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REVIEW ARTICLE

Hepatitis B resistance in Iran

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ABSTRACT

Emerging new medications in the treatment of HBV produces hope and promises for curing of HBV. Over the past decade, the development of oral nucleoside/nucleotide analogs (NAs) with favorable potencies and tolerabilities has led to substantial advances in chronic hepatitis B (CHB) therapy. The oral anti-HBV agents currently approved are lamivudine, adefovir dipivoxil , entecavir , telbivudine, clevudine, and tenofovir. Treatment algorithms have been developed to assist in identification of suitable candidates for treatment and to determine and initiate appropriate treatment. In this review the problem of drug resistance in the course of chronic hepatitis B treatment are discussed in detail from both aspects of clinical and genetics.

Keywords: *Chronic hepatitis B, Drug resistance.*
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INTRODUCTION

Since discovery of HBsAg (Australia Antigen) in 1963 By Dr. B. S. Blumberg much progress has been achieved so far, beside of virology standpoints such as HBVDNA viral load, genotyping and mutations, each of them has definite role as risk factors in pathogenesis for developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). Emerging new medications in the treatment of HBV, many hope and promises for curing of HBV and related liver disease attract attention. Two billion people worldwide have evidence of hepatitis B virus exposure, and an estimated 400 million are actively infected (1). Worldwide, the prevalence of hepatitis B virus varies greatly. In hyperendemic areas, such as China, Southeast Asia, Western Pacific, and sub-Saharan Africa, the carrier rate exceeds 8% and transmission occurs mainly from mother to infant during parturition, as well as by horizontal transmission among children less than 5 years of age, and to a lesser extent between sexually active adults. In North America and Europe less than 1% are chronically infected, the result of injection drug use, sexual transmission, nosocomial infection, or emigration from endemic areas. In 30%, no clear mode of transmission is found (1).

At the beginning Interferon (INF) was used as a first line therapy for many years, but later on standard interferon alfa-2a has largely been replaced by peginterferon alfa-2a in routine practice. Over the past decade, the development of oral nucleoside/nucleotide analogs (NAs) with favorable potencies and tolerabilities has led to substantial advances in chronic hepatitis B (CHB) therapy. The oral anti-HBV agents currently approved are lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine,

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clevudine, and tenofovir (2). These NAs necessitate long, and in many cases, indefinite treatment to achieve sustained viral suppression. Unfortunately, because the duration of NA treatment is prolonged, the risk of development of drug resistance increases.

The goal of therapy for hepatitis B is to eliminate or significantly inhibit the replication of HBV and prevent the progression of liver disease toward cirrhosis, liver failure, HCC, eventually leading to transplant or death. Therefore, primary aim of treatment is to reduce HBVDNA by suppression of viral replication, which results in histological improvement and alanine aminotransferase (ALT) normalization and finally seroconversion of HBeAg and HBsAg, the highly desirable goal of antiviral therapy. Beside that, host factors has also definite role in developing liver disease and its complications. Although the national strategy with massive vaccination to prevent and eliminate transmission in the community has great impact on community acquired HBV. Due to high prevalence of HBV in many developing countries, including Iran, chronic HBV infection is considered a health problem influencing health economy in these countries. Even following Hepatitis B mass vaccination in the HBsAg positivity rate was 1.7% in first survey (95% CI: 1.6%-1.8%), and 17% (95% CI: 1.6%-1.9%) in the second (3).

Definition of HBV resistance to antiviral drugs

The selection of antiviral-resistant mutations is a major concern with long-term NA treatment. The rate of resistance depends on a number of factors, including pretreatment HBV DNA levels, potency and rapidity of HBV DNA suppression, prior exposure to oral antiviral nucleosides or nucleotide therapy, duration of treatment, and the degree of genetic barriers to resistance to the individual drug. The development of antiviral resistance is associated with loss of initial response and rebound of HBV DNA, which is followed by biochemical breakthrough and reversion of histological improvement, in some patients, resistance leads to sever exacerbation and progression of liver disease. There are several major risk factors for the development of resistance to NA, especially to LMV. These include a high level of HBV DNA, high serum levels of ALT, and high body mass index (4, 5). Prior therapy with NA, as well as inadequate viral suppression during therapy, has also been shown to induce drug resistance (4, 6). Transmission of drug-resistant mutants in newly infected patients is also likely to predispose to more rapid resistance once treatment is initiated, as it was shown for HIV infection. The long-term rates of resistance are highest for lamivudine (65-70% at 4-5 years) (7), intermediate for telbivudine (25% in HBeAg-positive patients and 11% in HBeAg-negative patients at 2 years) (7), lower for adefovir (29% at 5 years) (8), and lowest for entecavir in the absence of prior lamivudine resistance (1.2% at 5 years), and for tenofovir in treatment-naïve patients (0% at 1 year) (9). Patients with lamivudine resistance have a 51% rate of novel mutations after 5 years of entecavir therapy. Thus, when possible, it is beneficial to use the most potent NAs that possess the lowest risk of genotypic resistance as initial therapy for patients with nucleoside-naïve (7).

Mechanisms of selection and emergence of HBV drug-resistant

The main factors involved in the selection of escape mutants are: (i) the long half-life of hepatocytes and viral cccDNA; (ii) the HBV genome variability leading to a complex viral quasispecies and mutant archiving in cccDNA. The composition of the viral quasispecies evolves over time depending on the selective pressure including antiviral therapy and the host immune

response. Escape mutants may then spread in the liver and become the dominant species depending on their fitness (ie, their capacity to replicate and dominate wild-type strain in the presence of antiviral pressure) and the replication space available for their dissemination in the liver (10-13).

Relationship exists between the level of viral suppression with a drug and the probability of resistance development. If viral load suppression is low, the chance of resistance development is also low; with complete viral load suppression, the chance of resistance is low; but if we have moderate viral suppression, the chance of resistance is high. (14). And also the inverse correlation between serum levels of HBV DNA and CTL escape mutations of the core protein in HBeAg seroconverted patients, supports the notion that selection of CTL escape mutations consolidates the persistence of HBV infection despite reducing viral fitness (15).

However, compensatory mutations that can restore replication fitness frequently emerge during continued treatment leading to a progressive increase in serum HBV DNA which may exceed pretreatment levels. Thus, early detection and intervention can prevent hepatitis flares and hepatic decompensation, and this is particularly important in patients who are immunosuppressed and those with underlying cirrhosis. Another potential consequence of antiviral-resistant mutations is cross-resistance with other nucleotides. Serial changes in serum HBV DNA and ALT levels are in association with emergence of antiviral-resistant HBV mutants (16).

Detection and monitoring of resistance

Two types of mutations have been associated with treatment failure to NA: primary resistance mutations which are directly responsible for drug resistance, and secondary (compensatory) mutations, which promote or enhance replication competence. Compensatory mutations emerge the reason is that the selection of resistance-associated changes in the viral polymerase is usually associated with some cost in replication fitness for the virus (17).

Genotypic resistance

The first manifestation of antiviral resistance is the detection of resistant mutation in HBV genome, known to confer resistance that develops during antiviral therapy. Antiviral-resistant mutations can be detected at the same time or prior to virology breakthrough (increase in serum HBV DNA by >1 log above nadir), months and sometimes years before biochemical breakthrough (16).

Virologic breakthrough

Following the development of genotypic resistance, the viral rebound during continued treatment occurs after achieving virologic response, and there will be an increase in serum HBV DNA by >1 log (10-fold) above pretreatment level reaching up to 20,000 IU/mL. Resistant mutations may be detected with time, serum HBV DNA levels continue to increase (viral rebound) and ALT becomes abnormal (biochemical breakthrough). Measurement of viral load is important for monitoring and confirming the presence of drug-resistant virus because nearly all instances of resistance to NA are initially identified by a sustained rise in viral load that occurs despite continuing antiviral therapy (10).

Biochemical breakthrough

Virologic breakthrough is usually followed by biochemical breakthrough, which is defined as elevation in ALT during treatment in a patient who had achieved initial response. In some patients, emergence of antiviral resistance leads to a marked increase in ALT (hepatitis flare) after

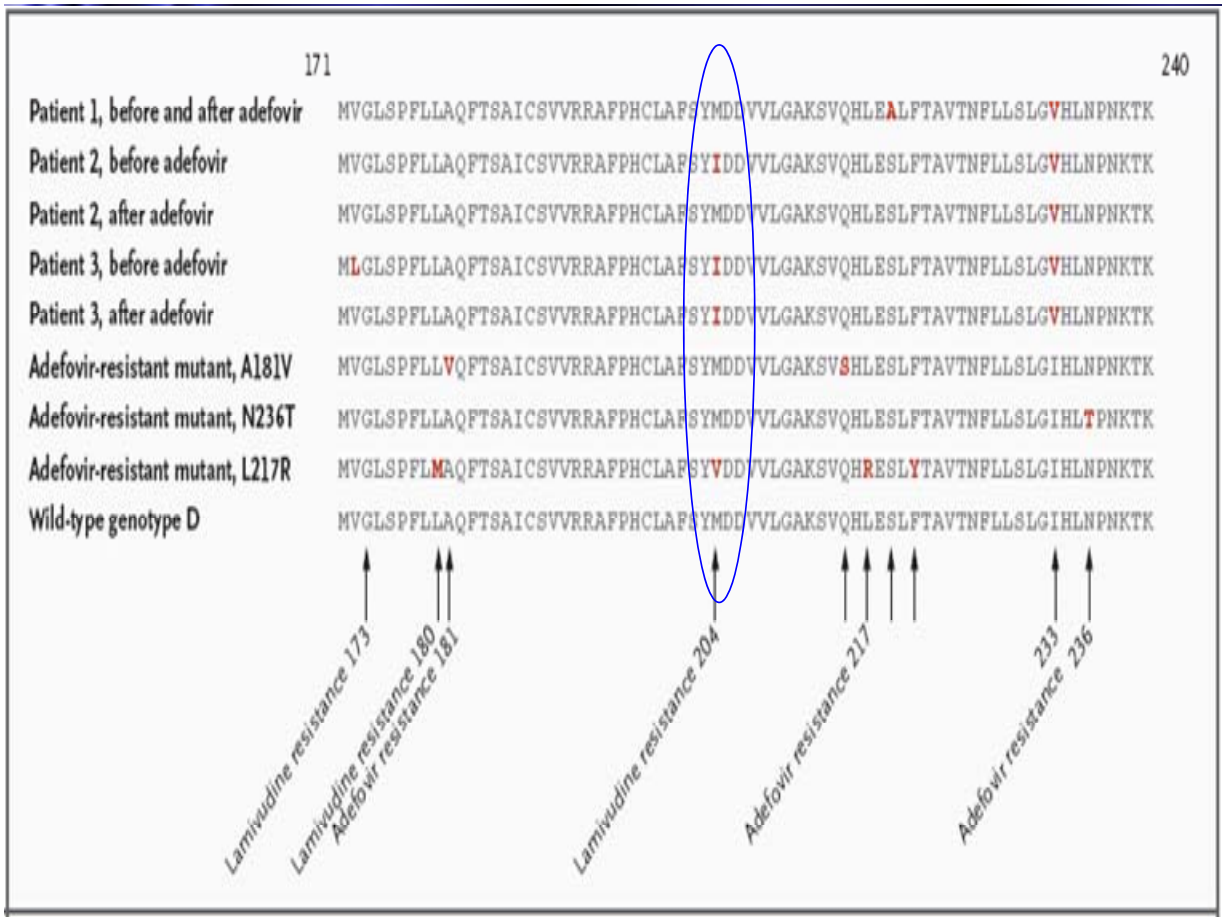

Figure 1. Antiviral resistance mutations

achieving normalization, during continued treatment (12).

Phenotypic resistance

It can be defined as decreased susceptibility (in vitro testing) to inhibition by antiviral drugs associated with genotypic resistance (13).

Cross-resistance

It means the presence of mutants selected by one agent that also confer resistance to other antiviral agents (10).

Clinical outcome of resistance

Resistant mutants have great impact on clinical outcome of patients on antiviral therapy. Consequence of drug-resistance is loss of clinical benefits including reversion of virologic and histologic improvement, increased rate of disease progression, severe exacerbation in patients with liver cirrhosis, decreased rate of HBeAg seroconversion, and risk of graft loss and death in liver transplant patients, It also has potential impacts on public health through transmission of drug-resistance strains and vaccine failure due to HBsAg mutations (18).

Location and terminology of antiviral resistant mutation

The pattern of development of HBV resistant mutants varies by chemical class of nucleoside analogues which can be categorized as:

1. L-nucleosides, such as lamivudine, emtricitabine, telbuvidine and clevudine (19-21).

2. Acyclic phosphonates such as adefovir and tenofovir.

3. Cyclopente(a)nes such as entecavir.

Nomenclature in discussing HBV resistance uses an abbreviation for the gene region in lower case (rt for reverse transcriptase, c for HBcAg, s for HBsAg) followed by the wild-type amino acid symbol, its position in the gene region, and finally the mutant or variant amino acid symbol (22).The typical lamivudine resistant mutations involve the conserved "YMDD" motif of the polymerase gene, changing it to YVDD or YIDD, the standardized nomenclature being rtM204V and rtM204I. The rtM204V/I mutation is usually accompanied by a compensatory mutation upstream of the YMDD motif at rtL180M and/or rtV173L. The rtM204V/I mutations are considered primary resistant mutations that lower the susceptibility of HBV to lamivudine, while the rtL180M and rtV173L mutations are considered secondary or compensatory, allowing for the resistant mutant to replicate at a higher rate. Generally, development of the lamivudine resistant HBV effectively makes other L-nucleosides ineffective. However, rates of development and proportions of various mutants may vary with different L-nucleosides. Typical antiviral resistance mutations are shown in figure 1 (21). These mutations that have been associated with a decrease in activity of the antiviral agent are found in domains A, B, C and D of the polymerase (rt) gene at the amino acid positions listed. A more thorough discussion of antiviral resistance has been provided in recent reviews (21, 22). Adefovir and tenofovir have potent activity against lamivudine-resistant strains in vitro and in vivo (23, 24) whereas entecavir has reduced efficacy against rtM204V/I mutants (25).The most common resistant mutations associated with adefovir therapy have been rtA181V/T and rtN236T, but several other single or multiple mutations have been described (26-28). Resistance to entecavir has been encountered mainly in patients with pre-existing lamivudine- resistance and include multiple changes, typically rtI169T, rtT184S/A/I/LG/C/M, rtS202G/C/I, or rtM250I/V and one or more lamivudine-resistant mutation sites, typically rtL180M and rtM204V (25, 29). Detection of resistant mutations usually requires sequencing of the polymerase gene, but various assays including reverse hybridization and restriction fragment length polymorphism have been developed that detect the more common mutations (30).

In our study in Iran, 54 from 249 patients (21.6%) had received lamivudine (100 mg⁄ day) for more than 1 year. Thirty-one treated patients (57%) had HBV isolates with drug resistance mutations in the HBV pol region. Twenty-eight of 31 isolates (90.32%) had lamivudine resistance mutations, and three patients had isolates with both lamivudine resistance and adefovir dipivoxile resistance mutations (9.67%). Analysis of the RT region of the pol gene revealed M204I in 19 patients (61.29%), L180M + M204I in six patients (19.35%), L180M + M204V in two patients (6.45%) and V173L + L180M + M204V in one patient (3.22%). The L180M + M204V + A181V mutations and V173L + L180M + M204I + A181T mutations (lamivudine and adefovir resistance) were observed in one and 2 patients, respectively (31).

Polymerase gene mutations conferring resistance to nucleos(t)ide analogs are depicted. Resistance to lamivudine (LMV) and telbivudine (LdT) is conferred by mutations in the YMDD motif within the C domain of the polymerase, ie, rtM204V or rtM204I, often associated with compensatory mutations in the B domain restoring a higher replication capacity, ie, rtL180M and/or rtV173L. Resistance to adefovir (ADV) is conferred by a rtA181V or rtA181T substitution or a rtN236T substitution. The rtA181V/T substitution can also confer decreased

susceptibility to LMV and LdT. Resistance to entecavir (ETV) is conferred by a combination of mutations in the B, C, or D domain of the viral polymerase, in addition to a background of substitutions at position rt204. Resistance to tenofovir (TDF) may be conferred by amino acid substitution at position rt194, which needs to be confirmed.

Strategy and management of drug-resistant HBV infection

At present time, seven therapeutic agents have been approved for the treatment of adults with chronic hepatitis B in the United States. Treatment of chronic HBV can be initiated with any of these 7 approved antiviral medications, but in view of the high rate of drug resistance during long-term treatment, lamivudine and telbivudine are not preferred except where only a short course of treatment is planned. Since adefovir is less potent than other NAs and is associated with increasing rate of antiviral resistance after the first year of therapy, it is best utilized as a second line drug in the treatment-naïve patients after pegIFN, tenofovir or entecavir. The first-line drugs recommended for treatment of hepatitis B are pegIFN, entecavir or tenofovir. De novo combination therapy seems to be a logical approach but none of the combination regimens tested to date is clearly superior and it remains to be shown if a clinically significant decrease in the rate of antiviral-resistance results from combination therapy as compared to entecavir or tenofovir monotherapy. Key issues in antiviral therapy choice are efficacy, durability of response and drug resistance.

One of the most important points is the prevention of resistance following long-term antiviral therapy. We need to avoid unnecessary treatment and starting with a potent antiviral drug that has low rate of resistance or with combination therapy and in patients with primary non-response, switching to alternative therapy. Patients who develop breakthrough infection while receiving NA therapy compliance should be ascertained, and treatment resumed in patients who have had long lapses in Medications. A confirmatory test for antiviral-resistant mutation should be performed if possible to differentiate primary non-response from breakthrough infection and to determine if there is evidence of multi-drug resistance (in patients who have been exposed to more than one NA treatment). Monitoring is very important and all patients with virologic breakthrough should be tested for serum HBVDNA viral load every 3-6 months during treatment and confirm antiviral resistance with genotyping testing be considered for rescue therapy. For patients in whom there is no clear indication for hepatitis B treatment and who has compensated liver disease, withdrawal of therapy may be considered but these patients need to be closely monitored and treatment reinitiated if they experience severe hepatitis flares. Assays for serum levels of HBV DNA and ALT should be performed 3–6 months after beginning of therapy, to check for efficacy and compliance; lack of compliance is the most common cause of primary treatment failure. Additional assays, performed at 6-month intervals during the first 2 years of treatment, are recommended for patients with mild liver disease. Patients should then be assessed for viral load and ALT level every 3 months after 2 years of therapy: this is the time during which the probability of developing resistance increases. The consequences of resistance appear more rapidly and can become life-threatening in patients with advanced disease; these patients should be tested for viral load and ALT level every 3 months. Once the viral load increases to 1.0-log10 IU/ mL, HBV Pol should be sequenced to identify resistance mutations and determine the next therapeutic approach, based on cross-resistance information. There are 2 strategies for treating patients who have a partial virologic response to LMV, ADV, or LdT at week 24: change to a more potent drug

(ETV or TDF) or add a more potent drug that does not share cross-resistance. Tenofovir should not be added to ADV therapy if the patient is infected with an HBV mutant that is resistant to ADV (ie, rtA181T/V, rtN236T) because these drugs belong to the same chemical group of NA, the alkyl phosphonates. In cases of resistance, an appropriate rescue therapy should be initiated that has the most effective antiviral effect and minimal risk for selection of MDR strains. Therefore, adding a second drug that is not in the same cross-resistance group as the first is the recommended strategy. Treatment adaptation should be performed accordingly and is summarized as follows:

- LMV resistance: add TDF (add ADV if TDF not available);
- ADV resistance: it is recommended to switch to TDF if available AND add a second drug without cross-resistance. If an rtN236T substitution is present, add LMV, ETV, or LdT or switch to TDF plus emtricitabine. If an rtA181V/T substitution is present, it is recommended to add on ETV or to switch to TDF plus ETV or TDF plus emtricitabine (as a single tablet: Truvada);
- LdT resistance: it is recommended to add TDF (or ADV if TDF is not available);
- ETV resistance: it is recommended to add TDF;
- TDF resistance: primary resistance to TDF has not been confirmed so far.

It is recommended that genotyping and phenotyping be done by a reference-type laboratory to determine the cross-resistance profile. Entecavir, LdT, LMV or emtricitabine could be added but would depend on the profile Note that the safety of some combinations in the longer term is presently unknown and that add-on therapy is not always successful in achieving adequate viral inhibition (PCR undetectability) (32, 33, 34).

Peginterferon

There are two forms of peginterferon alpha, alpha-2a and alpha-2b by adding a polyethylene glycol molecule to IFN alpha-2a and alpha-2b. Peginterferon is different in pharmacologic actions comparing to standard interferon and has longer half life for once a week dosing, better maintenance and effectiveness. Soon after several randomized studies in CHB with peginterferon, was found to have limitation in efficacy. Data from long-term follow-up studies revealed that the virology response to peginterferon was sustained after therapy in patients with HBeAg-positive and HBeAg-negative chronic hepatitis. Multiple studies showed that peginterferon are superior to lamivudine in efficacy with respect to HBeAg seroconversion in HBeAg-positive patients, HBV DNA suppression, and HBsAg seroconversion in patients with HBeAg-positive and HBeAg-negative patients especially in patients with low HBV DNA viral load and high ALT level. The durability of response to peginterferon alfa-2a depends on duration of therapy. In Lau et al. study, 44 of the 58 (83%) patients with seroconvertion at 6 months 36 maintained seroconversion at 12 months after treatment. In this analysis sustained HBeAg seroconversion was associated with higher baseline ALT and lower baseline HBV DNA level. Overall finding from these studies demonstrate that peginterferon offers superior efficacy to lamivudin, resulting in a great incidence of HBeAg seroconversion, HBV DNA suppression, and HBsAg seroconversion in patients with HBeAg positive and HBeAg negative chronic hepatitis B. The addition of Lamivudin to peginterferon alfa-2a did not improve post-therapy response rate. The most important point with peginterferon therapy was the lack of resistance in many clinical trials comparing with other modalities of therapy such as NAs (35-38).

Lamivudine (Epivir-HBV, 3TC)

Lamivudine is the negative enantiomer of 2-3 dideoxy- 3-thiacytidine. Incorporation of the active triphosphate (3TC-TP) into growing DNA chains results in premature chain termination thereby inhibiting HBV DNA synthesis. Lamivudine monotherapy is effective in suppressing HBV replication and in ameliorating liver disease. HBeAg seroconversion after a 1-year course of lamivudine treatment is similar to that of a 16-week course of standard IFN-α, but lower than that of a 1-year course of pegIFN- α (39). In one study in Iran over half (53.5%) of chronic hepatitis B patients with HBeAg negative have normal liver enzyme level at 12-mo lamivudine therapy (40).

Factors associated with an increase rate of lamivudine resistance include long duration of treatment, high pretreatment serum HBV DNA level, and a high level of residual virus after the first course of treatment. One study reported that the rate of lamivudine resistance was significantly higher in patients whose serum HBV DNA level exceeded 200 IU/mL (1000 copies/mL) after 6 months of treatment compared to those with lower HBV DNA levels (63% vs. 13%) (41) The emergence of lamivudine resistance had also been reported to be after initial response and even associated with HBeAg seroconversion, possibly via immune mediated mechanism and in some patients may be associated with acute exacerbations of liver disease and rarely hepatic decompensation and death (42-43). Exacerbations and flares of hepatitis may also occur after withdrawal of treatment due to rapid outgrowth of wild-type virus, but two studies in Asia found that the occurrence of hepatitis flares and hepatic decompensation were similar among patients with lamivudine breakthrough who stopped or continued lamivudine treatment (44,45). Due to selection of drug-resistant mutants with time, patients who receive and continue lamivudine therapy will be presenting the increasing rates of virologic and biochemical flare during maintenance treatment. The durability of response after cessation of treatment is expected to be 70% to 90%. Viral relapse and exacerbations of hepatitis may occur after discontinuation of lamivudine therapy (46) even in patients who developed HBeAg seroconversion, and with a delay up to 1 year after cessation of treatment. Thus, all patients should be closely monitored after treatment is discontinued (every 1-3 months for the first 6 months, and every 3-6 months thereafter). Reinstitution of lamivudine treatment is usually effective in patients who have not developed resistance. In patients who have breakthrough infection, testing for lamivudine-resistant mutants should be performed when possible. The vast majority of patients with confirmed lamivudine-resistance should receive rescue therapy with antiviral agents that are effective against lamivudine-resistant HBV mutants. In terms of salvage therapy for LAM-resistant or ADV resistant CHB, the American Association for the Study of Liver Diseases practice guideline has recommended switching to ETV as an optimal strategy and this protocol has been widely applied in clinical practice. Recent reports have shown that switching to ETV therapy in LAM-refractory patients with CHB has resulted in superior viral suppression compared with continued LAM therapy, with a comparable safety profile. However, the cumulative probability of genotypic ETV resistance development over 4 years was 43% in LAM-refractory patients, which is considerably greater than the 1.2% probability seen in NA-naïve patients. In fact, a new treatment algorithm does not recommend ETV monotherapy as a rescue therapy for patients with LAM resistance CHB (39).

Adefovir resistance

Adefovir dipivoxil is an orally bioavailable pro-drug of adefovir, a nucleotide analog of adenosine monophosphate. It can inhibit both the reverse transcriptase and DNA polymerase activity and is incorporated into HBV DNA causing chain

termination. In vitro and clinical studies showed that adefovir is effective in suppressing wild-type as well as lamivudine-resistant HBV.

Adefovir Resistance occurs at a slower rate during adefovir treatment compared to lamivudine and no adefovir-resistant mutations were found after 1 year of treatment in the patients who participated in the Phase III trials (47). Aggregate data from 5 studies including 3 studies using the combination of lamivudine and adefovir in patients with lamivudine resistant HBV estimated the cumulative rate of adefovir resistance to be 15% by 192 weeks (48). The phase III trial in HBeAg-negative patients found that the cumulative probabilities of genotypic resistance to adefovir at 1, 2, 3, 4, and 5 years were 0, 3%, 11%, 18%, and 29%, respectively. Cumulative rate of genotypic resistance to adefovir in the phase III trial in HBeAg-positive patients was estimated to be 20% after 5 years of treatment (50). Recent studies using more sensitive methods have reported detection of adefovir-resistant mutations after 1 year of treatment and rates of genotypic resistance exceeding 20% after 2 years of treatment (50, 51). In these studies, adefovir resistance was predominantly found in patients with prior lamivudine resistance switched to adefovir monotherapy. In vitro studies showed that adefovir-resistant mutations decrease susceptibility by 3-15- fold (47, 52). Nevertheless, clinical studies found that viral rebound, hepatitis flares and even hepatic decompensation can occur (53). Risk factors for adefovir resistance that have been identified include suboptimal viral suppression and sequential monotherapy (50, 51). Sequential treatment with lamivudine followed by adefovir had also been reported to select for dual-resistant HBV mutants (53). In vitro and clinical studies showed that adefovir-resistant HBV mutants are susceptible to lamivudine and entecavir (52). However, in patients with prior lamivudine resistance, who developed adefovir resistance after being switched to adefovir monotherapy, re-emergence of lamivudine-resistant mutations has been reported soon after reintroduction of lamivudine. With adefovir monotherapy cumulative probability of virologic outcome after 5 year genotypic resistance was 29%, virologic reboud 16% and clinical breakthrough 11%. Higher levels of HBV DNA at one year predict adevofir resistance in long-term therapy (53). There are anecdotal cases where switching from adefovir to tenofovir resulted in a decrease in serum HBV DNA levels. This may be related to a higher dose of tenofovir being used 300 mg versus adefovir 10 mg. However, serum HBV DNA remained detectable and adefovir-resistant mutations persist after switching to tenofovir monotherapy indicating that these two drugs are cross-resistant (54). By contrast, rescue therapy with combination of lamivudine or emtricitabine and tenofovir resulted in suppression of serum HBV DNA to undetectable levels (54). One case series reported that two patients with adefovir-resistant HBV responded to entecavir with a decrease in serum HBV DNA to undetectable levels (50). There have been few reports on the effect of ETV switching in patients with multidrug resistance that developed after switching to ADV monotherapy for LAM resistant HBV. The virological, serological, and biochemical outcomes of ETV monotherapy were evaluated over 48 weeks in patients with compensated CHB who developed resistance to both LAM and ADV after sequential therapy, compared with patients showing resistance to LAM. The 48-week ETV treatment was less effective in LAM/ADV-resistant than in LAM-resistant patients. Continuing ETV monotherapy could be determined based on the virological response at 12 weeks in LAM/ADV-resistant patients (55).

In patients with adefovir resistance with no prior exposure to other NAs, lamivudine, telbivudine or entecavir may be added. Alternatively, adefovir may be stopped and

tenofovir plus lamivudine or emtricitabine may be used. In patients with prior lamivudine resistance in whom lamivudine had been stopped when treatment was switched to adefovir, adefovir may be stopped and tenofovir plus lamivudine, emtricitabine or entecavir may be used but the durability of response to this combination is unknown (56).

Telbivudine resistance

Telbivudine (deoxythymidine) is an L-nucleoside analogue with potent antiviral activity against HBV. Clinical trials showed that telbivudine is more potent than lamivudine in suppressing HBV replication. Although telbivudine is associated with lower rate of drug resistance than lamivudine, the resistance rate is substantial and increases exponentially after the first year of treatment. In the phase III clinical trial, genotypic resistance after 1 and 2 years of treatment was observed in 5.0% and 25.1% of HBeAg positive and in 2.3% and 10.8% of HBeAg-negative patients who received telbivudine compared to 11.0% and 39.5% of HBeAg-positive and 10.7% and 25.9% of HBeAg-negative patients who received lamivudine. Thus telbivudine is associated with a high rate of resistance and telbivudine resistant mutations are cross-resistant with lamivudine. Therefore, telbivudine monotherapy has a limited role in the treatment of hepatitis B (57).

Tenofovir (Viread) resistance

Tenofovir disoproxil fumarate is a nucleotide analogue that was first approved for the treatment of HIV infection as Viread (tenofovir only) or Truvada (tenofovir plus emtricitabine as a single pill) and was approved for the treatment of chronic hepatitis B (39).Tenofovir is structurally similar to adefovir. In vitro studies showed that tenofovir and adefovir are equipotent. Because tenofovir appears to be less nephrotoxic, the approved dose is much higher than that of adefovir, 300 mg versus 10 mg daily. This may explain why tenofovir has more potent antiviral activity in clinical studies. During 96 weeks of treatment In the two phase III clinical trials, 7 patients were observed to have virologic breakthrough. It should be emphasized that 17 patients who had persistent detection of serum HBV DNA at week 72 and were at the greatest risk of tenofovir resistance received additional treatment with emtricitabine but tenofovir- resistant HBV mutations were not detected in any of these patients (58). Therefore, data on resistance to tenofovir monotherapy beyond 72 weeks cannot be determined from the two pivotal trials.

Entecavir (Baraclude) resistance

Entecavir, a carbocyclic analogue of 2-deoxyguanosine, inhibitsHBVreplication at three different steps: the priming of HBV DNA polymerase, the reverse transcription of the negative strand HBVDNAfrom the pregenomic RNA, and the synthesis of the positive strandHBVDNA. In vitro studies showed that entecavir is more potent than lamivudine and adefovir and is effective against lamivudine-resistant HBV mutants although the activity is lower compared to wild-type HBV (54). Virologic breakthrough was rare in nucleoside-naı¨ve patients, and was observed in only 3.6% of patients by week 96 of entecavir treatment in the phase III clinical trial of HBeAg-positive patients (59). Resistant mutations to lamivudine and entecavir were detected in only two (1%) patients while resistant mutations to lamivudine only were found in three patients (60). Preliminary data suggest that the rate of entecavir resistance remained at 1.2% in nucleoside-naı¨ve patients, after up to 5 years of treatment (6). However, virologic breakthrough was detected in 7% of patients after 48 weeks and in 16% after 96 weeks of treatment in the phase III trial of lamivudine

refractory patients (63,64). Preliminary data indicate that entecavir resistance increased to 51% of patients after 5 years of entecavir treatment in lamivudine-refractory patients (62). Lamivudine should be discontinued when patients are switched to entecavir to decrease the risk of entecavir resistance. In vitro studies have shown that entecavir-resistant mutations are susceptible to adefovir and tenofovir, but there are very little clinical data on the efficacy of adefovir or tenofovir in patients with entecavir-resistant HBV.

Emtricitabine (Emtriva, FTC) resistance

Emtricitabine is a potent inhibitor of HIV and HBV replication. FTC has been approved for HIV treatment as Emtriva (FTC only) and as Truvada (in combination with tenofovir as a single pill). Because of its structural similarity with lamivudine (3TC), treatment with FTC selects for the same resistant mutants. FTC-resistant mutations in the YMDD motif were detected in 13% of patients. In one study of 248 patients (63% were HBeAg positive) FTC 200 mg daily resulted in a significantly higher rate of histologic (62% vs.. 25%), virologic [undetectable HBV DNA by PCR assay] (54% vs.. 2%) and biochemical (65% vs. 25%) responses at week 48 compared to placebo but HBeAg seroconversion rates were identical (12% in the two groups) (63).

Clevudine resistance

Clevudine is a pyrimidine nucleoside analogue that is effective in inhibiting HBV replication in vitro and in animal models. Clinical trials showed that clevudine in doses of 30 mg daily for up to 24 weeks was well tolerated. Serum HBV DNA levels were undetectable by PCR assay at the end of treatment in 59% of HBeAg-positive and in 92% of HBeAg-negative patients (64, 65). A unique feature of clevudine is the durability of viral suppression, persisting for up to 24 weeks after withdrawal of treatment in some patients. Nonetheless, clevudine has not been shown to increase the rate of HBeAg seroconversion compared to placebo controls and in vitro studies suggest that it can select for mutations in the YMDD motif. Clinical trials found that rtA181T mutation which is associated with resistance to lamivudine and adefovir can be selected after only 24 weeks of clevudine treatment (64). Clevudine has been reported to be associated with myopathy in patients who have been treated for longer than 24 weeks, the onset of symptoms typically occurred after 8 months and mitochondrial toxicity has been documented in some patients (66, 67). These reports have led to discontinuation of the global phase III clinical trial on clevudine.

Conclusion

Prolonged antiviral therapy with the oral NAs is associated with the development of resistance, and as long as treatment continues resistance will be part of therapy.

Because of the unusual replication strategy used by HBV, viral populations are genetically heterogeneous, so even treatment-naïve patients have drug-resistant mutants that constitute only a minor component of the population in the absence of selection pressure from antiviral drugs. The spread of drug-resistant HBV mutants can be reduced by avoiding unnecessary drug use, choosing drugs and combinations more carefully, and continually monitoring or carrying out targeted surveillance for drug resistance (68). A majority of patients may not require antiviral therapy. Several professional bodies publish regularly updated guidelines to assist clinicians with recognition, diagnosis, prevention, and management of CHB.

Treatment algorithms have been developed to assist in identification of suitable candidates for treatment and to determine when to initiate

treatment. Because drug-resistant mutant HBV populations are established and expand through replication, antiviral therapy, once initiated, should aim to suppress viral replication as completely and rapidly as possible. The lower risk of resistance to TDF and ETV (compared with LMV, LdT, and ADV) supports their use as first-line therapy, especially in patients who have received liver transplants and those with cirrhosis or decompensated liver disease because development of drug resistance is more likely to precipitate clinical deterioration in these groups. Combination chemotherapy is being used more frequently to treat CHB. It is effective when the appropriate combinations are employed and can reduce the risk of drug resistance. Although HBV mutants that are resistant to single drugs exist before therapy starts and can evolve rapidly in patients, HBV mutants with MDR are much less likely to exist before treatment. Ideally, drugs used in combination should have different mechanisms so that they have additive synergistic effects. Combination therapy using NA with a complementary cross-resistance profile prevents the development of resistance but does not have increased antiviral effects, compared with single-drug therapy (69). Use of interferon in combination with NA is probably the next logical step. Although initial clinical trials of such combinations were disappointing, results from later trials are more encouraging. However, the added benefit of the combination tends to be lost after treatment cessation (70, 71).

Combinations of L-nucleosides are unlikely to be more effective than therapy with single L-nucleosides and can have antagonistic effects (because they compete for cellular activation mechanisms and viral targets). The lack of cross-resistance of HBV mutants to LMV and ADV observed in vitro (except for rtA181T/V) and in some clinical studies indicates that these drugs could be effective in combination. Preliminary data also support the use of ETV in combination with ADV or TDF, but definitive recommendations will require further clinical trials and cost-benefit studies. Each patient's response to treatment should be monitored carefully so that drug resistance can be detected early, before viral breakthrough and disease progression. Ideally, treatment for CHB should begin at diagnosis; this is not feasible because of limitations of drugs. Clinical trials and concurrent improvements in diagnostic technology ensure the fact that treatment options and expert opinion on patient management will continue to evolve.

REFERENCES

1. Mast EE, Mahoney FJ, Alter MJ, Margolis HS. Progress toward elimination of Hepatitis B transmission in the United States. Vaccine 1998; 16(Suppl): S48-S51.

2. McQuillan GM, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, Margolis HS. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994. Am J Public Health 1999; 89: 14-18.

3. Zali MR, Mohammad K, Noorbala AA, Noorimayer B, Shahraz S. Rate of hepatitis B seropositivity following mass vaccination in the Islamic Republic of Iran. East Mediterr Health J 2005; 11: 62-67.

4. Nafa S, Ahmed S, Tavan D, Pichoud C, Berby F, Stuyver L, et al. Early detection of viral resistance by determination of hepatitis B virus polymerase mutations in patients treated by lamivudine for chronic hepatitis B. Hepatology 2000; 32: 1078–88.

5. Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. Clin Infect Dis 2003; 36: 687–96.

6. Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 2001; 34: 785–91.

7. Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused

by chronic infection with hepatitis B virus. Gastroenterology 1995; 109: 908-16.

8. Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, et al. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003; 37: 756-63.

9. Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001; 121: 101-109.

10. Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev 2000; 64: 51–68.

11. Kock J, Baumert TF, Delaney WEth, Blum HE, von Weizsäcker F. Inhibitory effect of adefovir and lamivudine on the initiation of hepatitis B virus infection in primary tupaia hepatocytes. Hepatology 2003; 38: 1410–18.

12. Delmas J, Schorr O, Jamard C, Gibbs C, Trépo C, Hantz O, et al. Inhibitory effect of adefovir on viral DNA synthesis and covalently closed circular DNA formation in duck hepatitis B virus-infected hepatocytes in vivo and in vitro. Antimicrob Agents Chemother 2002; 46: 425–33.

13. Zhou T, Saputelli J, Aldrich CE, Deslauriers M, Condreay LD, Mason WS, et al. Emergence of drug-resistant populations of woodchuck hepatitis virus in woodchucks treated with the antiviral nucleoside lamivudine. Antimicrob Agents Chemother 1999; 43: 1947-54.

14. Zoulim F. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antiviral Res 2004; 64: 1–15.

15. Sendi H, Mehrab-Mohseni M, Shahraz S, Norder H, Alavian SM, Noorinayer B, et al. CTL escape mutations of core protein are more frequent in strains of HBeAg negative patients with low levels of HBV DNA.J Clin Virol 2009; 46: 259-64.

16. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol 2005; 42: 302–308.

17. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, et al. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007; 46: 254–65.

18. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. Hepatology 2001; 33: 1527-32.

19. Tipples A, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 1996; 24: 714-17.

20. Ling R, Mutimer D, Ahmed M, Boxall EH, Elias E, Dusheiko GM, et al. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 1996; 24: 711-13.

21. Locarnini S. Molecular virology and the development of resistant mutants: implications for therapy. Semin Liver Dis 2005: 25:9-19.

22. Lok AS, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky J-M, et al. Antiviral-resistant HBV: standardization of nomenclature and assays and recommendations for management. Hepatology 2007; 45: 254-65.

23. Zoulim F. In vitro models for studying hepatitis B virus drug resistance. Semin Liver Dis 2006; 26: 142-52.

24. Ono-Nita SK, Kato N, Shiratori Y, Lan KH, Yoshida H, Carrilho FJ, et al. Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. J Clin Invest 1999; 103: 1635-40.

25. Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. Antimicrob Agents Chemother 2004; 48: 3498-507.

26. Bartholomeusz A, Locarnini S, Ayres A, Thompson G, Sozzi V, Angus P, et al. Molecular modeling of hepatitis B virus polymerase and adefovir resistance identifies three clusters of mutations. Hepatology 2004; 40 (Suppl 1): 246A.

27. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006; 44: 283-90.

28. Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 2006; 44: 703-12.

29. Colonno R, Rose R, Levine S, Baldick J, Pokornowski K, Plym M, et al. Entecavir two year resistance update: no resistance observed in nucleoside naıve patients and low frequency resistance emergence

in lamivudine refractory patients. Hepatology 2005; 42(Suppl 1): 573A.

30. Stuyver L, Van Geyt C, De Gendt S, Van Reybroeck G, Zoulim F, Leroux-Roels G, et al. Line probe assay for monitoring drug resistance in hepatitis B virus-infected patients during antiviral therapy. J Clin Microbiol 2000; 38: 702-707.

31. Amini-Bavil-Olyaee S, Herbers U, Mohebbi SR, Sabahi F, Zali MR, Luedde T, Trautwein C, Tacke F Prevalence, viral replication efficiency and antiviral drug susceptibility of rtQ215 polymerase mutations within the hepatitis B virus genome. J Hepatol 2009; 51: 647-54.

32. European Association for the Study of the Liver. EASL clinical practice guidelines: management of chronic hepatitis B. J Hepatol 2009; 50: 227–42.

33. Keeffe EB, Zeuzem S, Koff RS, Dieterich DT, Esteban-Mur R, Gane EJ et al. Report of an international workshop: roadmap for management of patients receiving oral therapy for chronic hepatitis B. Clin Gastroenterol Hepatol 2007; 5:890–97.

34. Chan HL, Heathcote EJ, Marcellin P, Lai CL, Cho M, Moon YM, et al. Treatment of hepatitis B e antigen positive chronic hepatitis with telbivudine or adefovir: a randomized trial. Ann Intern Med 2007; 147: 745–54.

35. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. A randomized, double blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001; 34: 395–403.

36. Reddy KR, Wright TL, Pockros PJ, Shiffman M, Everson G, Reindollar R, et al. Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. Hepatology 2001; 33: 433–38.

37. Janssen HL, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAgpositive chronic hepatitis B: a randomised trial. Lancet 2005; 365: 123–29.

38. Chan HL, Leung NW, Hui AY, Wong VW, Liew CT, Chim AM, et al. A randomized, controlled trial of combination therapy for chronic hepatitis B: comparing pegylated interferon-alpha2b and lamivudine with lamivudine alone. Ann Intern Med 2005; 142: 240–50.

39. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology 2009; 50: 661-62.

40. Mohammad Alizadeh AH, Ranjbar M, Karimi B, Hatami S. Biochemical response to lamivudine treatment in HBeAg negative chronic hepatitis B patients in Iran. World J Gastroenterol 2006; 12: 4203-205.

41. Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 2001; 34: 785-91.

42. Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999; 30: 567-72.

43. Bartholomew MM, Jansen RW, Jeffers LJ, Reddy KR, Johnson LC, Bunzendahl H, et al. Hepatitis B virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 1997; 349: 20-22.

44. Liaw YF, Chien RN, Yeh CT. No benefit to continue lamivudine therapy after emergence of YMDD mutations. Antivir Ther 2004; 9: 257-62.

45. Wong VW, Chan HL, Wong ML, Tam JS, Leung NW. Clinical course after stopping lamivudine in chronic hepatitis B patients with lamivudine- resistant mutants. Aliment Pharmacol Ther 2004; 19: 323-29.

46. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000; 32: 635-39.

47. Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003; 125: 292-97.

48. Locarnini S, Qi X, Arterburn S. Incidence and predictors of emergence of Adefovir resistant HBV during four years of Adefovir Dipivoxil (ADV) Therapy for patients with chronic hepatitis B (CHB). J Hepatol 2005; 42(Suppl 2): S17.

49. Marcellin P, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S, et al. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2008; 48: 750-58.

50. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. J Hepatol 2006; 44: 283-90.

51. Lee Y, Suh D, Lim Y, Jung S, Kim K, Lee H, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. Hepatology 2006; 43: 1385-91.

52. Villeneuve JP, Durantel D, Durantel S, Westland C, Xiong **S**, Brosgart CL, et al. Selection of a hepatitis B virus strain resistant to adefovir in a liver transplantation patient. J Hepatol 2003; 39: 1085-89.

53. Fung SK, Andreone P, Han SH, Rajender Reddy K, Regev A, Keeffe EB, et al. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. J Hepatol 2005; 43:937-43.

54. Ono SK, Kato N, Shiratori Y, Kato J, Goto T, Schinazi RF, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest 2001; 107: 449-55.

55. Shim JH, Suh DJ, Kim KM, Lim YS, Lee HC, Chung YH, et al. Efficacy of entecavir in patients with chronic hepatitis B resistant to both lamivudine and adefovir or to lamivudine alone. Hepatology 2009; 50: 1064-71.

56. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006; 354: 1001-10.

57. Tillmann HL, McHutchison JG. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 2008; 358: 1517.

58. Snow-Lampart A, Chappell BJ, Curtis M, Zhu Y, Heathcote EJ, Marcellin P, et al. Week 96 resistance surveillance for HBeAg positive and negative subjects with chronic HBV infection randomized to receive tenofovir DF 300 mg qd. Hepatology 2008; 48(Suppl): 745A.

59. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. Gastroenterology 2007; 133: 1437-44.

60. Colonno R, Rose R, Baldick C, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naive patients with hepatitis B. Hepatology 2006; 44: 1656-65.

61. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology 2009; 49: 1503-14.

62. Sherman M, Martin P, Lee W, Silva M, Liaw YF, Rustgi VK, et al. Entecavir results in continued virologic and biochemical improvement and HBeAg seroconversion through 96 weeks of treatment in lamivudine-refractory, HBeAg chronic hepatitis B patients (ETV-026). Gastroenterology 2006; 130(Suppl 2): A765.

63. Lim SG, Ng TM, Kung N, Krastev Z, Volfova M, Husa P, et al. A double-blind placebo-controlled study of emtricitabine in chronic hepatitis B. Arch Intern Med 2006; 166: 49-56.

64. Yoo BC, Kim JH, Chung YH, Lee KS, Paik SW, Ryu SH, et al. Twenty-four-week clevudine therapy showed potent and sustained antiviral activity in HBeAg-positive chronic hepatitis B. Hepatology 2007; 45: 1172-78.

65. Yoo BC, Kim JH, Kim TH, Koh KC, Um SH, Kim YS, et al. Clevudine is highly efficacious in hepatitis B e antigen-negative chronic hepatitis B with durable off-therapy viral suppression. Hepatology 2007; 46: 1041-48.

66. Kim BK, Oh J, Kwon SY, Choe WH, Ko SY, Rhee KH, et al. Clevudine myopathy in patients with chronic hepatitis B. J Hepatol 2009; 51: 829-34.

67. Seok JI, Lee DK, Lee CH, Park MS, Kim SY, Kim HS, et al. Long-term therapy with clevudine chronic hepatitis B can be associated with myopathy characterized depletion of mitochondrial DNA. Hepatology 2009; 49: 2080-86.

68. Richman DD. The impact of drug resistance on the effectiveness of chemotherapy for chronic hepatitis B. Hepatology 2000; 32: 866–67.

69. Sung JJ, Lai JY, Zeuzem S, Chow WC, Heathcote EJ, Perrillo RP, et al. Lamivudine compared with lamivudine and adefovir dipivoxil for the treatment of HBeAg positive chronic hepatitis B. J Hepatol 2008; 48: 728–35.

70. Marcellin P, Lau GK, Bonino F, Farci P, Yurdaydin C, Piratvisuth T, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004; 351: 1206–17.

71. Lau GK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005; 352: 2682–95.