Hepatitis B Virus Drug Resistance and Combination Therapy

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Abstract

Despite the development of new nucleoside analogs, antiviral therapy of chronic hepatitis B remains a major clinical challenge. Indeed, because of both viral genome persistence and hepatitis B virus genome heterogeneity, these antivirals may select for drug-resistant mutants. The development of drug resistance is associated with liver disease progression. The progress made in virologic assays also allows a better monitoring of antiviral therapy, which enables an early treatment adaptation based on the knowledge of cross resistance between antivirals before clinical deterioration. The management of antiviral drug resistance relies on the addition of a second drug having a complementary cross-resistance profile. This has evolved towards earlier intervention at the time of virologic breakthrough, and currently it is even recommended to adapt antiviral treatment in case of incomplete viral load suppression during the first year of therapy. The concept of de novo combination therapy to significantly prevent the delay of the occurrence of hepatitis B virus drug resistance is discussed and awaits confirmation by clinical trials. (Hepatology Rev. 2007;4:34-47)

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Key words

Introduction

The emergence of viral resistance during treatment is becoming an important clinical issue for hepatitis B virus (HBV) antiviral therapy. Considerable progress has been achieved in the efficacy of treatment, with the development of new drugs that allow a sustained suppression of HBV replication, or at least maintaining the viral load below a clinically relevant threshold. However, although most of the drugs currently registered for the treatment of chronic hepatitis B are effective in suppressing viral load, long-term therapy is required to avoid viral reactivation and progression of liver disease. Because of the variability of the HBV genome, such long-term treatments are associated with the emergence of resistant viral strains, which may compromise the initial clinical benefit of the treatment.

Mechanisms of HBV Drug Resistance

A number of factors are involved in antiviral drug resistance during HBV treatment, including the level of viral replication, the frequency of mutation in the HBV genome, the replication fitness of the mutants, and the available space for viral replication. The genetic barrier for resistance defined by the number of mutations required on the same viral genome to confer drug resistance is also an important parameter, as the addition of mutations in the viral polymerase gene and the overlapping surface gene may result in less-fit mutants.

Viral Persistence

Viral persistence is responsible for treatment failure and for most of the clinical complications of the disease. One of the major determinants in the slow kinetics of HBV clearance is the presence of a replicative form of HBV-DNA, termed covalently closed circular DNA (cccDNA). The cccDNA is generated from viral DNA contained in the incoming virions after cell entry, and from new synthesized viral DNA contained in newly produced nucleocapsids, which are recycled back to the nucleus of infected cells. Viral DNA enters into the nucleus where it is transformed into supercoiled cccDNA. During chronic HBV infection, cccDNA is maintained in the hepatocyte nuclei at a level of 30-50 copies and persists with a long half-life in infected cells. Furthermore, it was shown that antiviral therapy with nucleoside or nucleotide analogs cannot prevent the initial formation of cccDNA in infected cells, indicating that persistent viremia during therapy leads to the infection of new cells, therefore, the use of more potent antiviral drugs may provide a clinical benefit by decreasing the rate of infection of new hepatocytes during therapy. This pool of DNA represents the transcriptionally active form of viral genome. This viral genome form acts as a reservoir for the reactivation of viral genome replication and is responsible for viral relapse after withdrawal of antiviral therapy or in case of immune suppression. Therefore, it is clearly established that the stability and replenishment of cccDNA in infected cells play a key role in the difficulty in eradicating chronic HBV infection with current antiviral agents. For instance, results of quantification of intrahepatic cccDNA during adefovir dipivoxil therapy showed that the complete clearance of viral cccDNA may require more than 10 years of therapy.

In addition to the critical role of cccDNA, host-related factors may have an impact on viral persistence. Since HBV is not directly cytopathic, chronic hepatitis B results from an inadequate adaptive host immune response against infected cells that is not sufficient to control active viral replication and that leads to a chronic inflammatory activity. Due to this defective antiviral immune response, hepatocytes have usually a long half-life, which contributes to the persistence of the virus in the liver. It was shown by mathematical modeling that the half-life of hepatocytes may vary widely from 30 to 100 days, depending on the vigor of the individuals’ immune response.

Another major factor involved in viral persistence during the chronic phase of the disease and in the selection of viral-resistant strains is the viral genome variability. Molecular studies of HBV have shown that simple mutants preexist in the overall population of HBV prior to therapy. This intrinsic genetic variability is the consequence of spontaneous errors occurring during the reverse transcriptase step at each replication cycle. It is due to the fact that HBV does not possess DNA repair mechanisms that maintain the stability of viral DNA during replication by the elimination of incorrectly inserted nucleotides. Thus, considering the high levels of virus production that characterize HBV infection, this results in an important heterogeneity of the viral genome, and the generation of numerous viral mutants which circulate in the patients’ blood and which are called quasispecies.

Viral quasispecies within the same patient evolve during the course of infection, different variants or mutants being selected at different stages of infection in response to the host immune response or antiviral therapy, depending on their intrinsic fitness. A classification system was developed to define HBV genotypes. A genetic variability of more than 8% of the overall genome results in the classifica-
Selection of HBV Mutants

Long-term antiviral therapy with nucleoside analogs is associated with the selection of viral strains carrying specific mutations in the polymerase gene, which render them insensitive to the drug. Among genetic variants of HBV emerging under the selection pressure, polymerase gene variants appear to have the most important clinical implications in the development of resistance to antivirals. Different mechanisms are involved in drug resistance under antiviral therapy.

First, as described above, a complex mixture of genetically distinct variants develops under selective pressure. A newly acquired or a preexisting mutation conferring a selective advantage to a variant will generate a virus that is more viable and can spread more rapidly in the liver, allowing the corresponding mutant to accumulate and become the dominant species in the infected liver in the presence of the antiviral drug. The kinetics of replacement of wild-type virus in liver cells by a dominant mutant is generally slow. Indeed, resistant mutants mainly infect uninfected cells. The spread of the dominant mutant therefore depends on the availability of a free liver space for its replication. Therefore, several months of antiviral therapy may be needed for the immune system to remove the hepatocytes containing wild-type virus and to generate new cells that are susceptible to infection by viral drug-resistant mutants. On the other hand, the infectivity of antiviral drug-resistant mutants may have a major impact on the rapidity of selection of these strains during therapy. For instance, some mutations in the overlapping surface gene may result in reduced viral fitness. The level of resistance to a drug conferred by a given mutation may have profound implication on the fitness of the mutant. This may explain the difference in drug resistance rates observed with the different antivirals.

The Main Drug-Resistant Mutants

Nucleotide substitutions are the result of mutations in the viral genome. They may remain silent or lead to a decreased susceptibility to a given antiviral agent. The main mutations conferring resistance to nucleoside analogs have been characterized. A model for the selection of the main drug-resistant mutants is presented in figure 1.

HBV Mutants Resistant to Lamivudine and Other L-nucleoside Analogs

As lamivudine (3TC) was the first HBV reverse transcriptase inhibitor to be approved for HBV chronic infection, the mechanism of emergence of lamivudine resistance has been extensively studied. The main mutations associated to lamivudine resistance have been mapped within the catalytic site in the C sub-domain of the YMDD motif, corre-
HBV Mutants Resistant to Adefovir Dipivoxil

Long-term administration of adefovir dipivoxil (ADV) has been shown to select mutations conferring phenotypic resistance to adefovir: rtA181T/V in the B domain of the polymerase, and rtN236T in the D domain. Phenotypic analysis of the adefovir-resistant strains showed that the rtN236T and rtA181V mutations confer only a weak decrease in drug susceptibility \textit{in vitro} (usually 4- to 14-fold for the rtN236T and 2- to 3-fold for the rtA181V mutation). These levels of resistance to adefovir are much lower than that seen for lamivudine resistance mutations, indicating that these mutants are less susceptible to adefovir but not fully resistant. This is likely to explain the slower rate of resistance and the delay observed in the emergence of adefovir-resistant strains \textit{in vivo}. Information on the true fitness of the rtN236T and rtA181V mutants, which may impact on the rate of resistance, is still lacking; it is known that these mutants do not exhibit significant replication impairment \textit{in vitro}, but their capacity to spread in noninfected cells has not been studied so far. Because of the \textit{in vivo} pharmacodynamics of adefovir leading to a lower antiviral efficacy of the 10 mg dosage compared to the 30 mg dosage, these changes in drug susceptibility are significant enough to be responsible for viral breakthrough, hepatitis flares, and hepatic decompensation in some patients. Another mutation leading to an rtI233V change was described to confer primary resistance to adefovir, but this was not confirmed in another study suggesting that this mutation may represent only a genetic polymorphism (K. Borroto-Esoda, et al., EASL 2007).

HBV Mutants Resistant to Entecavir

There are recent reports of HBV resistance to entecavir (ETV). Several genotypic profiles have been characterized. First, entecavir resistance has been observed in patients who previously failed lamivudine treatment. In this case, resistant mutations occur on viral genomes already harboring “lamivudine-resistant” mutations. These latter strains have a reduced susceptibility to entecavir, but still remain partially sensitive to the drug; they can be considered as “primary” resistance mutations. However, additional substitutions render them fully resistant to entecavir by restoring viral replication capacities in the presence of the drug. Susceptibility to entecavir may be decreased by > 100-fold, particularly when entecavir “secondary” resistance mutations (including I169T and M250V, or T184G and S202I/G) are present. In contrast, these “secondary” resistance mutations, when present alone, are not associated with resistance. This suggests that an accumulation of primary and then secondary mutations is required for the development of entecavir resistance. A few cases of entecavir resistance have been observed in nucleoside-naive patients, and the same process of selection of primary and secondary resistance mutants was described.

Definition of Resistance

Since early detection of viral drug resistance has important clinical implications, standardized definitions were proposed. These terminologies are mandatory to compare the antiviral efficacy of the different drugs across the trials and also to help clinicians in the early detection of antiviral drug resistance and tailoring of antiviral therapy. Clinically, one may define resistance to antivirals in patients with chronic hepatitis B at different levels as follows (Fig. 2).

Genotypic Resistance

This corresponds to the detection of mutations in the HBV genome that have been previously demonstrated to confer resistance to the drug during antiviral therapy. Since appearance of amino acid substitutions in the reverse transcriptase region is considered a good predictor of subsequent virologic breakthrough, monitoring of genotypic resistance...
is considered an important tool for the detection of resistant strains before the development of true drug resistance. This is especially true for patients who present incomplete viral load suppression during antiviral therapy.

Virologic Breakthrough

This usually follows genotypic resistance. It corresponds to a rise in serum HBV-DNA levels of at least 1 log_{10} copies/ml compared to the lowest value during therapy (nadir value), in two consecutive samples one month apart, in patients who have previously responded and have good treatment compliance.

Clinical Breakthrough

This is defined by an elevation in serum alanine aminotransferase (ALT) levels following virologic breakthrough in patients who previously showed transaminases normalization under treatment. It has been reported that there is a lag period between the appearance of a virologic breakthrough and elevation in serum ALT levels, which may remain normal for several weeks or months after the rise in viral load. It may result sometimes in hepatitis flares, but in all cases worsening of liver histology results from this clinical breakthrough.

Phenotypic Resistance

This refers to a decreased susceptibility to inhibition by antiviral drugs measured in vitro in tissue culture of HBV strains isolated from patients showing virologic breakthrough, and which harbor polymerase gene mutations responsible for treatment failure\textsuperscript{51}.

Diagnosis of Resistance

Viral Load Monitoring

The level of viral load was found to be an important risk factor for the progression of liver disease, including the development of liver cirrhosis and hepatocellular carcinoma\textsuperscript{52,53}. During antiviral therapy, a rise of 1 log or more in serum HBV-DNA levels in treatment-compliant patients is usually the consequence of the selection of drug-resistant viral strains.

With the rapid development of molecular technologies, new tests are becoming available. Assays that are both sensitive and quantitative must be used to determine the antiviral efficacy and to detect as soon as possible a rise in HBV-DNA levels reflecting the emergence of drug-resistant viral strains\textsuperscript{54,55}. Real time polymerase chain reaction (PCR) assays appear to be the most sensitive among the current methods to quantify viral copy number and provide an accurate monitoring of antiviral efficacy and virologic breakthrough\textsuperscript{56}.

Due to the diversity of methods used to quantify HBV-DNA levels, significant variations were observed in viral load results. In order to enable standardization of viral load results and allow comparison between studies, the World Health Organization (WHO) has established an international reference standard for HBV-DNA units, set as IU/ml\textsuperscript{57}.

\textbf{Figure 2.} Rationale for de novo combination therapy. MDR: multidrug resistant.
Genotypic Assays

The detection of polymerase mutations by sequencing, line probe assay, or DNA chip technologies is the best way to identify resistant strains of hepatitis B to new drugs. As discussed above, the emergence of drug resistance is due to the selection of a given mutant that was not detectable prior to treatment and that has become dominant among the quasispecies during antiviral therapy. Since there is a lag between the spread of the mutant virus in the liver and the rise in viral load, it may prove useful to detect mutations selected during treatment before viral breakthrough and the appearance of clinical symptoms. Genotypic assays are based on the amplification of HBV genome by PCR, and the detection of mutations by sequencing or hybridization techniques. They should be performed at the time of virologic breakthrough or even before when viral load is incompletely suppressed after several months of therapy. This allows the tailoring of antiviral therapy to the virologic situation of the patient to facilitate the individualized treatment of each patient. Ideally, the HBV genomic sequence of treated patients who have developed virologic breakthrough should be compared with the HBV sequence from the same patient before therapy or from known sequences of the same HBV genotype, i.e. HBV resistance databases.

Sequencing assays allow the detection of new mutations. They can identify HBV mutants when they represent at least 20% of the whole viral quasispecies. Specific hybridization assays, such as the line probe assay, allow the detection only of known mutations, but are usually more sensitive; they can detect minor mutants representing as low as 5% of the viral quasispecies. Genotypic assays provide important information on the major mutants circulating in the patients at a given time point of antiviral therapy. With the knowledge of cross resistance for each known mutant, antiviral treatment can be adapted to the pattern of HBV mutations. This is particularly important, as an increasing number of patients have been exposed to multiple drugs and are at risk of developing multidrug-resistant mutants.

Up to now, HBV genotyping remains expensive, and health insurance providers in most countries do not cover its cost. Efforts should be made to convince the health authorities of the utility of HBV genotyping technologies, as this was the case with monitoring anti-HIV therapy. On the other hand, new tools based on DNA chip technologies are in development and may become available in the future, allowing an automated determination of HBV genotypes and of the most clinically relevant mutants.

Phenotypic Analysis of HBV Clinical Isolates

Phenotypic assays are used to demonstrate that a given mutation selected in vivo is indeed conferring resistance to antiviral drugs. These assays detect in vitro a difference in drug susceptibility by comparison with wild-type HBV. The standardized classification determines several degrees of antiviral drug resistance, defined as high (> 100-fold increase), intermediate (10- to 100-fold increase) or low-level (2- to 10-fold increase) in effective concentration of the drug required to inhibit 50% of the target (EC)
comparing the wild-type HBV reference. Unfortunately, the classification does not always translate to the in vivo situation as it does not take into account the in vivo pharmacokinetics/pharmacodynamics of the drug. For example, it has been shown that, in the case of adefovir, a low-level increase in EC50 in vitro is sufficient to drive treatment failure. Phenotypic assays can also be used to determine whether a given resistance mutation is conferring cross resistance to other drugs (Table 1). This information has major clinical implications for treatment adaptation in case of incomplete viral load suppression or virologic breakthrough.

With the rapid development of new drugs increasing the number of drug-resistant mutants that can be selected, this drug susceptibility testing may become an important tool for the recognition of resistance mutations and for the evaluation of the effect of the available drugs on the newly characterized mutants. Used in addition to genotypic assays, phenotypic assays may help select the best treatment strategy in patients infected with drug-resistant strains.

Incidence of Resistance

Lamivudine administration may be associated with a rapid development of mutants. The incidence of resistance to lamivudine increases with the duration of treatment, with resistance being observed in 22% of treated patients after one year, rising to 38% after two years, 53% after three years, and 66% after four years.

Available data concerning resistance to adefovir dipivoxil were provided by a five-year cohort study in HBeAg-negative patients. The rate of genotypic resistance to adefovir was found to be lower than with lamivudine, while the time lag between the beginning of treatment and outbreak of resistance was longer. The cumulative rate of adefovir resistance was 0% after one year, 2% after two years, 5.9% after three years, 18% after four years, and 29% after five years.

The resistance rate to entecavir has been evaluated from studies covering three years of treatment. The results of clinical trials have shown a low rate of resistance (<1%) in nucleoside-naïve patients. However, the cumulative rate of resistance is much higher in patients treated by entecavir for lamivudine failure, reaching up to 30% of patients after three years of therapy.

Due to the lack of long-term clinical studies, the resistance rate to other antiviral drugs is difficult to estimate. The frequency of resistance to emtricitabine is 13% after one year, and 18% after two years. With telbivudine, clinical trials reported a resistance rate of 4.5% after 48 weeks of therapy. Resistance to tenofovir has been described by one group, but this was not confirmed by another study. It is noteworthy that most clinical studies with tenofovir have been performed in HIV/HBV-coinfected patients who received a combination of tenofovir and lamivudine or emtricitabine as part as their HAART regimen, which may explain the lack of resistance to tenofovir observed up to now.

Clinical Consequences of Resistance

Most of the data concerning the clinical consequences of HBV resistance have been obtained from studies performed with lamivudine. Results of various studies clearly demonstrated that resistance to antiviral treatment was associated with clinical deterioration with a wide spectrum of presentations, ranging from slow progression to acute exacerbations and liver disease decompensation. Studies with lamivudine have shown that this deterioration often followed the same scenario with firstly, outbreak of genotypic resistance, followed by increase of viral load, then by increase in ALT levels and worsening of liver histology. It was also shown that the duration of infection with a lamivudine resistance mutant was associated with an increased risk of ALT elevation or flare.

Some studies also suggest that patients with precore mutants and/or liver cirrhosis tend to develop more severe symptoms at the time of lamivudine resistance development. It has been shown also that immunocompromised patients (HIV/HBV-coinfection or liver transplantation) have a higher rate of lamivudine resistance accompanied by clinical breakthrough with acute exacerbations or rapid progression of liver disease. It has been suggested that combination therapy should be used in such populations of patients in order to minimize the risk of resistance development and of clinical deterioration.

The same scenario was shown to occur with adefovir resistance, although at a slower rate, first with detection of adefovir resistance mutants, followed by the increase in viral load and ALT elevation; some patients presented a liver decompensation.

Treatment of HBV Drug Resistance and its Prevention

Although antiviral therapy of chronic hepatitis B has significantly evolved over the last decade with...
the development of new antiviral agents, it is still not possible to eradicate the virus. As the emergence of virologic resistance during therapy is becoming an issue for long-term anti-HBV therapy, the management of patients should involve effective monitoring of them, an appropriate choice of the initial drug, and early treatment adaptation, as soon as resistance has emerged to maintain patients in remission. More proactive strategies to prevent the development of drug resistance are also discussed.

Monitoring of Antiviral Therapy and Detecting Drug Resistance

The major goal in the treatment of chronic hepatitis B is to obtain sustained viral suppression and eventually HBe and HBs seroconversion. Since HBV infection can remain clinically silent for long period of time, it is important to identify early changes in serum viral DNA levels in treated patients, which may reflect the selection of drug-resistant mutants, before clinical deterioration. The risk of emergence of drug-resistant mutants mandates that patients receiving antiviral treatment for chronic hepatitis B should be closely monitored.

The timing of monitoring is also determined by the clinical state of the patient. A three-monthly assessment is required for patients to follow the response to antiviral therapy and to allow a prompt detection of primary suboptimal response or secondary emergence of drug resistance. In patients with severe disease, more frequent monitoring might be appropriate.

Predictive Factors of Resistance

The occurrence of HBV drug resistance is influenced by several factors, which can be divided into pretreatment and on-treatment risk factors, which are related to the infected host or to the virus itself. These predictive factors are well known for lamivudine; less information is available for newer drugs such as adefovir, entecavir, or telbivudine. Pretreatment factors of lamivudine resistance include high viral load, increased body mass index, high serum ALT level (> three-times the upper limit of normal value) and high liver inflammatory score index on liver biopsy examination. Other factors have been associated with a more rapid selection of resistant strains, such as high viral replication levels (high HBV-DNA and HBeAg positivity), ALT elevation, and previous treatment with nucleoside analogs. However, the main independent parameter found with multivariate logistic analysis was high HBV-DNA level at baseline. Regarding entecavir and adefovir, as the resistance rates are lower, no study has reported on baseline predictive factors of resistance.

Once therapy has been initiated, assessment of several factors during therapy should allow to detect promptly a primary or secondary treatment failure and to tailor antiviral therapy accordingly. These parameters include virologic markers (viral load suppression or viral breakthrough), serology (HBeAg or HBsAg loss and seroconversion), liver function tests, and ideally, genotypic assays. All four should be considered for an optimal management of drug resistance.

When available, genotypic assays should be performed at the time of virologic breakthrough in order to identify the selected mutant responsible for treatment failure and tailor antiviral therapy to this new viral strain. Interestingly, it has been clearly demonstrated that among these markers, viral load is a major determinant of therapeutic outcome. Although viral suppression below 4-5 log_{10} copies/ml was considered indicative of remission of liver disease activity, recent reports have shown that disease progression might occur in patients with a viral load as low as 4 log_{10} copies/ml. Furthermore, results of clinical trials have shown that persistent viremia during therapy is associated with an increased risk of HBV drug resistance development.

These findings are critical for the management of chronic hepatitis B treatment, emphasizing the importance of choosing the best first-line therapy, and in the case of incomplete viral load suppression during treatment, the importance of an early intervention to prevent the subsequent occurrence of antiviral drug resistance.

Impact of Viral Load Monitoring: On-treatment Prediction of Treatment Efficacy and Emergence of Drug Resistance

The objective of viral load levels measurement is twofold: firstly to monitor the magnitude of viral load suppression, and secondly to detect viral breakthrough as early as possible. Early during therapy, viral load assessment allows to confirm the initial antiviral response. At week 12, a failure to reduce HBV-DNA by 1 log_{10} IU/ml indicates primary treatment failure and is an indication to change the treatment regimen.

The next monitoring time point should be at week 24 of therapy. This measurement is considered essential in the management of both HBeAg-positive and HBeAg-negative patients because it was found to be the main predictor of subsequent treatment efficacy. Week 24 viral load allows the classification of virologic responses into complete (HBV-DNA < 60 IU/ml), partial (residual HBV-DNA levels < 2000 IU/ml or < 4 log_{10} copies/ml) or in-
adequate virologic responses (HBV-DNA levels \( \geq 2000 \text{ IU/ml} \) or \( \geq 4 \log_{10} \text{ copies/ml} \));55,58 In the phase III GLOBE trial, 921 patients were randomized to treatment for two years with lamivudine versus telbivudine and evaluated for response to antiviral therapy. The results of the study suggest that the magnitude of viral suppression at week 24 was strongly associated with clinical and virologic efficacy outcomes (HBeAg loss, suppression of HBV-DNA, ALT normalization, and viral breakthrough) at year one, irrespective of HBeAg serology at baseline. Among patients with undetectable HBV-DNA levels at week 24, 90% remain HBV-DNA negative (< 300 copies/ml) at week 52, and less than 1% developed resistance.68 Recent studies reported that week 24 HBV-DNA levels were also highly predictive of efficacy and resistance outcomes after two years of treatment.67

Regarding the emergence of HBV drug-resistant mutants, it is currently thought that the main on-treatment risk factor predicting resistance is persisting viremia. The correlation between on-treatment viral load levels and emergence of drug-resistance has been demonstrated with lamivudine, adefovir and telbivudine. In an Asian study, Yuen et al.18 showed that in patients receiving prolonged lamivudine therapy, viral load levels at week 24 of treatment predicted the subsequent occurrence of resistance. Genotypic resistance was detected in 63% of patients with HBV-DNA levels > 1000 copies/ml compared to 12% in patients with levels < 1000 copies/ml. These data strongly suggest that patients with low HBV-DNA levels (< 1000 copies/ml) after six months of therapy had a significantly lower risk of developing resistance compared to those with a higher viral load.66

The antiviral response at week 24 of therapy was also found to be a predictor of resistance in patients treated with telbivudine or lamivudine in the Globe trial. Higher rates of resistance at two years were observed when the week 24 viral load was > 1000 copies/ml compared to patients with a lower viral load at the same time point, whatever their initial HBeAg status.67 The rate of HBeAg seroconversion at year 2 was 46% in patients with undetectable viral DNA at week 24 versus 6% for those having viral DNA levels > 6 \( \log_{10} \) copies/ml at week 24. The rate of resistance to telbivudine at year 2 was 4% in HBeAg-positive and 2% in HBeAg-negative patients with undetectable HBV-DNA levels at week 24, versus 30 and 60% for patients with viremia levels > 4 \( \log_{10} \) copies/ml at week 24.

Adefovir dipivoxil was shown to suppress viremia levels with a slower effect by comparison with other nucleoside analogs, i.e. lamivudine, entecavir, or telbivudine. Therefore, the week 24 time point should not be used for predicting resistance to adefovir therapy. Interestingly, it was demonstrated in HBeAg-negative patients treated with adefovir dipivoxil for 192 weeks, that patients with HBV-DNA levels > 1000 copies/ml after 48 weeks of therapy had a higher risk of developing adefovir resistance at week 192. Seventeen out of 35 patients (49%) developed adefovir-resistant mutants, as compared to 6% (5/89) of patients with a viral load < 1000 copies/ml at week 48.42

With entecavir, the rate of resistance reported so far in nucleoside-naive patients did not allow to perform the analysis of on-treatment. Studies need to be performed in patients with lamivudine resistance who are treated with entecavir.

Altogether, a precise monitoring of viral load during antiviral therapy allows the diagnoses of primary treatment failure, insufficient viral suppression predicting a high likelihood of drug resistance development, or virologic breakthrough. In all these situations, an early intervention is mandatory to adapt antiviral therapy and control viral replication to prevent clinical deterioration.

Impact of Cross Resistance on HBV Drug Resistance Management

Cross resistance refers to the situation in which a decreased susceptibility to more than one antiviral drug is conferred by the same amino acid substitution or combination of amino acid substitutions. Results of in vitro cross-resistance testing have shown that lamivudine-resistant mutants (rtM204V or rtM204I mutants) are not sensitive to other L-pyrimidine analogs such as emtricitabine, clevudine, telbivudine and torcitabine, while they remain susceptible to purine analogs such as adefovir, tenofovir, and alamafivir; these mutants also have an intermediate susceptibility to emtricitabine, clevudine, telbivudine, and entecavir, while the rtA181V mutant has a reduced susceptibility to lamivudine.38-40,61,65,89 Although some level of cross resistance has been observed in vitro, rtN236T adefovir-resistant mutant is susceptible to lamivudine, emtricitabine, clevudine, and entecavir, while the rtA181V mutant has a reduced susceptibility to lamivudine.38-40,61,65,89

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The current knowledge suggest that the development of entecavir resistance follows a “two-hits” model, with the first selection of primary resistance mutations at position rt204, followed by the addition of secondary resistance mutations conferring full resistance to entecavir.46-48,90-92
This may explain why the development of entecavir resistance is more rapid in patients with lamivudine failure who already have selected the primary resistance mutations, by comparison with the nucleoside-naive patients in whom the whole process of selection of primary and secondary mutations needs to take place. Although strains harboring classic lamivudine resistance mutations exhibit an intermediate susceptibility to entecavir, they remain sensitive initially to entecavir in vivo when this is administered at a higher dose (1.0 mg daily). If additional secondary mutations occur, full resistance to entecavir is observed and is followed by viral breakthrough. This suggests that entecavir may not be considered as an optimal treatment for patients infected with lamivudine-resistant HBV, but a good treatment option in nucleoside-naive patients. If entecavir has to be prescribed in patients with lamivudine failure, lamivudine should be discontinued and entecavir prescribed at double dose.

Telbivudine is ineffective in vitro against the rtM204I mutant as well as the rtL180M + M204V mutant, but remains active against the rtM204V single mutant. This may explain why the single rtM204I mutant has been the only resistant mutant detected during the phase III trials of telbivudine.

Results of in vitro cross-resistance data are summarized in table 1.

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**Figure 3. Rationale for an add-on strategy instead of a switching strategy in case of HBV resistance.**

LAM: lamivudine; ADV: adefovir; R: resistant.

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**The Choice of First-Line Therapy to Prevent HBV Drug Resistance**

Since a limited number of drugs are available for the management of HBV infection, one of the most important treatment decisions is the choice of the first-line therapy prescribed to naive patients. Unfortunately, although drugs with a high genetic barrier for resistance are now available, it is clear that all antiviral monotherapies may be associated with the emergence of resistant strains. This represents the main challenge of long-term management in chronic HBV infection, although the incidence of antiviral drug resistance varies according to the antiviral drugs. Physicians have to prevent viral resistance as much as possible by choosing an appropriate regimen in which the risk of emergence of resistance is minimized. Current standard treatment practice is to start with monotherapy, using the most potent antiviral with the lowest rate of genotypic resistance. In any case, antiviral response should be closely monitored over time.

**Treatment of Lamivudine Resistance by Add-On Therapy**

In the past, salvage therapy was proposed to patients with lamivudine resistance and clinical breakthrough (high viral load and ALT elevation). There...
was a debate on whether adefovir dipivoxil switch or add-on to ongoing lamivudine was the best strategy. The knowledge of cross-resistance data and of the viral genome diversity advocates for an add-on therapy at an early stage, i.e. viral breakthrough, to control rapidly viral replication and prevent clinical deterioration (Fig. 3). Several studies have shown that switching from lamivudine to adefovir monotherapy was associated with a high incidence of adefovir resistance. A large, multicenter, Italian cohort study showed that rescue therapy with the addition of adefovir after development of virologic breakthrough in HBeAg-negative patients treated with lamivudine led to viral suppression for three years in most patients. None of the patients receiving the add-on strategy developed genotypic resistance to adefovir, by contrast to those who received adefovir monotherapy.

Another Italian study compared two groups of patients with known resistance to lamivudine: in one group, adefovir was added at the time of clinical breakthrough with high viremia levels, while in the other group, adefovir was introduced earlier at the time of viral breakthrough. The results clearly showed that treatment efficacy was improved when adefovir was started earlier, at the time of virologic escape.

Therefore, the current standard-of-care for lamivudine resistance is to add adefovir dipivoxil as early as virologic breakthrough occurs.

**Prevention of Drug Resistance by Early Add-On Therapy**

Several studies have shown the clinical impact of viral load suppression during therapy in terms of drug resistance development. The checkpoint was suggested to be week 24 for lamivudine and telbivudine, and week 48 for adefovir dipivoxil, as the latter is a slower acting antiviral. All clinical studies performed with these drugs showed that if the viral load is not sufficiently suppressed (i.e. > 3 log copies/ml), the subsequent risk of resistance is significantly high. It was therefore suggested that a second drug with a complementary cross-resistance profile should be added to prevent the subsequent development of drug resistance and maintain the control of viral replication. It should be noted that several clinical studies are ongoing to demonstrate the efficacy of this strategy.

**De Novo Combination Therapy to Prevent Drug Resistance**

The concept of combination therapy is emerging as a critical issue to prevent the selection of drug-resistant mutants. The rationale is based in part on the fact that simple mutants preexist in the viral quasispecies and are archived in viral cccDNA within infected cells, and that complex mutants harboring several mutations have less chance to preexist or to occur. Using a single agent followed by switching or add-on of another drug may exert a selective pressure on the viral quasispecies, allowing the selection of a first resistant mutant with a single resistance mutation. If viral load is not suppressed, additional mutations may occur in the quasispecies and in the preselected mutant, favoring the emergence of variants with multiple mutations conferring multidrug resistance, as already observed.

The current knowledge indicates that the add-on strategy would be efficient in preventing subsequent drug resistance only if (i) add-on is done early, (ii) using drug with a complementary cross-resistance profile, and (iii) viral replication is suppressed following treatment adaptation. The development of new therapeutic concepts based on *de novo* combinations requires clinical studies, as the clinical endpoint is the prevention of drug resistance. Indeed, none of the currently available drugs for combination have shown a synergistic antiviral effect in patients except with the combination of pegylated interferon (PEG-IFN) and lamivudine, which, however, cannot be prescribed for long periods of time because of side effects. Moreover, the combination of PEG-IFN and lamivudine has failed to demonstrate higher rates of sustained HBeAg seroconversion or of sustained HBV-DNA suppression after discontinuation of treatment in HBeAg-negative patients. The existing data on combination therapy with drugs having a complementary cross-resistance profile, i.e. lamivudine plus PEG-IFN or lamivudine plus adefovir, have shown a decreased rate of lamivudine resistance. It is interesting to note that *in vitro* experiments have shown that IFNα was equally effective on the main drug-resistant mutants as on wild-type HBV. Combination of nucleoside analogs with a complementary cross-resistance profile is currently the preferred option as it may prevent or delay significantly the development of drug resistance (Fig. 2). In this case, a synergistic antiviral effect is not expected with the currently available drugs which target the same viral enzyme, but the main goal will be the long-term prevention of drug resistance development. More clinical studies are therefore required with the newer drugs and with the appropriate control groups to demonstrate, in the long-term, the safety profile of the combination and its cost-effectiveness in preventive drug resistance.
Conclusion

Despite the development of new nucleoside analogs, antiviral drug resistance remains a clinical challenge. However, the availability of better tools for the monitoring of HBV infection and of drugs with complementary cross-resistance profiles has allowed to improve dramatically the management of antiviral therapy and that of drug resistance. Currently, it is possible to maintain the majority of patients in remission, provided that careful virologic monitoring and judicious treatment tailoring are followed. The main objective is to obtain a profound and sustained viral suppression, which is a prerequisite for liver histology improvement and prevention of drug resistance. Future clinical trials will tell if de novo combination, or very early add-on therapy when viral suppression is not achieved, is the best treatment option for chronic hepatitis B. Indeed, the management of antiviral drug resistance has evolved towards earlier combination or add-on therapy. The difficulty to obtain viral clearance and the long-term risk of antiviral drug resistance and development of hepatocellular carcinoma advocate the search for new drugs targeting other steps of the viral lifecycle to improve the results of antiviral therapy.

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References


Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:676-86.


