Current status of antiviral therapy for hepatitis B

Therapeutic Advances in Gastroenterology
(2008) 1(1) 61–75
DOI: 10.1177/1756283X08093944
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***Abstract*:** Chronic hepatitis B (CHB) is a major public health problem affecting up to 400 million people globally. Complications of CHB including liver failure and hepatocellular carcinoma result in 1.2 million deaths per year, making CHB the 10th leading cause of mortality worldwide. The natural history of CHB is variable and complex. The past decade witnessed important developments for the therapy of hepatitis B and marked the new era of oral therapy.
The ultimate goal of CHB therapy is to arrest the progression of liver injury and to prevent the development of liver failure and hepatocellular carcinoma. Currently, six agents are approved for the treatment of CHB. Each of these agents, given as monotherapy, has been shown to produce virological, biochemical, and histological benefits for both HBeAg positive and negative CHB. There are, however, limitations in spite of their efficacy. The significant side-effect profile of interferon, for example, limits its long-term use. The approved oral agents are tolerable with prolonged use but drug resistance could limit long-term monotherapy. To date, combination therapy with nucleoside analogue and pegylated interferon or two nucleos(t)ide analogues given for one year does not show superiority in durability of response compared to monotherapy. Ongoing research effort is critical to identify the ideal hepatitis B therapy that is safe, effective, and produces durable response with a finite course of therapy. It is equally important to conduct a well designed, prospective natural history study to identify predictors of disease progression. This will accurately guide treatment strategy for this important disease.

***Keywords*:** Hepatitis B virus, pegylated interferon, nucleos(t)ide analogues, drug resistance

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Introduction

Hepatitis B virus (HBV) infection is a major public health problem worldwide, responsible for significant morbidity and mortality from chronic liver disease. It is estimated that there are 350 to 400 million HBV carriers globally [Lee, 1997]. In the United States, approximately 1.5 million people are infected and 50,000–100,000 new cases are reported annually despite the availability of effective vaccines [McQuillan *et al.* 1999]. This is likely an underestimate since prevalence of chronic hepatitis B (CHB) among immigrants from HBV endemic areas is much higher than that in the general population [Margolis *et al.* 1991].

HBV is a DNA virus in the family of Hepadnaviridae [Tacke *et al.* 2004]. There are eight major genotypes of HBV and their prevalence varies amongst geographic regions (Table 1) [Magnius and Norder, 1995]. The compact genome of HBV consists of four partially overlapping open reading frames encoding for the envelope (pre-S/S), core (precore/core), polymerase, and X proteins [Tacke *et al.* 2004]. Through the process of endocytosis, HBV gains entry into the hepatocyte. However, its surface receptor has not been identified [Doo and Liang, 2001]. After uncoating, the relaxed circular genome is converted in the nucleus to a covalently closed circular (ccc) DNA that is the template for viral replication [Locarnini and Mason, 2006; Doo and Liang, 2001]. The persistence of HBV in the liver, despite antiviral therapy, is due to the maintenance of HBV cccDNA in the nuclei of infected cells. HBV replicates asymmetrically via reverse transcription of an RNA intermediate. Since its polymerase/reverse transcriptase (Pol/Rt) lacks proofreading activity, spontaneous mutations are estimated to occur at a rate of one error

per 10^4–10^5 nucleotides daily [Locarnini and Mason, 2006]. The resulting random mutations at the polymerase/reverse transcriptase active site may overlap with the antiviral-induced mutations and facilitate drug resistance.

CHB is defined by the persistence of serum hepatitis B surface antigen (HBsAg) for six months or longer [Hollinger and Lau, 2006]. The natural history of CHB can be classified into four major clinical phases based on levels of serum alanine aminotransferase (ALT) and HBV DNA, presence of HBeAg, and suspected immune status [Lok *et al.* 2001]. These phases are: (1) immune tolerance, (2) HBeAg-positive CHB, (3) inactive carrier, and (4) HBeAg-negative CHB. The disease, however, can be variable and the patients may not proceed through all phases of the disease during the course of infection (Table 2) [Lok *et al.* 2001]. The emergence of pre-core [nucleotide 1896 mutation from guanine (G) to adenine (A)] and basal core promoter (BCP) [adenine (A) to thymine (T) transversion at nucleotide 1762 together with a guanine (G) to adenine (A) transition at nucleotide 1764] mutants lead to HBeAg-negative CHB [Hunt *et al.* 2000; Okamoto *et al.* 1994; Okamoto *et al.* 1990]. These patients continue to have moderate HBV replication and active liver disease. The frequency of these HBV mutants varies worldwide as a result of the different geographic distribution of the HBV genotypes. Patients with HBeAg-negative CHB typically have heterogeneity of disease activities characterized by fluctuating levels of serum aminotransferases and HBV DNA [Hadziyannis and Vassilopoulos, 2001]. These observations underscore the importance of regular assessments of HBsAg positive patients over time in order to confirm the diagnosis of HBeAg-negative CHB versus inactive HBV carrier. HBeAg positive and HBeAg negative-CHB patients with persistent or intermittent elevation of aminotransferases and HBV DNA levels, and histological evidence of active hepatitis should be considered for antiviral therapy.

The past decade witnessed important developments for the therapy of hepatitis B. The availability of lamivudine in 1998 not only marked the new era of oral therapy, it also represents a paradigm shift in the management of this important disease (Figure 1). The focus of this review is to discuss both the advances and the unmet needs with the current paradigm.

Table 1. Global distribution of HBV genotypes.

Genotype	Geographical distribution
A	North America, Pandemic
B and C	Asia
D	Middle East, South Europe (Mediterranean), India
E	Africa, especially Sub-Sahara
F	Native Americans and Polynesians
G	USA, Europe (France, Germany, The Netherlands)
H	South and Central America

Therapy for chronic hepatitis B

There are six agents approved for the treatment of CHB by the U.S. Food and Drug Administration (FDA) [Hoofnagle *et al.* 2007; Hollinger and Lau, 2006]. They are peginterferon and standard interferon-alpha (pegIFN-α, IFN-α), nucleoside (lamivudine, entecavir, and telbivudine) and nucleotide (adefovir) analogues. Tenofovir disoproxil fumarate and the combination of tenofovir and emtricitabine (Truvada™) both have potent activity against HBV, and are currently approved for use in the treatment of human immunodeficiency virus (HIV). It is

Table 2. Phases in the natural history of chronic hepatitis B (CHB).

	Spontaneous recovery	Immune tolerance	HBeAg positive CHB	HBeAg negative CHB	Inactive carrier
HBsAg	Absent	Present	Present	Present	Present
HBeAg	Absent	Present	Present	Absent	Absent
ALT	Normal	Normal	Elevated	Elevated (fluctuate)	Normal
HBV DNA	Undetectable in serum	High 10^8–10^{11} C/mL	High 10^6–10^{10} C/mL	Moderate (fluctuate) 10^3–10^8 C/mL	Low $<10^4$ C/mL

C/mL, Copies/mL. It is estimated that 1IU/ml is approximately 5.6 copies/mL.

anticipated that tenofovir will become FDA approved for CHB in 2008.

The ultimate goal of therapy for CHB is to arrest the progression of liver injury and to prevent the development of liver failure and hepatocellular carcinoma (HCC). The most important short- and intermediate-term objective of therapy is to maximize HBV DNA suppression. Complete eradication of the HBV is difficult for it has a tendency to integrate into the host genome or remain latent as cccDNA [Laras *et al.* 2006]. Patients who beco-me HBsAg negative and develop anti-HBs generally have resolution of liver disease. Thus, HBsAg seroconversion should be considered a complete therapeutic response, the most desired endpoint of therapy [Werle-Lapostolle *et al.* 2004; Lau *et al.* 1997]. A significant reduction in serum HBsAg titer has been observed with antiviral therapy, which correlated with changes in cccDNA, total intracellular HBV DNA and serum HBV DNA [Werle-Lapostolle *et al.* 2004]. The cccDNA is the major template for transcription and translation of viral antigens, including HBsAg. Changes in serum HBsAg titer might be used as a surrogate for liver cccDNA level, especially the latter requiring a liver biopsy [Zoulim, 2005].

Each of these agents, given as monotherapy, has been shown to produce virological, biochemical, and histological benefit for both HBeAg positive and negative CHB. The biochemical and histological responses usually parallel HBV DNA suppression. Comparison of the potency of these medications for both HBeAg positive and negative CHB during the first year of therapy from representative publications is shown in Figure 2 and Table 3 [Lai *et al.* 2007; Lok and McMahon 2007; Lai *et al.* 2006; Marcellin *et al.* 2004;

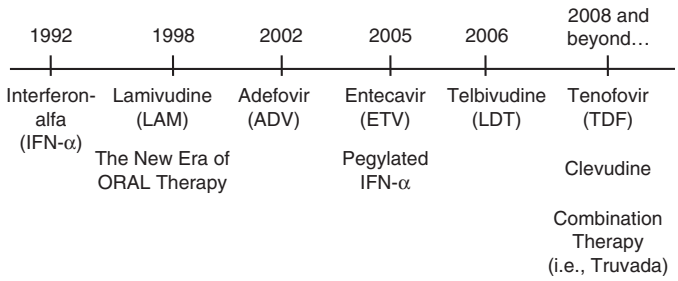

Figure 1. Timeline of the FDA-approved therapy for chronic hepatitis B in the United States.

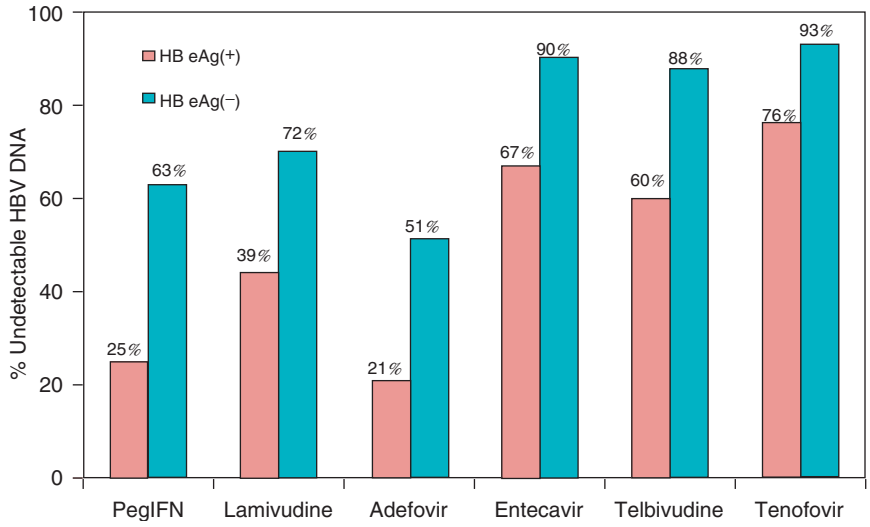

Figure 2. Virological response with undetectable HBV DNA by RT-PCR at week 48–52. In all of these studies, the lower limit for HBV DNA detection was 300 copies/ml with the exception of tenofovir (LLD 400 copies/mL). Tenofovir data for Figures 2 and 3 derived from Marcellin *et al.* and Heathcote *et al.*, AASLD 2007 Abstract nos. LB2 and LB6.

Hadziyannis *et al.* 2003; Lok *et al.* 2001]. Patients with HBeAg-negative CHB tend to have lower baseline serum HBV DNA level compared to HBeAg-positive patients. As a result, there was a higher rate of complete viral suppression for HBeAg-negative CHB with every antiviral agent. Nucleos(t)ide analogues are more potent in HBV DNA suppression compared to interferon for both HBeAg positive and negative CHB.

Interferons

Standard IFN-α was the first drug available for treatment of CHB. More recently, long-acting, once weekly pegIFN α-2a (40 kD branched pegylated molecule) was approved by FDA of the United States in 2005. It has similar safety profiles and is more effective compared to standard IFN. The recommended regimen for CHB is pegIFN α-2a 180 µg subcutaneously weekly for one year. The therapeutic effects of IFN are secondary to its direct antiviral function, antiproliferative effect (anti-angiogenic and anti-tumor), immunomodulatory properties, and control of apoptosis. The immunomodulatory effects of IFN can be recognized clinically as flares of hepatitis that often precedes a virological response [Perrillo, 2001].

Table 3. Biochemical and histological response at week 48–52.

Drug	Normalization of ALT		Improvement in Histology	
	HBeAg+ CHB (%)	HBeAg- CHB (%)	HBeAg+ CHB (%)	HBeAg- CHB (%)
Peginterferon	39	38	38	48
Lamivudine	66	74	59	63
Adefovir	48	72	53	64
Entecavir	68	78	72	70
Telbivudine	77	74	65	66
Tenofovir	69	77	74	72
Placebo	21	24	25	28

Tenofovir data was derived from Marcellin *et al.* and Heathcote *et al.* AASLD 2007 Abstract nos. LB2 and LB6, respectively. ALT, alanine aminotransferase, CHB, chronic hepatitis B.

Traditionally, one of the most important treatment endpoints for patients with HBeAg-positive CHB is the loss of HBeAg. PegIFN α-2a has the highest HBeAg seroconversion rate (30% at one year) in spite of its lower antiviral potency compared to the nucleos(t)ide analogues (Figure 3). Long-term follow-up studies of IFN-α therapy from North America and Europe reported that 95–100% of those who cleared HBeAg continued to be HBeAg negative after 5 to 10 years of follow-up and 30-86% of them eventually lost HBsAg [Lau *et al.* 1997; Niederau *et al.* 1996]. Liver-related complications and mortality were greater in nonresponders compared to responders, especially among those with pre-existing cirrhosis [Lau *et al.* 1997]. These studies demonstrated that the loss of HBeAg is a reliable treatment end-point that is associated with

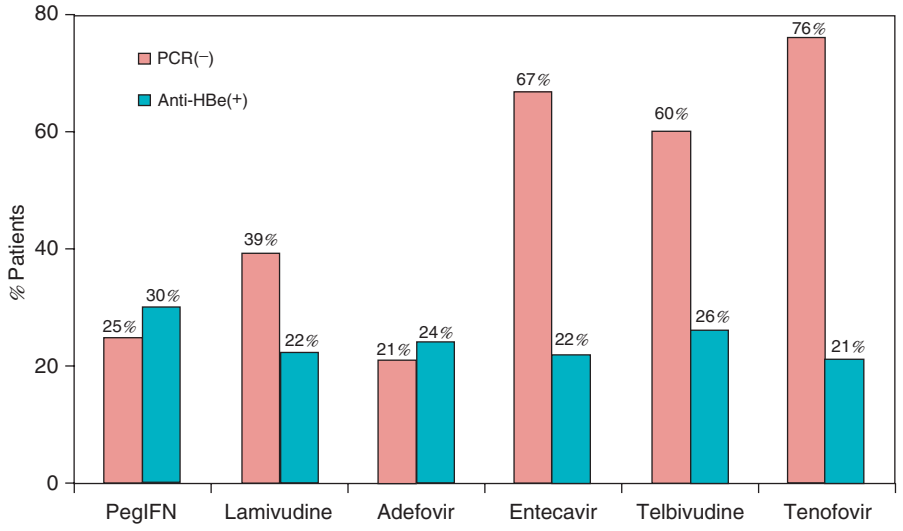

Figure 3. Relationship between antiviral potency and rate of HBeAg seroconversion at week 48–52. There is no positive correlation between potency in HBV DNA suppression and HBeAg seroconversion among the medications. The higher rate of HBeAg seroconversion associated with pegIFN-α is consistent with the evidence that HBeAg and HBsAg clearance is an immune-mediated phenomenon.

long-term disease remission. In contrast, long-term follow-up of patients in Asian studies generally showed a lower rate of durable responses to IFN-α, and inconsistent rates of HBeAg and HBsAg clearance [Yuen *et al.* 2001; Lin *et al.* 1999; Lok *et al.* 1993]. These differences in long-term IFN-α treatment outcomes noted in the Eastern and Western countries could reflect differences in viral factors such as genotypes and in the natural history of the disease in high *vs* low endemic areas [Alward *et al.* 1985; McMahon, *et al.* 1985]. There is evidence that patients with HBV genotype A have the highest rate of IFN-induced HBeAg loss compared to the other genotypes and genotype A is most common in North America and Europe [Janssen *et al.* 2005; Lau *et al.* 2005; Cooksley *et al.* 2003]. In contrast, the HBeAg clearance associated with nucleos(t)ide analogues appears independent of HBV genotype.

The major disadvantage of IFN therapy is its significant side-effect profile that limits its long-term use. It is contraindicated in decompensated cirrhosis and tends to be ineffective in patients with normal aminotransferases. The therapy for HBeAg-negative CHB is particularly challenging due to its high relapse rate and typically requires a prolonged, indefinite course of therapy [Hadziyannis *et al.* 2003; Manesis and Hadziyannis, 2001]. However, sustained virological response, defined as HBV DNA levels <20,000 copies/mL at 24 weeks after cessation of a 48-week course of therapy, was higher with pegIFN α-2a compared to lamivudine (43% *vs* 29%, $p = 0.007$) [Marcellin *et al.* 2004]. Loss of HBsAg associated with pegIFN α-2a therapy was 4% compared to 0% with lamivudine at one year. Despite its limitations, IFN therapy is associated with the highest rates of both HBeAg and HBsAg seroconversion at one year of therapy, underscoring the importance of immunomodulatory properties on viral clearance.

Nucleoside and nucleotide analogues

Nucleoside or nucleotide analogues compete with naturally occurring purines and pyrimidines for binding to HBV DNA polymerase. They require intracellular phosphorylation for their activity. Analogues lacking a 3'-OH group on the sugar moiety result in immediate chain termination. Many of these compounds are unnatural L-enantiomers [Leemans *et al.* 2006]. One of the significant impacts of these oral agents is their beneficial effects on end stage liver disease [Arora and Keeffe, 2007]. Unlike IFN, nucleos(t)ide analogues are well tolerated by patients with decompensated liver disease and significant improvement of hepatic synthetic function has been documented [Arora and Keeffe, 2007]. Among the available nucleos(t)ide analogues, entcavir, telbuvudine and tenofovir are most potent in HBV DNA suppression (Figure 2). At one year, ≥60% of HBeAg-positive and >85% of HBeAg-negative CHB patients achieved undetectable HBV DNA by RT-PCR assays with these three agents [Lai *et al.* 2007; Lai *et al.* 2006]. Adefovir and tenofovir are both structurally related nucleotides. The clinical dosage of tenofovir 300 mg has significantly greater antiviral effect than adefovir dosed at 10 mg[Del Poggio *et al.* 2008; Tan *et al.* 2008]. Adefovir 10 mg is associated also with a high rate of primary nonresponse in up to 30% of the patients with HBeAg-positive CHB. Although, adefovir at 30 mg has higher antiviral potency, it is not recommended for its potential nephrotoxicity, a Fanconi-like syndrome with phosphaturia and proteinuria [Perazella, 2003]. Of note, nucleos(t)ide analogues are renally excreted so dose adjustment is essential in accordance with creatinine clearance [Izzedine *et al.* 2005].

An important question is whether the potency of the antiviral agent is associated with an increased rate of HBeAg and HBsAg seroconversion. The one-year HBeAg seroconversion rate is similar across the nucleoside and nucleotide analogues (between 21% and 26%) regardless of their antiviral potency (Figure 3) [Lai *et al.* 2007; Lai *et al.* 2006; Marcellin *et al.* 2004; Hadziyannis *et al.* 2003]. Similarly, the one-year HBsAg seroconversion rates are <1% for all the nucleos(t)ides. For each agent evaluated, there is a trend toward increased rates of undetectable HBV DNA with prolonged therapy beyond the first year in the absence of drug-associated resistance. Similarly, the rate of HBeAg seroconversion increased to ~30% for lamivudine, adefovir, entecavir, and telbivudine at year two of continuous therapy for patients with HBeAg-positive CHB [Lok and McMahon, 2007; Gish *et al.* 2007; Hollinger and Lau, 2006; Leung *et al.* 2001; Liaw *et al.* 2000]. The durability of HBeAg seroconversion, however, is variable and relapse rates of up to 60% after nucleos(t)ide analogue therapy [Lok *et al.* 2001]. HBsAg loss also increases with prolonged monotherapy but at a very low rate. Continuous therapy with entecavir for two years is associated with a 5% HBsAg loss and only 2% HBsAg

seroconversion [Gish *et al*. 2007]. Unlike IFN, the nucleos(t)ide analogues are well tolerated even with long-term therapy. The effectiveness and durability of response, unfortunately, could be compromised by the emergence of mutations in the HBV DNA polymerase which confers to the HBV mutants a selective resistance to the drug. To date, IFN-induced resistance has not been reported and HBV resistance to tenofovir has not been well characterized. The primary site(s) of mutations associated with the nucleos(t)ide antiviral agents are showed in Figure 4 [Hollinger *et al*. 2006; Locarnini *et al*. 2004; Hoofnagle *et al*. 1987].

Drug resistance and cross-resistance

Antiviral resistance is defined as the selection of HBV mutants conferring reduced susceptibility to a drug that results in primary or secondary treatment failure. While resistance is more likely the cause of secondary treatment failure, it may cause primary treatment failure due to transmission of resistant HBV mutants or due to cross-resistance resulting from previous therapies [Pawlotsky *et al*. 2008]. The risks of the emergence of drug-resistant mutants in the HBV DNA polymerase/reverse transcriptase increases with duration of therapy, high baseline serum HBV DNA level, incomplete viral suppression during the first six months of therapy, noncompliance to therapy and prior exposure to nucleos(t)ide analogues [Kim *et al*. 2007; Hollinger *et al*. 2006]. The first clinical manifestation of antiviral resistance is virologic breakthrough that is defined as a >1 $\log_{10}$ increase in serum HBV DNA from nadir in a patient who had an initial virologic response [Pawlotsky *et al*. 2008]. Depending on the sensitivity of the genotyping assay, drug-resistant mutations can be detected months prior to the rise of the serum HBV DNA. The subsequent biochemical breakthrough with increased serum aminotransferases tends to occur 3 to 6 months after virologic breakthrough [Lau *et al*. 2000]. Antiviral resistance can be associated with acute hepatitis flare with decompensation of liver disease especially among those with advanced fibrosis [Liaw *et al*. 2004]. These observations underscore the importance of regular monitoring for early virologic breakthrough and adjust antiviral therapy accordingly to prevent biochemical breakthrough.

Lamivudine is associated with the highest rate of resistance, reaching near 70% by year four of continuous therapy [Lok *et al*. 2001]. The primary mutations associated with lamivudine resistance are located in the YMDD catalytic motif of the C domain of the HBV reverse transcriptase (RT) (rtM204V/I) while compensatory mutations (rtV173L, rtL180M) are identified in domain B [Locarnini, 2005]. By phenotypic analysis, the rtM204V and rtL180M combined mutations induce a 1000-fold decrease of susceptibility to lamivudine *in vitro* by comparison with wild-type (wt) HBV [Locarnini, 2005; Liu *et al*. 2001] The main effect of the compensatory mutations is to restore replication fitness of the drug-associated HBV mutant. Thus, HBV DNA level usually increases with continuous therapy after the emergence of the primary mutation [Locarnini, 2005; Liu *et al*. 2001]. Adefovir is generally effective against both wild type HBV and lamivudine resistant mutants [Ono-Nita *et al*. 1999]. There is evidence to support the 'addition' of adefovir to lamivudine in the presence of lamivudine resistance to prevent the subsequent development of adefovir resistance. Fung *et al*. initially reported a 22% adefovir resistance rate at year-two if lamivudine was 'switched' to adefovir monotherapy after the emergence of lamivudine resistance

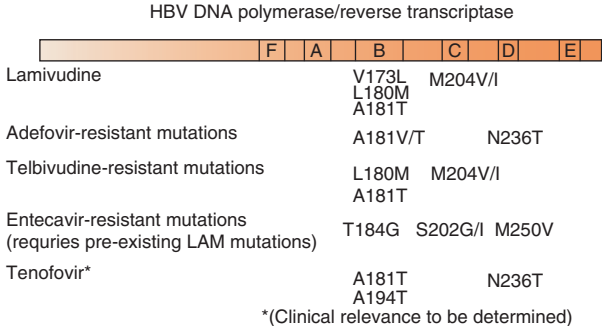

Figure 4. Anti-viral-induced mutations at HBV DNA polymerase/reverse transcriptase. Only the major primary mutations and clinically relevant compensatory mutations are shown.

[Fung *et al.* 2006]. More recently, Lampertico and coworkers compared the efficacy between adefovir monotherapy and adefovir, lamivudine combination on 588 HBeAg-negative patients with lamivudine-resistance CHB. They reported 0% adefovir resistance rate at three years with combination therapy compared to 16% with the switch from lamivudine to adefovir monotherapy [Lampertico *et al.* 2007].

Despite the initial low resistance rate with adefovir, the cumulative resistance rate increased to 29% by year five [Hadziyannis *et al.* 2005; Papatheodoridis *et al.* 2005]. The primary site of adefovir-associated resistance mutation, rtN236T, is located in domain D of the HBV reverse transcriptase. This mutation results in a 3- to 6-fold reduction in susceptibility to ADV *in vitro* and remains susceptible to nucleoside analogues such as lamivudine, telbivudine, and entecavir. In contrast, the rtA181V/T mutation of adefovir in domain B was found to have reduced responsiveness to lamivudine and telbivudine in phenotypic assays. It remains susceptible to entecavir and tenofovir (Table 4) [Zoulim, 2006; Locarnini, 2005; Ono-Nita *et al.* 1999].

A number of recent studies reported that lamivudine monotherapy can promote the emergence of rtA181T mutation in adefovir treatment-naïve patients [Villet *et al.* 2008; Locarnini, 2005]. This single substitution at position rt181 appears to be sufficient to induce cross-resistance between lamivudine and adefovir. In the specific setting of lamivudine resistance with the presence of both rtM204V/I and rtA181T substitutions, the addition of adefovir will not be effective. The addition of tenofovir to lamivudine or switch to Truvada will be the authors' therapy of choice in this case based on the available *in vitro* data and limited clinical presentations (Table 4). These observations with lamivudine and adefovir therapy highlight the important roles of both genotypic and phenotypic assays in identifying the antiviral drug associated mutations and in informing the selection of the subsequent salvage therapy.

The development of entecavir resistance requires pre-existing lamivudine resistance mutations and additional changes in the HBV polymerase/reverse transcriptase: T184 in domain B, S202 in domain C or M250 in domain E (Figure 4) [Colonno *et al.* 2006; Tenney *et al.* 2004]. The relatively low resistance rate of entecavir at <1% in five years among previous treatment-naïve patients can be explained by a combination of its high genetic barrier requiring multiple mutations to reduce its efficacy, and its antiviral potency[63] (Tenney *et al.* APASL 2008) (Figure 5). In contrast, for patients with pre-existing lamivudine resistance who were subsequently switched to entecavir, entecavir resistance rate increased to 43% after five years of continuous therapy [Tenney *et al.* APASL 2008]. This illustrates the important concept of

Table 4. Antiviral resistance, cross resistance and salvage therapy.

	Antiviral resistant mutation						
	Lamivudine-R		Adefovir-R		Entecavir-R		Telbivudine-R
	M204V/I ± L180M	A181T	N236T	A181V/T	M204V/I + L180M + T184G or S202I or M250V	[4]M204V/I ± L180M	A181T
TDF *In Vitro* Cross Resistance	ETV, LdT	ADV, LdT	LAM, ETV	LAM, LdT	LAM, LdT	LAM, ETV	LAM, ADV
Remain sensitive	ADV, TDF	[3]TDV, ETV	LdT	[3]TDF, ETV	ADV, TDF	ADV, TDF	[3]TDF, ETV
[2]Salvage therapy	Add ADV *or* add [1]TDF *or* switch to [1]Truvada	Add [1]TDF *or* switch to [1]Truvada	Add LAM *or* add ETV *or* add LdT	Add ETV	Add ADV *or* add [1]TDF	Add ADV *or* add [1]TDF	Add [1]TDF *or* switch to [1]Truvada

ADV = adefovir, TDF = tenofovir, LAM = lamivudine, ETV = entecavir, LdT = telbivudine, Truvada = TDF plus emtricitabine.
[1]TDF and Truvada are not currently FDA-approved for chronic hepatitis B.
[2]The suggested salvage therapy is based on both *in vitro* cross resistance profiles and clinical findings. They reflect the experience and opinions of the authors.
[3]1-fold decrease in TDF susceptibility for rtA181V/T *in vitro* (van Bömmel et al, poster #960, AASLD 2007).
[4]rtM204V and rtL180M, in addition to rtM204I, have also been associated with telbivudine use.

the emergence of drug resistance in the setting of reduced genetic barrier.

Even though both entecavir and telbivudine have excellent antiviral potency, telbivudine monotherapy is associated with much higher rate of resistance, up to 22% for HBeAg-positive CHB at two years [Keeffe *et al.* 2008; Lai *et al.* 2007]. This could be partially explained by the difference in genetic barrier in the development of resistance between the two drugs. Unlike entecavir, telbivudine only requires the single mutation to confer resistance. Cross-resistance between lamivudine and telbivudine is unavoidable since both drugs induce mutations at HBV reverse transcriptase position 204. Similar to lamivudine, the presence of telbivudine resistance would likely predispose to the emergence of entecavir resistance based on the *in vitro* data [Zoulim, 2006; Yang *et al.* 2005]. Similar to lamivudine, there is evidence that telbivudine can promote the emergence of rtA181T in treatment-naïve patients.

Monitoring and management of antiviral resistance

Antiviral resistance is the major limitation of prolonged nucleos(t)ide analogue therapy. Careful consideration is needed to select first-line therapy in order to avoid the emergence of resistance; especially that may limit future treatment choices due to cross resistance with other agents. Lamivudine, in the authors' opinion, is no longer considered a first-line monotherapy because of its high rate of resistance. Even though the wild-type HBV repopulates and becomes the dominant viral species after the discontinuation of antiviral therapy in the setting of resistance, the drug resistant mutants will persist indefinitely in low level. Upon rechallenged with the same drug or drugs with cross-resistant profiles, the resistant mutants will have growth advantage and replicate in high levels [Lau *et al.* 2000].

HBV DNA quantification is important for initial patient evaluation, for monitoring treatment response and for early detection of virological breakthrough on therapy. The ideal HBV quantification assay should be sensitive, reproducible and have a broad dynamic range of at least 5 $\log_{10}$. The real-time PCR quantification assays possess these properties and, therefore, are recommended for HBV DNA baseline determination and monitoring during therapy [Lole and Arankalle, 2006; Gordillo *et al.* 2005]. All patients should have baseline serum HBV DNA, ALT, liver function tests, HBeAg/anti HBe prior to initiating the treatment. Thereafter, serum HBV DNA and ALT should be checked every 3–6 months to ensure adequate response to the treatment and early detection of treatment failure [Pawlotsky *et al.* 2008].

For nucleos(t)ide analogue, its antiviral effect is defined as ≥ 1 $\log_{10}$ decrease in HBV DNA within three months of starting the treatment while its antiviral efficacy is the quantitative $\log_{10}$ reduction in viral load when compared to pretreatment level [Pawlotsky *et al.* 2008]. Treatment failure can be primary and secondary.

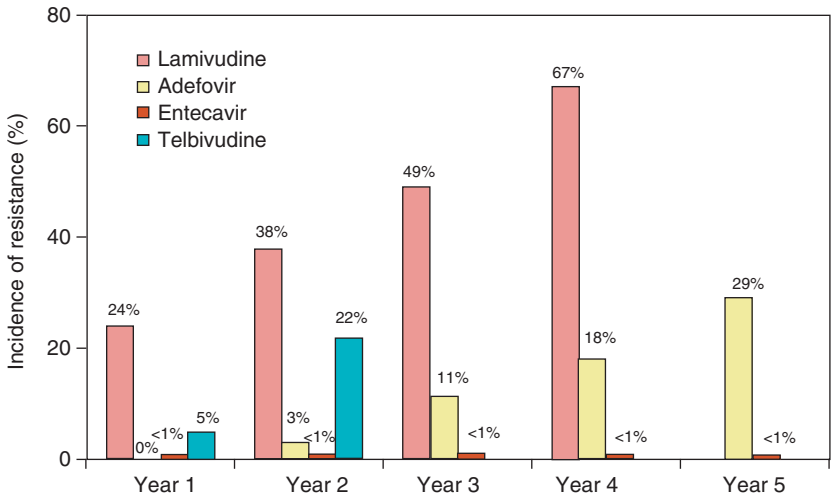

Figure 5. Rate of antiviral resistance with virologic breakthrough on continuous monotherapy.

Primary treatment failure is defined as a decrease in serum HBV DNA of ≤ 1 $\log_{10}$ IU/mL from baseline after three months of starting therapy [Pawlotsky *et al.* 2008]. Secondary treatment failure is a rebound of serum HBV DNA resulting in an increase of ≥ 1 $\log_{10}$ IU/mL in patients with initial antiviral treatment effect [Pawlotsky *et al.* 2008; Lok and McMahon, 2007]. This should be confirmed by two consecutive determinations at a one-month interval. For patients with primary or secondary treatment failure, medication non-compliance should be excluded and if drug resistance is suspected, resistance testing should be performed [Pawlotsky *et al.* 2008; Lok and McMahon, 2007].

The sensitivity of the different genotypic assays for the detection of drug resistance varies significantly in their ability to identify minor strains of viruses (Table 5)[Sablon and Shapiro, 2005; Sablon *et al.* 2003]. Direct sequencing, for example, has relative low sensitivity and can only detect mutant viruses if they exceed 15–50% of the total viral population. The advantage of direct sequencing is its ability to identify new mutations. LiPA and MALDI-TOF assays, in contrast, can identify very low levels (5%) of the mutant viruses. It is, therefore, important to know which resistance assay was applied. Standardization of the genotypic resistance assays is necessary to determine the incidence and prevalence of nucleos(t)ide-induced resistant HBV mutations.

Combination therapy

Mathematical modeling of the HBV kinetics with nucleotide analogue therapy showed a biphasic decline of the HBV levels. The initial, faster phase of viral load decline reflects the clearance of HBV particles from plasma. The second, slower phase of viral load decline closely mirrors the rate-limiting process of infected cell loss [Tsiang *et al.* 1999]. Since the second phase of viral decline is likely to be induced by an immune-mediated process, the immune clearance of the virus should be up-regulated by immunomodulators such as the IFNs. This suggests that there may be at least a theoretical advantage to the use a combination of a nucleoside or nucleotide analogue with IFN.

Nucleoside analogues and pegIFN

There are a number of published multicenter clinical trials using a combination of lamivudine and pegIFN-α. In the study conducted by Janssen *et al.*, 307 HBeAg-positive patients were randomized to receive either a combination of pegIFN α-2b, 100 µg/week for 32 weeks then 50 µg/week for 20 weeks in combination with lamivudine 100 mg/day, or pegIFN α-2b with placebo [Janssen *et al.* 2005]. At 26 weeks follow-up, no difference in efficacy endpoints was found between the pegIFN monotherapy and combination therapy which used a relatively low dose of pegIFN. Besides elevated baseline ALT levels, HBV genotype also was identified to be a predictor of response: 60% of the genotype A patients responded compared to 42% for genotype B, 32% for genotype C and 28% for genotype D. Lau *et al.* and Marcellin *et al.* reported results of large randomized controlled trials comparing the efficacy and safety of pegIFN α-2a (180 µg weekly), pegIFN α-2a (180 µg weekly) with lamivudine (100 mg daily) and lamivudine (100 mg daily) alone for 48 weeks in HBeAg-positive and negative patients, respectively [Lau *et al.* 2005; Marcellin *et al.* 2004]. At 24 weeks of follow-up, the two pegIFN treatment arms (with or without lamivudine) showed the same efficacy in HBV DNA suppression and HBsAg seroconversion, and were superior to that observed with lamivudine alone in both studies. There was a higher rate of lamivudine resistance in the lamivudine monotherapy arm (18%) compared with the pegIFN α-2a plus lamivudine combination arm

Table 5. Comparison of genotypic assays for detection of drug resistance.[1]

Method	Sensitivity	Information details	Commercial	Complexity of interpretation
Direct sequencing	15–50%	High	Yes	High
RFLP	5–10%	Low	No	Intermediate
RT-PCR	5–10%	Low	No	Intermediate
LiPA	5%	Low	Yes	Low
Florescence	Not determined	Intermediate	No	Intermediate
[2]MALDI-TOF	<5%	Intermediate	No	High

[1]Modified from Erwin Sablon and Fred Shapiro, [Sablon and Shapiro, 2005].
[2]matrix-assisted laser desorption and ionization time-of-flight mass.

(<1%) at week 48 ($p < 0.001$). It is important to emphasize that in both studies combination therapy was associated with at least a 1 $\log_{10}$ greater HBV DNA suppression at the end of the 48-week treatment period compared to either monotherapy. This finding raises the possibility that with prolonged therapy, the durability of combination therapy will increase.

Combined nucleoside and nucelotide analogues

To date, there has been limited data on the efficacy of combining nucleoside and nucleotide analogues. Lau *et al*. evaluated combination therapy with lamivudine and famciclovir in 21 HBeAg-positive Chinese patients [Lau *et al*. 2000]. They found that patients who received lamivudine 150 mg daily and famciclovir 500 mg three times daily had a more rapid fall in HBV DNA levels and a higher rate of HBeAg loss compared to those on lamivudine monotherapy. A recent study compared the efficacy of adefovir with lamivudine *vs* lamivudine alone in 112 treatment-naïve, predominantly HBeAg-positive patients [Abstract, Sung *et al*. J Hepatol 38(suppl), A4313. 2003]. The rates of undetectable HBV DNA by PCR (39 and 41%) and HBeAg loss (19 and 20%) were similar in the two arms. However, there was a significantly lower rate of lamivudine resistance in the combination group (2%) compared to lamivudine monotherapy (20%) ($p \leq 0.003$).

Although, the combination regimens evaluated so far for 48 to 52 weeks did not appear to improve efficacy, they did reduce the rates of resistance to nucleoside or nucleotide monotherapy (see above). Currently, there are no data on prolonged combination therapy beyond a year. An optimal combination regimen should work synergistically in viral suppression, increase rates of HBeAg and HBsAg seroconversion, and prevent the occurrence of viral resistance.

Who should be treated?

Complete eradication of HBV is not achievable with the currently available agents. Most of the patients with CHB require long-term treatment that can be associated with increased risk of developing antiviral resistance and potential side effects. Until ideal therapy becomes available, it is logical to provide therapy for selective patients who are at risk of developing complications from CHB. A number of recently published practice guidelines provided important framework to manage patients with HBeAg-positive and negative CHB [Keeffe *et al*. 2007; Lok and McMahon, 2007]. The recommendations on therapy are largely based on serum levels of ALT and HBV DNA. There are, however, continuous debates on the optimal ALT and HBV DNA cut-off values to initiate therapy. For patients who do not meet the clear HBV and ALT criteria for therapy, liver biopsy is essential to determine the degree of hepatic inflammation and fibrosis and to treat if there is evidence of disease. The degree of liver injury and its rate of progression vary significantly among patients with CHB. Factors such as serum ALT, HBV DNA, HBV genotypes, naturally occurring HBV mutants and hepatic steatosis have been implicated in disease progression but their accuracy is imperfect. A better understanding of the natural history and identification of predictors of disease progression are crucial for the selection of patients for therapy.

Although, serum ALT levels have traditionally been used as an indicator of the severity of hepatic necroinflammatory activity, emerging data suggest that it does not always reflect the degree of underlying disease in CHB. While the REVEAL Study Group noted that patients with higher baseline ALT levels had increased rates of liver disease progression, more than 80% of the cases of cirrhosis and HCC occurred in patients with ALT activity lower than 45 U/L[Chen *et al*. 2006; Iloeje *et al*. 2004]. Other studies have shown that 30–40% of patients with normal serum aminotransferases may have significant degree of liver disease on biopsy [Kim *et al*. 2004]. Taken together, these findings suggest that serum ALT activity within the normal laboratory range may not be a reliable prognostic predictor for CHB. Limitations of these studies are the lack of serial ALT measurements during the follow-up period and the lack of detailed patient characterizations. Since serum aminotransferases fluctuate over time, especially among those with HBeAg-negative CHB, a single, baseline value cannot be expected to reliably predict the course of a chronic disease. In addition, patients with advanced cirrhosis usually have normal or near-normal ALT. Thus, ALT values must be evaluated in the context of other lab results and clinical features.

The serum concentration of HBV DNA is a measure of the level of viral replication in the liver. Chen *et al*. conducted a long-term observational study on over 3000 HBV carriers in Taiwan for a mean follow-up period of eleven years and found

that the risk of cirrhosis and HCC increased significantly proportional to the levels of serum HBV DNA $\geq 10^4$ copies/mL [Chen *et al.* 2006; Iloeje *et al.* 2003]. The incidence of cirrhosis increased from 4.5% (relative risk, 1.4) for patients with baseline serum HBV DNA concentrations <300 copies/ml to 36.2% (relative risk, 9.8) for patients with serum concentrations of $\geq 10^6$ copies/mL. The relationship between serum HBV DNA concentration and cirrhosis remained independent of HBeAg status and ALT level. Likewise, a high baseline and persistently elevated serum HBV DNA concentration increases the risk of HCC. Of the 164 patients in whom HCC developed, the incidence rates of HCC increased in a dose-response relationship beginning with a baseline serum HBV DNA concentration of 10^4 copies/mL. The findings of this study are important, however; it suffers from a number of limitations similar to those of smaller retrospective studies. The patients in these studies did not have liver biopsies at baseline or during follow-up, so that the subset of patients who developed cirrhosis or HCC within each of the HBV DNA categories could not be assessed as to risk based on histological criteria. For the majority of the cohort, there was no monitoring of serial ALT, HBV DNA levels, or HBeAg serology during follow-up. Other important viral factors such as HBV genotype and HBeAg-negative mutants were not factored into the analysis. In addition, 85% of this study population had HBeAg-negative CHB. These findings require confirmation before they can be generally applied, especially to young subjects in the immune tolerance phase of the disease.

Available data suggest that HBV genotype may be related to disease outcome. Due to the variability of the HBV genotypes in different regions of the world, a comprehensive comparison of disease outcome based on genotypes using standardized study criteria is unavailable. In Asia where genotype B and C predominant, genotype C is found to be associated with a higher risk of reactivation of hepatitis B and progression to cirrhosis than genotype B [Chan *et al.* 2004; Yuen *et al.* 2004]. In Europe, genotype D is associated with more active disease than genotype A [Kidd-Ljunggren *et al.* 2002]. Genotype F was recently implicated in conferring increased risk of HCC in Alaskan natives [Livingston *et al.* 2007] To date, there has been little information regarding disease outcomes associated with genotype E, G, and H. Besides HBV genotypes, naturally occurring HBV mutants have been implicated in disease progression. A number of Asian studies have provided evidence that BCP mutants increase the risk of liver disease progression and HCC development. Since genotype C has a higher prevalence of BCP mutation than genotype B (odds ratio, 5.18), it is uncertain whether BCP mutant alone is an independent risk. Deletions in the pre-S gene of HBV genome have also been implicated in progressive liver injury and hepatocarcinogenesis [Sugauchi *et al.* 2003; Fan *et al.* 2001].

These naturally history studies collectively provide evidence that high HBV DNA, genotype C, BCP mutation and pre-S deletion are associated with liver disease progression and HCC development in patients with CHB. It is possible that a combination of these viral factors synergistically increases risk for disease complications. Large-scale prospective studies are needed to confirm the causal relationship between these viral factors and clinical outcomes of chronic HBV infection.

There is strong evidence that nonalcoholic fatty liver disease (NAFLD) and insulin resistance are associated with increased fibrogenesis in chronic hepatitis C [Hu *et al.* 2007; Leandro *et al.* 2006; Patton *et al.* 2004]. The impact of NAFLD and metabolic syndrome on CHB is not well understood. In a retrospective study, Bondini and colleagues found that among 64 HBV patients with available liver biopsies, 8 had nonalcoholic steatohepatitis (NASH) and 4 had simple steatosis [Bondini *et al.* 2007]. They reported that patients with hepatitis B superimposed with NASH were older, more likely to have hypertension, dyslipidemia, and increased waist circumference. Interestingly, Chu *et al.* found that increased body mass index (BMI) and hepatic steatosis are associated with HBsAg clearance [Chu *et al.* 2007]. The authors compared BMI and ultrasound grading of hepatic steatosis between 54 patients with documented HBsAg clearance and 108 age- and gender-matched HBsAg carriers. High BMI (>26) and moderate to severe hepatic steatosis were significantly more prevalent among those with HBsAg clearance. The major limitation of the study is the lack of histological evaluation of steatosis, inflammation and fibrosis. In spite of these limitations, the impact of metabolic syndrome and hepatic steatosis on hepatitis B merits careful evaluation in a prospective study since the prevalence of obesity and NAFLD is increasing among Asian Americans.

Conclusions

HBV continues to be a major cause of significant morbidity and mortality despite the availability of effective vaccines and improved therapeutic options. The natural history of hepatitis B is not well understood. A number of viral and host factors have been implicated in disease progression and development of HCC. However, histological data is lacking in most published studies. Furthermore, viral and host factors may work additively or even synergistically in modifying disease status. A large, prospective clinical study with uniform histological assessment and well-represented populations of CHB will be instrumental to accurately establish predictors and confounders of disease outcomes. This will not only extend our understanding of the natural history of hepatitis B but will also accurately guide treatment strategy for this important disease. The ultimate goal of therapy for CHB is to arrest the progression of liver injury and to prevent the development of hepatic complications such as liver failure and HCC. Sustained inhibition of HBV replication has been shown to be associated with normalization of aminotransferases and histological improvement, while HBsAg seroconversion is the best surrogate marker for viral clearance. Choice of first-line therapy taking into account antiviral potency, safety, and low risk of antiviral resistance is critical. The ideal hepatitis B therapy should be safe, effective with a finite course of therapy and associated with a sustained and durable response. Ongoing research is essential to evaluate the new and currently available agents, not only as monotherapy, but as combination therapy to identify the synergies necessary to reach the ultimate goal of therapy.

Conflict of interest statement

Daryl Lau: Bristol-Myers Squibb (research support), Gilead Sciences (consultant), Idenix Pharmaceuticals (consultant), Roche Pharmaceuticals (consultant and research support).

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