

Emerging drugs for hepatitis B

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Abstract

Chronic hepatitis B remains a treatment challenge despite the availability of new nucleoside analogs. This is due to the persistence of viral infection during therapy, which exposes the patient to the risk of developing antiviral drug resistance. Therefore, new polymerase inhibitors are needed to manage resistance to currently available drugs and to design new trials of combination therapy to delay drug resistance. In the future, antiviral agents targeting other steps of the viral life cycle will be needed to achieve antiviral synergy and prevent antiviral drug resistance. Immune modulators are also expected to enhance antiviral response and to achieve sustained response. Discovery of new antiviral drugs and design of new treatment strategies are therefore needed to manage this disease which is still the main cause of cirrhosis and hepatocellular carcinoma worldwide.

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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 is the first cause of hepatocellular carcinoma world wide (1 , 2). 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 (3 , 4).

The hepatitis B vaccine was shown to be effective and cost-efficient. Several studies have shown that mass vaccination program, especially when implemented in neonates or infants who are exposed to the highest risk of chronicity after HBV exposure, can decrease dramatically the incidence of new infection cases, the prevalence of chronic carriers, and the incidence of hepatocellular carcinoma (5 -7). However, despite the existence of efficient vaccines, W.H.O estimates that there are still more than 350 million chronic carriers worldwide, some of whom may need antiviral therapy to prevent the complications of the disease. Therefore, the treatment of chronic hepatitis B remains a major public health concern.

Antiviral treatment strategies rely either on the stimulation of the specific anti-HBV immune response or on the inhibition of viral replication (Figure 1).

Interferon alpha based therapies show a direct antiviral effect as well as a stimulation of the anti-HBV immune response. These treatments are associated with a sustained response in approximately 30% of patients, even with their pegylated forms (8 , 9). Because of this non satisfactory response rate and the side effects associated with IFN therapy, alternative treatments based on nucleoside analogs have been evaluated in clinical trials.

Despite the development of new nucleoside analogs that effectively inhibits hepatitis B virus (HBV) replication, antiviral therapy of chronic hepatitis B remains a clinical challenge mainly because of the slow kinetics of viral clearance and the subsequent emergence of drug resistant mutants. Nucleoside analogs mainly target the different viral polymerase activities, i.e. RNA dependent - DNA synthesis (reverse transcription) and DNA dependent - DNA synthesis (Figure 2). This results in the inhibition of infectious virion production and a decreased rate of infection of new hepatocytes. However, none of the available polymerase inhibitors have been shown to prevent infection of uninfected hepatocytes and the de novo formation of covalently closed circular (ccc) DNA. Antivirals have a modest indirect effect on cccDNA by inhibiting the intracellular recycling of nucleocapsids, but the long half-life of infected hepatocytes and cccDNA necessitates very long duration of therapy placing patients at risk of developing HBV drug resistant mutants (10).

Medical Need

Chronic hepatitis B remains a treatment challenge because of the long-term endpoint of antiviral therapy. Since the complications of the disease including cirrhosis decompensation and development of hepatocellular carcinoma (HCC) usually occur after 20 to 30 years of infection, the decision of when to treat is important. Furthermore, it was shown that antiviral therapy in patients with compensated liver cirrhosis provides a clear clinical benefit in terms of prevention of disease progression as compared to patients receiving placebo (11). It is therefore important to treat all patients with active disease who are at risk of liver disease progression. On the other hand, there is not yet a large scale clinical study showing the benefit of long-term antiviral therapy in terms of HCC prevention, although it might be hypothesized that early antiviral intervention may reduce the risk of HCC as suggested in the woodchuck model of hepadnavirus infection (12).

There is clearly a need for improved antivirals with a better antiviral potency and a lower resistance rate to control viral replication and the liver disease. Furthermore, in patients who have developed antiviral drug resistance, new antivirals that are active on the drug resistant strains selected by the approved treatments are needed to combat treatment failure. It is now debated whether de novo combination therapy or early add-on therapy is the best strategy for the management of antiviral drug resistance.

Existing Treatment and Therapeutic Class review

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. This is especially important in healthcare workers (13) and in chronically HBV infected pregnant women in whom antiviral treatment during the last trimester of pregnancy increases the protection of new-borns who receive Hepatitis B Immune Globulins and vaccine at birth (14).

In the review of antiviral drug efficacy, it is important to distinguish the two main forms of chronic hepatitis B. In patients with **wild type virus infection (HBeAg positive)**, the primary goal of antiviral therapy is to achieve seroconversion from HBeAg to the homologous anti-HBe antibody (referred hereafter as **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 (referred hereafter as **HBs seroconversion**).

In patients with an **HBeAg negative chronic hepatitis B**, available antiviral agents are effective in suppressing HBV replication but in most cases are not capable of eradicating the virus. Therefore, the main objective of therapy is to control viral replication to prevent ALT flares and/or induce remission of disease. The only event marking a sustained response to therapy would be HBs seroconversion to anti-HBs, but unfortunately this occurs only seldom.

The main treatment classes are presented in Figure 3.

Indications of antiviral therapy

Based on the present knowledge of the natural history of chronic HBV hepatitis and on the efficacy of antiviral drugs, treatment is indicated only in HBsAg carriers with liver disease, as this was recommended in the guidelines proposed by the different international liver disease societies (AASLD, EASL, and APASL) (15–17).

HBsAg carriers with chronic hepatitis B who should be treated

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 $10^E 4$ 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 (18, 19), 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 (20). **The HBeAg positive form** is associated with a so called wild type virus infection, HBsAg and HBeAg positivity, high HBV DNA levels usually $> 10^E 6$ 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 $> 10^E 4$ copies/mL and elevated ALT levels that may also be fluctuating over time. Treatment endpoints differ depending on the form of chronic hepatitis B.

Chronic HBsAg carriers who should not be treated

Patients with chronic HBV infection who are in the **immunotolerance phase** should not be treated. These individuals represents a minority among chronic HBsAg carriers. 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 risk of disease progression is low as long as ALT levels remain within the normal range. 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 responding to therapy, i.e. HBeAg seroconversion. However, patients should be monitored carefully on a regular basis to diagnose a break in immune tolerance characterized by an elevation in ALT levels and a decline in viral load. These events reflect the onset of liver damage and may represent an indication for antiviral therapy, unless HBeAg seroconversion (which is the ultimate goal of therapy, see below) occurs spontaneously.

A 6 monthly analysis of ALT levels and HBV DNA is recommended in chronic carriers who are in the immunotolerance phase. Patients' relatives and household contacts should be screened for HBV markers and vaccinated against HBV if not protected.

The other category of patients with chronic HBV infection who should not be treated are the **HBsAg inactive carriers**. These individuals are the majority among HBsAg 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, should these occur. A 6 monthly follow-up of ALT levels and HBV DNA is suitable to make sure that the inactive state is maintained over time.

Treatment of HBeAg positive chronic hepatitis B

There are currently two treatment options: the use of a finite course of standard or pegylated IFN, or long-term therapy with nucleoside analogs (Figure 1). The choice depends on the evaluation of factors predictive of treatment response, and on the medical history of the patient.

Results of pegylated IFN alpha administration

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 (9, 21). 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 syndrome, 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 (22–24). 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 inflammatory activity index (HAI). An improvement of liver fibrosis has also been observed during lamivudine therapy (25). 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 (26). 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 (27). Lamivudine resistance develops in up to 70% of patients after 4 years of therapy (28, 29). 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 (11). 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 (28, 30, 31). It is therefore necessary to make an early diagnosis of drug resistance to adapt rescue antiviral therapy prior to the degradation of liver functions (31, 32).

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 (9, 21). 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. Twenty-four 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. However, the study design for such combination treatments might be improved in the future, as in these previous trials a fixed course of lamivudine added to a fixed course of interferon was evaluated, while usually nucleoside analogs are prescribed either indefinitely or until HBe or HBs seroconversion. Furthermore, delayed administration of interferon after prolonged viral suppression induced by nucleoside analogs should be evaluated, as this treatment schedule may enhance the restoration of the specific anti-HBV immune response.

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 (33). After 48 weeks of therapy with a 10mg daily dose, the median viral load decline was approximately 3.5 log₁₀ copies/ml by comparison with pre-treatment values. Noteworthy the 30mg daily dose of adefovir dipivoxil induced a better viral suppression than with the 10mg dosing, but this higher dose was associated with kidney toxicity. The 10mg dosing was therefore used for adefovir dipivoxil registration. 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. Recent data of extended adefovir dipivoxil administration for 5 years showed probabilities of HBeAg loss of 60%, HBe seroconversion of 48%. Four patients (2%) had an HBs seroconversion. HAI and fibrosis scores improved in 67% and 60% of patients, respectively. Adefovir resistance mutations were not observed after one year of treatment but developed in 20% of patients after 4 years.

Results of entecavir administration

Entecavir was evaluated in phase II trials (34, 35) 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 (36–38). 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 (39). Entecavir was approved, in 2005 by the US FDA and in 2006 by the EMEA, 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 mostly in patients treated for lamivudine resistance (40). Approximately 38% of patients treated with entecavir for lamivudine failure develop resistance to entecavir after three years of therapy. The resistant mutants are then resistant to both lamivudine and entecavir (41). A recent study showed that entecavir was effective in lamivudine refractory patients, with resistant sequences arising from a subset of patients harboring preexisting resistant variants and with approximately half of the patients experiencing a virologic rebound (42–44).

Results of Telbivudine administration

The safety, antiviral activity, and pharmacokinetics of Telbivudine have been assessed in 43 adults with hepatitis B e antigen-positive chronic hepatitis B (45). 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 (46). Telbivudine resistance was observed in approximately 5% of patients after one year of therapy and associated with a M204I mutation in the viral polymerase (47). When combining the whole cohort of HBeAg positive and negative patients who have received telbivudine for two years, the incidence of resistance was 9% and 14% after one and two years, respectively. Telbivudine has been approved in the USA at the end of 2006, and should be approved in 2007 in Europe.

Results of new drugs tenofovir, emtricitabine, clevudine

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 significantly decreased HBV load both in lamivudine naive patients and those with lamivudine resistant mutants (48–51). Several non randomized studies suggest that tenofovir may be more potent than adefovir in reducing HBV load (52). Ongoing phase III trials are comparing the anti-HBV activities of tenofovir and adefovir in HBV mono-infected patients and in HIV-HBV co-infected patients; the first results confirm the superiority of tenofovir over adefovir in suppressing viral replication (53).

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. HBV resistant mutants were detected in 18% of patients after 96 weeks of therapy (54).

Clevudine was assessed in clinical trials which showed its potent anti-HBV activity and lack of toxicity during the treatment period (55). Interestingly, the rebound of serum viral DNA after treatment withdrawal seems to follow slower kinetics than for other nucleoside analogs. This was consistent with previous data obtained in WHV chronically infected woodchucks treated with clevidine (56, 57). This drug has been approved in Korea and is currently phase III trials in other countries.

Other nucleoside or nucleotide analogs, such as pradefovir, valtorcitabine, ANA 380/LB80380 and others, are in clinical development; some of them have already shown clinical activity in early phase trials and may therefore represent interesting drugs for the future (58–60). Publications of the results from phase II trials are urgently awaited.

Treatment of HBeAg negative chronic hepatitis

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 (61). 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 recent 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 (30). Long term studies showed that the antiviral efficacy and histological improvement is progressively lost with time, as the prevalence of resistance mutations is increasing (62). After 3 to 4 years of therapy, the percentage of lamivudine resistance offsets the percentage of patients initially responding (63–66). ALT levels increase progressively with the duration of infection with the lamivudine resistant mutants: no patient who developed lamivudine resistance mutation for 24 months had normal ALT levels (64, 65). 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 lamivudine resistant mutants were noted in patients with Child B and C cirrhosis but not in patients with non-cirrhotic chronic hepatitis (63), in agreement with previous studies (30, 31).

Adefovir dipivoxil administration given for 48 weeks in HBeAg negative patients (67) 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 log₁₀ 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, $P < 0.001$) (68). 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 (68). 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 histological improvement. Development of adefovir resistant mutations was observed in 29% of patients (69).

Entecavir administration in HBeAg negative chronic hepatitis. Among patients with HBeAg-negative chronic hepatitis B who had not previously been treated with a nucleoside analogue, the rates of histologic improvement, virologic response, and normalization of ALT levels were significantly higher at 48 weeks with entecavir than with lamivudine (36). The safety profile of the two agents was similar, and there was no evidence of viral resistance to entecavir during the study period.

A study evaluated the efficacy of a **combination of pegIFN alpha 2a with lamivudine**, in comparison with pegIFN alone and lamivudine alone (8). Treatment was administered for 48 weeks and end points were analyzed 24 weeks post-treatment. During therapy, there was a greater benefit in the combination treatment 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% of 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.

Treatment of severe forms of chronic hepatitis B

In patients with advanced liver disease including cirrhosis and decompensation of liver disease, the use of standard interferon or its pegylated form is limited by its side effects (including neutropenia and thrombopenia), and by the risk of hepatitis flare and liver failure by the induction of the immune response. The use of nucleoside analogs has changed dramatically the management of these patients, as these drugs induce a profound inhibition of viral replication accompanied by the improvement of liver functions allowing some patients to be withdrawn from the liver transplantation list (70–72).

In the setting of liver transplantation, interferon alpha is usually contra-indicated because of the risk of liver graft rejection. The use of nucleoside analogs in combination with Immune globulins has also improved dramatically the management of these patients by decreasing the risk of HBV recurrence to below 10%. In patients who present an HBV recurrence on their liver graft, early intervention with nucleoside analogs allows to control viral replication and prevent the accelerated progression of the disease in these immunosuppressed patients. (73).

Nucleoside analogs have also helped in the management of challenging patient populations such as those receiving chemotherapy, bone marrow or kidney transplantation, or immunosuppressive treatments (74).

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 (Table 1). HBV resistance to antivirals can be defined at different levels (75): 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 lowest 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, including liver failure in some patients.

Lamivudine resistance

Mutations conferring resistance to lamivudine are mainly located in the C domain of the reverse transcriptase within the YMDD motif, leading to amino acid changes rtM204V or rtM204I, and may be associated with compensatory mutations in the C domain with the rtV173L or rtL180M amino acid changes. 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 (28, 29) (Figure 4). The emergence of HBV drug resistant mutants can be associated with severe ALT flares, disease progression, cirrhosis decompensation, liver failure and death, depending on the underlying liver condition and on the rapidity of treatment adaptation (11, 29, 31). *In vitro* studies showed that the main lamivudine resistance mutants remain sensitive to adefovir and tenofovir and have a reduced susceptibility to entecavir (10, 76). 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 (77, 78). 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, as early as possible to prevent or delay the subsequent selection of new resistant mutants. It was shown in cohort studies that an early add-on therapy with adefovir and lamivudine controlled viral replication and maintained normal ALT levels in the majority of patients (79, 80).

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 treatment naïve 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 (81). Noteworthy, cases of entecavir resistance were described so far only in patients with lamivudine

resistant HBV, suggesting that some level of cross-resistance between these two drugs is responsible for the selection of mutants resistant to both drugs (40 , 82). 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 (Figure 4). 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 (83 –85). This may be accompanied by liver failure (86). 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 (85 , 86).

Entecavir resistance

Entecavir resistance was observed mostly during therapy of lamivudine refractory patients. The resistance rate appears to be approximately 10% after two years in patients with lamivudine failure, and increases significantly with duration of treatment (Figure 4) (43). Recent reports suggest that entecavir resistant strains may also be selected in nucleoside naive patients treated with entecavir (42 , 44). The main resistance mutations are rtT184G, rtS202I, rtM250V on a background of lamivudine resistance mutations (40 , 43). The current concept is that these entecavir resistance mutants are selected in a stepwise manner with the first selection of mutants in the YMDD motif of the reverse transcriptase (« primary resistance » mutations), followed by the addition of mutations conferring a high replication advantage in the presence of entecavir (« secondary resistance » mutations) (42). These mutants are resistant to lamivudine but appear to be susceptible to adefovir and tenofovir in vitro (87). Clinical data are awaited to provide recommendation for the treatment of entecavir resistant patients.

Multidrug resistance has been described in some patients who received sequential therapy with insufficient viral suppression (82 , 88 , 89). The impact of cross-resistance testing is important in these situations to tailor antiviral therapy to the viral strains circulating in the patients(90).

Current Research Goals

The current research goals follow several aspects with the definition of predictive factors of response or resistance during therapy, the evaluation of combination therapy strategies to manage or prevent drug resistance, the evaluation of new nucleoside analogs and their association with immune modulators (vaccine therapy etc...) to clear or control viral infection on the long term.

Scientific Rationale

Evaluation of treatment strategies

Until now the improvement in liver histology parameters, as measured by different scores (Knodel, Ishak, or METAVIR scores), has been used as the primary end-point in clinical trials (33 , 36 , 37 , 67 , 91). All the results of these trials have shown a correlation between viral load suppression and the improvement of liver histology parameters and/or the decreased risk of progression of liver disease as compared to patients who received placebo (92). It is therefore likely that viral suppression will become the primary end-point of clinical trials as this is associated with an improvement of liver histology, an increased chance of HBe seroconversion and a lower risk of drug resistance development (93). Furthermore, as the future trials will not include placebo groups any more, it will be more difficult to show differences in liver histology improvement when comparing potent antiviral drugs. The use on non-invasive markers of liver damage may also be part of the assessment criteria (94). In any case, the recent advances in this field will move towards the long-term evaluation of viral load suppression and all additional relevant markers for the assessment of new drugs or new strategies.

Some groups also claim that with entecavir the risk of drug resistance is so low after three years of therapy, that monotherapy with this drug should be the gold standard in nucleoside naive patients. However, data beyond one year of therapy have to be analyzed with caution as the study protocol allowed only a selected proportion of patients to continue entecavir therapy beyond one year (42 , 44). It will be interesting to see the results of long-term cohort studies in patients treated with such potent drugs with a relatively low risk of resistance, i.e. entecavir and tenofovir.

With the development of new antiviral drugs with a more potent antiviral activity which are less prone to drug resistance at least on the short-term, there is a debate on whether a de novo combination therapy with drugs lacking cross-resistance or an early add-on therapy would be the best strategy to prevent or manage drug resistance and prevent the progression of liver disease. In this view, recent studies have been performed and showed that the magnitude of viral load suppression during the first months of therapy may predict the subsequent treatment outcome, i.e. seroconversion in patients with maximal viral suppression or emergence of drug resistance in patients

with sub-optimal antiviral response (93). Some authors recommend to start with a potent anti-HBV drug having a high genetic barrier for resistance (i.e. resistance being the consequence of the coexistence of multiple mutations) and evaluate antiviral response after 6 months of therapy ; if viral load is not suppressed adequately, then a second drug lacking cross-resistance with the initial one is added.

However, one may argue that with such a strategy, the lack of viral suppression may already be an indicator of selection of drug resistant strains that may then favor the emergence of multidrug resistant mutants when the second drug is added. The other approach would be to start with two potent antiviral drugs lacking cross-resistance and exhibiting a high genetic barrier of resistance to combat the drug resistant mutants that already pre-exist prior to therapy. With such an approach, the risk of selecting multidrug resistant strains would be lower, as already demonstrated in the case of HIV (10 , 75). Now that several anti-HBV agents have been approved and belong to these categories of drugs with a good anti-HBV profile, it is becoming urgent to evaluate these two strategies, as only long-term studies will provide a final answer.

Viral replication and new targets for antiviral therapy

HBV is a DNA virus that belongs to the hepadnavirus family and infects mainly hepatocytes, although other extrahepatic reservoirs have been described. All the viruses of this family share the same replication strategy [for more detail see (95 , 96)]. The major characteristics of the replication cycle that are relevant to antiviral therapy are the following : i) HBV replication does not induce a cytopathic effect in infected cells, which in turn is one of the factors involved in viral persistence ; ii) viral covalently closed circular DNA (cccDNA), is the transcriptionally active form of viral genome and was shown to have a long half-life in infected cells ; iii) viral genome replication occurs via a reverse transcription step that leads to the production of viral DNA genome within nucleocapsids which are then enveloped prior to virion release. The reverse transcriptase activity also generates viral mutants at each replication cycle. Furthermore, viral nucleocapsids may also be recycled back to the nucleus to initially amplify and then maintain a stable pool of viral cccDNA in the liver. The viral polymerase has been the main target for the development of antivirals of the nucleoside analog family. Most of these compounds inhibit the RNA-dependent DNA polymerase activity of the viral polymerase while other inhibitors are more specific for the DNA-dependent DNA polymerase activity (97). Although these agents are potent inhibitors of the viral polymerase activity, tissue culture and in vivo experiments showed that their long-term administration does not lead to complete clearance of viral cccDNA from infected cells (98 , 99). Other steps of the replication cycle are potentially relevant for the development of specific inhibitors such as viral entry into the cell, the priming of reverse transcription, the RNaseH activity of the viral polymerase, nucleocapsid assembly, virus packaging and morphogenesis, etc. (see Figure 2).

As the selection of drug resistant mutants is a major clinical concern, the search for new polymerase inhibitors with different cross-resistance profile is still very important for the rescue of treatment failure and the development of combination therapy strategies (100). Other viral target for antiviral therapy should be evaluated as the combination of drugs with different mechanisms of action may help to obtain antiviral synergy and to prevent the selection of drug resistant mutants. Several viral targets are being evaluated in experimental models with : 1) entry inhibitors, such as myristylated pre-S1 peptides that inhibit HBV infection in tissue culture and animal models (101), 2) agents that inhibit nucleocapsid assembly and/or pre-genomic RNA packaging (102 , 103), 3) compounds that inhibit viral morphogenesis such as iminosugars which inhibit the glycosylation of viral envelope glycoproteins (104).

Another challenging target for viral replication inhibition is the formation of the recalcitrant cccDNA. Indeed, cccDNA is one of the major determinants of viral persistence requiring long-term treatment and therefore exposing patients to the risk of drug resistance (105 , 106). However, none of the approved nucleoside analogs has been shown to inhibit directly the formation of cccDNA from incoming virions. These polymerase inhibitors have an indirect effect on cccDNA formation by inhibiting viral DNA synthesis and therefore reducing nucleocapsid recycling to the nucleus and in turn cccDNA amplification. It is now possible to quantify cccDNA in liver biopsies of patients undergoing antiviral therapy (107) and to determine its transcriptional activity (108). Efforts are still needed to find strategies that can specifically inhibit its formation or its transition from an transcriptionally inactive state to an transcriptionally active state. The persistence of low levels cccDNA in the liver of treated patients may not be deleterious if the residual viral replication is controlled by the specific anti-HBV immune response, especially by the TH1 response (109 –112).

The use of immune modulators in the therapy of chronic hepatitis B

The understanding of the mechanisms involved in the spontaneous viral clearance are critical for the design of new regimens or novel concepts of antiviral therapy. In the transgenic mice and chimpanzee models, it was shown that a non cytolytic TH1 response decreases viral replication and the number of cells supporting viral gene expression and replication. This antiviral effect is mediated by the in situ expression in the infected liver of cytokines such as interferon gamma, interleukine 12, and tumor necrosis factor alpha, leading to the curing of infected cells (113 –115). On the other hand, in other hepadnavirus infection models, ie the duck and woodchuck models, it was shown that spontaneous and antiviral mediated viral clearance mainly involves the lysis of infected cells and cell turnover. The latter generates non infected cells and dilutes the remaining infected cells (116 –119). It was also shown that the rapid resolution of viral infection involves the production of anti-envelope antibodies that may neutralize the circulating virions.

Whether or not, the differences observed are due to the specific animal models that have been studied remains to be determined. However, based on all these data as well as clinical observations, it seems that a concerted and timely action of a cytotoxic and non cytotoxic CD8 response, with hepatocyte turn-over and production of neutralizing antibodies is required to clear acute viral infection from the liver (115 , 118). In chronically infected patients, resolution of chronic hepatitis B likely represents a long-term host immune control and not a cure or complete clearance of infection as viral cccDNA was shown to persist in serologically cured individuals (107 , 120 , 121). This may have important implications in terms of antiviral therapy of chronic hepatitis B in the clinical setting for the design of improved protocols using nucleoside analogs or antiviral cytokines to achieve a sustained control of viral replication.

So far, the combination of polymerase inhibitors and interferon alpha (pegylated or not) has not proven to be more effective than either regimen alone in terms of sustained virologic response (9 , 21). This may be due to the fact that the vigor and time of restoration of immune response during nucleoside analog therapy may greatly vary from patient to patient and that interferon alpha may have not been administered in a timely manner. Other approaches are under evaluation, such as the combination of nucleoside analogs and immunomodulatory strategies based on vaccine therapy or the administration of TH1 cytokines. Interestingly, it was shown in experimental models of HBV infection that envelope protein recombinant vaccine therapy may increase the rate of sustained immunological response in woodchucks treated with clevudine (122), and that a viral envelope expressing DNA vaccine administration may enhance the antiviral activity of adefovir leading to an increased rate of viral clearance in the duck model (123 , 124). DNA vaccine in a transgenic mouse model also induced a specific antiviral response : CD8+ or CD4+ T lymphocytes from immunocompetent DNA-immunized animals were sufficient to control viral gene expression in the livers of the recipient transgenic mice. This effect was mediated by a cytokine-dependent mechanism common to both T cell subpopulations. This mechanism did not require cell lysis, but involved the production of IFN-gamma by the activated T cells (125). On the other hand, it was shown that the intrahepatic delivery of IFN gamma, a TH1 cytokine, in woodchucks in which viral load was significantly decreased by a combination of two nucleoside analogs (clevudine and emtricitabine), did not increase the rate of clearance of cccDNA and infected cells from the liver of these animals (57). However, in the woodchuck model, it was also shown by other investigators that IFN gamma does not deplete viral replicative intermediates within infected cells (126). Other studies using DNA based vaccine in the woodchuck model of hepadnavirus infection also led to interesting results in terms of protective immunity but results in terms of therapeutic response in chronically infected animals are not yet satisfactory (127 , 128). More studies are therefore required to gain more insight in the immunological response against HBV infected cells and its therapeutic consequences.

Competitive Environment

There are now several cytokines and nucleoside analogs which are approved for the treatment of chronic hepatitis B. Other nucleoside or nucleotide analogs are under experimental evaluation or in early stage of clinical trials. The novel immune modulation strategies rely mainly on vaccine therapy, and several studies are ongoing. The main drugs or compounds commercially available or in development are shown in Figure 3 .

Potential Development Issues

Chronic hepatitis B is a major disease which is the leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. The demonstration that the control of viral replication leads to an improved clinical outcome even in patients with advanced liver disease has led to the development and approval of new drugs in the past 10 years. These new antiviral agents are most welcome because of the risk of emergence of drug resistant mutants. It was also shown that the occurrence of antiviral drug resistance is associated with a worsening of the liver disease. This is becoming a major concern as long-term therapy is needed for chronic hepatitis B. The availability of these new drugs has been a major advance in this field as it is now possible to combat antiviral drug resistance at early stages and adapt antiviral treatment to maintain patients in clinical remission. This allows to propose « à la carte » therapy depending on the treatment history of the patients, and on the pattern of mutations in the circulating viral strains (75). This is of particular importance, since several cohort studies have shown that the development of liver cirrhosis and hepatocellular carcinoma is associated with high viremia levels.

The evaluation of nucleoside analog combinations is also discussed to prevent the development of drug resistance as the efficacy of the newer drugs is increasing, indicating that long-term studies on large cohort of patients will be required to demonstrate the efficacy of the combination therapy versus tailored add-on therapy in case of treatment failure. Furthermore, cost-effective analysis taking into account the cost of several factors such as liver disease management, drugs, treatment monitoring, and the cost of drug resistance, are urgently needed. On the long-term, the treatment of this disease will need novel antivirals as the risk of HBV drug resistant mutants is increasing with the duration of therapy. These drugs will need to target other steps of the viral life cycle, i.e. different from the viral polymerase activity, and to have an excellent safety profile since most patients will require long-term therapy. This should be kept in mind, with the Fialuridine catastrophe which led to mitochondrial toxicity and the death of several patients with lactic acidosis (129 , 130). Another challenge will be the development of novel approaches to stimulate the anti-HBV immune response and obtain a long-term control of viral replication after treatment withdrawal (109).

Expert Opinion

Antiviral therapy of chronic hepatitis B remains a major challenge. The long-term control of viral replication will need more research effort to better understand the viral life cycle and identify new targets for the inhibition of viral replication (Figure 2). A more detailed understanding of cccDNA formation and persistence is needed to determine whether specific inhibitors might be developed without interfering with cellular functions. The role of the viral X protein which has been shown to be required for the establishment of viral infection needs to be deciphered. Indeed, a better knowledge of its functions might lead to novel HBV inhibitors. Other targets are under experimental investigation such as viral entry into the cell and viral morphogenesis. This may lead to new classes of antivirals which will be important for the management of the disease. Indeed, the identification of new classes of inhibitors may help to obtain antiviral synergy and prevent drug resistance.

The understanding of viral persistence and escape to the anti-HBV immune response is another key issue. Indeed, a better knowledge of the innate and adaptive immune response against HBV might help to develop strategies to overcome the immune tolerance state which is often associated with chronic hepatitis B and is one of the reason why long-term therapy with nucleoside analogs is required. However, this will be a challenge as most chronic carriers have been infected for decades at the time of diagnosis and the chances to overcome immune tolerance might be thin even with new approaches relying on DNA vaccine.

A goal that is realistically achievable in a near future is a prolonged control of viral replication using combination of antivirals in a rational way. This implies a detailed knowledge of the cross-resistance profile of each antiviral available on the market and the characterization of the viral strains circulating in the patients at the time of treatment adaptation (Table 1). In this respect, the development of robust genotypic and phenotypic assays will be more and more needed for the treatment of chronic hepatitis B. Furthermore, standardization of definitions of treatment response and failure in clinical trials and in clinical practice is mandatory. Treatment recommendations using algorithms based on in vitro data of the antiviral activity profile of the drugs and clinical experience are urgently needed to guide clinicians in the treatment of patients at individual level.

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Figure 1

the main antiviral agents and their mode of action

Treatment options for chronic hepatitis B

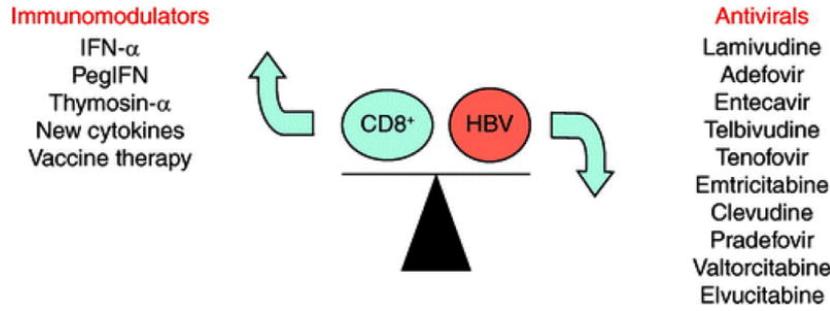


Figure 2

the HBV life cycle and the main targets for antiviral therapy CccDNA : covalently closed circular DNA ; mRNA : messenger RNA ; pg RNA : pregenomic RNA ; RC DNA : relaxed circular DNA.

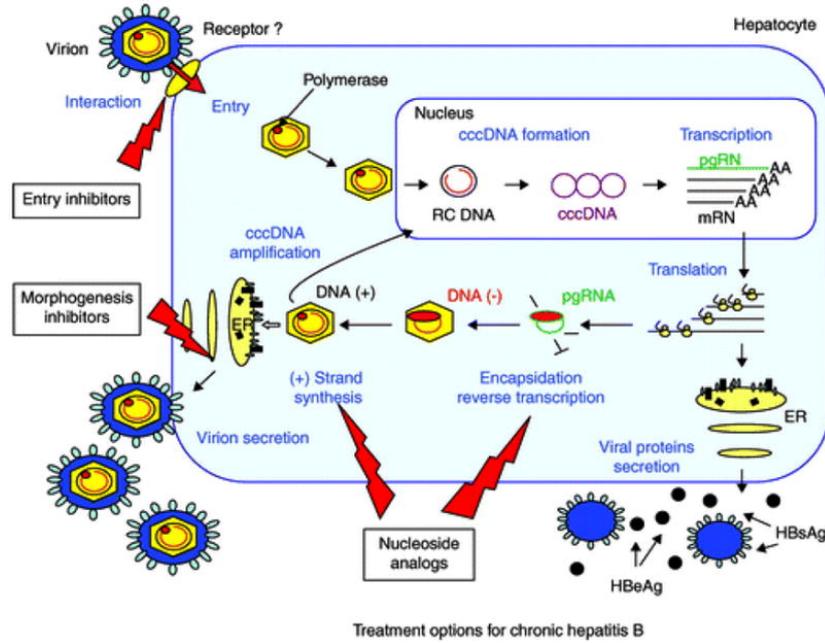


Figure 3

the main classes of antiviral agents and their chemical structures The structures can be found on the PubChem Compound website at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>

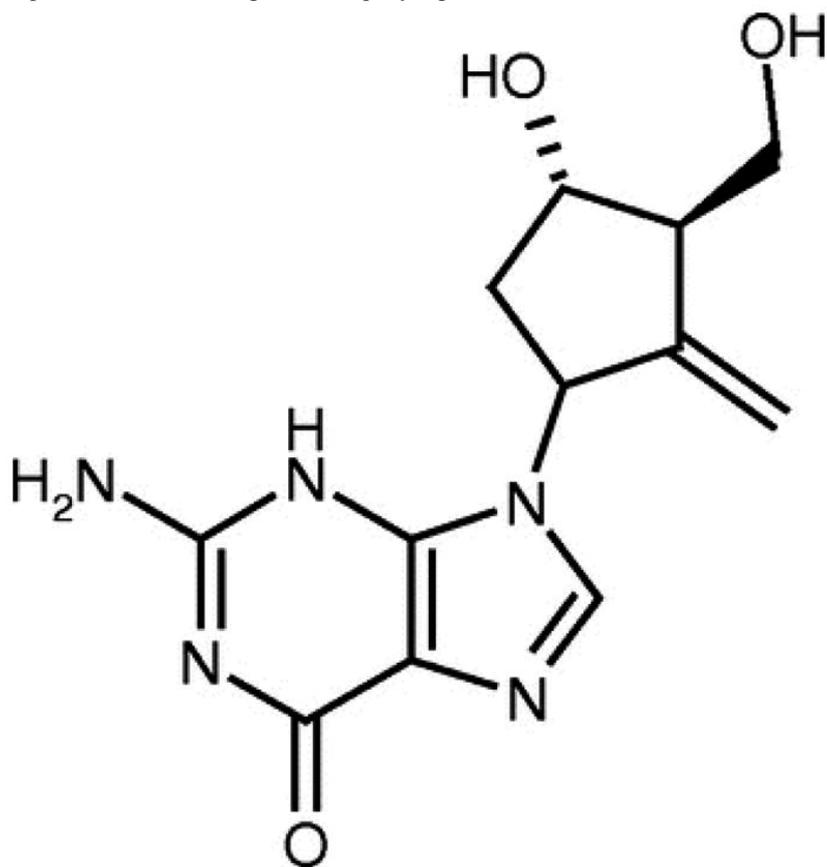


Figure 4

the rate of resistance to antivirals in nucleoside naive patients, and in lamivudine refractory patients The resistance rates are derived from published clinical studies (29 , 43 , 44 , 46 , 69 , 93 , 131)

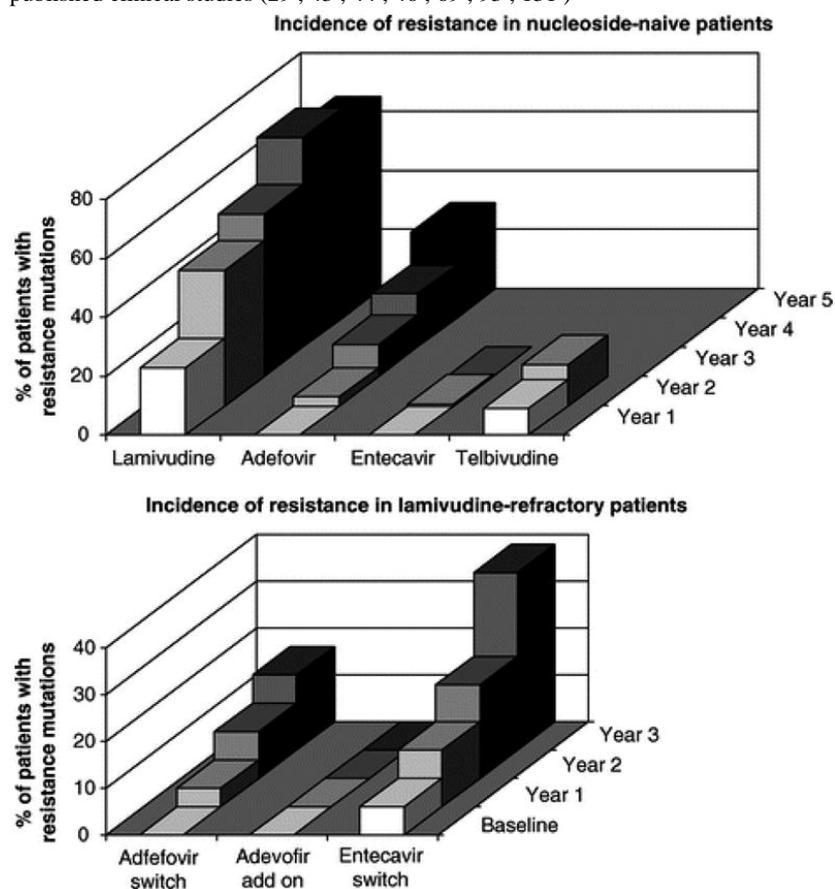


Table 1

Results of in vitro cross-resistance of hepatitis B virus mutants to antiviral agents.

	Resistance mutation				
	L180M + M204V	M204I	N236T	A181V	S202G/I* + M250I*
Drugs with reduced activity	Lamivudine	Lamivudine	Adefovir	Adefovir	Entecavir
	Emtricitabine	Emtricitabine			Lamivudine
	Clevudine	Telbivudine			Telbivudine
	Telbivudine				
	Elvucitabine				
Drugs with intermediate activity	Entecavir	Entecavir		Lamivudine	
Drugs that remain active	Adefovir	Adefovir	Lamivudine	Tenofovir	Adefovir
	Tenofovir	Tenofovir	Emtricitabine	Entecavir	Tenofovir
			Entecavir		
			Telbivudine		

* In the presence of primary resistance mutations (L180M + M204V).

Results from phenotypic assays published in the literature allow drugs to be assigned as having reduced activity, intermediate activity or activity against the different hepatitis B virus mutants depending on their relative activity as compared with wild-type virus [40, 43, 82, 87-89, 130-133]. The thresholds to distinguish these different categories are still debated because phenotypic assays are not commercially available and may vary from one laboratory to another, and because the translation of in vitro results to the clinic also depends on the specific pharmacodynamics of each drug. For instance, in vitro studies may show an intermediate activity against a given mutant, but the drug may still show antiviral efficacy in patients, at least initially; as we do not have a long-term clinical follow up for all mutants and drugs, it is, therefore, difficult to know if such drugs with intermediate activity may then select these mutants in vivo or select even more complex mutants with secondary mutations, or if the drug will overcome this intermediate activity by its antiviral potency in vivo.