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Antiviral therapy of chronic hepatitis B

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“Dedicated to Prof. Erik De Clercq at the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.”

Abstract

Treatment of chronic hepatitis B remains a clinical challenge. Long-term viral suppression is a major goal of antiviral therapy to improve the clinical outcome of the patients. Antiviral treatment of chronic hepatitis B relies currently on immune modulators such as interferon alpha and its pegylated form, and viral polymerase inhibitors. Because of the slow kinetics of viral clearance and the spontaneous viral genome variability, viral mutants resistant to nucleoside analogs may be selected. However, the development of new antiviral agents is rapidly improving the offering of therapy of chronic hepatitis B. These new therapeutic advances are reviewed in this manuscript.

Background

Chronic hepatitis B virus infections remain a major public health problem worldwide. The main clinical outcome is the development of chronic hepatitis, followed by liver cirrhosis and hepatocellular carcinoma. HBV replication does not induce directly a cytopathic effect, liver damage is induced the specific anti-HBV immune response against infected hepatocytes. Cohort studies have shown a clear link between the persistence of viral replication and the severity of liver disease. Therefore, long-term viral suppression is a major goal of antiviral therapy to improve the clinical outcome of the patients. Antiviral treatment of chronic hepatitis B relies currently on two categories of medications : 1) immune modulators such as interferon alpha and its pegylated form, 2) viral polymerase inhibitors that belong to the nucleoside and nucleotide analog family.

Goals of antiviral therapy

The main goal of antiviral therapy is to suppress HBV replication to induce the remission of liver disease activity. In addition, the inhibition of HBV replication decreases patients' infectivity and the risk of HBV transmission. In patients with **wild type virus infection**, the primary goal of antiviral therapy is to achieve seroconversion from HBeAg to the homologous anti-HBe antibody (i.e. HBe seroconversion) as this immunologic event is associated with a reduction of the risk of progression of the liver disease. Noteworthy, a prior decline in viral load is mandatory to obtain HBe seroconversion which is subsequently required to achieve seroconversion from HBsAg to the homologous anti-HBs antibody (i.e. HBs seroconversion). In patients with an **HBeAg negative chronic hepatitis B**, mainly associated with a pre-core mutant infection, available antiviral agents are effective in suppressing HBV replication but in most cases are not capable of inducing 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.

Different types of response have been defined during the European consensus conference in 2002 (de Franchis et al., 2003), i.e. the initial response, the 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 10^4 or 10^3 copies/mL, the biochemical response by the normalization of ALT levels, and the histological response 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 depending on the timing during therapy. The initial response is characterized by a decrease in viral load, at week 12 of therapy, by at least one \log_{10} copies/mL compared to the baseline value. The maintained response is defined by a low viral load during therapy. Depending on the use of nucleoside analog 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 is, the less is the risk to develop viral drug resistance. The end of treatment response is defined by the response observed at the end of therapy, if it was decided to stop treatment. A relapse is defined by the increase in viral load after treatment cessation. The sustained response is conventionally defined by the maintainance of the response 6 months after drug withdrawal.

Indication of antiviral therapy

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 immunoactive phase. As this phase is characterized by high levels of viral replication and immunomediated damage of HBV containing hepatocytes, these HBsAg-positive carriers usually have levels of viral DNA in serum higher than 10^4 copies/mL, and exhibit elevated serum ALT levels. 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 imply a significant risk of disease progression towards liver cirrhosis and hepatocellular carcinoma (Realdi et al., 1994; Yang et al., 2002; Yuen et al., 2005), 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 (Lok and McMahon, 2001). 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 usually $>$

10^4 copies/mL and elevated ALT levels that may also be fluctuating 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 immunotolerance phase. They are defined serologically by HBsAg positivity, HBeAg positivity, high HBV DNA levels (usually higher than 10^8 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 of 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.

The other category of patients with chronic HBV infection who should not be treated are the HBsAg inactive carriers. Their virologic profile is characterized by HBsAg positivity, HBeAg negativity, anti-HBe antibody positivity, low HBV DNA levels ($<10^4$ 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 viremia below 10^4 copies/mL. It is currently recommended that these patients should not be treated, but followed carefully to promptly diagnose reactivation of viral replication and ALT exacerbations.

Antiviral Agents

The main antiviral agents that have been approved or are in clinical development belong to two main categories depending on their mechanism of actions (Lok and McMahon, 2004): 1) immune modulators such as interferon alpha 2a and 2b, and their pegylated forms, 2) viral polymerase inhibitors that belong to the nucleoside and nucleotide analog family.

Treatment of Wild type virus infection, i.e. HBeAg positive chronic hepatitis B (See table 1)

There are currently two treatment options: the use of a finite course of standard or pegylated IFN, or long-term therapy with nucleoside analogs. The choice should depend on the evaluation of factors predictive of treatment response, the past history of the patient (contra-indications to IFN for instance), his/her lifestyle, the desire of a pregnancy, as well as the personal choice of the patient.

Results of standard IFN alpha. A sustained response, defined by HBe seroconversion 24 weeks post-treatment is induced by subcutaneous administration of standard IFN in 20 to 40% of patients depending on patients characteristics ; only 5 to 10% of patients seroconvert in the placebo group (Perillo et al., 1990; Wong et al., 1993). 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%). Response is marked by the clearance of serum HBV DNA, and an increase of ALT levels during the second or third month of therapy; the latter reflects the immunological response leading to clearance of the virus. 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 has varied widely (7% to 65%) for follow-ups of 3 to 4 years (Korenman et al., 1991; Lok et al., 1993). The European consensus conference recommended to use a regimen of 5 MU daily or 10 MU thrice weekly for 24 weeks (de Franchis et al., 2003). However, due to the frequency of side effects at these high doses of IFN, 5-6 MU IFN 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.

Results of pegylated IFN alpha. Phase III trials evaluating the antiviral effect of pegylated IFN alpha 2a or 2b administration for 48 weeks have shown HBe seroconversion rates of approximately 30% 6 months post-treatment (Janssen et al., 2005; Lau et al., 2005). Interestingly, an 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. Tolerance of pegylated IFN alpha was generally similar to that of standard IFN and side effects were also similar in nature and frequency. Flu like syndrom, inflammatory skin reaction at the injection site and neutropenia were more frequent with pegylated than with standard IFN.

Results of lamivudine administration. Several phase III trials have evaluated the antiviral efficacy of lamivudine administration in patients with HBeAg positive chronic hepatitis B (Dienstag et al., 1999; Lai et al., 1998; Zoulim, 2002). Advantages of Lamivudine are the oral administration, an excellent safety profile, the rapid antiviral effect, and the relatively low cost of therapy. Viral load declines by 3 to 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 (Dienstag et al., 2003). However, the primary goal of therapy, i.e. HBe seroconversion is obtained only in approximately 20% of patients after one year of treatment, which was nevertheless significantly higher than in patients receiving placebo (5-10%). Continuous lamivudine therapy is indicated in the 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 (Leung et al., 2001). A factor influencing the durability of HBe seroconversion is the duration of lamivudine therapy after seroconversion.

The major problem of long-term lamivudine therapy is the occurrence of drug resistance. The spontaneous variability of 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 (Zoulim et al., 2006). Lamivudine resistance develops in up to 70% of patients after 4 years of therapy (Lai et al., 2003; Lok et al., 2003). Lamivudine resistance leads to an increase in viral load (viral breakthrough) which is followed by an increase in ALT levels (biochemical breakthrough), a reduced HBe seroconversion rate, and a progression of liver disease (Liaw et al., 2004). 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 which may precipitate liver failure (Hadziyannis et al., 2000; Lai et al., 2003; Nafa et al., 2000). It is therefore necessary to make an early diagnosis of drug resistance to adapt rescue antiviral therapy prior to the degradation of liver functions (Lampertico et al., 2005; Nafa et al., 2000).

Results of adefovir dipivoxil administration. A large phase III trial has evaluated the antiviral efficacy of adefovir dipivoxil administration in 515 patients with HBeAg positive chronic hepatitis B (Marcellin et al., 2003). After 48 weeks of therapy, the median viral load decline was approximately 3.5 log₁₀ copies/ml by comparison with pre-treatment values. HBe seroconversion was achieved only in a minority of patients, i.e. 14% in the group of patients receiving adefovir dipivoxil 10 mg daily versus 6% in the placebo group. ALT levels normalized in 48% of patients receiving adefovir, versus 16% in the placebo group. Liver histology improved in 53% of patients, versus 25% in the placebo group. With a daily dose of 10 mg, tolerance 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 (Marcellin et al, Meeting of the European Association for the Study of the Liver, 2005).

Results of entecavir administration. Entecavir was evaluated in phase II trials (Lai et al., 2002; Wolters et al., 2002) and in 3 controlled phase III trials involving 1633 patients with chronic HBV infection, detectable HBV DNA, persistently elevated ALT levels and chronic inflammation on liver biopsy. In 2 randomized studies involving nucleoside naive patients (HBeAg positive or negative), entecavir administered 0.5 mg orally once daily for 52 weeks was superior to lamivudine (100 mg orally once daily for 52 weeks) on the primary efficacy endpoint of histological improvement and on secondary endpoints, such as the reduction in viral load and normalization of ALT (Chang et al., 2006; Lai et al., 2006; Zoulim, 2006). After two 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 (Gish et al., 2005a). 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 (Chang et al., 2005). 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 resistance mutants have been described mostly in patients with lamivudine resistance (Tenney et al., 2004). Approximately 9% of lamivudine resistant patients treated with entecavir develop resistance to entecavir after two years of therapy. The resistant mutants are then resistant to both lamivudine and entecavir (Colonno et al., 2005).

Results of combination therapy. Several studies have evaluated the efficacy of a combination of pegIFN alpha 2a or 2b with lamivudine, in comparison with pegIFN alone and/or lamivudine alone (Janssen et al., 2005; Lau et al., 2005). Treatment was administered for 48 weeks and end points were analyzed 24 weeks post-treatment. During therapy, the decline of viral load was higher in the combination group than in the single treatment group. The rate of lamivudine resistance was lower in patients who received the combination of lamivudine with pegIFN by comparison with lamivudine monotherapy. 24 weeks post-therapy the rate of HBe seroconversion was similar, i.e. approximately 30%, both in patients who received pegIFN alone or the combination with lamivudine. The HBe seroconversion rate was lower in patients who

received lamivudine monotherapy, i.e. approximately 20%. In agreement with combination studies with standard IFN and lamivudine, these studies did not show an added benefit of the combination.

Results of new drugs : emtricitabine, telbivudine, tenofovir.

Emtricitabine was evaluated in phase II and phase III trials. 98 patients were randomized to receive emtricitabine at 25mg, 100mg, or 200mg daily for 48 weeks and then 200mg until week 96. The dose of 200mg daily gave the best results. After 2 years, 53% 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 weeks of therapy (Gish et al., 2005b).

The safety, antiviral activity, and pharmacokinetics of Telbivudine have been assessed in 43 adults with hepatitis B e antigen-positive chronic hepatitis B (Lai et al., 2004). This placebo-controlled dose-escalation trial investigated 6 telbivudine daily dosing levels (25, 50, 100, 200, 400, and 800 mg/d); treatment was given for 4 weeks. 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 telbivudine doses of 400 mg/d or more. In the 800 mg/d cohort, the mean HBV DNA reduction was 3.75 log₁₀ copies/mL at week 4, comprising a 99.98% reduction in serum viral load. Subsequently, large phase III studies have shown the superiority of telbivudine compared to lamivudine in the suppression of viral load (by 6.5 log₁₀ versus 5.5 log₁₀) and improvement of liver histology (Lai et al., 2005a). Telbivudine resistance was observed in approximately 5% of patients after one year of therapy and associated with a M204I mutation in the viral polymerase (Lai et al., 2005b).

Tenofovir is already approved for the treatment of HIV infection. Its anti-HBV activity has been studied mainly in HIV-HBV co-infected patients. In this patient population, tenofovir administration decreased significantly HBV load both in lamivudine naive and lamivudine resistant patients (Bani-Sadr et al., 2004; Benhamou et al., 2003; Dore et al., 2004; Lacombe et al., 2005). Several non randomized studies suggest that tenofovir may be more potent than adefovir in reducing HBV load (van Bommel et al., 2004). 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.

Predictive factors of treatment response.

Pre-treatment factors predictive of therapy response have been identified. They may be useful for the decision to treat and the choice of drug to use. The results of clinical trials have shown that high ALT values

(> 3xULN), and low viral load (<10^{E7}copies/ml) are predictive of a favorable response to standard or pegylated IFN. Recent studies suggested that HBV genotypes A (versus D) and B (versus C) are associated with a better response to IFN therapy. In patients treated with nucleoside analogs (lamivudine or adefovir), the only established baseline predictor of HBe seroconversion is the level of serum ALT. In patients with ALT higher than 3xULN or even 5xULN, the likelihood of HBe seroconversion after one year of lamivudine therapy is significantly increased (Chien et al., 1999).

Treatment of pre-core mutant infection, i.e. HBeAg negative chronic hepatitis B (see table 2)

Standard IFN administration was the only treatment available until the end of the nineties. Trials using 6-12 months of IFN therapy showed that, regardless of IFN 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 therefore that therapy should not rely on courses of IFN shorter than one year. Long-term administration for at least two 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 IFN course was sufficiently long to maintain the suppression of viral replication (Lampertico et al., 1997; Manesis and Hadziyannis, 2001). However, side effects and poor tolerance to IFN administration limit its prolonged use in this form of chronic hepatitis B.

Lamivudine administration has been evaluated in patients with HBeAg negative chronic hepatitis B in randomized trials and in cohort studies. Given at a dose of 100-150 mg daily for 52 weeks, lamivudine induces a marked suppression of serum HBV-DNA accompanied by normalization of ALT in approximately 80 % of the patients, and by liver histology improvement. However, with a few exceptions, treated patients do not clear HBsAg and are subject to disease reactivation after discontinuing therapy (Tassopoulos et al., 1999). Long-term therapy is therefore recommended. Unfortunately, prolonged lamivudine administration is hampered by the emergence of drug resistance. Long-term lamivudine studies have shown that after reaching a peak between 6 and 12 months of therapy, the response rate decreases because of virological breakthroughs associated with the emergence of lamivudine-resistant HBV mutants. In a study, the virological response diminished from 68% at month 12 and 24 to 52% and 41.6%, respectively at month 18 and 24 of therapy (Hadziyannis et al., 2000). Long term studies showed that the antiviral efficacy and histological improvement is progressively lost with time, as the prevalence of resistance mutations is increasing (Rizzetto et al., 2005). After 3 to 4 years of therapy, the percentage of lamivudine resistance offsets the percentage of

patients initially responding (Buti et al., 2001; Di Marco et al., 2004; Papatheodoridis et al., 2002; Rizzetto, 2002). ALT levels increase progressively with the duration of infection with the YMDD mutants : no patient who developed lamivudine resistance mutation for 24 months had normal ALT levels (Papatheodoridis et al., 2002; Rizzetto, 2002). In a retrospective nationwide analysis of lamivudine therapy in Italy, the development of clinically important events after virologic 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 (Di Marco et al., 2004), in agreement with previous studies (Hadziyannis et al., 2000; Nafa et al., 2000).

Adefovir dipivoxil administration given for 48 weeks in HBeAg negative patients (Hadziyannis et al., 2003) induced in 64% of patients an improvement of histologic liver abnormalities, compared with 33% of patients who received placebo ($P < 0.001$). Serum HBV DNA levels were reduced to < 400 copies/mL in 51% of patients in the adefovir dipivoxil group (63 of 123) and in 0% in the placebo group ($P < 0.001$). The median decrease in log-transformed HBV DNA levels was greater with adefovir dipivoxil treatment than with placebo (3.91 vs. 1.35 log copies/mL, $P < 0.001$). ALT levels had normalized at week 48 in 72% of patients receiving adefovir dipivoxil (84 of 116), compared with 29% of those receiving placebo (17 of 59, $P < 0.001$) (Hadziyannis et al., 2005b). A longer duration study for 144 weeks showed a median decrease in serum HBV DNA of 3.47 log₁₀ copies/ml at 96 weeks and 3.63 log₁₀ copies/ml at week 144 (Hadziyannis et al., 2005b). HBV DNA was below 1000 copies/ml in 71% and 79% patients after 96 and 144 weeks respectively. 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 has to be prolonged in this patient population to avoid viral reactivation and ALT flares. Resistance mutations rtN236T and rtA181V were identified in 3% and 5.9% of patients after 96 and 144 weeks respectively. Side effects after 144 weeks were similar to those observed at week 48. Recent studies showed the clinical response after 5 years of therapy : 70% of patients had a suppression of viral load below the limit of detection of PCR assay, which was accompanied by ALT normalization and histology improvement. Development of adefovir resistant mutations was observed in 29% of patients (Hadziyannis et al., 2005a).

A study evaluated the efficacy of a **combination of pegIFN alpha 2a with lamivudine**, in comparison with pegIFN alone and lamivudine alone (Marcellin et al., 2004). Treatment was administered for 48 weeks and end points were analyzed 24 weeks post-treatment. During therapy, there was a benefit in the combination by

comparison with the single treatment in terms of viral load decline. The rate of lamivudine resistance was lower in patients who received the combination of lamivudine with pegIFN by comparison with lamivudine monotherapy. However, 24 weeks post-therapy, there was no difference in the rate of ALT normalization (approximately 60%) or virologic response (approximately 20% patients) between the groups who received pegIFN alone or in combination with lamivudine. The two groups of patients who received pegIFN had a better response rate 24 weeks post-therapy compared to the group who received lamivudine alone. In view of the fluctuating nature of HBeAg-negative disease, long-term follow-up studies are necessary to determine whether the response is indeed sustained.

Management of patients with Drug resistance

The rescue treatment of patients with drug resistance has improved significantly in recent years. New drugs are available, and the knowledge of the *in vitro* cross-resistance profile has provided the rationale for their use in patients with treatment failure. HBV resistance to antivirals can be defined at different levels : 1) genotypic resistance is the detection of polymerase gene mutations known to confer resistance to the drug, 2) virologic breakthrough is defined by an increase of at least one \log_{10} copies/mL compared to the lower value during treatment, associated with the presence of resistance mutations ; it usually follows genotypic resistance, 3) clinical failure is defined by viral breakthrough and increase in ALT levels and subsequently progression of liver disease.

Lamivudine resistance. 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 one year of treatment, lamivudine resistant mutants emerged in 22% of patients, increasing to 38% after 2 years, 53% after 3 years, and 66% after 4 years (Lai et al., 2003; Lok et al., 2003). *In vitro* studies showed that the main lamivudine resistance mutants remain sensitive to adefovir and tenofovir and have a reduced susceptibility to entecavir (Zoulim, 2004a; Zoulim, 2004b). Adefovir has a proven clinical benefit in the treatment of lamivudine resistance with a significant inhibition of viral mutant replication and improvement in liver function after 1 year of therapy. Several studies compared the addition of adefovir to ongoing lamivudine and the switch from lamivudine to adefovir (Perrillo et al., 2004; Peters et al., 2004). After 48 weeks of therapy there was no difference in viral load decline in these two treatment groups. Indeed, in most clinical trials of adefovir administration for lamivudine failure, virological endpoints were examined at week 48 of therapy, while adefovir resistance starts to occur during the second year. 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 1mg daily instead of 0.5mg given to naïve patients. Entecavir induced a significant decline in viral load in these lamivudine refractory patients (Chang et al., 2005). 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 (Tenney et al., 2004). Based on these findings, follow-up studies are required to better determine the indication of entecavir in patients with prior lamivudine resistance.

Adefovir resistance. In patients treated continuously with ADV 10 mg/day drug-resistant mutants emerge in 2%, 5.9%, 18%, and 29% of patients after 2, 3, 4, and 5 years respectively. Resistance to adefovir dipivoxil is 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 (Angus et al., 2003; Brunelle et al., 2005; Villeneuve et al., 2003). This may be accompanied by liver failure (Fung et al., 2005). *In vitro*, the rtN236T mutations is sensitive to both lamivudine and entecavir; the rtA181V showed a decreased susceptibility to lamivudine. Few reports showed the benefit of lamivudine administration in patients adefovir resistance (Fung et al., 2005; Villeneuve et al., 2003).

Entecavir resistance. Entecavir resistance was observed so far only during therapy of lamivudine refractory patients. The resistance rate appears to be approximately 10% after two years in patients with lamivudine failure. The main resistance mutations are rtT184G, rtS202I, rtM250V on a background of lamivudine resistance mutations (Tenney et al., 2004). These mutants are resistant to lamivudine but appear to be susceptible to adefovir *in vitro* (Villet et al., 2005). Clinical data are awaited to provide recommendation for the treatment of entecavir resistant patients.

Monitoring of antiviral therapy

The risk of the emergence of drug-resistance mandates the monitoring of antiviral therapy when patients are treated with nucleos(t)ides. The rationale for the timing of monitoring derives from the consideration that the biochemical breakthrough usually occurs with 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 usually has no major clinical consequences, in the latter it may precipitate liver failure and death. Monitoring should be performed by measuring the viral load with quantitative HBV-DNA testing whenever possible and affordable.

Early during therapy (week 8 or 12) viral load monitoring allows to assess the initial response which may predict the treatment outcome. In HBeAg positive patients treated with lamivudine or adefovir dipivoxil, the magnitude of HBV DNA decline early on during therapy correlates with the trend of subsequent HBe seroconversion (Werle et al., 2004). The antiviral response at week 24 of therapy was also 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 (*Lai et al., 2005a*). In the 5 year study of adefovir dipivoxil administration in HBeAg negative chronic hepatitis, patients with a viral load lower than 3 log₁₀ copies/mL after one 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 > 6log₁₀ copies/mL respectively (*Hadziyannis et al., 2005a*). These results suggest that patients in whom a significant viral load suppression could not be achieved, 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 drug resistance is likely to occur. It is important to assess drug compliance, as any drug interruption may lead to a rebound of viral replication and ALT flares. The detection of polymerase mutations by sequencing, line probe assay, DNA chip technologies, or other tools will be important in the future to target new treatment to the profile of mutations in the polymerase gene (Lok et al., 2002; Nafa et al., 2000). Indeed the cross resistance profile is different from one mutant to another (Durantel et al., 2004; Yang et al., 2004).

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 (Sung et al., 2005; Werle-Lapostolle et al., 2004; Zoulim, 2005). 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 to tailor antiviral therapy to the virological situation of the patient, as already shown in HIV (Durantel et al., 2004; Yang et al., 2004).

Conclusions

Currently, patients with a minimal disease, whether in the immunotolerance phase or with inactive infection should not be treated. 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 favorable response, a finite course of pegylated interferon should be tried as a first

line option. In other cases (including non responders to IFN, patients intolerant to IFN and those with factors of poor response to IFN) , long-term therapy with nucleoside analogs is usually needed.

Long term therapy is required in patients who are HBeAg negative. Nucleoside analogs are better tolerated than pegylated interferon, but the therapeutic choice must take into account the risk of drug resistance. In patients with severe liver disease, i.e. decompensated liver cirrhosis or HBV recurrence on the liver graft, one might consider to combine nucleoside analogs lacking cross-resistance to provide the best chance of long-term control of viral replication and disease progression.

In the situation of treatment failure, the knowledge of the resistance profile is important to adapt antiviral therapy. One of the major questions in the future will be to delay or prevent drug resistance. Combination therapy, though currently very expensive, will probably become part of the treatment paradigm. Indeed, results of cross-resistance studies on the main lamivudine-, adefovir-, entecavir- resistant strains are now becoming available (Angus et al., 2003; Delaney et al., 2001; Fu and Cheng, 2000; Levine et al., 2002; Menne et al., 2002; Seigneres et al., 2002; Villeneuve et al., 2003). This should allow the evaluation of rational combinations taking into account the cross resistance profile of antiviral agents, to better suppress viral replication and prevent the selection of resistant mutants within the viral quasi-species (Zoulim, 2003; Zoulim, 2004a). Whether de novo combination therapy or very early add-on therapy when viral suppression is not achieved rapidly, is the best strategy in terms of prevention of treatment failure remains to determine by clinical trials.

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TABLE 1 : TREATMENT OF HBeAg POSITIVE CHRONIC HEPATITIS B

	pegIFN	Lamivudine	Adefovir	Entecavir
Viral suppression	4.5 log ₁₀ copies/mL	5-6 log ₁₀ copies/mL	3-4 log ₁₀ copies/mL	6-7 log ₁₀ copies/mL
HBe seroconversion	30%	15-20% at 1 year 25-30% at 2 year	10-15% at 1 year	20% at 1 year 30% at 2 years
Predictive factors	High ALT levels Low HBV DNA levels	High ALT	High ALT	?
Side effects	+	-	-	-
Resistance	No resistance But Non Response	20% per year	0% at 1 year	0% at 1 year (10% at 2 years in lamivudine resistant patients)

TABLE 2 : TREATMENT OF HBsAg NEGATIVE CHRONIC HEPATITIS B

	pegIFN	Lamivudine 100 mg/day	Adefovir 10 mg/day	Entecavir
Long term therapy	+	+ (continuous)	+ (continuous)	+ (continuous)
Side effects	+	-	-	-
Résistance	-	20 % per year	29 % at 5 year	0 % at 1 year
Sustained or maintained response	Sustained response \leq 30 %	Maintained response 30 – 35 % (3 years)	Maintained response \geq 70 % (5 years)	Maintained response ?
Clearance of HBsAg	+	-	-	?
Cost	++	±	+	+
