Antiviral therapy and resistance with hepatitis B virus infection

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INTRODUCTION

Hepatitis B virus (HBV) infection is still the most common cause of hepatocellular carcinoma and liver cirrhosis worldwide. Recently, however, there has been quite dramatic improvement in the understanding of HBV-associated liver disease and its treatment. It has become clear that high viral replication is a major risk factor for the development of both cirrhosis and hepatocellular carcinoma. Early studies have shown lamivudine lowers the risk of HBV-associated complications. There are currently three nucleos(t)ides licensed, in addition to interferon, and there are more drugs coming to the market soon. Interferon or its pegylated counterpart are still the only options for treatment with defined end points, while nucleos(t)ides therapy is used mostly for long term treatment. Combination therapies have not been shown to be superior to monotherapy in naive patients, however, the outcome depends on how the end point is defined. Interferon plus lamivudine achieves a higher viral suppression than either treatment alone, even though HBe-seroconversion was not different after a one year treatment. HBV-genotypes emerge as relevant factors, with genotypes "A" and "B" responding relatively well to interferon, achieving up to 20% HBsAg clearance in the case of genotype "A". In addition to having a defined treatment duration, interferon has the advantage of lacking resistance selection, which is a major drawback for lamivudine and the other nucleos(t)ides. The emergence of resistance against adefovir and entecavir is somewhat slower in naive compared to lamivudine resistant patients. Adefovir has a low resistance profile with 3%, 9%, 18%, and 28% after 2, 3, 4, and 5 years, respectively, while entecavir has rarely produced resistance in naive patients for up to 3 years.

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viral entry by using a peptide that is competitive with HBV binding.

Among the earliest substances to be used in man were interferon, acyclovir (previously shown to ameliorate Herpes Simplex Virus I associated encephalitis), as well as vidarabine and ARA-A\(^{[3-5]}\). Even though promising, these agents, except for interferon, were subsequently shown to have minor potency in controlled trials and/or had significant side effects prohibiting further development\(^{[6,7]}\). Similarly, a combination of interferon with these “antiviral” agents did not improve the efficacy of interferon\(^{[8]}\). Ganciclovir, which is effective against cytomegalovirus, has also been evaluated for effectiveness against HBV\(^{[9]}\), but was subsequently not developed further because more potent drugs were emerging.

**GOALS OF THERAPY FOR HBV INFECTION**

The main goal of antiviral therapy is to prevent the development of liver failure, due to either acute fulminant hepatitis or chronic hepatitis B with subsequent liver cirrhosis, the emergence of hepatocellular carcinoma, and HBV transmission. All of these can likely be achieved by suppressing HBV replication, which thereby leads to the remission of liver disease activity and infectivity.

In patients with wild type virus infection, the primary goal of antiviral therapy is to achieve seroconversion from HBcAg to the corresponding anti-HBe antibody (i.e. HBe seroconversion) because this immunologic event is associated with reduced risk for progression of liver disease\(^{[9]}\). Noteworthy, a prior decline in viral load is mandatory to obtain HBe seroconversion, which is subsequently required to also achieve seroconversion from HBsAg to the homologous anti-HBs antibody (i.e. HBs seroconversion). This, however, is achieved less frequently and its likelihood, as that of HBe-Seroconversion, might be genotype related (Figure 1)\(^{[10]}\).

HBcAg can be negative in the presence of ongoing high viral replication. In patients with HBcAg negative chronic hepatitis B, pre-core mutants can be detected, which are characterised by an inability to produce HBeAg in detectable quantities (Core-promoter mutations) or show a failure to produce HBeAg (start codon mutations or mutations towards a stop codon typically in the second to last codon of the pre-core region). Available antiviral agents are effective in suppressing HBV replication but in many cases, they are not capable of inducing a sustained response after treatment cessation. Therefore, the main objective of therapy is to control viral replication to prevent ALT flares and/or induce remission of disease.

**TREATMENT OUTCOME PARAMETERS**

Treatment responses have been poorly defined in the past and different studies use different endpoints, thereby making clear comparisons troublesome. In an approach to unify treatment outcome measurements, the European consensus conference in 2002 defined different types of responses\(^{[12]}\); i.e., an initial response, an on-treatment or maintained response, and the sustained response when antiviral treatment has been stopped. The virological response is defined by the decline in HBV DNA below \(10^3\) or \(10^4\) copies/mL, the biochemical response by the normalization of ALT levels, and the histological response (HAI score) by the improvement in the inflammatory activity or fibrosis indices. The combined response is defined by the improvement in ALT levels and decrease in viral load while the complete response is characterized by the combination of the decrease in viral load, the normalization of ALT levels, the occurrence of an HBe- or HBs-seroconversion, and an improvement of liver disease at histology.

The treatment response is also defined based on the duration of therapy. An initial response is characterized by at least \(1 \log_{10}\) copies/mL decrease in viral load compared to the baseline value at wk 12 of therapy. The maintained response is defined by a low viral load during therapy. Depending on the use of nucleoside analogue or interferon, there is no agreed threshold to define the maintained response. Usually, a decrease of viral load below \(10^4\) copies/mL is associated with an improvement of liver histology. However, with nucleoside analogs, the lower the viral load, the lower the risk to develop viral drug resistance. It seems to emerge that viral load shall decrease to \(< 3 \log_{10}(10^3)\) copies/mL. The end of treatment response is defined by the response observed at the end of therapy; if there was a decision to stop treatment. A relapse is defined by the increase in viral load after treatment.
cessation. The sustained response is conventionally defined by the maintenance of the response 6 mo after drug withdrawal. Finally, a breakthrough is an increase of the viral load of at least 1 log after initial response (see also resistance).

To enable better comparison of different studies in the future the following data should always be reported within a given study: HBeAg loss & HBeAg seroconversion to anti-HBe; HBsAg loss & HBsAg seroconversion to anti-HBs; End of treatment results, and if applicable at 6 mo follow-up; HBV-DNA log reduction within defined time points e.g. at wk 12 and 24, Number of patients not achieving a 1 or 2 log reduction within 12 and 24 wk; Mean and median log reduction; Achieved HBV DNA reduction to absolute values, such as below 400 copies (100 IU/mL) and below 50 copies (12.5 IU/mL); HBV-Genotypes.

If new assays become available, the studies should report data in a way that is comparable to former studies.

**INDICATION OF ANTIVIRAL THERAPY**

Treatment goals and knowledge of the natural history of disease are important for deciding who needs treatment. Two studies have shown that Asian males who are older than 30 years and HBeAg positive have a 10% risk of developing a hepatocellular carcinoma or cirrhosis. In these patients, antiviral strategies seem justified even in the absence of liver disease. In contrast to HCV, HBV can lead to hepatocellular carcinoma in absence of advanced fibrosis/cirrhosis. However, whether these data that were derived from an Asian population can be translated to other regions of the world with different HBV genotypes and ethnic backgrounds appears questionable. There was no difference in survival and liver related death in European HBsAg positive blood donors vs HBsAg negative blood donors. Based on the present knowledge of the natural history of chronic HBV hepatitis and on the efficacy of antiviral drugs, antiviral therapy of chronic HBV infection is indicated in patients with chronic hepatitis B in the immunotolerant phase characterized by high levels of viral replication and elevated serum ALT levels (Table 1). Liver histology usually shows inflammatory activity and variable degrees of liver fibrosis depending on the duration of the disease. Since continuing HBV replication and elevation of ALT levels reflect a significant risk of disease progression towards liver cirrhosis and hepatocellular carcinoma, antiviral therapy is indicated to decrease viral load, normalize ALT levels and induce a remission of the liver disease.

There are two main forms of chronic HBsAg positive hepatitis, which are distinguished by their HBeAg status. The HBeAg positive form is associated with a so-called wild type virus infection, HBsAg and HBeAg positivity, high HBV DNA levels, usually > 10^6 copies/mL, and elevated ALT levels. The HBeAg negative form is associated with core promoter and/or pre-core mutant virus infection, HBsAg positivity and HBeAg negativity (most patients have anti-HBe antibody), HBV DNA levels that are fluctuating but are usually > 10^5 copies/mL, and elevated ALT levels that may also fluctuate over time.

Treatment endpoints differ depending on the form of chronic hepatitis B.

It is currently not recommended to treat patients who are in the immunotolerant phase. They are defined serologically by HBsAg positivity, HBeAg positivity, high HBV DNA levels (usually higher than 10^6 copies/mL), and normal serum ALT levels. They usually have no liver damage or only minimal liver disease at liver biopsy examination, but they are highly infectious. The results of clinical trials for interferon alpha or nucleoside analogs indicate that patients with high HBV DNA load and normal ALT levels have almost no chance of HBeAg seroconversion. However, patients should be monitored on a regular basis to diagnose a break in immune tolerance characterized by an elevation in ALT levels and a decline in viral load, which may reflect the onset of liver damage and represent an indication for antiviral therapy. In addition, being a 30 years old Asian male with a viral load above 10^6 might also serve as an indication because of the 10% chance of developing a HCC or liver cirrhosis in ten years. However, this prediction probably cannot be transferred to women and to European patients.

The other category of patients with chronic HBV infection who should not be treated are HBsAg inactive carriers. Their virologic profile is characterized by HBsAg positivity, HBeAg negativity, anti-HBe antibody positivity, persistently low HBV DNA levels (< 10^5 copies/mL), and normal ALT levels. Liver histology usually shows no or minimal damage and the risk of progressing liver disease is considered to be minimal as long as ALT levels remain normal and viraemia is below 10^5 copies/mL. It is currently recommended that these patients should not be treated but should be followed carefully every 3 to 6 mo to promptly diagnose reactivation of viral replication and ALT exacerbations. When their values have been stable for 2 years, one can consider extending their monitoring intervals to 12 mo intervals.

<table>
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is to initiate every patient on antivirals to prevent further deterioration of their liver disease independent of disease activity at least in the presence of HBV-DNA. Certainly, when fibrosis regresses during treatment an interruption might be considered.

Another clear indication for antiviral treatment is prevention of reactivation on chemotherapy, where lamivudine has been associated with lower frequency and lower disease severity of hepatitis (Figure 2)[18,19]. To a lesser extent, antiviral therapy seems indicated in late pregnancy in women with a high viral load[20,21], but formal clinical studies have not yet been published.

General outcome predictors

Some pre-treatment factors have been identified that predict responses to therapy. They may be useful in treatment decisions and drug choices. The results of clinical trials have shown that high ALT values (> 3 × ULN) are always predictive of a higher chance of HBeAg-seroconversion. In addition, a low viral load (< 10⁷ copies/mL equivalent to 35 pg/mL) is predictive of a favourable response to standard or pegylated interferon. In addition, likewise to HCV though to a lesser extent, there is emerging evidence that HBV-genotypes are associated with treatment responses. While genotypes seem to be of no relevance for nucleos(t)ide therapy, there is ample evidence that the HBV genotypes A (versus D) and B (versus C) are associated with a better response to interferon therapy. HBsAg-seroconversion might be strongly associated with genotype A[22].

STANDARD INTERFERON ALPHA

A sustained response, defined by HBe seroconversion 24 wk post-treatment, is induced by subcutaneous administration of standard interferon in 20% to 40% of patients depending on patient characteristics; while only 5% to 10% of patients seroconvert in the placebo group[21,22]. Spontaneous HBe seroconversion is part of the natural history of the disease and is believed to be driven by the host immune response; in all clinical trials the spontaneous rate of HBe-seroconversion ranges from 5% to 10% per year. Patients with high ALT levels, a high HAI score, and low HBV DNA levels have a higher chance of HBe seroconversion (> 40%). While responses to HCV associated interferon therapy are usually associated with an immediate drop in both HCV-RNA and ALT, response to interferon with HBV is, especially in responders, associated with a marked increase of ALT in conjunction with a decrease of serum HBV DNA during the second or third month of therapy. The former reflects the immunological response leading to clearance of the virus and might also be associated with the vanishing immunosuppression caused by HBV itself. Clearance of HBsAg and seroconversion to anti-HBs is a late event; the percentage of patients who became HBsAg-negative after seroconverting to anti-HBe varied widely (7%-65%) for follow-ups of 3-4 years[21,24]. The European consensus conference recommended using a regimen of 5 MU daily or 10 MU thrice weekly for 24 wk[25]. However, due to the frequency of side effects at these high doses of interferon, 5-6 MU interferon thrice weekly may be an optimal choice to allow the continuation of therapy. Side effects are frequent and numerous but usually mild and reversible after treatment withdrawal.

HBeAg negative patients with active hepatitis B are mostly infected with the so called pre-core mutant. Trials using 6-12 mo of interferon therapy in that patient population showed that, regardless of interferon dosage, there was a good response while on therapy (inhibition of HBV-DNA, normalization of ALT) but relapses post-therapy were common and observed in a majority of patients. These initial studies indicated that therapy, therefore, should not rely on courses of interferon less than 1 year. Long-term administration for at least 2 years showed clinical benefit in terms of viral suppression and ALT normalization. Approximately 30% of patients may present a sustained response after treatment withdrawal when the interferon course was sufficiently long to maintain the suppression of viral replication[26]. However, side effects and poor tolerance to interferon administration limit its prolonged use in this form of chronic hepatitis B.

RESULTS OF PEGYLATED INTERFERON ALPHA

Pegylation is binding a pegylated side chain to interferon leading to a 12 or 40 kD molecule, i.e., PEG-IFN-α2b, and Peg-IFN-α2a respectively, which increases the half life of interferon making a once weekly application feasible and sufficient. These pegylated interferons have proven
to not only increase convenience by enabling once weekly dosing but they have also improved efficacy dramatically in hepatitis C. Thus, it was a logical consequence to also evaluate them in Hepatitis B. A phase II study evaluated the efficacy of 90, 180 and 270 µg of PEG-IFN-α2a for 24 wk in comparison to a standard interferon-α2a 4.5MU three times per week\textsuperscript{[20]}. This dose of standard interferon 3 times per week, however, has to be considered to be inappropriate at that time.

In the subsequent phase III trials the antiviral effect of pegylated IFN-α2a (40 kDa) or -α2b (12 kDa) administration was evaluated for 48 wk (versus 24 wk used previously and considered as standard care). These trials have shown a HBe seroconversion rate of approximately 30% 6 mo post-treatment\textsuperscript{[11,27]}. However, standard interferon was not a comparator in that study; only lamivudine was tested. Interestingly, a HBs seroconversion rate of 3%-5% was observed at the end of follow-up, while clearance of HBsAg was observed in up to 7% of patients with high genotype dependence (Figure 1). Tolerance and the nature and frequency of side effects for pegylated interferon alpha were generally similar to that of standard interferon in historic controls. Flu like syndrome, inflammatory skin reaction at the injection site and neutropenia were more frequent with pegylated than with standard interferon. Interestingly, depression, which occurs in about 30% of HCV patients during treatment, was reported to be lower than 3%. Even though viral suppression at the end of follow-up was similar for Peg-IFN-α2a and Peg-IFN-α2a plus lamivudine, the end of treatment viral suppression was significantly more pronounced in combination therapy (Figure 3) based on the given confidence intervals.

**NUCLEOS(T)IDE ANALOOGUES**

While interferon usually leads to some side effects, such as flu like symptoms, the various nucleos(t)ides are characterised by few side effects, at least at the licensed dose.

**Famciclovir**

The first nucleos(t)ide studied in a larger trial was famciclovir, which was developed as a treatment for acyclovir resistant herpes simplex virus I infection. Famciclovir was subsequently shown to also have some HBV-activity in vitro and was thus developed for HBV therapy. In both liver and heart transplant patients, famciclovir has proven to ameliorate liver disease, despite only moderate virological response in most of the patients\textsuperscript{[28-30]}. Basically three different patterns of response were determined in our transplant patients. A third of the patients responded well, a third showed slow response and another third did not show any response (Figure 4). Interestingly, the responders showed a clear virological response, which rarely exceeded one log reduction within three months, while transaminases and liver function deterioration were ameliorated.

A controlled trial also proved efficacy\textsuperscript{[31]}, but the drug is relatively expensive to produce and lamivudine was emerging as a more potent and less expensive drug. Nevertheless there was a clinical response despite relatively moderate viral suppression of 70%, which is equivalent to less than a 1 log reduction. Some of the patients showed marked and clear improvement in liver function after having had a continuous decrease in liver function prior to initiation of famciclovir (Figure 5).

Despite promising results in liver\textsuperscript{[28,29]} and heart transplant\textsuperscript{[30]} patients, famciclovir was not developed further after the potency of lamivudine became evident. All of the patients responding slowly or not responding to famciclovir showed immediate and more marked response to lamivudine, as did the patients developing resistance on famciclovir\textsuperscript{[32]}.

**Lamivudine**

Lamivudine (3TC) has been developed for inhibiting the reverse transcriptase of HIV\textsuperscript{[33]}, but as HBV’s life cycle also requires a functioning reverse transcriptase, lamivudine was investigated and proven to be effective in inhibiting HBV as well, both in vitro and in vivo\textsuperscript{[34-36]}. Several phase III trials have demonstrated the antiviral efficacy of lamivudine administration in patients with HBeAg positive\textsuperscript{[35,36]} and HBeAg negative chronic hepatitis B in doses of 100 to 300 mg/d\textsuperscript{[37]}. The higher doses used in HIV have not proven to be more efficacious\textsuperscript{[38-40]}, even though their potential in preventing resistance has not been determined. Advantages of lamivudine are oral administration, an excellent safety
profile, a rapid antiviral effect, and a relatively low cost of therapy. Viral load declines by 3-5 log10 copies/mL after a year of therapy compared to baseline values. The antiviral effect is accompanied by a significant decrease in ALT levels, and an improvement in the histology activity index. An improvement of liver fibrosis has also been observed during lamivudine therapy[43]. However, the primary goal of therapy, i.e. HBe seroconversion, is obtained only in approximately 20% of patients after 1 year of treatment, which was nevertheless significantly higher than in patients receiving placebo (5%-10%). Continuous lamivudine therapy is indicated in patients who do not seroconvert. It avoids a rebound of viral replication and exacerbations of liver disease. Continuing lamivudine therapy is associated with a progressive increase in the number of patients who undergo HBe seroconversion, reaching approximately 50% after 4 years of therapy[44]. A factor influencing the durability of HBe seroconversion is the duration of lamivudine therapy after seroconversion. In HBeAg negative patients, long-term lamivudine therapy is required because rebound is immediate after cessation[45].

Emtricitabine

Emtricitabine (FTC) is a L-deoxycytidine analogue as is lamivudine. Emtricitabine was also developed for HIV therapy, where it is often used in a fixed combination with tenofovir. Emtricitabine was evaluated in phase II and phase III trials. In a study randomising 98 patients to receive emtricitabine at 25, 100, or 200 mg daily for 48 wk and then 200 mg until wk 96, the dose of 200 mg daily provided the best results. After 2 years, 53% of the patients had serum HBV DNA below 4700 copies/mL, 33% seroconverted to anti-HBe and 85% had normal ALT levels. Resistance mutations were observed in 18% of patients after 96 wk of therapy[46].

A 200 mg dose of emtricitabine has been shown to be superior to placebo for histologic improvement (103 of 167 (62%) patients receiving FTC vs 20 of 81 (25%) receiving placebo; P < 0.001). Serum HBV DNA less than 400 copies/mL was achieved in 91 of 167 (54%) patients in the FTC group vs 2 of 81 (2%) in the placebo group (P < 0.001). Resistance towards FTC was detected in 20 of 159 FTC treated patients (13%, with a 95% confidence interval of 8%-18%). The safety profile of emtricitabine was found to be similar to that of placebo during treatment[47]. Being an L-nucleoside, FTC shows cross resistance to Lamivudine[48].

Telbivudine

Telbivudine is also an L-analogue, such as lamivudine, and it shares a similar resistance profile to lamivudine. However, resistance to telbivudine is associated with the YIDD mutation, leaving entecavir fully active. The safety, antiviral activity, and pharmacokinetics of telbivudine have been assessed in 43 adults with hepatitis B and antigen-positive chronic hepatitis B[49]. This placebo-controlled dose-escalation trial investigated six telbivudine daily dosing levels (25, 50, 100, 200, 400, and 800 mg/d); treatment was given for 4 wk. There was more than a 2 log reduction in all dose groups within one week, with disclosing higher potency of the > 400 mg dose only in the second phase. Telbivudine was well tolerated at all dosing levels, with no dose-related or treatment-related clinical or laboratory adverse events. Antiviral activity was dose-dependent, with a maximum at doses of 400 mg/d and or more. In the 800 mg/d cohort, the mean HBV DNA reduction was 3.75 log10 copies/mL at wk 4, comprising a 99.98% reduction in serum viral load. Subsequently, large phase III studies have shown the superioriority of telbivudine compared to lamivudine in the suppression of viral load (by 6.5 log10 versus 5.5 log10) and improvement of liver histology[50]. A 24 wk study also showed telbivudine to be more active than adefovir with a 6.3 vs 4.97 log reduction of HBV-DNA[50]. Telbivudine resistance was observed in

approximately 5% of patients after 1 year of therapy and associated with a M204I mutation, as expected, within the “YMDD”-motif in the viral polymerase\(^{[49]}\). However, the M204V mutation, which is frequently associated with additional mutations at 180 and 173, has not been detected with telbivudine\(^{[50]}\). This might be another advantage in addition to its higher antiviral activity. Importantly, pharmacokinetics indicate no alteration with impaired hepatic function\(^{[51]}\).

**Adefovir**

In the early 1990s, adefovir was shown to inhibit HBV and HIV in cell cultures\(^{[52,53]}\). Its development for HIV was halted because the dose required for HIV inhibition was associated with significant nephrotoxicity beyond 24 wk of treatment\(^{[54]}\). However, HBV was inhibited with lower doses of adefovir and it could even be used safely in renal impaired patients\(^{[55]}\). It was shown that a 10 mg dose provided a smaller decrease in viral load than a 30 mg dose (Figure 6) but there was a higher creatinine increase with the 30 mg dose and therefore only the 10 mg dose was developed further\(^{[56]}\).

In a large phase III trial, 515 patients with HBeAg positive chronic hepatitis B were treated with adefovir 10 mg \((n = 171)\), adefovir 30 mg \((n = 173)\) or placebo \((n = 167)\) for 48 wk. HBe seroconversion was achieved only in a minority of patients, i.e. 14% in the 30 mg daily and 12% in the 10 mg daily dosing group of patients receiving adefovir dipivoxil versus 6% in the placebo group. ALT levels normalized in 48% and 55% of patients receiving adefovir 10 and 30 mg adefovir respectively, versus 16% in the placebo group. Reduction of HBV-DNA and liver inflammation and fibrosis improved significantly in patients given adefovir (Figure 6)\(^{[57]}\). Tolerance for the daily dose of 10 mg adefovir was comparable to placebo. Extended administration of adefovir dipivoxil showed an increased rate of HBe seroconversion over time: 14% of 296 patients, 33% of 231 patients, and 46% of 84 patients after 1, 2, and 3 years of therapy, respectively\(^{[57,58]}\).

Similar to HBeAg positive patients, adefovir administration for 48 wk in HBeAg negative patients induced histologic improvement more frequently in adefovir treated \((64%) vs\) placebo treated patients \((33%\), \(P < 0.001\)), and reduced serum HBV DNA below < 400 copies/mL \([51% (63 of 123) \versus 0%\); \(P < 0.001\)]\(^{[59]}\). HBV DNA was below 1000 copies/mL in 51%, 71% and 79% patients after 48, 96 and 144 wk, respectively\(^{[59,60]}\). Interestingly, in the majority of patients who were switched from adefovir to placebo, the benefit of treatment was lost, indicating that antiviral therapy with nucleoside analogs must be prolonged in this patient population to avoid viral reactivation and ALT flares. Side effects after 144 wk were similar to those observed at wk 48. It has been presented recently that 22/33 anti-HBeAg negative patients showed sustained response when adefovir was stopped after 4 to 5 years of continues adefovir therapy\(^{[125]}\).

**Entecavir**

Entecavir was developed as an anti-herpes drug, but proved to display only moderate activity, which eventually led to discontinuation of development for this indication. However, Bristol-Myers Squibb discovered that entecavir was extremely potent against HBV through inhibition of HBV-DNA polymerase, with relatively low toxicity. Entecavir is the first HBV-specific antiviral to be licensed that seems to lack both HIV and herpesvirus cross-reactivity\(^{[61]}\), which is especially attractive for HIV-HBV co-infected patients not yet requiring HIV-treatment. Entecavir has been evaluated for naïve patients in two controlled phase III trials involving 715 HBeAg positive and 648 HBeAg-negative patients with chronic HBV infection, detectable HBV DNA, persistently elevated ALT levels and chronic inflammation on liver biopsy. Entecavir administered 0.5 mg orally once daily for 52 wk was shown to be superior to lamivudine (100 mg orally once daily for 52 wk) for the primary efficacy endpoint of histological improvement and for secondary endpoints, such as the reduction in viral load \((6.9log vs 5.4log, P < 0.001\) for HBeAg\(^+\); \(5.0 \versus 4.5log, P < 0.001\) for HBeAg\(^-\)) and normalization of ALT \((68 \versus 60%, P = 0.02\) for HBeAg\(^+\); \(78 \versus 71%; P = 0.045\) for HBeAg\(^-\))\(^{[62,63]}\). After 2 years of treatment, 81% of patients receiving entecavir had a viral load below 300 copies/mL versus only 39% of patients receiving lamivudine, 31% seroconverted to anti-HBe versus 26% in the lamivudine group, and 5% showed a clearance of HBsAg versus 3% in lamivudine treated patients\(^{[64]}\). The second year, however, was limited to 307 of the initial 709 patients. In lamivudine refractory
patients, entecavir administered at 1 mg once daily induced a significant viral load reduction and histological improvement, by comparison with the control group treated with lamivudine. Entecavir was approved in 2005 by the US FDA for the treatment of chronic HBV infection in adults with evidence of active viral replication and either evidence of persistent elevation in serum ALT or histologically active disease. Entecavir resistant mutants have been described mainly in patients with lamivudine resistance. Approximately 9% of lamivudine resistant patients treated with entecavir develop resistance to entecavir after 2 years of therapy. The resistant mutants are then resistant to both lamivudine and entecavir.

**Tenofovir**

Tenofovir is licensed for the treatment of HIV infection but has known activity against HBV as well. It is less nephrotoxic and therefore it can be used in a higher dose (300 mg) unlike adefovir, which is licensed for a 10 mg dose.

Tenofovir’s anti-HBV activity has been studied in vitro and in vivo mostly in HIV infected patients coinfected with HBV. In this patient population, tenofovir administration decreased HBV load significantly both in lamivudine naïve and lamivudine resistant patients. There is good evidence from non-randomised, but also a small randomised study, that tenofovir is more potent than adefovir in reducing HBV load. Phase III trials are ongoing to compare the anti-HBV activities of tenofovir and adefovir in HBV mono-infected patients and in HIV-HBV co-infected patients. Currently, even though it has higher potency and lower cost compared to adefovir, tenofovir cannot be prescribed. This can be considered as a drawback of modern bureaucratic medicine, which prohibits using a drug that has a better safety profile and higher activity at lower costs, but has not been specifically evaluated for that indication.

**Clevudine**

Clevudine is an artificial beta-L nucleoside analogue that shows cross-resistance to lamivudine. It seems to have an advantage in that viral load rebound after therapy cessation is not immediate. A specific attractive aspect of clevudine is its activity against delta virus infection, at least in the woodchuck model.

**Pradefovir (Removofir)**

There is evidence that the safety of ADV could be improved if liver-specific targeting could be achieved, thereby allowing higher liver-associated concentration without increase of systemic exposure with nephrotoxic consequences. One such prodrug is pradefovir, formerly removofir, which is under clinical development. 10, 20 and 30 mg of pradefovir seem to be more potent than 10 mg of adefovir. However, it needs to be determined whether it is more effective than tenofovir.

**ANA380**

ANA380 is a prodrug of ANA317, another recently reported substance with activity against lamivudine resistant HBV. Patients treated with ANA 380 at 30 mg, 60 mg, 90 mg, 150 mg and 240 mg dose levels experienced reduction in plasma HBV viral DNA at 12 wk of 2.8 log, 3.2 log, 3.9 log10, 3.9 log10 and 4.1 log10 units, respectively.

**Myleran B: an acylated PreS1 peptide**

It was found that the preS1 amino acids 2-48 mediate attachment of the virus to its target cells. Furthermore amino-terminally acylated peptides containing amino acids 2-18, and even more efficiently with 2-48 of the PreS1 domain, can be used to block hepatitis B virus infection. Using this concept, Urban et al, developed a peptide that was shown to inhibit HBV-infection in vitro and in animal models and is currently being developed as an antiviral approach. It is currently not clear whether it will inhibit infection in a post exposure approach, i.e. after needle stick injury or liver transplantation of HBV-positive patients, to prevent re-infection or whether it might have antiviral activity in chronic hepatitis B.

**COMBINATION THERAPY**

Several studies have evaluated the efficacy of a combination of interferon alpha 2a or 2b with lamivudine and more recently a combination of Peg-IFN alpha 2a or 2b with lamivudine in comparison with pegIFN alone and/or lamivudine alone. It was concluded that the efficacy of combined Peg-IFN plus lamivudine is not different from Peg-IFN alone if both are given for 48 wk. However, this depends on what you are observing. HBeAg seroconversion actually was even lower, though not significantly different, 24 wk post-treatment. Similarly the viral load reduction and normalisation of transaminases was similar between Peg-IFN plus lamivudine vs Peg-IFN monotherapy 24 wk after the end of therapy.

The decline of viral load was higher, however, in the combination group than in the single treatment group during therapy (Figure 3). The rate of lamivudine resistance was lower in patients who received a combination of lamivudine with pegIFN compared to lamivudine monotherapy, and following the state of art, one would not have stopped lamivudine therapy at 48 wk. Thus, it may be premature to state that Peg-IFN should not be combined with lamivudine, but this certainly would need further study.

Likewise, a very small study suggested that ccc-DNA reduction was augmented when adefovir is combined with Peg-IFN vs adefovir alone. Whether this can eventually lead to higher HBsAg seroconversion rates needs to be determined in future studies.

A combination therapy approach is also suggested with use of some of the more recently developed antivirals. It had been shown that adefovir plus emtricitabine is superior to adefovir alone. Given the similarity but superiority of tenofovir versus adefovir, the combination of tenofovir with emtricitabine appears especially promising, and since this combination is one of the backbones of HIV-antiretroviral therapy and there is already an excellent track record.

Recently, emtricitabine was also combined with 10 mg clevudine (1.8 to 2.3 log reduction) and results showed a superiority for the combination versus emtricitabine alone.
These viral load reductions, however, were still lower than those reported on 50 mg clevudine monotherapy (s. clevudine above). Thus, in the combination trials each drug should also be tested as monotherapy to exclude the “combination effect” of higher potency for one of the drugs.

**DRUG RESISTANCE**

HBV replicates via an error prone viral reverse transcriptase resulting in a large pool of quasispecies with mutations interspersed throughout the genome. During antiviral drug selection pressure (e.g., lamivudine, adefovir, or entecavir), HBV mutants are selected from the preexisting pool of quasispecies and over time become the dominant species.

Drug resistant mutants emerge as a function of at least six factors: (1) the viral mutation frequency (annual error rate), (2) the intrinsic mutability of the antiviral target site (some mutants are lethal and cannot replicate), (3) the selective pressure exerted by the drug (the stronger the more likely a resistance emerges), (4) the magnitude and rate of virus replication (the higher the viral load, the more likely resistance emerges), (5) the overall replication fitness of the mutant (some mutants are replicating very poorly and some require addition compensatory mutations), and (6) the availability of replication space (the amount of cccDNA harboured in a cell is limited; if there is no space for new cccDNA the likelihood of resistance is reduced).

HBV resistance to antivirals can be defined at different levels, which usually develop sequentially: (1) genotypic resistance is the detection of polymerase gene mutations known to confer resistance to the drug, (2) virologic breakthrough has been defined as an increase of at least one log₁₀ copies/mL compared to the lowest value during treatment, associated with the presence of resistance mutations following genotypic resistance, (3) clinical failure is defined as viral breakthrough and increase in ALT levels and subsequently progression of liver disease usually following the virological breakthrough. Very rarely increased replication of viruses can be observed after emergence of resistance, which was first described 40 years ago for enteroviruses, and some years ago for HIV, which has, however, minor clinical relevance because of the multiple drug approach in HIV.

One of the clear advantages of interferons is their inability to significantly induce mutations, which would subsequently abolish interferon activity. In contrast, all nucleos(t)ide given for more than 48 wk have been shown to induce mutations with various frequencies after 1 to 2 years and during longer treatment (Figure 7A and B), which leads to impaired sensitivity towards the appropriate antiviral. The first antiviral leading to some clinical improvement but also to mutations was famciclovir. It’s signature mutation was L528M (now corresponding to rtL180M, as numbering was changed to start at the start of the reverse transcriptase of HBV-DNA polymerase with the highly conserved EDWGPCDEHG motif) thereby eliminating different numbering for different HBV
genotypes. Patients with such a L528M/rtL180M mutation were shown to be sensitive to lamivudine, at least until YMDD mutations due to lamivudine became known as well[98].

New drugs have become available and knowledge of the in vitro cross-resistance profile has provided the rationale for their use in patients with treatment failure. The rescue treatment of patients with drug resistance has improved significantly in recent years.

The major problem of long-term lamivudine therapy is the occurrence of drug resistance. The spontaneous variability of the HBV genome and the slow kinetics of viral clearance, are the biological basis for the selection of drug resistant mutants. The results of phase III clinical trials and of cohort studies have shown an incidence of lamivudine resistance of approximately 20% per year[99]. Lamivudine resistance develops in up to 70% of patients after 4 years of therapy[99,100], leading to an increase in viral load (viral breakthrough) that is followed by an increase in ALT levels (biochemical breakthrough), a reduced HBe seroconversion rate in HBeAg positive patients, and a progression of liver disease[91]. In some patients, especially those with liver cirrhosis or severe fibrosis, the biochemical breakthrough that follows lamivudine resistance may cause a severe and acute exacerbation of liver disease that may precipitate liver failure[99,92,94].

Long-term studies have shown that antiviral efficacy and histological improvement is progressively lost with time because the prevalence of resistance mutations increases as liver disease continues. This was observed in some patients with YMDD mutations but none without those mutations[93]. ALT levels increase progressively with the duration of infection with the YMDD mutants and it was reported that no patient who developed lamivudine resistance mutation for 24 mo had normal ALT levels[90]. It is therefore necessary to make an early diagnosis of drug resistance to adapt rescue antiviral therapy prior to the degradation of liver functions[94,95].

In a retrospective nationwide analysis of lamivudine therapy in Italy, the development of clinically important events after virological breakthroughs depended on the severity of the underlying liver disease; severe hepatitis flares at the emergence of YMDD were noted in patients with child B and C cirrhosis but not in patients with non-cirrhotic chronic hepatitis[98], which is in agreement with previous studies[93,94]. Interestingly, the rate of HCC was diminished even in multivariate analysis in patients with maintained response to those with breakthrough[98].

Mutations conferring resistance to lamivudine are mainly located in the C domain of the reverse transcriptase within the YMDD motif, i.e. M204V or M204I, and may be associated with compensatory mutations in the C domain, i.e. V173L or L180M. After 1 year of treatment, lamivudine resistant mutants emerged in 22% of patients, increasing to 38% after 2 years, 53% after 3 years, 66% after 4 years, and 69% after 5 years[91,99,100]. However, this also means that approximately 30% of patients seem to never develop resistance against lamivudine. In vitro and in vivo studies showed that the main lamivudine resistant mutants remain sensitive to adefovir[101,102], tenofovir[103], and entecavir[104,105], even though susceptibility to entecavir was reduced in vitro[106].

Comparing the addition of adefovir to ongoing lamivudine and the switch from lamivudine to adefovir did not reveal any difference in viral load decline in these two treatment groups. However, recently Lampertico et al presented data showing very pronounced viral load reduction if adefovir was added when viral resistance emerged instead of when clinical resistance with elevated liver enzymes was evident[108]. In addition, the risk of resistance to adefovir was significantly less frequent in patients receiving adefovir in addition rather than as a substitute for lamivudine[104]. Thus, because of the lack of cross-resistance between the two drugs, there is now a consensus among experts that adefovir should be added to lamivudine in patients with lamivudine failure to prevent or delay the subsequent selection of new resistant mutants.

Because of the reduced susceptibility of the lamivudine resistance mutant to entecavir in vitro, entecavir was given to patients with lamivudine failure at a dose of 1 mg daily instead of 0.5 mg, which was given to naïve patients. Entecavir induced a significant decline in viral load in these lamivudine refractory patients[99]. Noteworthy, cases of entecavir resistance were described so far only in lamivudine resistant patients, suggesting that some level of cross-resistance between these two drugs is responsible for the selection of mutants resistant to both drugs. Based on these findings, follow-up studies are required to better determine the indication of entecavir in patients with prior lamivudine resistance.

Telbivudine and emtricitabine share the same resistance mutations as lamivudine except that telbivudine seems to not induce mutations at L180M and 173 as frequently. The year one resistance data within the GLOBE-study indicate that the telbivudine resistance is associated with a M250I mutation and not with a M250V mutation.

In patients treated continuously with adefovir 10 mg/d as a monotherapy, drug-resistant mutants emerged in 2%, 5.9%, 18%, and 29% of patients after 2-5 years, respectively. Resistance to adefovir is most frequently conferred by the selection of a rtN236T mutation in the D domain of the HBV polymerase or a rtA181V mutation in the B domain of the polymerase[107,109]. This may be accompanied by liver failure[110]. In vitro, the rtN236T mutation is sensitive to both lamivudine and entecavir and the rtA181V showed a decreased susceptibility to lamivudine, which can be confirmed in vivo[109,110]. Adefovir resistance can probably be significantly reduced if treatment is combined with lamivudine. In addition, it has been suggested that resistance to adefovir is more likely to develop in lamivudine resistant patients with 10% vs 0% after one year adefovir, which is in agreement with another Korean study reporting a resistance rate of 6.4% and 25.4% after 1 and 2 years adefovir therapy, respectively[112]. Mutant HBV with resistance against both adefovir and lamivudine can emerge[109].

Recently, it was suggested that a mutant/variant rtL233V is naturally occurring even before therapy in some HBV patients[113] and might be associated with reduced susceptibility to adefovir. Resistance was proven for that
mutation in vitro after it had been observed in three patients not responding to adefovir. Surprisingly, this mutation has not been observed in patients developing virological breakthrough. This mutation has not, however, been seen in any of the 20 patients with insufficient response to adefovir from another institution. Since 10% of patients with normal rt sequence have an insufficient response to adefovir, this may be more related to drug transporter polymorphisms, since they have been related to nephrotoxicity, but could also account for the insufficient response to adefovir.

Entecavir resistance was observed mainly in the therapy of lamivudine refractory patients. The resistance rate appears to be approximately 10% after 2 years and 25% after 3 years in patients with lamivudine failure and 0.8% in naive patients over 3 years. The main resistance mutations are rtT184G, rtS202I, rtM250V on a background of lamivudine resistance mutations. These mutants are resistant to lamivudine but appear to be susceptible to adefovir in vitro. Clinical data are awaited to provide recommendation for the treatment of entecavir resistant patients. The emergence of entecavir resistance seems to be bound to the presence of a M250V mutation, thus leaving entecavir as a full option in case of telbivudine resistance.

Monitoring of antiviral therapy

Monitoring during antiviral therapy could serve different purposes: (1) estimation of the response based on early viral kinetics; and (2) early recognition of the development of viral resistance with an increase in the viral load after initial reduction or by mutation monitoring.

It was initially shown by Puchhammer-Stockl et al that patients showing an early viral response are less likely to develop resistance with lamivudine, which was recently confirmed prospectively for telbivudine. Nevertheless, monitoring viral resistance by observing the emergence of mutations known to confer resistance would be the most sensitive way to monitor patients who remain viraemic on current treatments. Based on experience, it is not recommended to wait until an increase in viral load associated with ALT-elevation is evident, since these patients are less likely to respond as well as those placed on an alternative additional antiviral therapy earlier. In addition, this approach harbours the risk of hepatic decompensation. With any nucleos(t)ide there will always be a risk for the emergence of drug-resistance, which mandates monitoring patients on antiviral therapy. The rationale for the timing of monitoring derives from the consideration that the biochemical breakthrough usually occurs within a delay of several weeks after the virological breakthrough and that the clinical impact is usually different in non-cirrhotic than in cirrhotic disease. In the former, the ALT breakthrough most often has no major clinical consequences and in the latter it may precipitate liver failure and death. Monitoring should be performed by measuring the viral load with quantitative HBV DNA testing and certainly transaminases. If the residual viraemia remains high (see below) treatment should be switched to alternative therapy, if possible.

The antiviral response at wk 24 of therapy was found to be a predictor of subsequent efficacy (HBeAg loss, HBV DNA < 200 copies/mL, ALT normalization, and viral breakthrough) in patients treated with lamivudine or telbivudine. In the 5-year study of adefovir administration in HBBeAg negative chronic hepatitis, patients with a viral load lower than 3 log10 copies/mL after 1 year of therapy had a significantly lower risk of developing resistance by year 3 of treatment (< 3%) compared to a risk of 26% and 66% for those having a viral load between 3 and 6 log10 copies, and > 6 log10 copies/mL, respectively. On the other hand, this suggests that those who do not achieve a viral load reduction should be given rescue therapy before the development of true resistance.

During long-term treatment, a 3 or 6 monthly assessment of viral load and serological markers is required to monitor antiviral treatment efficacy and determine whether the response is maintained or whether drug resistance is developing. Certainly drug compliance is important, as any drug interruption may lead to a rebound of viral replication and ALT flares. The detection of polymerase mutations can be performed by sequencing, line probe assay, and DNA chip technologies. Detection may become more complex when additional treatment options become available, since there is emerging evidence that the cross-resistance profile is different from one mutant to another. The line probe assay is more sensitive than sequencing of PCR products but cannot detect new mutations.

New tools may become available in the future to monitor the efficacy of antiviral therapy, such as the quantification of intrahepatic cccDNA or the quantification of serum HBsAg as a surrogate marker. Furthermore, with the development of new drugs and the increasing complexity of the resistance profile, phenotypic assays to determine drug susceptibility of the clinical isolates may prove useful in tailoring antiviral therapy to the virological situation of the patient, as already shown in HIV.

CONCLUSIONS

Patients with minimal disease, whether in the immuntolerant phase or with inactive infection, should not be treated. However, if it is confirmed that the risk of HCC is 10% within 10 years for patients with more than 106 viral load, these patients should receive antiviral treatment irrespective of the activity of their liver disease. In patients with chronic hepatitis proven by ALT elevation and abnormal liver histology, antiviral therapy is indicated because all studies have shown that antiviral therapy decreases the risk of liver disease progression compared to the natural history of the disease. In patients who are HBeAg positive, the primary goal of antiviral therapy is to obtain HBe seroconversion. If the patient is young and has predictive factors of favourable response, a finite course of pegylated interferon should be tried as a first line option in genotype A and B patients. In other cases (including non-responders to IFN, patients intolerant to interferon and those with factors of poor response to interferon), long-term therapy
with nucleos(t)ide analogues is usually needed.

Long-term therapy is probably required in patients who are HBcAg negative. Nucleoside analogues are better tolerated than pegylated interferon, but the therapeutic choice must take into account the risk of drug resistance (Table 2).

Likely future therapy is to begin with an inexpensive antiviral and then adding or switching to another in the case of insufficient response. In patients with severe liver disease, i.e. decompensated liver cirrhosis or HBV recurrence on the liver graft, one might consider combining nucleoside analogues lacking cross-resistance from the start to provide the best chance of long-term control of viral replication and disease progression.

Finally, it is recommended that physicians should be brought back into the position of prescribing licensed drugs, even if they are only licensed for another treatment, when there is evidence for superiority of such an approach. One such example is tenofovir, which has been licensed for HIV and displays higher efficacy and a better safety profile than adefovir.

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**Table 2 Treatment options for chronic hepatitis B and their profile**

<table>
<thead>
<tr>
<th>Standard Interferon PegIFN</th>
<th>Lamivudine</th>
<th>Adefovir</th>
<th>Entecavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral suppression 4.5 log10 copies/mL</td>
<td>5-6 log10 copies/mL</td>
<td>+ (continuous)</td>
<td>3-4 log10 copies/mL</td>
</tr>
<tr>
<td>Long term therapy (6 to) 12 mo in HBcAg pos. Patients</td>
<td>24 mo in HBcAg neg. patients</td>
<td>- to very low</td>
<td>- to very low</td>
</tr>
<tr>
<td>Side effects</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HBe seroconversion</td>
<td>30%</td>
<td>15%-20% at 1 yr, 25%-30% at 2 yr</td>
<td>10%-15% at 1 yr</td>
</tr>
<tr>
<td>Predictive factors for seroconversion</td>
<td>High ALT, low HBV DNA levels</td>
<td>High ALT</td>
<td>High ALT</td>
</tr>
<tr>
<td>Clearance of HBsAg</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cost</td>
<td>++ per months</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Sustained response</td>
<td>&lt; 30% HBsAg - ca. 30% in HBcAg +</td>
<td>Maintained response</td>
<td>Maintained response</td>
</tr>
<tr>
<td>Maintenance response</td>
<td>Not applicable</td>
<td>Maintained response</td>
<td>Maintained response</td>
</tr>
<tr>
<td>Clearance of HBsAg</td>
<td>+ (Genotype dependent)</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>Resistance</td>
<td>No resistance but non-response</td>
<td>20% per year</td>
<td>0% at 1 yr up to 29% at 5 yr</td>
</tr>
</tbody>
</table>
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