitis B. This prevents potential risks of HBIg therapy and discomfort for the patients and reduces treatment costs substantially.

In conclusion: several factors have been identified which influence treatment outcome. Although the mechanisms behind these factors are only partly understood, the factors are of clinical significance. Both patient and viral characteristics have to be taken into account to determine the optimal treatment and follow-up tailored to the patient. Patient tailored treatment is feasible, but at present is far from perfect. In the forthcoming years new drugs will be available and more will be learned about factors influencing response. Old treatment schedules and new regimes need to be evaluated in different groups of patients. Such large scale clinical studies result in a better understanding of hepatitis B infection and will help optimizing patient-tailored treatment.

Samenvatting en conclusies

Sinds de ontdekking van het hepatitis B virus in 1967 zijn de behandelingsmogelijkheden en inzichten zijn enorm toegenomen. Verscheidene behandelingen zijn ter beschikking gekomen en de kennis van de epidemiologie, natuurlijk beloop, genetica, de interactie met het immuunsysteem en de respons op behandeling is verruimd. Als gevolg hiervan zijn de behandelingsuitkomsten verbeterd. Tegenwoordig kan de ziekte bij de meeste patiënten onder controle worden gehouden. De ideale behandeling is nog ver weg. Genezing wordt slechts in een minderheid van de met interferon behandelde patiënten bereikt en deze behandeling gaat gepaard met de nodige bijwerkingen. De respons van middelen die de virale replicatie remmen ligt hoger en ze hebben weinig bijwerkingen. De behandelingsduur is echter onbekend en moeten derhalve gedurende lange tijd worden gecontinueerd. De veiligheid van deze geneesmiddelen op de lange termijn is onbekend. Bovendien wordt de effectiviteit aangetast door het ontstaan van resistente stammen.

Het hepatitis B virus heeft zich goed aangepast aan menselijk lichaam en is in staat het immuunsysteem te omzeilen op manieren die grotendeels onbekend zijn. Het virus is in staat een stabiele pool van genetisch materiaal in de vorm van covalent gesloten circulair DNA (cccDNA) te handhaven, welke het virus beschermd tegen irradicatie ten tijde van een krachtige immuunrespons of sterke onderdrukking van de virale replicatie. Zelfs bij patiënten met HBsAg seroconversie kan nog cccDNA worden aangetoond. Dit kan aanleiding geven tot terugkeer van ziekte in immuungecompromiteerde patiënten. Het aantal fouten tijdens replicatie is hoog, waardoor er vele virusvarianten ontstaan en het virus zich zo kan aanpassen aan veranderende omstandigheden en zodoende therapie resistent wordt. De combinatie van grote diversiteit van de gastheer en het virus maakt elke infectie uniek. In vroeger tijden waren de behandelingsopties gelimiteerd, maar deze zijn de laatste jaren toegenomen. Nieuwe dilemma's ontstaan met onze toegenomen kennis van de ziekte. In dit proefschrift worden enkele van deze dilemma's besproken.

Behandeling met PEG-interferon-α lijkt minder effectief in patiënten die resistentie hebben ontwikkeld tegen lamivudine. Slechts 12% van de patiënten werden HBeAg negatief. Bovendien hadden de patiënten die HBeAg negatief werden vage bandjes in de resistentie bepaling wat suggereert dat er slechts een geringe hoeveelheid resistent virus was. Hoewel het de grootste groep die beschreven is zijn de aantallen te klein om een definitieve conclusie te trekken. Dit fenomeen suggereert dat veranderingen in het virale genoom en hiermee in de structuur van het virus die geassocieerd zijn met resistentie ook de immuunrespons van de gastheer kunnen beïnvloeden.

Patiënten die resistent worden vormen een nieuwe groep, welke snel diverser wordt doordat er nieuwe geneesmiddelen op de markt komen en er nieuwe mutaties ontstaan.

Een complicerende factor bij de behandeling is kruisresistentie waardoor ook de effectiviteit van andere middelen beïnvloed kan worden. De incidentie van resistentie bij nucleoside naïeve patiënten is erg laag wanneer ze behandeling met entecavir krijgen. De incidentie van resistentie is veel hoger in patiënten die reeds lamivudine resistent zijn door gedeeltelijke kruisresistentie. Zelfs langdurige krachtige onderdrukking van de virale replicatie door entecavir in lamivudine resistente patiënten geeft geen volledige bescherming tegen het ontstaan van entecavir resistentie. Behandeling met tenofovir bleek zeer effectief te zijn in het geval van entecavir resistentie. Het veranderen van het behandelingsregime in lamivudine resistente patiënten goed reagerend op behandeling met tenofovir naar adefovir resulteerde in virale relapse. Na omzetten hadden 6 van de 10 patiënten een virale load van boven de 10^4 kopieën/ml. Alle patiënten met een virale load van meer dan 10^3 kopieën/ml op het moment van therapie omschakeling hadden een relapse. Dit is extra bewijs dat tenofovir een krachtiger antiviraal middel is dan adefovir. Het toont ook aan dat patiënten niet van tenofovir over gezet moeten worden op adefovir.

Het overtuigenste bewijs wordt geleverd door adequaat gepowerde, dubbelblind gerandomiseerde studies. De keerzijde van deze studies is dat dit een geselecteerde populatie is die geen afspiegeling van de huidige populatie hepatitis B patiënten is, omdat de meesten reeds behandeling in het verleden hebben gehad. Bij velen faalde deze behandeling, vaak ook door het ontstaan van resistentie. De uitkomsten van adefovir in een ongeselecteerde populatie zijn minder dan vergeleken met die van de gerandomiseerde trials. Velen hebben geen virologische response en de incidentie van resistentie is veel hoger. Vooral patiënten met levercirrhose bleken een lagere response op behandeling te hebben en vaker resistent te worden. Patiënten met cirrose hebben een hoog risico om een hepatocellulair carcinoom te krijgen of hepatologisch te decompenseren en virale onderdrukking is daarom zeer belangrijk in deze groep. Met adefovir behandelde patiënten met een levercirrose dienen nauwgezet vervolgd te worden.

Dankwoord

"Last, but not least" is een vaak gehoorde uitdrukking als het gaat om het dankwoord. En niet ten onrechte. Neem de opbouw van dissertaties. Een titelblad met de naam van de promovendus. Dit wordt gevolgd door een persoonlijk gekleurde visie op het onderwerp met hierin de doelstellingen van het proefschrift. De hoofdmoot wordt gevormd door de bevindingen, dikwijls in de vorm van publicaties, waarbij de promovendus steevast op de eerste of tweede plaats in het rijtje van auteurs staat. Het op de voorgrond plaatsen van de promovendus wordt gecomplementeerd door een discussie van de hand door de 'auteur' van de dissertatie. De gemotiveerde lezer moet zich eerst door specialistische teksten worstelen bijna geheel in het teken van de promovendus. Het getuigt dikwijls van een groot doorzettingsvermogen om een proefschrift van voor tot achter te lezen. Pas als het boek bijna uit is ontvouwt zich het plot. De rechtlijnigheid en schijnbare simpliciteit maakt plaats voor een complex samenspel tussen de promovendus en zijn omgeving. De plaats van de promovendus wisselt in het raderwerk en nieuwe verbanden worden aangegaan. In plaats van een persoonlijke prestatie is het een collectieve prestatie. Na het lezen van het dankwoord moet je dan de dissertatie in een ander licht zien. Er zou recht gedaan worden wanneer het dankwoord aan het begin van het proefschrift geplaatst wordt en de lezer het geheel direct in het juiste perspectief leest.

Zonder plezier in het werk geen motivatie en dus ook geen inspiratie of verfrissende ideeën. Ik heb een hele plezierige tijd gehad op de dakpoli, ook wel bekend als het Picasso-gebouw. Op de eerste plaats mijn kamergenoten Monica en later Madeleen en Martijn. Vaak hebben we elkaars vorderingen besproken onder het genot van een goed glas wijn. Gelukkig hadden we het niet alleen over het werk. Het senseo-apparaat zorgde voor de nodige toeloop en gezelligheid van de rest. Ik zou zeggen; blijf koffie drinken. Beste Annemiek. Dank voor alles wat je me hebt nagelaten om mee verder te gaan. Helaas heeft de lamivudine database een tweede promovendus overleeft.

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door verloren gewaande statussen weer boven water te krijgen. Hartelijk dank voor de gezellige samenwerking.

Zonder virologische data zou dit proefschrift niet tot stand zijn gekomen. Wegens het retrospectieve karakter van veel van de studies betekende dit voor de laboranten van de afdeling virologie een gang naar de vriezer om tussen de vele duizenden monsters de juiste te vinden. Gelukkig kon ik altijd weer bij jullie komen met de befaamde groene kaarten, waarna de uitslagen na enkele dagen beschikbaar waren.

De ontwikkeling van nucleoside en nucleotide analogen is in een stroomversnelling geraakt. Hiervan getuigen de grote multicenter trials die liepen tijdens mijn promotie-onderzoek en die ik mocht begeleiden. Participeren is wellicht een beter woord, want er was al een goed geoliede machinerie in de vorm van de research nurses; Anneke, Heleen en Cocki. Zij verzorgden de gehele logistiek en deden het datamanagement van de door ons geïncludeerde patiënten. De hoogwaardige kwaliteit werd bevestigd door een officiële audit, waarbij men zeer lovend was over de gang van zaken in het Erasmus MC. Dames chapos en heel hartelijk bedankt voor alle gezelligheid.

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Curriculum Vitae

De auteur van dit proefschrift werd op 9 mei 1979 geboren te Amstelveen. Van 1991 tot 1997 volgde hij de opleiding voorbereidend wetenschappelijk onderzoek aan het Hermann Wesselink College te Amstelveen.

In 1997 startte hij met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam.

Na het behalen van zijn artsexamen in 2003, begon hij als arts-onderzoeker in het Erasmus MC te Rotterdam aan het onderzoek over de behandeling van hepatitis B, dat is beschreven in dit proefschrift (supervisor Prof. Dr. S.W. Schalm, in 2006 opgevolgd door Prof. dr. H.L.A. Janssen. Tijdens zijn promotie-onderzoek had hij een eigen poli alwaar patiënten met chronische hepatitis B behandeld werden. Ook begeleidde hij de dagelijkse gang van zaken van enkele grote internationale multi-center studies voor hepatitis B in het Erasmus MC.

Van februari 2007 tot december 2007 werkte hij als arts-assistent bij de Interne Geneeskunde in het Spaarne Ziekenhuis te Hoofddorp. Hierna kreeg hij een aanstelling als arts-assistent bij de Cardiologie in het Sint Lucas Andreas Ziekenhuis te Amsterdam. Als toekomstig beroep heeft hij gekozen voor de functie van bedrijfsarts.

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Abbreviations

HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis Delta virus
HIV	human immunodeficiency virus
HR	hazard ratio
i.v.	intravenous
IFN-a	interferon-α
IU/l	international unit/ liter
LAM	lamivudine
L-dC	valtorcitabine
LDD	lower limit of detection
L-dT	telbuvidine
L-FMAU	clemuvidine
LLD	lower limit of detection
MEIA	microparticle enzyme immune assay
MU	mega units
OLT	orthotopic liver transplantation
ORF	open reading frame
PCR	polymerase chain reaction
PEG-interferon	pegylated interferon
PMEA	adefovir
qPCR	quantitative polymerase chain reaction
RNA	Ribonucleic acid
TDF	tenofovir disoproxil fumerate
ULN	upper limit of normal
ULN	upper limit of normal
VR	virologic response
WHO	World Health Organisation
xULN	times upper limit of normal
YMDD	Special sequence in HBV genome (YMDD-motif)

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