

Review Article

Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009

Osamu Yokosuka,¹ Masayuki Kurosaki,² Fumio Imazeki,¹ Yasuji Arase,³ Yasuhito Tanaka,⁴ Kazuaki Chayama,⁵ Eiji Tanaka,⁶ Hiromitsu Kumada,³ Namiki Izumi,² Masashi Mizokami⁷ and Masatoshi Kudo⁸

¹Department of Medicine and Clinical Oncology, Postgraduate School of Medicine, Chiba University, Chiba,

²Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, ³Department of

Hepatology, Toranomon Hospital, Kawasaki, ⁴Department of Virology and Liver Unit, Nagoya City University

Graduate School of Medical Sciences, Nagoya, ⁵Department of Medicine and Molecular Science, Hiroshima

University, Hiroshima, ⁶Department of Medicine, Shinshu University School of Medicine, Matsumoto, ⁷Research

Center for Hepatitis and Immunology, International Medical Center of Japan Kounodai Hospital, Ichikawa, and

⁸Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Recently, much progress has been made in the field of hepatitis B, such as natural history of the disease in relation to the amount of hepatitis B virus (HBV) DNA, genotypes of HBV influencing the natural course and treatment effects, mutations of HBV influencing the severity of the disease and development of hepatocellular carcinoma, and antiviral treatment such as nucleos(t)ide analogues and pegylated interferon. To make the consensus for the diagnosis, management and treatment of hepatitis B, a meeting was held during 45th annual meeting of Japan Society of Hepatology (JSH) in June 2009. In the meeting, recommendations and informative statements were discussed on the following subjects: (i) natural history of HBV infection; (ii) clinical implication of HBV genotypes; (iii) HBV mutations and their potential impact on

pathogenesis of HBV infection; (iv) indications for antiviral treatment of chronic hepatitis B; (v) nucleos(t)ide analogues for chronic hepatitis B; and (vi) interferon therapy for chronic hepatitis B. The presenters reviewed the data on these subjects and proposed the consensus statements and recommendations. These statements were discussed among the organizers and presenters, and were approved by the participants of the meeting. In the current report, the relevant data were reviewed and the 12 consensus statements and nine recommendations on chronic hepatitis B were described.

Key words: genotype, hepatitis B virus, interferon, mutation, natural history, nucleotide analogue

Hepatitis B virus (HBV) is one of the most distributed viruses which infect humankind. More than 3 billion people, one half of the world's population, have been exposed to HBV during their life.¹ Acute infection in adults is self-limited in general whereas infection during early childhood will develop into persistent chronic infection in most individuals.² More than 400 million people worldwide are chronically infected with HBV and are at risk of developing life-threatening complications

including liver cirrhosis and hepatocellular carcinoma (HCC).¹ HBV is a major public health problem worldwide especially in East Asia and Africa. In Japan, approximately 1.5 million people are infected with HBV and it is one of the major causes of HCC and chronic hepatic failure. Other complications of HBV infection include fulminant hepatitis and acute liver failure.

The consensus meeting for diagnosis, management and treatment for hepatitis B was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M Kudo), where the recommendations and informative statements were discussed. Although the JSH consensus meeting of hepatitis B had been held four times so far, recommendations were hitherto published only in Japanese and this is the first report in English. Established

Correspondence: Professor Osamu Yokosuka, Department of Medicine and Clinical Oncology, Postgraduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Email: yokosukao@faculty.chiba-u.jp

Received 6 April 2010; revision 25 August 2010; accepted 20 September 2010.

information for pathogenesis and contributing factors for disease progression which was agreed by the organizers and presenters are shown as “consensus statements”, and clinically useful consensus are shown as “recommendations”. The quality of recommendations or informative statements are required to show a “level” (assessing strength or certainty) of evidence and “grading” of recommendations or assessment according to a standard reporting system of clinical guidelines.³

NATURAL HISTORY OF HBV INFECTION

AN EVALUATION OF studies on the natural history of HBV infection was done using the scoring system proposed by MacMahon *et al.*⁴ in the present analysis because scoring systems for treatment studies cannot always be applied directly to those using natural history. The proposed scoring system consists of levels 1 (1a, 1b), 2 (2a, 2b, 2c), and 3. Level 1a is defined as a population-based longitudinal cohort study with a hepatitis B surface antigen (HBsAg) negative comparison group. Level 1b is identical to level 1a, but with no comparison group. Level 2a is defined as a clinic-based longitudinal cohort study, level 2b is a population-based or clinic-based cohort nested case-control study, and level 2c is a cross-sectional clinic-based study. Level 3 is defined as an observation study case series.

The natural history of chronic HBV infection can be classified into several phases based on levels of alanine aminotransferase (ALT), hepatitis B e-antigen (HBeAg) status, amounts of HBV DNA, and estimated immunological states.^{4–9} A representative classification of these phases is shown in Table 1. In the immune tolerance phase, HBeAg is positive, serum levels of ALT are normal, histological activities of hepatitis are absent or minimal, and levels of HBV DNA are elevated. The

immune tolerance phase is thought to occur most frequently in individuals who are infected through perinatal transmission, and this phase usually lasts until adolescence or young adulthood.^{10–12}

The chronic hepatitis B phase is characterized by elevated ALT and HBV DNA levels. In this phase, the host's immune system recognizes HBV as being foreign and initiates an immune response that results in hepatitis. In cases who are HBeAg positive, active hepatitis can be prolonged and may result in cirrhosis. However, chronic hepatitis B eventually transitions into an inactive phase with a loss of HBeAg positivity in the majority of patients. Seroconversion to anti-HBe and the fall of serum HBV DNA to low levels result in the disappearance of disease activity, despite persisting HBsAg and low levels of HBV DNA.^{13–16} Seroconversion rates range 7–16% per year according to reports with higher evidence levels (levels 1b, 2a).^{16–19} Factors associated with seroconversion are age (level 1b),²⁰ ALT levels (level 1b), occurrence of acute exacerbation of hepatitis (level 1b),^{19,21} and genotype (level 2c).^{22,23}

The seroconversion of HBeAg results in the transition from hepatitis phase to inactive carrier phase, which is generally thought to be a benign course for HBV carrier, but sometimes hepatitis can be reactivated spontaneously.²⁴ Patients experiencing reactivation undergo another transition, with increases in HBV DNA and ALT levels and disease activity without reappearance of HBeAg.²⁴ This phase is referred to as HBeAg negative chronic hepatitis B. Occasional severe hepatitis B flare-ups with middle range HBV DNA levels (3–8 log copies/mL) occur in this phase.^{8,25} HBeAg negative chronic hepatitis B is caused by mutant strains of HBV unable to produce HBeAg,^{25,26} and tends to develop into cirrhosis and complicate HCC more than HBeAg positive chronic hepatitis B.^{27–30}

Table 1 Phases in the natural history of HBV carriers (modified from ⁴)

Phase	Hepatitis	Blood			Liver
		DNA	HBeAg	HBsAg	cccDNA
Immune tolerance	–	8–11	+	+	+
HBeAg positive	Usually	6–10	+	+	+
Chronic hepatitis	Persistent				
HBeAg negative	Often	3–8	–	+	+
Chronic hepatitis	Fluctuating				
Inactive carrier	–	<4	–	+	+
Recovery	–	–	–	–	+

HBV DNA: log copies/mL. cccDNA, covalently close circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Many factors that are associated with the development of HCC have been reported so far. Higher age (level 1a), male sex (level 1a), presence of cirrhosis (level 2a) and familial cluster of carriers (level 2c) are reported as host factors.^{31,32} Viral factors include high viral load (level 1b),^{33–36} existence of pre-core and core promoter mutations (level 2a), genotype C and high ALT levels (level 1b). High viral load should be considered as a factor in patients over 35–40 years of age. Co-infection with hepatitis C virus, hepatitis D virus or HIV (level 2a), drinking habit (level 2c) and exposure to aflatoxin (level 2c) are reported as social and environmental factors.^{37–39} Other lifestyle-related factors, such as smoking habit, obesity and complications from diabetes mellitus, have been documented as well.

Consensus 1

In patients with chronic hepatitis B, seroconversion of HBeAg usually results in the transition from hepatitis phase to inactive carrier phase, which generally has low HBV replication and normal ALT levels. However, reactivation of chronic hepatitis can spontaneously occur without the reappearance of HBeAg. At this point, active hepatitis continues and the risk of complicating cirrhosis and HCC is high in patients with HBeAg negative chronic hepatitis B. (Level 1b.)

In the inactive carrier phase, HBV replication is continuously suppressed as a result of predominantly host immunological pressure against HBV. Patients in the inactive carrier phase generally have a benign course because active hepatitis subsides and the risk of HCC decreases.^{19,20,24,40} However, regular follow up is required because reactivation of HBV sometimes occurs spontaneously or as a result of immunosuppressive therapy.^{19,24}

Hepatitis B surface antigen is known to fall to undetectable levels in some inactive carriers. This HBsAg negative phase, referred to as the recovery phase, has no hepatitis and a low risk of HCC. Still, caregivers must be aware that patients who are old or cirrhotic have a relatively higher risk of HCC.^{41,42} Disappearance of HBsAg in the recovery phase does not indicate complete eradication of HBV because the HBV genome remains as covalently close circular DNA (cccDNA) in the nucleus of hepatocytes.

Consensus 2

2-1 HBV can not be completely eradicated using any currently existing treatment measures. (Level 2a.)

2-2 Patients in the inactive carrier or recovery phase have a benign clinical course. However, regular follow up of such patients is required because reactivation of hepatitis B and ensuing HCC can occur. (Level 1b, 2a.)

Clinicians have to consider two types of hepatitis B reactivation: one during the inactive carrier phase and the other in the recovery phase.⁴ Both types of reactivation have been attributed with increasing incidence to strong immunosuppressive therapies. De novo hepatitis B, a reactivation of hepatitis B in the recovery phase, tends to develop into fulminant hepatitis, which has a very high mortality rate.^{43–46} Thus, establishment of effective measures to prevent reactivation of hepatitis B is necessary.

Consensus 3

3-1 Reactivation of hepatitis B can occur during the inactive carrier or recovery phases and stems mainly from strong immunosuppressive treatment courses. (Level 2a.)

3-2 Recent advances in medical care have increased the use of immunosuppressive agents and thus the incidence of hepatitis B reactivation. (Level 2a.)

3-3 Reactivation of hepatitis B tends to develop into fulminant hepatitis. (Level 2a.)

Recommendation 1

In addition to the loss or seroconversion of HBeAg, a substantial decrease in HBV viral load and subsequent disappearance of hepatitis are the primary targets in the treatment of patients with chronic hepatitis B. (Level 1b.)

Recommendation 2

The main goals of HBV carrier treatment are patients in the inactive carrier and recovery phases. However, caregivers should be aware that reactivation of hepatitis B and complication of HCC can occur even in these benign phases. (Level 1b.)

Recommendation 3

Reactivation of hepatitis B due to immunosuppressive therapy tends to develop into severe hepatitis, thus requiring the establishment of effective preventative measures. (Level 2a.)

CLINICAL IMPLICATION OF HBV GENOTYPES

DISTINCT CLINICAL AND/OR virological characteristics of the HBV infection have been reported in different geographical parts of the world and are increasingly associated with host factors, environmental factors and the genetic diversity of the infecting virus.⁴⁷ HBV is classified into at least eight genotypes (A–H) based on an intergroup divergence of 8% or more in the complete nucleotide sequence and a number of subgenotypes (Aa/A1, Ae/A2, Bj/B1, Ba/B2, Cs/C1, Ce/C2, D1, D2, and so forth) that are currently known to have distinctive association with ethnic and/or geographical distribution.⁴⁸

Association between HBV genotype and clinical manifestation

Acute hepatitis

The universal vaccination program against HBV has significantly reduced the number of new infection cases in most countries with levels of endemicity estimated from intermediate to high.⁴⁹ However, efficiency of universal vaccination in countries with a low level of endemicity still remains controversial. Japan is one of the countries with a low level of endemicity and mainly vertical (mother to baby) transmission route.⁵⁰ In Japan, HBV vaccination in combination with HBV immunoglobulin treatment is the only recommended measure for infants born to HBsAg positive mothers. Studies in Japan indicated genotype C (subgenotype Ce/C2) to be the major type in the country and genotype B (subgenotype Bj/B1) is the second distributed. Surveillance studies have shown a recent trend toward increase in number of acute hepatitis B infection among young adults mainly through sexual contacts.^{51,52} Although most cases are associated with genotype C infection, there is a continuous trend toward increase in prevalence of genotype A among acute hepatitis cases.^{51,53–56} Patients infected with genotype C have been known to be rarely associated with development of chronic persistence after acute infection in immune competent adults in Japan (1%) in contrast to the higher rates of those infected with genotype A (6–23%).^{53,54} A recent multicenter study in Japan indicated a trend among chronic hepatitis B patients toward increase in prevalence of genotype A (from 1.7% in 2002 to 3.5% in 2006), whereas other genotypes remained stable at their prevalence during the same period.⁵⁷ The shift in genotype prevalence with the increase of genotype A among chronically infected carriers can be explained by higher risk of genotype A to develop persistence. This is consistent with higher rates

of chronic persistence after acute infection in adults in European countries where genotype A is prevalent (10%).^{48,58} This is also consistent with results of *in vitro* and *in vivo* comparisons of different genotype strains showing different dynamics of replication: slow for genotype A and rapid by genotype C.^{59,60} The surveillance study indicated that all patients treated with lamivudine (LVD) recovered from acute hepatitis, whereas none of the three patients who developed a chronic outcome had received antiviral treatment during their acute phase of infection, indicating that LVD might be able to prevent the chronic outcome.⁵⁴ Cumulatively, these data indicate the clinical importance of routine genotyping for acute hepatitis B patients.

Fulminant hepatitis

One of the most serious complications of acute HBV infection is fulminant hepatitis. In Japan, the annual number of fulminant hepatitis reported was approximately 400 cases, with approximately half of these caused by HBV infection. Despite its rather low incidence, fulminant hepatitis is a national problem because the mortality rate is extremely high.⁶¹ It is important to understand factors predisposing for development of fulminant hepatitis. Viral factors associated with the development of fulminant hepatitis are mutations in the core promoter (T1762/A1764)⁶² and the pre-core region (A1896).^{54,63,64} However, these findings were not consistent with studies in Europe and the USA.^{65–67} A large-scale cross-sectional study in Japan revealed association between genotype B (subgenotype Bj/B1) infection and development of fulminant hepatitis; on the other hand, no cases of fulminant hepatitis were registered among those infected with genotype A (subgenotype Ae/A2).⁵⁴ Differences in genotypes circulating in Asia and Europe/USA may indicate that distinct viral factors are playing roles in manifestation of infection by different genotype.

Chronic hepatitis

Chronic HBV infection is the most common cause of HCC in Asia.⁶⁸ Efficient surveillance and early diagnosis of development of this life complication requires risk stratification of chronic hepatitis B patients. Older age, male sex and liver cirrhosis are well recognized factors associated with increased risk of HCC.^{69,70} In addition, recent large-scale population-based and clinical case-control studies carried out in Asia, have shown that infecting virus factors associated with a high risk of HCC, include HBV DNA levels,^{71,72} HBV basal core promoter mutations,³⁵ genotype C (vs B),^{22,36,73,74} and sub-

genotype Ce/C2.^{71,75} There are data indicating that genotype C infection associated with a higher viral load than genotype B.⁷⁶ Association of genotype F with HCC was found to be higher than that of genotype C in Alaskan natives.^{77,78} Unfortunately, there are few prospective studies examining other HBV genotypes for association with adverse outcomes. Genotype A (subgenotype Aa/A1) was found in association with development of HCC in young adults in South Africa.^{79,80} However, very high rates of detection of subgenotype Aa/A1 among asymptomatic carriers suggest contribution of environmental factors (aflatoxin contained in food) for the development of HCC. In comparison with Aa/A1, HCC associated with Ae/A2 is found primarily in older individuals. In addition, the rate of complications, including HCC, for those infected with subgenotype Ae/A2 appears to be less than that found in those infected with genotype D, C or F1.^{77,81} A prospective study in Spain showed that genotype A (presumably Ae/A2) infection was associated with a significantly higher cumulative rate of sustained biochemical remission, HBV DNA and HBeAg clearance in patients with chronic HBV infection than genotype D infection.⁸¹

Consensus 4

- 4-1 Recently, there is an increase of HBV genotype A proportion among acute hepatitis B infection cases in Japan. (Level 3.)
- 4-2 HBV genotype A acute infection has a tendency to evolve in chronic hepatitis compared to genotype B/C. (Level 3.)
- 4-3 Antiviral therapy of acute infection might be efficient in prevention of chronic carrier stage. (Level 3.)
- 4-4 Genotype C compared with genotype B is associated with higher risk of outcome in HCC in chronic carriers. (Level 2a, grade B.)
- 4-5 Genotype A compared with genotype D and F in chronic carriers is associated with better prognosis in terms of spontaneous ALT normalization and DNA clearance. (Level 2a, grade B.)

HBV MUTATIONS AND THEIR POTENTIAL IMPACT ON PATHOGENESIS OF HBV INFECTION

THE HBV GENOME consists of double-stranded DNA, 3200 bp in length. HBV replicates through reverse transcription of a RNA intermediate, the prege-

nome RNA, different from all known mammalian DNA viruses. HBV infection is characterized by high levels of virus production, however, the HBV reverse transcriptase is an error-prone enzyme lacking proof-reading capacity, resulting in a large number of nucleotide substitutions during replication. The misincorporation rate has been estimated to be of the order of 10^{10} incorrect nucleotide incorporations per day. As a result, HBV has a quasispecies distribution in infected patients.

Naturally occurring mutations identified in the HBV genome are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers. Among them, several specific mutations have been shown to be associated with the pathogenesis of HBV infection.

HBeAg seroconversion

A HBV strain harboring stop codon mutation in the precore region was first reported in anti-HBe positive patients with chronic hepatitis.²⁵ The precore region located upstream of the core region is involved in the production and secretion of HBeAg protein. HBeAg is secreted into blood after removal of N-terminal 19 amino acids (a.a.) and C-terminal 34 a.a. from HBeAg precursor protein composed of precore and core regions. Nucleotide substitution of G to A at nt 1896 confers stop codon (TAG) mutation from tryptophan (TGG) at codon 28 in the precore region, resulting in a failure to produce HBeAg protein.^{82–84} Although controversial, 10 genotypes have been identified tentatively so far⁸⁵ and genotypes affect the occurrence of stop codon mutation in the precore region. The stop codon mutation in the precore region (G1896A) is rarely encountered in HBV genomes of genotype A, some of genotype C and F, because they possess C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.⁸⁶

The HBV core promoter regions located upstream of core region are involved in the transcription of precore mRNA and pregenomic RNA. Nucleotide substitution of A to T at nt 1762 combined with substitution of G to A at nt 1764 in the core promoter region give rise to a reduced transcription of precore mRNA and increased level of viral DNA, resulting in a decreased production of HBeAg protein and enhanced viral replication.^{87–89}

Consensus 5

Nucleotide substitution G1896A confers stop codon mutation in the precore region. Nucleotide substitution A1762T combined with substitution G1764A in

the core promoter region give rise to a reduced transcription of precore mRNA. These nucleotide changes in combination with a reduction of HBeAg caused by suppressed replication of HBV are closely associated with HBeAg seroconversion. (Level 2b, grade B.)

Association between HBV mutations and clinical manifestation

Fulminant hepatitis

Precore and core promoter mutations are very frequent in patients with fulminant hepatitis from Asia^{62,63,90} and the Middle East.⁶⁴ However, these mutations were not detected in those from Western countries.^{65,67,91,92} This difference could be attributable to the difference of genotype prevalence, frequent genotype Ae and rare Bj in Western countries.⁸⁶ The patients infected with the former genotype rarely have precore mutant virus, while the latter frequently have the mutant virus. Stop codon mutation in the precore region is inhibited in genotype A because of C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.⁹³

Ozasa *et al.* analyzed the difference of host and viral factors between 40 patients with fulminant hepatitis B and 256 with acute self-limited hepatitis B in a multicenter cross-sectional study,⁵⁴ and showed that precore stop codon mutation of G1896A and genotype Bj are associated with fulminant hepatitis in Japan. They also reported the marked enhancement of viral replication by introducing either G1896A or A1762T/G1764A mutation into the Bj clone in *in vitro* transfection study. Because this type of HBV mutant is found not only in patients with fulminant hepatitis but also in asymptomatic HBV carriers,⁹⁴ the interaction between the virus and the host's immune response might influence the outcome of HBV infection.

In addition to the mutants mentioned above, pre-S2 defective virus or HBV defective in secretion because of surface gene mutations are reported in patients with fulminant hepatitis. These mutant viruses showed a characteristic feature of virus retention in hepatocytes and misassembly with high replication capacity.^{95–97}

HCC development

Evidence has been accumulating over the past decade that the risk of developing cirrhosis and HCC is influenced by the patient's viral status, such as genotype, viral load and genomic mutations. Naturally occurring

mutations have been identified in the structural and non-structural genes as well as the regulatory elements of the virus, and these mutations are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers.⁹⁸

A double mutation, A1762T/G1764A in the basal core promoter region has been found in patients with advanced liver disease and HCC. Several case-control studies,^{30,35,99–102} retrospective cohort studies^{103,104} and one prospective cohort study¹⁰⁵ confirmed this finding, while some conflicting results were also reported in the case-control studies^{106,107} and one prospective study.¹⁰⁸

The role of deletions in the pre-S region of the HBV genome has been shown to be associated with the development of progressive liver diseases including HCC. Several case-control studies confirmed this finding.^{27,107–110} A further mapping study of the pre-S region showed that all the deletion regions encompassed T- and B-cell epitopes and most of them lost one or more functional sites including the polymerized human serum albumin-binding site.¹⁰⁹ Deletion of these functional sites may cause intracellular retention of HBV envelope proteins and viral particles and contribute to more progressive liver damage and HCC development.

In addition to these common mutations, several other mutations, C1653T in the enhancer II region, T1753C/A/G in the core promoter region, and G1317A/T1341C/A/G in enhancer I region, have been reported to be associated with the development of HCC in some case-control studies.^{30,107,111}

Consensus 6

There is some evidence that emergence of HBV genomic mutations arising during the course of chronic infection influence the outcome of chronic liver disease. Among them, core promoter mutations A1762T/G1764A might have a potential for developing progressive liver disease and HCC. (Level 2a, grade B.)

HBsAg escape mutant

The HBsAg mutant was first described in a child born to a HBsAg positive mother who developed acute hepatitis B in spite of vaccination and passive immunization against HBV.¹¹² This viral strain contained a substitution of glycine to arginine at position 145 (sG145R) and was able to escape the immune surveillance, resulting in an infection despite the presence of anti-HBs antibodies, vaccine escape mutant. Similar mutants have been detected all over the world.^{113–115}

Patients after liver transplantation for HBV-related chronic liver disease who had received anti-HBs antibodies to prevent re-infection of the graft showed an “immune escape mutant”.^{116–118} Furthermore, “diagnosis escape mutants” have also been described because HBsAg detection assays are based on anti-HBs antibodies.¹¹⁹ The emergence of these variants may contribute to occult HBsAg negative HBV infection.¹²⁰

The HBV genome is organized in such a way that the envelope gene is overlapped by the polymerase gene; therefore, HBV with changes in the polymerase gene associated with resistance to the nucleos(t)ide analog which are described in detail in section 5 may have consequent changes in the envelope gene. A triple mutant causing LVD resistance (rtV173L + rtL180M + rtM204V), which have an enhanced replication capacity compared with rtL180M + rtM204V alone, causes two amino acid changes in the overlapping surface gene (sE164D + sI195M). This mutant reduces anti-HBs binding to levels seen only with the vaccine escape mutant sG145R.¹²¹ Some patients treated with LVD showed seroclearance of HBsAg with detectable circulating HBV DNA. An sP120A mutation was associated with HBsAg seroconversion in these patients and this mutation produces a reduced anti-HBs binding which causes the failure to detect HBsAg.¹²²

Consensus 7

Amino acid substitutions, deletions or insertions across the “a” determinant of HBsAg, such as a substitution sG145R, give rise to vaccine and immunoglobulin escape mutant. (Level 4, grade C.)

INDICATIONS FOR ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS B

ONCE THE LIVER is persistently infected with HBV, it is difficult to eradicate the virus. It is reported that the natural clearance rate of HBsAg in asymptomatic HBsAg carriers is approximately 1–2% per year.¹²³ Therefore, the first goal in treating chronic hepatitis B is to prevent patients from progression to cirrhosis and occurrence of HCC.

When the initiation of antiviral therapy for chronic hepatitis B is considered, it is very important to estimate the fibrosis stage of each patient. If possible, a liver biopsy should be performed in order to obtain sufficient information to determine the extent of hepatic fibrosis. When the fibrosis stage of patients with chronic hepatitis B is moderate to severe, or when the patients

have cirrhotic liver, the administration of antiviral therapy should be considered. When inflammatory activity is high and the fibrosis seems to be progressive, the introduction of antiviral therapy should also be considered.

In order to prevent the occurrence of hepatic fibrosis and HCC, virological factors as well as biochemical factors are important. A long-term follow-up study of untreated HBsAg positive individuals in Taiwan in which the cumulative incidence of HCC and cirrhosis were studied for 13 years revealed that high baseline HBV DNA was associated with increased risk of HCC and cirrhosis. Incidence rate of HCC in patients whose viral load of HBV DNA was less than 300 copies/mL was 1.3%, whereas in patients whose viral load was more than 1 000 000 copies/mL the incidence rate was 14.9%.³³ Moreover, incidence of cirrhosis in patients whose viral load was less than 300 copies/mL was 4.5%, whereas it was 36.2% in patients whose viral load was more than 1 000 000 copies/mL.¹²⁴ Therefore, the introduction of antiviral therapy should be considered based on biochemical and virological findings.

As mentioned above, although high viral load of HBV DNA is one of the strong risk factors in predicting poor prognosis of HBV carriers, low HBV DNA level does not rule out risk in Asian patients. Among HBeAg positive patients, HBV DNA levels of less than 10⁵ copies/mL predicted better histological outcome; however, 14.3% of patients still had established fibrosis.¹²⁵ The liver biopsy is also very useful for such cases.

Recommendation 4

- 4-1 Introduction of antiviral therapy should be considered on the biochemical and virological findings. (Level 2a, grade B.)
- 4-2 Antiviral therapy should be considered for patients with low virus load but progressed hepatic fibrosis. (Level 2a, grade B.)
- 4-3 Liver biopsy finding (if available) should be useful to determine the introduction of antiviral therapy. (Level 2a, grade B.)

On the other hand, when patients with HBV have obscure or mild fibrosis, a close observation without any medication could be considered for them. Once antiviral therapy with a nucleos(t)ide analogue is started, it is very difficult to stop. Therefore, for patients who are in an inactive carrier state and whose fibrosis stage is relatively mild, a coarse observation without any treatment could be a useful choice to treat the patients.

Young patients with chronic hepatitis B, especially those who are HBeAg positive, often face the flare-up of hepatitis. Because such patients are likely to achieve spontaneous HBe seroconversion and go into an inactive carrier state, unnecessary antiviral therapy should be avoided for them. A coarse observation without any medications should be considered for young patients or those with mild fibrosis.

Recommendation 5

Indication of antiviral therapy for chronic hepatitis B: Observation without therapy should be considered for young patients or those with mild fibrosis. (Level 3, grade B.)

NUCLEOS(T)IDE ANALOGUES FOR CHRONIC HEPATITIS B

AS STATED ABOVE, the goal of antiviral therapy in patients with chronic hepatitis B is to prevent cirrhosis and HCC. Maintaining persistent suppression of HBV replication reduces the development of cirrhosis and HCC. In the last decade, there has been a major advance in the treatment of chronic hepatitis B with nucleos(t)ide analogues such as LVD, adefovir (ADV), entecavir (ETV), telbivudine and tenofovir.^{126–132} In treatment by nucleos(t)ide analogues for chronic hepatitis B in Japan, LVD, ADV and ETV are mainly used at present. Nucleos(t)ide analogues are potent inhibitors of the polymerase/reverse transcriptase and are easy to administrate p.o. to chronic hepatitis B patients because of low adverse effects and strong efficacy to suppress HBV replication. Thus, nucleotide analogue therapy could rescue liver decompensation, reduce fibrosis progression and prevent the development of HCC.^{133–136} On the other hand, there are major disadvantages including requirement of prolonged or even indefinite therapy for most patients and the high incidence of antiviral resistance. Disadvantages of nucleos(t)ide analogues include the development of antiviral resistance.^{137–140} Drug-resistant viruses emerge during the treatment and could be associated with flare-up of hepatitis. Due to no proof of reading activity of HBV polymerase, the spontaneous substitution rate of HBV genome is high in the natural course of the disease. Through the selection of pre-existing resistant variants and gradual accumulation of new a.a. substitutions, the mutations exhibiting the best replication capacity in the presence of the drug are selected under the circumstance of antiviral pressure.

The level of intrinsic resistance and the replicative fitness determine the mutant spread and hence the annual incidence of drug resistance.

LVD

Lamivudine was the first nucleoside analogue licensed for the treatment of chronic HBV infection in Japan in 1999. LVD was given at a dose of 100 mg daily and has excellent safety and tolerability.^{141–143}

Liaw *et al.* reported that continuous treatment with LVD delays the clinical progression of chronic hepatitis B with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and risk of HCC (level 1b).¹³⁴ Matsumoto *et al.* also showed that LVD therapy effectively reduces the incidence of HCC in Japanese patients with chronic hepatitis B.¹⁴⁴ Thus, it is generally considered that control of viral load using nucleos(t)ide analogues is effective to prevent complicating HCC in patients with active chronic hepatitis B.

Consensus 8

The control of viral load using nucleos(t)ide analogues reduces the risk of complicating HCC in patients with chronic hepatitis B. (Level 1b, grade B.)

Lamivudine resistance is characterized by the mutation of the highly conserved tyrosine, methionine, aspartate, aspartate (YMDD) nucleotide-binding motif in the catalytic domain of the enzyme. YMDD to YIDD (rtM204I) or YVDD (rtM204V) mutations are associated with LVD resistance.^{142,145,146} These resistant mutants appear to replicate less efficiently than the wild-type virus *in vitro*, however, additional mutations such as rtV173L and rtL180M can restore partially the replication capacity *in vitro*.^{147,148} LVD resistance occurred in approximately 20% of patients after 1 year, which increased to approximately 70% after 5 years (Fig. 1).

A meta-analysis, which included Asian patients and North American/European patients, indicated that HBV subtype ayw (genotype D) appears to respond significantly better to LVD treatment than does HBV subtype adw (genotype A). Insufficient suppression of the adw subtype during the early phase of treatment may lead to the high incidence of LVD resistance in HBV subtype adw.¹⁴⁹ In a study comparing the virological outcome among infections with HBV genotypes A, B and C, patients infected with genotype A had the lowest rate of HBV DNA clearance than those with genotype B or C, and had the highest incidence of resistant mutations.¹⁵⁰

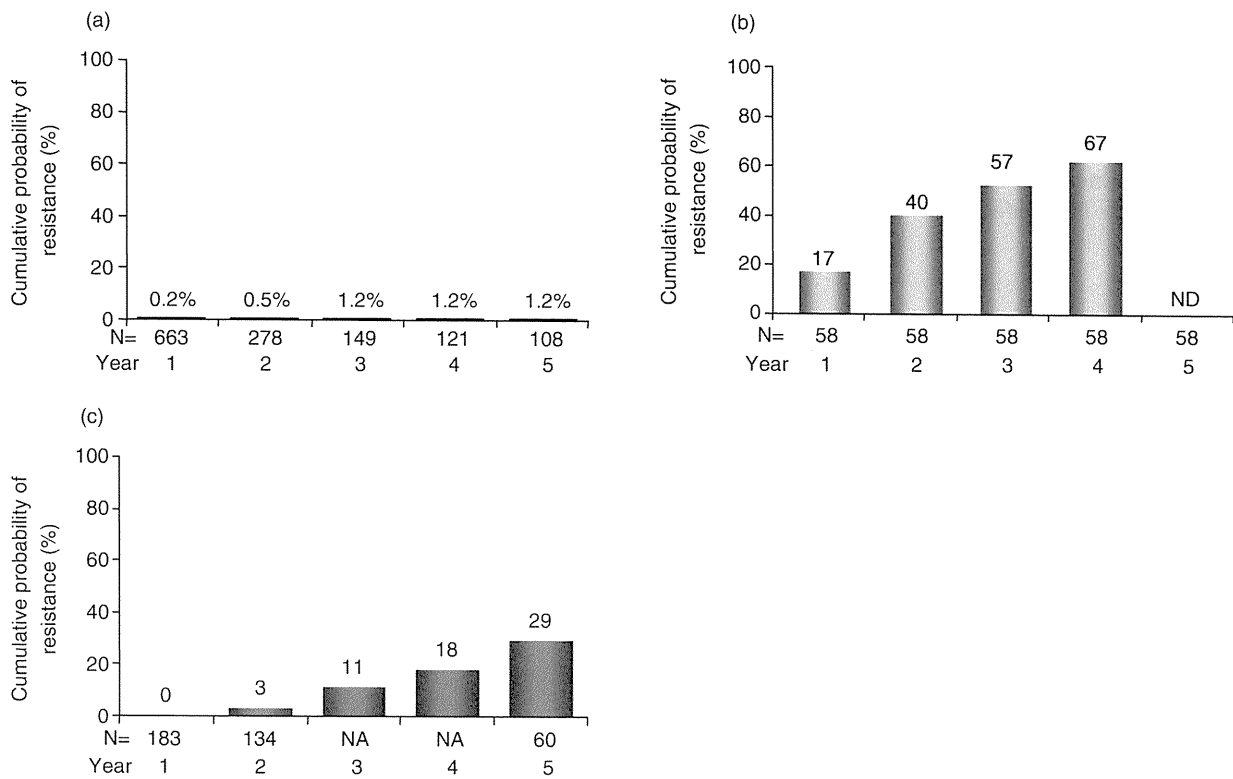


Figure 1 Cumulative probability of resistance after the initiation of entecavir (ETV), lamivudine (LVD) and adefovir (ADV) for patients with hepatitis B e-antigen. (a) Cumulative probability of resistance after the initiation of ETV.¹⁵⁹ (b) Cumulative probability of resistance after the initiation of LVD.¹³⁸ (c) Cumulative probability of resistance after the initiation of ADV.¹⁵³

Lamivudine or hepatitis B immunoglobulin (HBIG) treatment induced vaccine/HBIG-escape mutations sP120T and sG145R in combination with LVD-resistance mutations. These mutations are associated with rtI128N and rtW153Q in the polymerase protein and have been found to partially restore the *in vitro* replicative capacity of LVD-resistant HBV.¹²¹

Another LVD resistant mutation, rtA181T, concomitantly generates a stop codon in the surface antigen (sW172stop), resulting in impaired secretion of HBsAg.¹⁵¹ Neither the adefovir associated resistance mutation rtN236T nor the tenofovir associated resistance mutation rtA194T causes changes in the envelop protein.

ADV

Adefovir dipivoxil is a prodrug of ADV and has structural similarity to the natural substrate, dATP. Several studies have also been conducted using ADV.^{128,152–154} In HBeAg positive patients, treatment with ADV for 1 year resulted in HBeAg seroconversion in 12%, serum HBV DNA in less than 10³ copies/mL in 21% and normaliza-

tion of ALT in approximately 48% of patients.¹²⁷ The rate of HBeAg seroconversion increased to 29% after 2 years and 43% after 3 years of treatment. In HBeAg negative patients, serum HBV DNA of less than 10³ copies/mL and normalization of ALT were observed in 51% and 72%, respectively, after 1 year of ADV.¹⁵⁴ After 5 years of therapy, the serum HBV DNA were less than 10³ copies/mL in 67% of patients, and ALT level normalized in 69%. The reported incidence of ADV resistance is 0% after 1 year, 3% after 2 years and 29% after 5 years of antiviral therapy (Fig. 1).¹⁵⁴ The primary mutations associated with ADV resistance are rtN236T and rtI233V in the D domain and rtA181V in the B domain of HBV polymerase. In comparison with more than 100-fold decrease in sensitivity to LVD associated with the two primary mutations, the rtN236T mutation confers only a 5–10-fold decrease in sensitivity to ADV *in vitro*,¹⁵⁵ which may explain the delayed emergence of this mutant.

In LVD-resistant patients treated with ADV monotherapy, the rate of antiviral resistance was 6–18% after

1 year and 21–38% after 2 years.^{156,157} Switching therapy from LVD to ADV may enhance the acquisition of another mutation and induce replication of HBV DNA.^{158–160} On the other hand, combination therapy of LVD and ADV effectively suppressed viral replication and maintained high efficacy in LVD-resistant patients with chronic HBV infection.

ETV

Entecavir is a guanine analogue and Chang *et al.* have reported that ETV is effective in reducing the serum level of HBV DNA compared with LVD in HBeAg positive patients (Table 2).¹⁵⁹ The cumulative proportion of patients with undetectable HBV DNA (<300 copies/mL) increased to 81% after 1 year of therapy and 93% after 5 years of therapy.¹⁶⁰ After 1 year of treatment with ETV, the serum ALT level was normalized in approximately 70% of patients, and increased to 90% of patients after 5 years. Lai *et al.* have reported that ETV is more efficacious in HBeAg negative patients compared with LVD (Table 2).¹⁶¹ ETV is the most potent of the currently available anti-HBV drugs because it affects multiple functions of the polymerase, including priming, reverse transcription and DNA elongation.¹⁶²

Entecavir was licensed for the treatment of chronic hepatitis B in Japan in 2006. In nucleos(t)ide-naïve patients, ETV is given at dose of 0.5 mg/day.

The rate of ETV resistance was extremely low in nucleoside-naïve patients.^{160,163,164} The incidence of ETV resistance in nucleos(t)ide analogue-naïve patients was reported to be 1.2% at 3 years (Fig. 1).^{160,163,164} HBeAg loss was observed in 8% of these patients. The response to ETV was lower in LVD-resistant patients than in nucleos(t)ide analogue-naïve patients. In LVD-resistant patients, 20% of patients had undetectable HBV DNA levels after 48 weeks of ETV therapy, and the resistance rate to ETV was 26% at 3 years. Patients with HBeAg at the initiation of ETV had a resistance rate to ETV of 36% at 3 years. On the other hand, patients without HBeAg at the initiation of ETV did not have resistance to ETV at 3 years (Fig. 2).^{160,165} In LVD-resistant patients, the risk of the development of resistance to ETV is much higher than those without LVD resistance.^{160,165}

The resistance to ETV is principally associated with the mutations rtM250V, rtI169T or rtS202I in addition to the primary LVD resistance mutations rtM204V + rtL180M. The need for multiple mutations to induce ETV resistance suggests a higher genetic barrier to resistance and explains the low rate of resistance to ETV in nucleos(t)ide analogue-naïve patients.

Table 2 Efficacy of nucleoside analogues for chronic hepatitis B

		Subject: HBeAg positive patients ¹⁵⁹			
	<i>n</i>	Change of HBV DNA (log copies/mL)	Negativity of HBV DNA of <300 copies/mL	Normalization of ALT	SC
ETV 0.5 mg	354	-6.9	67%	68%	21%
LVD 100 mg	355	-5.4	36%	60%	18%
					<i>P</i> = 0.33
					<i>P</i> < 0.05
					<i>P</i> < 0.001
					<i>P</i> < 0.001
		Subject: HBeAg negative patients ¹⁶¹			
	<i>n</i>	Change of HBV DNA (log copies/mL)	Negativity of HBV DNA of <300 copies/mL	Normalization of ALT	
ETV 0.5 mg	325	-5.0	90%	78%	<i>P</i> < 0.05
LVD 100 mg	323	-4.5	72%	71%	

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; LVD, lamivudine; SC, seroconversion; VR, virological response.

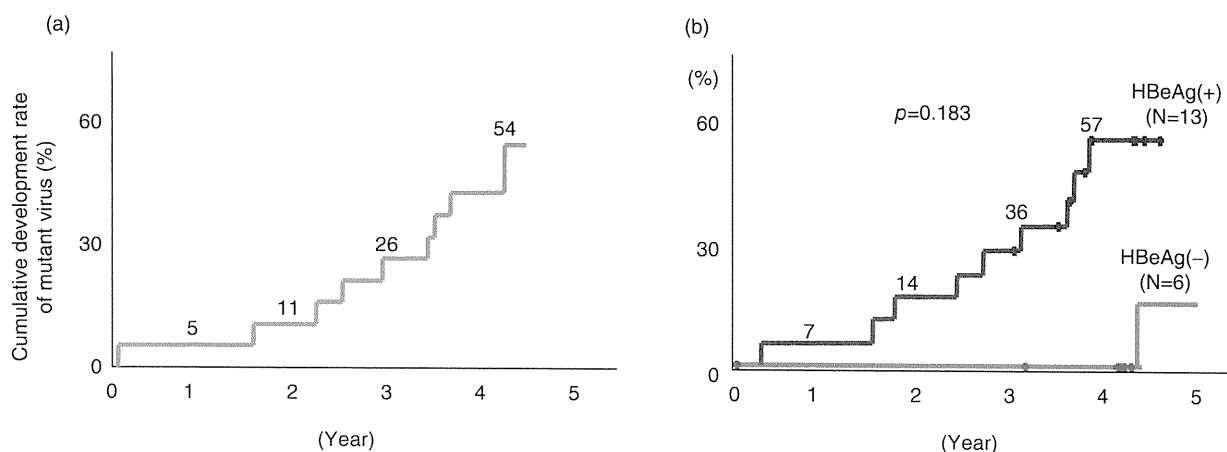


Figure 2 Cumulative development rate of mutant virus after the initiation of entecavir monotherapy in hepatitis B patients with resistance after the administration of lamivudine monotherapy.¹⁶⁴ (a) Cumulative development rate of mutant virus in all patients. (b) Cumulative development rate of mutant virus based on the difference of hepatitis B patients with positive hepatitis B e-antigen (HBeAg) and hepatitis B patients with negative HBeAg.

Consensus 9

Drug-resistant virus with specific mutations in the polymerase/reverse transcriptase gene emerges during nucleos(t)ide analogue therapy in chronic hepatitis B patients. The rtM204V/I and rtL180M mutations are associated with LVD resistance, the rtN236T and rtI233V or rtA181V with ADV resistance, and the rtM250V or rtT184G or rtS202I combined with rtM204V + rtL180M with ETV resistance. (Level 4, grade C.)

Recommendation 6

When patients with chronic hepatitis B are treated with nucleos(t)ide analogues, ETV should be given as the first-line drug because of its high efficacy and low emergence of viral resistant mutant. (Level 1b, grade A.)

Recommendation 7

The combination therapy of LVD and ADV is an effective treatment for LVD-resistant patients. (Level 1b, grade B.)

INTERFERON THERAPY FOR CHRONIC HEPATITIS B

INTERFERON (IFN) WAS the first antiviral treatment approved for chronic HBV infection. IFN- α and - β

have a predominantly antiviral effect but also have an immunomodulatory effect and antiproliferative effect which is in contrast to direct antiviral agents such as nucleos(t)ide analogues. The duration of treatment is defined (usually 24–48 weeks) in IFN therapy. This finite duration of therapy is an advantage over direct antiviral agents which are usually given indefinitely. The long-term outcome of therapy is more precisely described in IFN compared to LVD due to its longer history of clinical usage.

Selection of patients

Factors associated with favorable response to IFN therapy are vigorously studied (Table 3). For HBeAg positive patients, high pretreatment ALT levels,¹⁶⁶ high grade of necroinflammation on liver histology and low serum HBV DNA level have consistently been shown to be predictive of favorable response.¹⁶⁷ Other predictive factors include female sex,¹⁶⁶ younger age,^{168,169} and HBV genotype A versus D or B versus C.^{169,170} Patients fulfilling these predictors are the best candidates for IFN treatment. For HBeAg negative patients, there is no consistent predictor of response. Adverse events such as severe infection or exacerbations of liver disease were common when IFN was given for decompensated cirrhosis. Thus, patients with decompensated cirrhosis should not be treated with IFN due to a risk of precipitating hepatic failure and fatal complications.^{171,172}

Table 3 Predictive factors for response to interferon therapy

Predictive factors	HBeAg positive	HBeAg negative
Race	No correlation	No correlation
Age	No correlation or Younger	No correlation or Younger
Sex	No correlation or Female	No correlation or Female
ALT	Higher level	No correlation or Higher level
Activity	Higher grade	No correlation
Fibrosis	Conflicting	No correlation
HBV DNA titer	Lower titer	No correlation or lower titer
Genotype	A > D, B > C	A > D, B > C
Precore	Conflicting	No correlation
Core promoter	mutant	

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

Recommendation 8

Younger age, high ALT levels, low HBV load, genotype A or B and high inflammatory activity in liver biopsy are predictive of good response to IFN. IFN therapy should be considered in patients fulfilling these predictors. (Level 2a, 2b, grade B.)

Recommendation 9

Interferon should be avoided for patients with decompensated cirrhosis. (Level 4, grade D.)

Standard IFN therapy in HBeAg positive chronic hepatitis B

A meta-analysis of 16 randomized controlled studies have shown that treatment with IFN- α for 16–24 weeks versus an untreated control is associated with higher rate of HBeAg loss (33% vs 12%), HBeAg seroconversion (difference of 18%), undetectable HBV DNA by hybridization or branched chain assay (37% vs 17%), HBsAg loss (7.8% vs 1.8%) and ALT normalization (difference of 23%) (Table 4).¹⁷³ A controlled trial has shown that extending therapy for up to 32 weeks in patients who remained HBeAg positive at the end of 16 weeks of

therapy improved the rate of HBeAg seroconversion.¹⁷⁴ The durability of HBeAg seroconversion is more than 80%, and even delayed seroconversion could occur in 10–15% of patients 1–2 years after completion of therapy.^{175–177} The loss of HBsAg is reported to occur in 12–65% of patients who cleared HBeAg.^{175,178} However, this is a rare event in Asian patients.^{176,177}

Consensus statement 10

10-1 In HBeAg positive patients, treatment with IFN versus untreated control is associated with higher rate of HBeAg loss, HBeAg seroconversion, undetectable HBV DNA, HBsAg loss and ALT normalization. Extension of therapy improves the rate of HBeAg seroconversion. (Level 1a,1b.)

10-2 Durability of HBeAg seroconversion is more than 80%. The loss of HBsAg is rare in Asian patients. (Level 1b.)

Standard IFN therapy in HBeAg negative chronic hepatitis B

Although the rate of response at the end of therapy is 60–90%, the durability of long-term response is less

Table 4 Standard interferon therapy for HBeAg positive chronic hepatitis B. Meta-analysis of 16 randomized controlled trials

	Interferon	Control	P-value
Loss of HBV DNA	37%	17%	0.0001
Loss of HBeAg	33%	12%	0.0001
Loss of HBsAg	7.8%	1.8%	0.001
Seroconversion		Difference of 18%	0.002
ALT normalization		Difference of 23%	0.0001

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

than 50%.^{179,180} Longer duration of therapy is associated with improved durability of response: 10–15% with 4–6 months of therapy, 22–30% with 6–12 months of therapy and 30% with 24 months of therapy.^{181–184}

Consensus statement 11

- 11-1 Durability of response is less than 50% in HBeAg negative patients. (Level 1b.)
 11-2 Longer duration of therapy (>48 weeks) is associated with improved durability of response. (Level 2b.)

Pegylated IFN (PEG IFN)

Twenty four weeks of PEG IFN- α -2a monotherapy had higher rate of combined response (loss of HBeAg, suppression of HBV DNA <500 000 copies/mL and ALT normalization) compared to standard IFN- α -2a.¹⁸⁵ Another study with 24 weeks of PEG IFN- α -2b monotherapy also showed a higher rate of HBeAg loss and HBV DNA suppression compared to standard IFN- α -2b.¹⁶⁹

Controlled studies comparing the 48 weeks of PEG IFN- α -2a and LVD in HBeAg positive and negative patients revealed that PEG IFN had a higher rate of sustained response.^{170,171} Seroconversion of HBeAg (32% vs 19%), ALT normalization (41% vs 28% in HBeAg positives and 59% vs 44% in HBeAg negatives), HBV DNA suppression (HBV DNA <10 000 copies/mL, 32% vs 22% in HBeAg positives; HBV DNA <20 000 copies/mL, 43% vs 29% in HBeAg negatives) and negative HBV DNA (14% vs 5% in HBeAg positives and 19% vs 7% in HBeAg negatives) were more frequent in PEG IFN treated patients.

Differences were reported in outcome of the antiviral treatment of patients infected with different genotypes; genotype B is associated with a higher rate of antiviral response to IFN treatment than HBV genotype C among Asian patients with HBeAg positive chronic hepatitis B.^{169,186,187} In multicenter trials comparing combination therapy of PEG IFN- α -2b and LVD versus PEG IFN- α -2b alone, it was shown that treatment with PEG IFN- α -2b is the best therapy to achieve HBsAg clearance in patients with genotype A compared with D.^{188,189}

Combination or sequential therapy

Combination of two antiviral agents with different mechanisms of action seems a logical approach to improve efficacy. In fact, simultaneous combination of LVD and PEG IFN has a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone. However, there is no difference in treatment response between the simultaneous combination of LVD and IFN or PEG IFN compared to IFN or PEG IFN alone (Table 5).^{132,133,170}

There are several clinical trials of sequential therapy with LVD followed by IFN.^{190–194} Common to all studies is that the sequential therapy had no advantage over IFN alone. Some studies have shown the suggestive evidence that sequential therapy had a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone (Table 5).^{190–194} However, because the study protocols and their results are variable, a conclusive result could not be drawn.

Table 5 Sequential therapy of lamivudine and interferon

		BR	SC	VR	LVD-R
Manesis <i>et al.</i> 2006 (<i>n</i> = 36) ¹⁹⁰	Sequential	39%	NA	28%	
	IFN	22%	NA	19%	
Shi <i>et al.</i> 2006 (<i>n</i> = 162) ¹⁹¹	Sequential	53%	NA	14%	0%
	LVD	36%	NA	18%	23%
Yurdaydin <i>et al.</i> 2005 (<i>n</i> = 78) ¹⁹³	Sequential	51%	NA	54%	24%
	LVD	41%	NA	59%	53%
Sarin <i>et al.</i> 2005 (<i>n</i> = 75) ¹⁹⁴	Sequential	40%	40%	40%	15%
	LVD	14%	11%	16%	8%
Schalm <i>et al.</i> 2000 (<i>n</i> = 226) ¹⁹²	Sequential	50%	36%	55%	0%
	IFN	50%	22%	49%	0%
	LVD	63%	19%	63%	31%

BR, biochemical response; IFN, interferon; LVD, lamivudine; LVD-R, lamivudine resistant mutation; NA, not applicable because hepatitis B e-antigen patients are studied; SC, seroconversion; VR, virological response.

Long-term outcome

The end-point of antiviral therapy is to prevent liver cirrhosis and HCC. Meta-analysis of five studies including 935 patients revealed that IFN treatment significantly decreased the incidence of cirrhosis with the combined risk ratio of 0.65 (95% confidence interval [CI] = 0.47–0.91).¹⁹⁵ Meta-analysis of 11 studies including 2082 patients revealed that IFN treatment significantly decreased the incidence of HCC with the combined risk ratio of 0.59 (95% CI = 0.43–0.81).¹⁹⁵ These results suggest that IFN prevents progression of liver disease to liver cirrhosis or delays the development of HCC, as long as it is within 4–7 years of follow up which is the length of follow up in these studies. Sustained response to IFN therapy was associated with increased survival.^{175,181,196,197} To further elucidate the impact of IFN on the natural course of chronic hepatitis B, studies with larger populations followed for longer periods may be needed.

Consensus statement 12

- 12-1 IFN therapy prevents progression to cirrhosis or the development of HCC. (Level 1a.)
12-2 IFN therapy is associated with improved survival. (Level 1b.)

Adverse effects

The most frequent adverse effects are flu-like symptoms, fatigue, myelosuppression and dermal reaction at the injection site. Others include alopecia, depression and thyroid dysfunction. Less frequent but severe adverse events include interstitial pneumonitis, exacerbation of underlying autoimmune disorders, cerebral vascular events and flare of hepatitis.

REFERENCES

- Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; 11: 97–107.
- McMahon BJ, Alward WL, Hall DB *et al.* Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; 151: 599–603.
- Shiffman RN, Shenkelle P, Overhage JM, Drimshaw J, Deshpande AM. Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann Intern Med* 2003; 139: 493–8.
- McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; 49: S45–55.
- Fattovich G. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003; 23: 47–58.
- Chen DS. From hepatitis to hepatoma: lessons from type B viral hepatitis. *Science* 1993; 262: 369–70.
- Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; 45: 1056–75.
- Lok AS. Chronic hepatitis B. *N Engl J Med* 2002; 346: 1682–3.
- Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000 – summary of a workshop. *Gastroenterology* 2001; 120: 1828–53.
- Chang MH, Hsu HY, Hsu HC, Ni YH, Chen JS, Chen DS. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. *Hepatology* 1995; 22: 1387–92.
- Hui CK, Leung N, Yuen ST *et al.* Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. *Hepatology* 2007; 46: 395–401.
- Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. *Hepatology* 1988; 8: 1130–3.
- Bortolotti F, Guido M, Bartolacci S *et al.* Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. *Hepatology* 2006; 43: 556–62.
- Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF. Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; 116: 829–34.
- Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981; 94: 744–8.
- McMahon BJ. Epidemiology and natural history of hepatitis B. *Semin Liver Dis* 2005; 25 (Suppl 1): 3–8.
- Fattovich G, Rugge M, Brollo L *et al.* Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. *Hepatology* 1986; 6: 167–72.
- Liaw YF, Chu CM, Huang MJ, Sheen IS, Yang CY, Lin DY. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. *Liver* 1984; 4: 301–6.
- Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. *Gastroenterology* 1987; 92: 1839–43.
- McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; 135: 759–68.
- Yuen MF, Yuan HJ, Hui CK *et al.* A large population study of spontaneous HBeAg seroconversion and acute exacerbation.

- bation of chronic hepatitis B infection: implications for antiviral therapy. *Gut* 2003; 52: 416–19.
- 22 Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000; 118: 554–9.
 - 23 Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; 122: 1756–62.
 - 24 Hsu YS, Chien RN, Yeh CT *et al.* Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* 2002; 35: 1522–7.
 - 25 Carman WF, Jacyna MR, Hadziyannis S *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; 2: 588–91.
 - 26 Chan HL, Hussain M, Lok AS. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. *Hepatology* 1999; 29: 976–84.
 - 27 Chen CH, Hung CH, Lee CM *et al.* Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; 133: 1466–74.
 - 28 Marschenz S, Endres AS, Brinckmann A *et al.* Functional analysis of complex hepatitis B virus variants associated with development of liver cirrhosis. *Gastroenterology* 2006; 131: 765–80.
 - 29 Chen CH, Changchien CS, Lee CM *et al.* Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case-control study. *J Infect Dis* 2008; 198: 1634–42.
 - 30 Yuen MF, Tanaka Y, Shinkai N *et al.* Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer II/core promoter/precore regions and HBV DNA levels. *Gut* 2008; 57: 98–102.
 - 31 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
 - 32 Yu MW, Chang HC, Liaw YF *et al.* Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000; 92: 1159–64.
 - 33 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295: 65–73.
 - 34 Ishiguro S, Inoue M, Tanaka Y, Mizokami M, Iwasaki M, Tsugane S. Serum aminotransferase level and the risk of hepatocellular carcinoma: a population-based cohort study in Japan. *Eur J Cancer Prev* 2009; 18: 26–32.
 - 35 Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003; 124: 327–34.
 - 36 Yu MW, Yeh SH, Chen PJ *et al.* Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; 97: 265–72.
 - 37 Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut* 2004; 53: 744–9.
 - 38 Ohnishi K, Iida S, Iwama S *et al.* The effect of chronic habitual alcohol intake on the development of liver cirrhosis and hepatocellular carcinoma: relation to hepatitis B surface antigen carriage. *Cancer* 1982; 49: 672–7.
 - 39 Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. *Gastroenterology* 2004; 127: S72–78.
 - 40 Manno M, Camma C, Schepis F *et al.* Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; 127: 756–63.
 - 41 Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. *Gastroenterology* 2002; 123: 1084–9.
 - 42 Huo TI, Wu JC, Lee PC *et al.* Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. *Hepatology* 1998; 28: 231–6.
 - 43 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; 131: 59–68.
 - 44 Tanaka E, Umemura T. History and prevention of de novo hepatitis B virus-related hepatitis in Japan and the World. *Clin J Gastroenterol* 2008; 1: 83–6.
 - 45 Umemura T, Kiyosawa K. Fatal HBV reactivation in a subject with anti-HBs and anti-HBc. *Intern Med* 2006; 45: 747–8.
 - 46 Umemura T, Tanaka E, Kiyosawa K, Kumada H. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clin Infect Dis* 2008; 47: e52–56.
 - 47 Taylor BC, Yuan JM, Shamliyan TA, Shaikat A, Kane RL, Wilt TJ. Clinical outcomes in adults with chronic hepatitis B in association with patient and viral characteristics: a systematic review of evidence. *Hepatology* 2009; 49: S85–95.
 - 48 Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; 40: 14–30.
 - 49 Chen DS. Hepatitis B vaccination: the key towards elimination and eradication of hepatitis B. *J Hepatol* 2009; 50: 805–16.
 - 50 Lok AS. Natural history and control of perinatally acquired hepatitis B virus infection. *Dig Dis* 1992; 10: 46–52.
 - 51 Koibuchi T, Hitani A, Nakamura T *et al.* Predominance of genotype A HBV in an HBV-HIV-1 dually positive

- population compared with an HIV-1-negative counterpart in Japan. *J Med Virol* 2001; 64: 435–40.
- 52 Shibayama T, Masuda G, Ajisawa A *et al.* Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. *J Med Virol* 2005; 76: 24–32.
- 53 Kobayashi M, Arase Y, Ikeda K *et al.* Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. *J Med Virol* 2002; 68: 522–8.
- 54 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; 44: 326–34.
- 55 Sugauchi F, Orito E, Ohno T *et al.* Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. *Hepatol Res* 2006; 36: 107–14.
- 56 Suzuki Y, Kobayashi M, Ikeda K *et al.* Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J Med Virol* 2005; 76: 33–9.
- 57 Matsuura K, Tanaka Y, Hige S *et al.* Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. *J Clin Microbiol* 2009; 47: 1476–83.
- 58 Sherlock S. The natural history of hepatitis B. *Postgrad Med J* 1987; 63 (Suppl 2): 7–11.
- 59 Sugiyama M, Tanaka Y, Kato T *et al.* Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. *Hepatology* 2006; 44: 915–24.
- 60 Sugiyama M, Tanaka Y, Kurbanov F *et al.* Direct cytopathic effects of particular hepatitis B virus genotypes in severe combined immunodeficiency transgenic with urokinase-type plasminogen activator mouse with human hepatocytes. *Gastroenterology* 2009; 136: 652–62. e3.
- 61 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; 38: 646–57.
- 62 Sato S, Suzuki K, Akahane Y *et al.* Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 1995; 122: 241–8.
- 63 Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 1991; 324: 1699–704.
- 64 Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; 324: 1705–9.
- 65 Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. Nucleotide sequence analysis of the precore region in patients with fulminant hepatitis B in the United States. *Gastroenterology* 1993; 105: 1173–8.
- 66 Feray C, Gigou M, Samuel D, Bernuau J, Bismuth H, Brechot C. Low prevalence of precore mutations in hepatitis B virus DNA in fulminant hepatitis type B in France. *J Hepatol* 1993; 18: 119–22.
- 67 Liang TJ, Hasegawa K, Munoz SJ *et al.* Hepatitis B virus precore mutation and fulminant hepatitis in the United States. A polymerase chain reaction-based assay for the detection of specific mutation. *J Clin Invest* 1994; 93: 550–5.
- 68 Chan HL, Sung JJ. Hepatocellular carcinoma and hepatitis B virus. *Semin Liver Dis* 2006; 26: 153–61.
- 69 Lok AS. Hepatitis B: liver fibrosis and hepatocellular carcinoma. *Gastroenterol Clin Biol* 2009; 33: 911–15.
- 70 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.
- 71 Chan HL, Tse CH, Mo F *et al.* High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol* 2008; 26: 177–82.
- 72 Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. *Am J Gastroenterol* 2006; 101: 1797–803.
- 73 Chan HL, Hui AY, Wong ML *et al.* Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; 53: 1494–8.
- 74 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; 37: 19–26.
- 75 Tanaka Y, Mukaide M, Orito E *et al.* Specific mutations in enhancer II/core promoter of hepatitis B virus subgenotypes C1/C2 increase the risk of hepatocellular carcinoma. *J Hepatol* 2006; 45: 646–53.
- 76 Huang Y, Wang Z, An S *et al.* Role of hepatitis B virus genotypes and quantitative HBV DNA in metastasis and recurrence of hepatocellular carcinoma. *J Med Virol* 2008; 80: 591–7.
- 77 Livingston SE, Simonetti JP, McMahon BJ *et al.* Hepatitis B virus genotypes in Alaska Native people with hepatocellular carcinoma: preponderance of genotype F. *J Infect Dis* 2007; 195: 5–11.
- 78 Tseng TC, Kao JH. HBV genotype and clinical outcome of chronic hepatitis B: facts and puzzles. *Gastroenterology* 2008; 134: 1272–3. author reply 3.
- 79 Kew MC, Kramvis A, Yu MC, Arakawa K, Hodgkinson J. Increased hepatocarcinogenic potential of hepatitis B virus genotype A in Bantu-speaking sub-saharan Africans. *J Med Virol* 2005; 75: 513–21.
- 80 Kramvis A, Kew MC, Bukofzer S. Hepatitis B virus precore mutants in serum and liver of Southern African Blacks with hepatocellular carcinoma. *J Hepatol* 1998; 28: 132–41.
- 81 Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology* 2002; 123: 1848–56.

- 82 Ou JH, Laub O, Rutter WJ. Hepatitis B virus gene function: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. *Proc Natl Acad Sci U S A* 1986; 83: 1578–82.
- 83 Uly A, Bruss V, Gerlich WH, Köchel HG, Thomssen R. Precore sequence of hepatitis B virus inducing e antigen and membrane association of the viral core protein. *Virology* 1986; 155: 89–96.
- 84 Miyanohara A, Imamura T, Araki M, Sugawara K, Ohtomo N, Matsubara K. Expression of hepatitis B virus core antigen gene in *Saccharomyces cerevisiae*: synthesis of two polypeptides translated from different initiation codons. *J Virol* 1986; 59: 176–80.
- 85 Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; 40: 14–30.
- 86 Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus – large-scale analysis using a new genotyping method. *J Infect Dis* 1997; 175: 1285–93.
- 87 Okamoto H, Tsuda F, Akahane Y *et al.* Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994; 68: 8102–10.
- 88 Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J Virol* 1996; 70: 5845–51.
- 89 Parekh S, Zoulim F, Ahn SH *et al.* Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. *J Virol* 2003; 77: 6601–12.
- 90 Kosaka Y, Takase K, Kojima M *et al.* Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology* 1991; 100: 1087–94.
- 91 Karayiannis P, Alexopoulou A, Hadziyannis S *et al.* Fulminant hepatitis associated with hepatitis B virus e antigen-negative infection: importance of host factors. *Hepatology* 1995; 22: 1628–34.
- 92 Laskus T, Rakela J, Nowicki MJ, Persing DH. Hepatitis B virus core promoter sequence analysis in fulminant and chronic hepatitis B. *Gastroenterology* 1995; 109: 1618–23.
- 93 Laskus T, Rakela J, Persing DH. The stem-loop structure of the cis-encapsidation signal is highly conserved in naturally occurring hepatitis B virus variants. *Virology* 1994; 200: 809–12.
- 94 Liu CJ, Kao JH, Lai MY, Chen PJ, Chen DS. Precore/core promoter mutations and genotypes of hepatitis B virus in chronic hepatitis B patients with fulminant or subfulminant hepatitis. *J Med Virol* 2004; 72: 545–50.
- 95 Pollicino T, Zanetti AR, Cacciola I *et al.* Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. *Hepatology* 1997; 26: 495–9.
- 96 Kalinina T, Riu A, Fischer L, Will H, Sterneck M. A dominant hepatitis B virus population defective in virus secretion because of several S-gene mutations from a patient with fulminant hepatitis. *Hepatology* 2001; 34: 385–94.
- 97 Bock CT, Tillmann HL, Maschek HJ, Manns MP, Trautwein C. A preS mutation isolated from a patient with chronic hepatitis B infection leads to virus retention and misassembly. *Gastroenterology* 1997; 113: 1976–82.
- 98 Zhang K, Imazeki F, Fukai K *et al.* Analysis of the complete hepatitis B virus genome in patients with genotype C chronic hepatitis in relation to HBeAg and anti-HBe. *J Med Virol* 2007; 79: 683–93.
- 99 Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999; 29: 946–53.
- 100 Liu CJ, Chen BF, Chen PJ *et al.* Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case-control study. *J Infect Dis* 2006; 194: 594–9.
- 101 Guo X, Jin Y, Qian G, Tu H. Sequential accumulation of the mutations in core promoter of hepatitis B virus is associated with the development of hepatocellular carcinoma in Qidong, China. *J Hepatol* 2008; 49: 718–25.
- 102 Tong MJ, Blatt LM, Kao JH, Cheng JT, Corey WG. Basal core promoter T1762/A1764 and precore A1896 gene mutations in hepatitis B surface antigen-positive hepatocellular carcinoma: a comparison with chronic carriers. *Liver Int* 2007; 27: 1356–63.
- 103 Tong MJ, Blatt LM, Kao JH, Cheng JT, Corey WG. Precore/basal core promoter mutants and hepatitis B viral DNA levels as predictors for liver deaths and hepatocellular carcinoma. *World J Gastroenterol* 2006; 12: 6620–6.
- 104 Yang HI, Yeh SH, Chen PJ *et al.* REVEAL-HBV Study Group. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; 100: 1134–43.
- 105 Fang ZL, Sabin CA, Dong BQ *et al.* HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; 103: 2254–62.
- 106 Bläckberg J, Kidd-Ljunggren K. Mutations within the hepatitis B virus genome among chronic hepatitis B patients with hepatocellular carcinoma. *J Med Virol* 2003; 71: 18–23.
- 107 Zhang KY, Imazeki F, Fukai K *et al.* Analysis of the complete hepatitis B virus genome in patients with genotype C chronic hepatitis and hepatocellular carcinoma. *Cancer Sci* 2007; 98: 1921–9.
- 108 Fang ZL, Sabin CA, Dong BQ *et al.* Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case-control study. *J Gen Virol* 2008; 89: 2882–90.

- 109 Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; 130: 1153–68.
- 110 Mun HS, Lee SA, Jee Y *et al.* The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; 80: 1189–94.
- 111 Takahashi K, Akahane Y, Hino K, Ohta Y, Mishihiro S. Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. *Arch Virol* 1998; 143: 2313–26.
- 112 Zanetti AR, Tanzi E, Manzillo G *et al.* Hepatitis B variant in Europe. *Lancet* 1988; 2: 1132–3.
- 113 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; 336: 325–9.
- 114 Yamamoto K, Horikita M, Tsuda F *et al.* Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; 68: 2671–6.
- 115 Hsu HY, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; 30: 1312–17.
- 116 McMahon G, Ehrlich PH, Moustafa ZA *et al.* Genetic alterations in the gene encoding the major HBsAg: DNA and immunological analysis of recurrent HBsAg derived from monoclonal antibody-treated liver transplant patients. *Hepatology* 1992; 15: 757–66.
- 117 Carman WF, Trautwein C, van Deursen FJ *et al.* Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996; 24: 489–93.
- 118 Ghany MG, Ayola B, Villamil FG *et al.* Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. *Hepatology* 1998; 27: 213–22.
- 119 Jongerius JM, Wester M, Cuyper HT *et al.* New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion* 1998; 38: 56–9.
- 120 Chemin I, Trépo C. Clinical impact of occult HBV infections. *J Clin Virol* 2005; 34 (Suppl 1): S15–21.
- 121 Torresi J, Earnest-Silveira L, Civitico G *et al.* Restoration of replication phenotype of lamivudine-resistant hepatitis B virus mutants by compensatory changes in the “fingers” subdomain of the viral polymerase selected as a consequence of mutations in the overlapping S gene. *Virology* 2002; 299: 88–99.
- 122 Hsu CW, Yeh CT, Chang ML, Liaw YF. Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology* 2007; 132: 543–50.
- 123 Chu CM, Yeh CT, Tsai SL *et al.* HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. *Hepatology* 2007; 45: 1187–92.
- 124 Iloeje UH, Yang HI, Su J *et al.* Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; 130: 678–86.
- 125 Yuen MF, Ng IO, Fan ST *et al.* Significance of HBV DNA levels in liver histology of HBeAg and Anti-HBe positive patients with chronic hepatitis B. *Am J Gastroenterol* 2004; 99: 2032–7.
- 126 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–8.
- 127 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; 348: 800–7.
- 128 Marcellin P, Chang TT, Lim SG *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; 348: 808–16.
- 129 Marcellin P, Lau GKK, Bonino F *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.
- 130 Lau GK, Piratvisuth T, Luo KX *et al.* Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; 352: 2682–95.
- 131 Sherman M, Yurdaydin C, Sollano J *et al.* Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006; 130: 2039–49.
- 132 Lai CL, Gane E, Liaw YF *et al.* Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; 357: 2576–88.
- 133 Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. *J Hepatol* 2003; 38: 322–7.
- 134 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–31.
- 135 Leung NW, Lai CL, Chang TT *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.
- 136 Liaw YF, Chang TT, Wu SS *et al.* Long-term entecavir therapy results in reversal of fibrosis/cirrhosis and continued histologic improvement in patients with HBeAg(+) and (–) chronic hepatitis B: results from studies ETV-022, -027 and -901. *Hepatology* 2008; 48: 706A. abst 894.
- 137 Lok AS, Zoulim F, Locarnini S *et al.* Hepatitis B Virus Drug Resistance Working Group. *Hepatology* 2007; 46: 254–65.
- 138 Yuan HJ, Lee WM. Molecular mechanisms of resistance to antiviral therapy in patients with chronic hepatitis B. *Curr Mol Med* 2007; 7: 185–97.

- 139 Chang TT, Lai CL, Chien RN *et al.* Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004; 19: 1276–82.
- 140 Yatsuji H, Noguchi C, Hiraga N *et al.* Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif. *Antimicrob Agents Chemother* 2006; 50: 3867–74.
- 141 Gaia S, Marzano A, Smedile A *et al.* Four years of treatment with lamivudine: clinical and virological evaluations in HBe antigen-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2004; 20: 281–7.
- 142 Tipples GA, 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.
- 143 Chayama K, Suzuki Y, Kobayashi M *et al.* Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. *Hepatology* 1998; 27: 1711–16.
- 144 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173–84.
- 145 Ono-Nita SK, Kato N, Shiratori Y *et al.* YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: a study by *in vitro* full-length viral DNA transfection. *Hepatology* 1999; 29: 939–45.
- 146 Lok AS, Hussain M, Cursano C *et al.* Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; 32: 1145–53.
- 147 Ono-Nita SK, Kato N, Shiratori Y *et al.* Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. *J Clin Invest* 1999; 103: 1635–40.
- 148 Delaney WE 4th, Yang H, Westland CE *et al.* The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication *in vitro*. *J Virol* 2003; 77: 11833–41.
- 149 Zollner B, Petersen J, Schafer P *et al.* Subtype-dependent response of hepatitis B virus during the early phase of lamivudine treatment. *Clin Infect Dis* 2002; 34: 1273–7.
- 150 Kobayashi M, Akuta N, Suzuki F *et al.* Virological outcomes in patients infected chronically with hepatitis B virus genotype A in comparison with genotypes B and C. *J Med Virol* 2006; 78: 60–7.
- 151 Yeh CT, Chien RN, Chu CM, Liaw YF. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. *Hepatology* 2000; 31: 1318–26.
- 152 Fung SK, Chae HB, Fontana RJ *et al.* Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; 44: 283–90.
- 153 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir Dipivoxil 438 Study Group. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005; 352: 2673–81.
- 154 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir Dipivoxil 438 Study Group. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; 131: 1743–51.
- 155 Angus P, Vaughan R, Xiong S *et al.* Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* 2003; 125: 292–7.
- 156 Lee YS, Suh DJ, Lim YS *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.
- 157 Chen CH, Wang JH, Lee CM *et al.* Virological response and incidence of adefovir resistance in lamivudine-resistant patients treated with adefovir dipivoxil. *Antivir Ther* 2006; 11: 771–8.
- 158 Yeon JE, Yoo W, Hong SP *et al.* Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. *Gut* 2006; 55: 1488–95.
- 159 Chang TT, Gish RG, de Man R *et al.* BEHoLD A1463022 Study Group A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; 354: 1001–10.
- 160 Tenney DJ, Rose RE, Baldick CJ *et al.* Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; 49: 1503–14.
- 161 Lai CL, Shouval D, Lok AS *et al.* BEHoLD A1463027 Study Group. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; 9 (354): 1011–20.
- 162 Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob Agents Chemother* 1997; 41: 1444–8.
- 163 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology* 2006; 44: 1656–65.
- 164 Tenney DJ, Rose RE, Baldick CJ *et al.* Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother* 2007; 51: 902–11.
- 165 Suzuki F, Suzuki Y, Akuta N *et al.* Changes in viral loads of lamivudine-resistant mutants during entecavir therapy. *Hepatol Res* 2008; 9 (38): 132–40.
- 166 Hoofnagle JH, Peters M, Mullen KD *et al.* Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. *Gastroenterology* 1988; 95: 1318–25.

- 167 Perrillo RP, Schiff ER, Davis GL *et al.* A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. *N Engl J Med* 1990; **323**: 295–301.
- 168 Suzuki F, Arase Y, Akuta N *et al.* Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan. *J Gastroenterol* 2004; **39**: 969–74.
- 169 Zhao H, Kurbanov F, Wan MB *et al.* Genotype B and younger patient age associated with better response to low-dose therapy: a trial with pegylated/nonpegylated interferon-alpha-2b for hepatitis B e antigen-positive patients with chronic hepatitis B in China. *Clin Infect Dis* 2007; **44**: 541–8.
- 170 Janssen HL, van Zonneveld M, Senturk H *et al.* Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; **365**: 123–9.
- 171 Perrillo R, Tamburro C, Regenstein F *et al.* Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. *Gastroenterology* 1995; **109**: 908–16.
- 172 Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. *Gastroenterology* 1993; **104**: 1116–21.
- 173 Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; **119**: 312–23.
- 174 Janssen HL, Gerken G, Carreno V *et al.* Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 1999; **30**: 238–43.
- 175 Niederau C, Heintges T, Lange S *et al.* Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996; **334**: 1422–7.
- 176 Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. *Gastroenterology* 1993; **105**: 1833–8.
- 177 Lin SM, Tai DI, Chien RN, Sheen IS, Chu CM, Liaw YF. Comparison of long-term effects of lymphoblastoid interferon alpha and recombinant interferon alpha-2a therapy in patients with chronic hepatitis B. *J Viral Hepat* 2004; **11**: 349–57.
- 178 Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 1997; **26**: 1338–42.
- 179 Fattovich G, Farci P, Rugge M *et al.* A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. *Hepatology* 1992; **15**: 584–9.
- 180 Hadziyannis S, Bramou T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis type B. *J Hepatol* 1990; **11** (Suppl 1): S133–136.
- 181 Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2001; **34**: 306–13.
- 182 Brunetto MR, Oliveri F, Coco B *et al.* Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. *J Hepatol* 2002; **36**: 263–70.
- 183 Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. *Gastroenterology* 2001; **121**: 101–9.
- 184 Lampertico P, Del Ninno E, Viganò M *et al.* Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. *Hepatology* 2003; **37**: 756–63.
- 185 Cooksley WG, Piratvisuth T, Lee SD *et al.* Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. *J Viral Hepat* 2003; **10**: 298–305.
- 186 Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; **33**: 998–1002.
- 187 Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002; **36**: 1425–30.
- 188 Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 2006; **101**: 297–303.
- 189 Buster EH, Flink HJ, Cakaloglu Y *et al.* Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology* 2008; **135**: 459–67.
- 190 Manesis EK, Papatheodoridis GV, Hadziyannis SJ. A partially overlapping treatment course with lamivudine and interferon in hepatitis B e antigen-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2006; **23**: 99–106.
- 191 Shi M, Wang RS, Zhang H *et al.* Sequential treatment with lamivudine and interferon-alpha monotherapies in hepatitis B e antigen-negative Chinese patients and its suppression of lamivudine-resistant mutations. *J Antimicrob Chemother* 2006; **58**: 1031–5.
- 192 Schalm SW, Heathcote J, Cianciara J *et al.* Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. *Gut* 2000; **46**: 562–8.