

Current Treatment and Future Directions in the Management of Chronic Hepatitis B Viral Infection

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The World Health Organization places hepatitis B virus (HBV) in the top 10 causes of death worldwide [1]. It is estimated that there are over 400 million carriers of HBV as well [2]. At least 20% to 30% of hepatitis B surface antigen (HBsAg) carriers will die of complications of chronic liver disease, including cirrhosis and liver cancer [3,4]. The serious consequences of end-stage liver disease and liver cancer occur in 30% of chronic carriers and confront patients and physicians throughout the world [5–9]. Vaccination is the major form of treatment (prevention) that may eventually eliminate HBV worldwide [10]. This article discusses the currently available treatments as well as evolving treatments for chronic HBV infection.

Tools, terminology, and assays

Liver biopsy remains an important tool to evaluate patients with chronic HBV infections but is not required for all patients. The determination of disease stage and grade allows the patient and physician to decide on treatment strategy, timing of treatment, and the need for and frequency of liver cancer screening. Alternatives to performing a liver biopsy to determine who to treat include surrogate testing (platelet count, white blood cell count, aspartate aminotransferase [AST] > alanine aminotransferase [ALT], presence of varices or an increase in spleen

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Table 1
Treatment Box

	ALT \leq 30 ^m U/L	ALT >30 ^m U/L
DNA <10 ⁴ copies/mL	Observe [Ⓢ]	Biopsy and treat underlying disease [ⓔ]
DNA >10 ⁴ copies/mL	Biopsy and treat if active HBV	(Consider Biopsy) eAg (+): \geq 6 m post eAg seroC eAg (-): Prolonged Rx beyond NAT [ⓓ] negative [Ⓢ]

^m Upper limits of normal for a person with a normal BMI.

[Ⓢ] Treat any patient with cirrhosis who is NAT positive, refer to specialist.

[ⓔ] Rule out fatty liver and other causes of CLD.

[ⓓ] NAT=Nucleic acid testing, such as PCR, bDNA or TMA.

[Ⓢ] Consider 3–5 years.

size), or the use of specific HBV DNA and ALT thresholds [11–14]. Because more powerful therapies are currently in development, it may be reasonable to decide to wait for their arrival instead of starting existing therapies in patients who have mild liver disease according to biopsy information. However, it is probably not feasible to biopsy all patients who have chronic HBV. Consequently, it is reasonable to use oral or injectable therapies when there is an increased risk of liver failure or liver cancer according to defined liver enzyme and HBV DNA levels (Table 1).

Definitions

Chronic hepatitis B virus disease

Chronic HBV disease has been defined by several expert panels as hepatic necroinflammation due to the presence of HBV [11,12,14]. These patients are positive for HBsAg (detected on at least two occasions in a 6-month period) and HBV DNA with serum levels of DNA greater than 100,000 copies per mL or the presence of hepatitis B core antigen (HBcAg) staining in the liver. Liver enzyme levels are either persistently elevated or intermittently elevated over periods of 6 months or longer. Controversies continue to evolve concerning the threshold of HBV DNA [13] to determine the diagnosis of chronic liver disease and at what threshold is an ALT or AST considered abnormal [15]. Patients with chronic HBV disease can be divided into three subgroups: hepatitis B e antigen (HBeAg)-positive, HBeAg-negative, and patients who lack active replication. Patients who have normal ALT levels but high levels of HBV DNA and necroinflammation on liver biopsy may also be included in the group with minimally active disease due to low risk of progressive liver disease compared with patients who have persistent or intermittent ALT elevation [5,7–9]. Patients who have elevated ALT and high HBV DNA levels are the leading candidates for therapy.

Reactivation of HBV disease occurs when a person is in the inactive HBsAg carrier state and develops elevated liver enzyme and HBV DNA levels and, in some patients, detectable HBeAg if the wild type virus is the dominant infection. The liver biopsy typically has a HAI score of 4 or greater [16]. These patients are candidates for treatment if they meet liver biopsy or surrogate criteria for active disease.

Acute exacerbation or flare

Some patients develop intermittent elevations of liver enzymes to levels that may exceed 10 times the upper limit of normal and are twice the baseline level. This flare of activity may be associated with viral integration into the hepatocyte's genome, transition to other clinical states, as defined in this section, or drug resistance if the patient is receiving oral medications [11].

Resolved infection

Resolved HBV infection is defined by serologic tests showing negative HBsAg, positive antibody to HBsAg (anti-HBs), and negative serum HBV DNA results in patients who have a history of HBV infection and currently normal liver enzyme levels. These patients may remain anti-HBc positive for 5 to 10 years or longer and are at risk of transmitting disease on rare occasions (such as the donation of solid organ tissue) or of reactivating HBV disease if treated with immunosuppressive medications. During periods of immunosuppression, active intervention with antiviral agents is strongly advised [11,12,14].

HBeAg clearance, seroconversion or reversion

A key step in the control of wild-type HBV infection by either the immune system or therapeutic intervention is the transition from HBeAg-positive disease

to the presence of anti-HBe. This event may occur in a stepwise fashion beginning with clearance of HBeAg but an absence of anti-HBe. This is an unstable state and may revert to active replication and reappearance of HBeAg. The long-term clearance of HBeAg and the appearance of anti-HBe is a much more stable state with rare reversion to the HBeAg-positive state, either with natural immune pressure or during/after directed antiviral therapy [11,17–19]. However, this may signal the emergence of the HBV precore mutant, which is marked by HBV DNA > 10,000 and continued HBeAg negativity [20–22].

Coinfection

Coinfection defined by the presence of active replication of hepatitis Delta virus (HDV), hepatitis C virus (HCV), or HIV presents a serious risk of active liver disease. The dominant viral infections must be treated if progressive liver disease is evident on biopsy [23–30].

Hepatitis B virus variants and mutants

The reverse transcriptase/polymerase of HBV lacks the conventional proof-reading function of higher-order polymerases. Therefore, HBV exhibits a mutation rate more than 10-fold higher than other DNA viruses and more closely resembles retroviruses such as HIV [31,32]. The natural evolutionary rate for the HBV genome in chronic hepatitis B is approximately 1.4 to 3.2×10^{-5} substitutions per site per year, while it is almost 100-fold higher in the liver transplantation setting, which is possibly as a result of immunosuppressive therapy. The issue of drug resistance will be expanded upon in the section on nucleoside and nucleotide analogs.

Precore and core gene mutants

The HBV core gene contains two in-frame start codons that control the synthesis of HBcAg and HBeAg [31,32]. Both proteins are targets for immunomediated viral clearance. HBcAg is the major protein component of the nucleocapsid and is required for viral replication. The two major groups of mutations that affect HBeAg synthesis are mutations in the precore region (G1896A) and in the basal core promoter (BCP) at nucleotide (nt) 1762 and nt 1764, all resulting in diminished production of HBeAg and an increased host immune response [31]. Precore mutations frequently occur temporally related to core gene mutations/deletions [33]. Mutations in the precore and BCP are likely to increase viral replication and enhance disease activity [33–36]. Mutations in the BCP, particularly at nt 1762 and nt 1764, resulting in T1762 or A1764, have been detected in patients who have persistent infection and fulminant hepatitis, as well as in immunosuppressed patients, and may increase the risk of liver cancer. The double mutation at T1762 and A1764 together is associated with a decrease in HBeAg

and an increase in viral load [37,38]. These mutants may cause a more severe course of disease, are often resistant to antiviral therapy, and require long-term oral therapy or the use of interferon therapy [39]. They may also be associated with liver cancer [39].

Polymerase gene mutations

The HBV polymerase open reading frame (ORF) is the longest region, covering almost 80% of the viral genome and overlapping the other three ORFs [32]. The HBV polymerase protein mediates encapsidation of pregenomic RNA into core particles and synthesizes the HBV DNA genome therein. It has four enzymatic activities: priming of DNA synthesis, RNA-dependent reverse transcriptase, DNA-dependent DNA polymerase, and RNaseH (RNA degradation). Hepatitis B virus quasispecies with polymerase mutations have been detected in patients receiving antiviral therapy [40]. On exposure to lamivudine therapy, discrete polymerase nucleotide polymorphisms result in amino acid changes in codon L180M (the template binding site of the polymerase; domain B) as well as codon M204V/I of the YMDD motif (the catalytic site of the polymerase; domain C), or both [41]. These mutations significantly decrease the *in vitro* sensitivity of the polymerase to lamivudine-associated chain termination and competition for enzymatic inhibition [40]. Similarly, after famciclovir therapy, polymerase mutations in codon L180M markedly decrease famciclovir efficacy [42]. Other mutations in these regions have been associated with resistance to newer therapies such as adefovir, entecavir, and LdT. The frequency of mutations appears to be very common with famciclovir, rare with adefovir, very rare with entecavir (and require the pre-existence of lamivudine resistance mutations), and intermediate with LdT therapy [43–49].

Genotypes and serotypes

HBV has several genetic variants described as serotypes (adw, ayw, and adr) and genotypes (A, B, C, D, E, F, G, and H) [50]. Genotype can be specified by sequencing the HBV genome (TRUGENE 1.0, Bayer Diagnostics, Emeryville, CA) or using a line probe assay (InnoLIPA, Innogenetics, Belgium). Serotyping can be performed using a serologically based antibody testing platform, although this test has never been used in clinical practice. Serotyping and genotyping may allow epidemiologists to track the movement of HBV infections throughout the world, potentially establishing specific clinical behaviors to each subgroup. Patients who have genotype A or B appear to respond to interferon therapy much better than those who have genotype C or D [51–54]. There is preliminary evidence that genotype B has a higher rate of DNA and ALT suppression than genotype C during lamivudine therapy [55]. Furthermore, patients with genotype C may have more serious disease when YMDD mutants emerge during lamivudine therapy [55]. There is also a statistical difference in the appearance of lamivudine resistance between the subtypes of genotype B; Ba has a higher

to anti-HBe occurs in 1% to 10% of chronic carriers per annum, but seroconversion from HBsAg to anti-HBs, with clearance of HBV from the liver, is very uncommon ($\leq 1\%$ per year) [57]. The rate of HBsAg seroconversion is higher in patients with more active liver disease as measured by liver enzymes than in those with persistently normal liver enzymes, but this is generally less than 15% over any 3-year interval [6,7]. In patients who have a flare of liver enzymes and rapidly declining HBV DNA, antiviral therapy may be delayed in patients with wild type (HBeAg-positive) infection to see if the patient is undergoing natural seroconversion. Flares are very rarely associated with spontaneous seroconversion of HBV DNA in patients with the precore mutant variant. Thus, these patients are more likely candidates for antiviral therapy if there is any evidence of active liver disease. The chronic carrier with a persistently normal level of liver enzymes (< 30 IU/mL) is at a much lower risk of progressive liver disease and cancer, although the overall risk is still significant. These patients commonly have minimal liver disease on biopsy, although significant fibrotic liver disease is observed in more than 20%. The frequency of blood testing of these patients is an important issue because annual testing may be insufficient to document a stable patient or define inactive disease. For such patients, the frequency of screening with blood tests could be at least biannually and a more thorough evaluation, such as testing every 3 months, may be justified. To decide who to treat, knowledge of the various disease states is required as well as arranging regular laboratory analyses including liver function tests, liver enzymes, HBV DNA by nucleic acid testing, anti-HBe, and HBeAg. A liver biopsy should also be considered.

Hepatitis D virus

A patient with dual HBV and HDV infection has a typical serological profile of HBsAg and anti-HBe positive (rarely HBeAg positive) as well as IgG anti-hepatitis delta (anti-HD) seropositivity (Table 2). Specific immunoassays are

Table 2
Clinical tests used for hepatitis B virus

	HBsAg	anti-HBc	anti-HBs	HBeAg	anti-HBe	DNA	anti-Delta	Vaccine Response
Acute HBV	+	+IgM	-	+	-	+	-	-
Acute HBV/Delta	+	+IgM	-	-/+	-	-/+	+	-
Chronic HBV wild type	+	+IgG	-	-/+	-/+	-/+	-	-
Chronic HBV high replication	+	+IgG	-	+	-	+	-	-
Chronic HBV low replication	+	+IgG	-	-	+	-	-	-
Chronic HBV/Delta	+	+IgG	-	-	+	-	+	-
Chronic HBV precore mutant	+	+IgG	-	-	+	+	-	-
Chronic HBV core mutant	+	-	-	+/-	+/-	-/+	-	-
Chronic HBV sAg mutant	-	+IgG	-	-/+	-/+	+	-	-
HBV vaccine response	-	-	+	-	-	-	-	+
Isolated anti-HBc								
False positive	-	+IgG	-	-	-	-	-	low titer
Low level carrier	-	+IgG	-	-	-/+	+	-	-
anti-HBs senescence	-	+IgG	-	-	-/+	-	-	high titer

available for detection of both IgM- (for both acute and active infection) and IgG-specific anti-HD (seen in chronic disease as well as resolved infection). Serum assays for detecting HDAg and HDV RNA are typically only available from central reference laboratories. Delta infection when superimposed on HBV disease, either acute or chronic, leads to much more serious outcomes such as fulminant liver failure or accelerated disease to cirrhosis. Treatment usually means more than 1 year of interferon therapy [58].

Hepatitis B virus coinfection with HIV

HBV coinfection with HIV results in more aggressive liver disease (progression to cirrhosis and liver failure) in some patients, although other patients have minimally active liver disease with little or no evidence of progressive liver disease [59]. Why certain patients have progressive liver disease in this setting and others do not is unclear and is not correlated with any specific clinical factor or laboratory test. Treatment with lamivudine, adefovir, or tenofovir may slow the progression of liver disease but is problematic from the perspective of increased resistance compared with patients who are not coinfecting [60]. With the advent of combination therapy with tenofovir and emtricitabine, both of which are effective against HBV and HIV, control of HBV in this population will be much easier.

Hepatitis B virus coinfection with hepatitis C virus

HBV coinfection with HCV results in increased risk of cirrhosis and liver cancer. Patients often have one dominant viral disease. Treatment focused on the dominant virus, as revealed by nucleic acid testing for viral replication, is probably the best management strategy [61–66].

Treatment response definitions

Definitions of response are defined as biochemical (liver enzymes), serologic (HBeAg and HBsAg status), virologic (viral replication), and histologic (liver biopsy). Another important perspective on HBV treatment is the question of whether HBV infection is ever “cured.” Chronic HBV exists in a very stable form in the hepatocyte known as covalently closed circular DNA (cccDNA) that has a very long intracellular half-life. There have been recent reports of antiviral effects (reduced cellular levels) by nucleoside or nucleotide analogs on cccDNA, including adefovir [67,68] and entecavir [69]. Clearance of this stable intracellular form of the HBV genome has not been shown conclusively with any antiviral agent and probably explains why HBV cannot be cured.

Biochemical response is defined as a fall in liver enzyme levels into the normal range. These events, like the virologic definition below, can be initial (during therapy), end of therapy, or sustained (after therapy). Recently, normal ALT has been defined more precisely as simply ALT of < 30 IU/mL for men and 27 IU/mL for women [15]. A sustained response is indicated by liver enzymes that are normal at the end of therapy and at least 6 to 12 months following cessation of therapy. Partial response is indicated by a marked fall (greater than 50%) in liver enzyme levels to the near normal range, reductions which are typically associated with improvement in histology [70–75].

Virologic response is indicated by a decrease of HBV DNA to less than 100,000 to 700,000 copies per mL from pretreatment levels greater than 100,000 or by a greater than 2 log reduction from baseline [70–75]. More recent definitions have arisen from newer assays with viral detection levels of 2000 copies/mL (bDNA Versant 3.0; Bayer Corp, Emeryville CA). The Roche Amplicor COBAS measures down to 200, 300, 400, or 1000 copies/mL depending on the assay generation. With the advent of an international standard, viral replication levels are now being reported in international units by most laboratories [76–78]. Assays that will be available in the very near future will measure as low as 40 IU/mL (TaqMan; Roche Diagnostics, Pleasanton, CA) as well as the transcription-mediated amplification assay developed for blood screening by Chiron Corporation (Procleix Ultrio, Emeryville, CA) that measures as low as 11 IU/mL. The level of reduction in serum HBV DNA levels has correlated with HBeAg loss with lamivudine, LdT, and adefovir (Fig. 1) [70–75]. More recently, the further reduction in HBV DNA levels by 6 logs with LdT and by 7 logs with entecavir did not result in a higher rate of HBeAg to anti-HBe seroconversion in 1-year

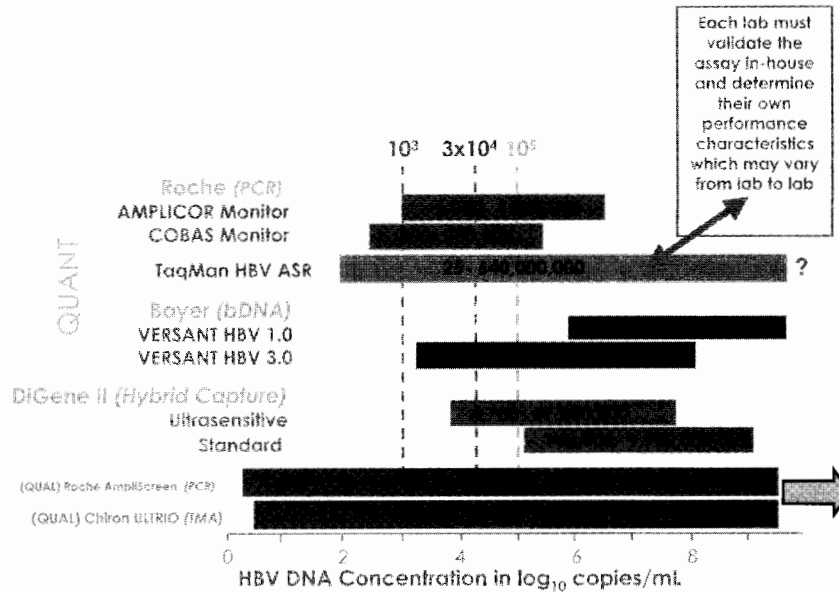


Fig. 1. Ranges of HBV DNA assays (copies/mL).

phase II and III clinical trials when compared with lamivudine or historical studies. This may indicate that the limit of HBeAg seroconversion has been reached with early DNA suppression and long-term data will be required to see if these high levels of viral suppression can result in better seroconversion rates at 2 to 5 years. An additional implication of the recent trial by Lau and colleagues [79] published in abstract form at the Boston 2004 meeting of the American Association for the Study of Liver Diseases showed a 32% HBeAg to anti-HBe seroconversion rate after 1 year of pegylated (PEG)-interferon therapy (PEGASYS; Roche Pharmaceuticals, Nutley, NJ) was that immune activation with interferon is required to further increase or accelerate seroconversion. Researchers and clinicians may also need to reach for a partner—specifically, immune activation (interferon or therapeutic vaccines)—to bring about further seroconversion in the case of wild-type virus and persistent DNA suppression in the case of the precore mutant. Virologic response can also be termed initial, end of treatment, or sustained if there is loss of viral markers or replication for 6 to 12 months after treatment.

Histologic response is defined as an improvement (improvement of ≥ 2 points of necroinflammation and no change in fibrosis) in liver histology on paired liver biopsies. It is the ultimate laboratory and patient goal of therapy and appears to correlate with the prevention of death, cirrhosis, and liver cancer. This has also been the primary goal of major licensing trials [70–75]. New medications that are currently being developed in phase III trials such as LdT are not required to

focus on this end point to be licensed after advocacy in the literature [80]. Improvement is usually defined by the Knodell HAI score and according to fibrosis by the Knodell, Ishak, Scheuer, or Metavir score or one of its modifications. There is no international consensus about which scoring system is best, but the Ishak fibrosis score is becoming the most commonly used system in clinical trials.

Complete response is defined as normalization of liver enzymes, serologic response, virologic response, and loss of HBsAg. Serum HBeAg must be negative, and HBV DNA must be negative by the most sensitive molecular test available. Complete response is synonymous with resolved HBV infection. This term does not require the presence of anti-HBs and does not require a follow-up liver biopsy. However, HBsAg to anti-HBs seroconversion is the best end point that can be measured by way of serologic testing and indicates the most stable form of viral suppression.

Current therapeutic options

Interferon

Interferon is a collective term for a large family of naturally occurring peptides that modulate immune function and are included within an even larger group of molecules known as *cytokines*. They are often involved in the host's control or elimination of acute and chronic viral infections. Interferon directly decreases viral replication by interference with DNA and RNA replication at the genome level. Interferon also indirectly modulates viral replication and viral protein synthesis by activating other immune pathways and modulating the levels of other cytokines.

Approximately 20 natural forms of interferon exist in humans, including alfa-2a (produced in lymphocytes), alfa-2b, beta (produced in fibroblasts), gamma (produced in T-cells), and omega. Many these interferons have been isolated and shown to have direct antiviral effects on HBV replication in humans [81]. Interferon is the only medication that has been proven to reduce and eliminate HBV infection (HBsAg to anti-HBs) in chronically infected patients in randomized controlled trials [79,82,83] and has not been associated with drug resistance. Patients with HBeAg-positive and HBeAg-negative chronic HBV infection have been shown to benefit from treatment according to a cumulative increase in patients who were HBV DNA-positive and HBeAg-negative and a decrease in the number of patients with decompensated liver disease [83–88]. Interferon alfa-2b (Intron-A; Schering, Kenilworth, NJ) has been approved for the treatment of chronic HBV infection in many countries throughout the world, including adults and children in the United States. Historically, HBeAg clearance occurs in 30% to 50% of selected patients treated with interferon, and HBsAg clearance has been seen in up to 30% of patients with long-term follow-up [89]. Interferon alfa-2a (Roferon; Hoffman La Roche Laboratories, Basel, Switzerland) and inter-

feron alfa-con1 (Infergen; Amgen, Thousand Oaks, CA) may also be used to treat HBV infection but have not been approved for this indication by the US Food and Drug Administration (FDA). New pegylated forms of interferon alfa-2a (PEGASYS; Roche Pharmaceuticals, Basel, Switzerland) and alfa-2b (PEG-Intron) have now completed their phase III trials and PEGASYS is FDA-approved in Europe and the United States as well as in other countries. The longer half-life of this compound appears to impart a clinical benefit over conventional interferon—although this is only implicated, directly, from the phase II dose ranging study because there was no standard interferon arm in any of the phase III studies. A large phase III study of PEG-alfa 2a in HBeAg-negative patients demonstrated a substantial rate of sustained viral suppression that was better than lamivudine alone but was not improved by the addition of lamivudine [83]. Interesting data on PEG-alfa 2b published by Janssen and colleagues [90] have shown a substantial benefit of interferon treatment in patients who have HBV genotype A and B when compared with genotypes B and C. A recent publication by Lau and colleagues [79] also shows improved HBeAg and HBsAg seroconversion rates as well as DNA suppression compared with lamivudine monotherapy and combination with interferon and lamivudine. Neither of these major licensing studies showed any benefit of combination therapy, except deeper viral suppression on therapy and <1% viral resistance compared with 17% for patients on lamivudine monotherapy.

The ideal dosing for the different forms of conventional interferon range from 15 to 30 million U weekly and PEGASYS at 180 µg per week. Interferon is administered via subcutaneous or intramuscular injection three times a week, though some clinical studies have used daily dosing regimens. Prolonged treatment for up to 32 weeks may be useful in patients who have an initial reduction in HBV DNA levels without DNA clearance. The side effect profile of interferon is significant; a wide variety of side effects are well described but hopefully will be reduced with newer forms of interferon or with lower doses used in combination with other forms of less toxic therapy. Patients who have the best antiviral response to interferon are those who have high liver enzyme levels, moderate serum DNA levels, and recent or adult-acquired infection with a normal immune response to HBV. Patients who fit this profile may have HBsAg seroconversion that may exceed 30% in long-term follow-up [89]. The advent of safe oral medications relegated interferon in many countries to a secondary position. However, due to increased awareness that resistance to currently available oral medications may lead to the development of severe and life-threatening disease, more short-term options with a defined treatment period (eg, PEG-interferon) are desirable. Markers to identify patients who will have long-term sustained response to PEG-interferon products are still being defined.

Nucleosides and nucleotides

From a historical perspective, nucleosides such as famciclovir and ganciclovir [91] can confer some level of anti-HBV activity. However, these medications

have been of little use to date due to low levels of efficacy (ganciclovir and famciclovir) and a very high level of viral resistance (famciclovir) [92,93]. Currently, lamivudine, adefovir, and entecavir are the only FDA-approved oral medications with proven efficacy against HBV, including HBeAg seroconversion and triple seroconversion. Lamivudine is also approved in many other countries, including China. Available data concerning efficacy include a rate of up to 25% to 35% HBeAg loss at 1 year of therapy and average seroconversion at 17%. Seventeen percent of patients underwent triple seroconversion in the major licensing studies, while HBsAg seroconversion has also been rarely reported (<6%) in patients remaining HBeAg-negative and HBV DNA-negative long-term [72,73,94–96]. Most patients relapse if therapy is stopped at 1 year, highlighting the need for long-term treatment in most patients [97–99]. Resistance to lamivudine is very common (>20%) after 1 year of treatment and as high as 70% to 90% after 3 to 5 years of treatment [74,100,101]. The initial emergence of resistant virus is associated with mild elevations of liver enzyme levels. Later, this emergence can be associated with more active liver disease, as measured by serum levels of liver enzymes, and the presence of progressive liver disease by liver biopsy [102], including a risk of flares and fulminant hepatitis [103,104]. Importantly, decreases in viremia can occur even after lamivudine resistance, although many patients are not stable and HBV DNA rebound is seen in the majority of cases whether or not therapy is continued. HBeAg seroconversion can also take place after lamivudine resistance. As long-term trials progressed, it became apparent that rebound could occur during treatment, the majority of which has been attributed to the development of drug resistance. This resistance was observed at a greater frequency and at an earlier time point in the transplant setting [105–107] than in immunocompetent patients [103,108–110]. Lamivudine-resistant populations of HBV were also detected in patients who were coinfecting with HBV and HIV and who were receiving lamivudine as antiretroviral therapy [111–113]. The incidence of resistance in immunocompetent and immunosuppressed patients correlates with treatment duration. During the first year of lamivudine therapy, resistance developed in 14% to 32% of cases studied [114–116], a frequency that increased to greater than 50% after 2 years [103].

Sequencing of the HBV polymerase ORF from drug-resistant isolates revealed mutations in a methionine codon, which caused substitution of isoleucine or valine for methionine 204 (M204V/I). These substitutions affect the catalytic site, changing the conserved YMDD tetrapeptide in motif C to YIDD or YVDD [117]. Mutations that alter the YMDD motif appear to have multiple effects on properties of the polymerase, but, in general, they seem to increase fidelity and reduce the ability to process the viral genomic code. A number of different mutational patterns appear to be responsible for lamivudine resistance, but the majority of cases of HBV resistance to lamivudine affect the M204 codon. One pattern is associated with two amino acid substitutions (L180M + M204V), while a second pattern causes a single substitution (M204I) [105,107].

Antiviral cross-resistance of hepatitis B virus variants

A variety of drug-resistant HBV mutants have emerged under the selective pressures of lamivudine or famciclovir therapy. Rational design of effective chemotherapeutic strategies for the future will require the determination of the sensitivities of these mutants to novel nucleoside and nucleotide analogs as they become available. Prominent leaders in the world of HBV and HIV treatment are advocating the use of combination therapy at the outset to block the development and possible serious consequences of HBV resistance such as resistance to subsequent therapies, vaccine escape mutants and severe flares of liver disease. Fortunately, convincing evidence indicates that adefovir dipivoxil and entecavir inhibit replication of lamivudine- and famciclovir-resistant HBV. Cell-free polymerase assays show that the sensitivity of wild-type and genetically engineered mutant HBV polymerases to adefovir diphosphate is not significantly different [118,119]. Analyses from several independent laboratories have confirmed that adefovir dipivoxil and entecavir are equally effective as inhibitors of wild-type virus and lamivudine-resistant HBV in cell culture. More recent data have shown that the only setting where entecavir resistance has been observed was in the presence of two or more point mutations associated with lamivudine resistance plus a further two- or three-point mutations associated with both phenotypic and genotypic resistance to entecavir. Thus it may require up to five-point mutations to see a rise in HBV DNA levels during treatment with entecavir. Furthermore, recent clinical experience shows that treatment with adefovir dipivoxil can suppress the replication of lamivudine-resistant HBV in vivo and vice versa [120,121].

Newer nucleos(t)ide treatments

Adefovir is a nucleotide analog of adenine and has a high binding affinity to the HBV polymerase. Adefovir is FDA-approved for the treatment of HBeAg-negative and HBeAg-positive liver disease as well as the treatment of the lamivudine-resistant mutant. Reductions in HBV DNA and ALT were seen in patients with the HBV precore mutant compared with placebo. The patients were treated with adefovir at 10 mg/d for 1 year in this placebo-controlled trial. The primary end point of treatment was improvement in the inflammatory score by at least 2 points and no worsening of fibrosis and was achieved in 67% of patients on adefovir and only 19% of patients on placebo. Data concerning the levels of DNA reduction, HBeAg loss, and HBeAg to anti-HBs seroconversion are shown in Figs. 2, 3, 4, and 5. A separate trial in HBeAg-positive patients demonstrated histologic improvement in 53% of the treatment group versus 25% of the placebo group [71]. To date, resistance has been observed in 5.9% of patients at 3 years and 18% at 4 years, which is remarkably less than the resistance rate seen with lamivudine, while no flares of severe liver disease or deaths have been observed to date. It is important to emphasize that patients who are treated with either lamivudine or adefovir need to undergo dose reduction if renal insufficiency is present, as described in the prescribing information. Renal injury due to adefovir

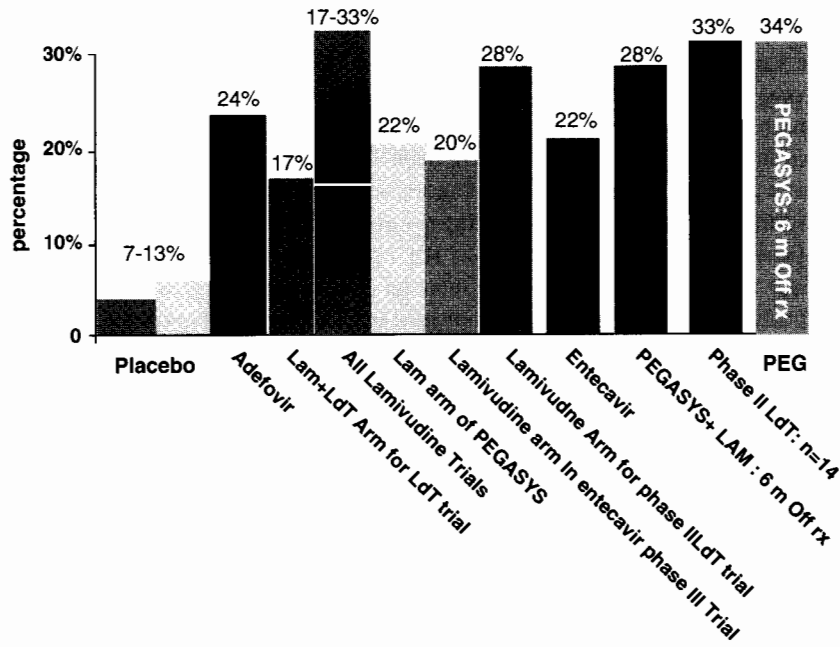


Fig. 2. HBeAg loss at 1 year.

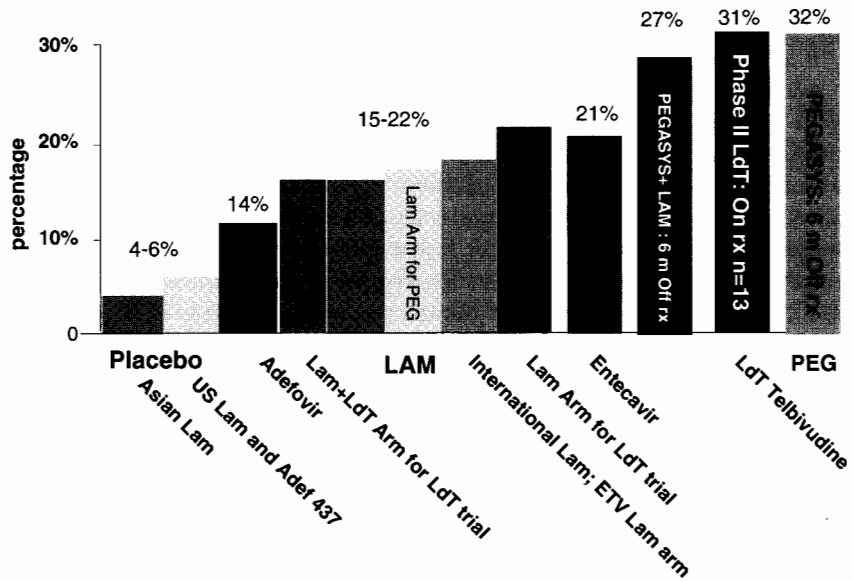


Fig. 3. HBeAg seroconversion at 1 year.

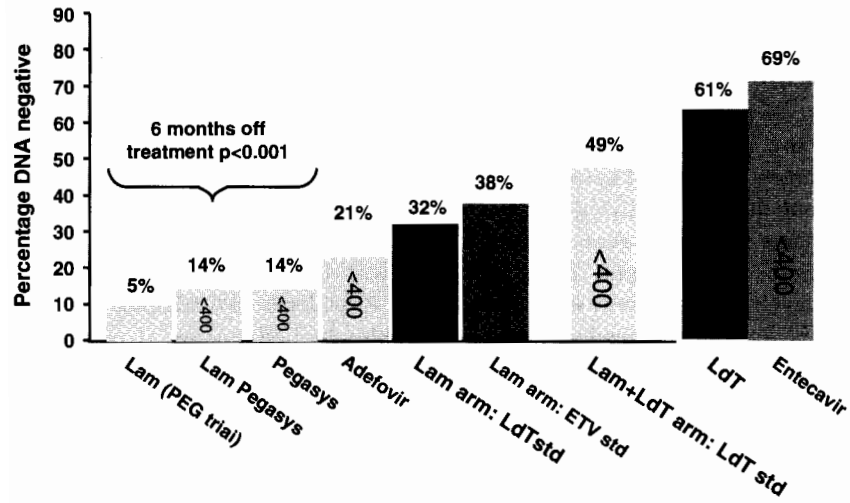


Fig. 4. DNA response at 1 year in HBeAg(+) studies.

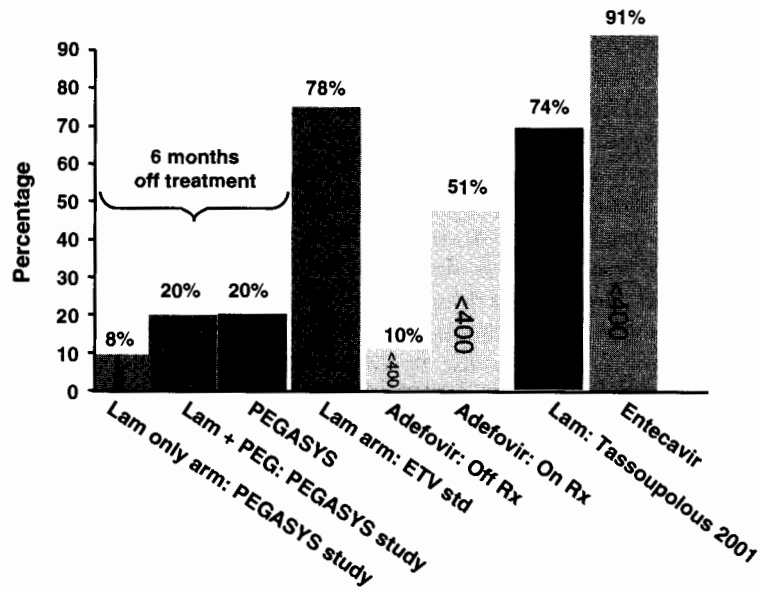


Fig. 5. DNA response at 1 year in HBeAg(-) studies.

Box 1. New hepatitis B virus therapies*Marketed*

Interferon alfa-2b
Lamivudine
Adefovir dipivoxil
Emtricitabine (HIV)
Tenofovir (HIV)
Truvada (HIV) (combination: tenofovir and emtricitabine)
Entecavir
PEG-interferon alfa-2a

Phase III

Emtricitabine (HBV)
Emtricitabine + tenofovir
PEG-interferon alfa-2b (investigator-initiated studies)
Telbivudine (LdT)

Phase II

Clevudine (Korea/Asia)
Valtorcitabine (LdC)
Amdoxovir
Racivir
BAM 205
HepX-B
HE2000
Thymosin-alpha
Pradefovir
EHT 899

Phase I/preclinical

MCC 478
MIC 210
Therapeutic HBV vaccines

future treatments may include dominant negative viral mutants, small interfering RNA, antisense DNA and RNA, intracellular antibodies, or delivery of current medications by way of new and advanced delivery methods such as liposomes or methods that include molecules that bind to specific cellular receptors. Treatment may also take place with DNA vaccines that may provide

ongoing internal immune suppression through production of HBV-derived proteins [100,132,133].

Post-liver transplantation management

Prevention of HBV recurrence post-orthotopic liver transplantation (OLT) with either passive immunoprophylaxis using hepatitis B immunoglobulin (HBIG) monotherapy or with antiviral agents such as famciclovir or lamivudine has improved the outcome for a number of patients. Fortunately, viral breakthrough is uncommon with combined therapy. However, breakthrough is common in monotherapy with nucleosides/nucleotides and the resulting liver disease that occurs from breakthrough associated with the emergence of drug-resistant virus can be more aggressive than wild type HBV. In post-OLT patients it has been observed that viral loads are at least 0.5 to 1.0 log greater after viral breakthrough compared with viral loads before introducing nucleoside treatment. The HBV characterized from these patients usually has multiple mutations in the polymerase and envelope genes and may lead to vaccine escape mutants [39]. It is important to highlight the need in HBV infections for therapeutic approaches that adequately suppress active replication to minimize the risk of selection of drug-resistant virus in the first place at a cost that is much less than the cost of HBIG and nucleos(t)ide therapy that may exceed \$50,000 in the first year after transplant. The preferred approach to achieve this goal is through appropriate combination oral antiviral chemotherapy when there is documentation of low levels of viral resistance and potential synergy has also been seen in terms of viral suppression [134,135]. In the future, we may also be able to replace standard HBIG with a mono- or biconal antibody that is undergoing early phase II trials (XTL Bio, Israel) that may allow much higher levels of antibody with lower doses (ie, micrograms) of antibody.

Management of special populations

Alcohol use in patients chronically infected with HBV increases the risk for progression to cirrhosis, results in more rapidly progressive liver disease, and also increases the risk of liver cancer [129,130,136–139]. Patients with chronic liver disease should not drink alcohol and patients with documented chronic HBV infection should be educated about the risks of alcohol use and advised not to drink any alcohol since the threshold for complications of alcohol use are unknown.

Treating chronic HBV infection in dialysis patients should focus on the presence or absence of progressive liver disease as demonstrated by biopsy. Interferon probably has lower efficacy in dialysis patients due to the relative state of immunosuppression in these patients. Lamivudine and adefovir can also be used but dose adjustments are required to take into account for the dominant renal clearance of these medications.

Other solid organ transplant recipients such as bone marrow, heart, and kidney recipients are at risk for reactivation of HBV disease after organ transplantation [140–144]. An expert in hepatitis B management must evaluate these patients before they undergo transplantation. If active HBV replication is present, avoiding or delaying organ transplantation is advised unless successful suppression of HBV disease can be achieved. Some transplant centers will not perform organ transplantations on patients who are HbsAg-positive. Another clinical setting is the transmission of HBV from the organ donor to recipient in which the risk of transmission is nearly 100% if the donor is HbsAg-positive. Donation is rarely performed in this circumstance. Another risk is the presence of anti-HBc in the donor, which poses a small (<3%) risk of HBV transmission for organ transplant recipients other than liver but is a significant risk to liver transplant recipients that ranges up to 70% [145,146]. Finally, recipients who are anti-HBc-positive can reactivate nascent HBV disease once immunosuppression is initiated [147].

Summary

Chronic HBV infection continues to pose a very serious health care problem worldwide. Exciting new treatments for the management of HBV continue to push viral levels to lower thresholds; improvements in histology, even in the short-term, are remarkable; and the safety of emerging agents remains very favorable. There are also emerging molecular tools and possibly treatments to further advance the therapeutic options for chronic HBV disease.

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