

responders showed that histological fibrosis and activity was improved in the assigned IFN-treated group. In contrast, in the untreated group, the fibrosis score did not decline.¹⁴⁴ In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.^{145–147} In the USA, an RCT of 53 patients in whom a histological response, but not a viral response was induced by 6 MIU of IFN showed that 3 MIU of IFN for 24 months improved the degree of hepatic fibrosis.

However, the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial found no difference in the progression of liver disease between a low-dose PEG-IFN group and a control group.¹³⁴ The large discrepancy in the effectiveness of IFN maintenance therapy between the HALT-C trial and Japanese trials might be attributed to several factors. First, the study designs differed. One of the most important differences was related to the patients' clinical characteristics. For example, patients enrolled in Japanese studies were older than those in the HALT-C trial. Elderly patients have a higher incidence of HCC than younger patients. It is suggested that the tumor-suppressive effect of IFN maintenance therapy might be more clearly demonstrated in a high-risk group, including elderly patients.¹³⁸

Until more data become available, the decision to perform IFN maintenance therapy should be made on an individual basis.

Recommendation 21: IFN maintenance therapy is a treatment option that can inhibit the progression of liver disease in patients with advanced hepatic fibrosis, especially in those who are elderly. However, the effect of monotherapy with IFN or PEG-IFN remains uncertain in non-responders to combination therapy with PEG-IFN plus RBV. (Level 2a, Grade C.)

CONSENSUS ON THERAPEUTIC STRATEGY FOR CH-C

Indication of antiviral therapy

IKEDA *ET AL.* elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.¹³² At 5 and 10 years, hepatocarcinogenesis rates in the intermediate ($100\text{--}140 \times 10^9/\text{L}$) and low platelet ($<100 \times 10^9/\text{L}$) groups were 10.9% and 21.6% in the IFN group ($n = 217$) and 19.5% and 43.0% in the untreated group ($n = 459$), respectively ($P = 0.0005$). IFN independently decreased the risk of carcinogenesis risk with a hazard ratio of 0.56 ($P = 0.035$). On the other hand, in the high platelet ($\geq 150 \times 10^9/\text{L}$) group,

no significant difference was found in 5- and 10-year carcinogenesis rates between the IFN-treated group ($n = 228$) and the untreated group ($n = 585$) ($P = 0.69$). Furthermore, IFN treatment significantly increased cumulative survival in the lower platelet subgroup ($P = 0.0001$) but did not affect the higher platelet subgroup ($P = 0.08$). Thus, the necessities of antiviral therapy are shown to be greater in elderly patients with advanced fibrosis, although adverse effects of IFN are reported to be more frequent and the efficacy of IFN to be lower in such patients.^{148–150}

Therefore, the indication of antiviral therapy should be considered in the following order: the necessity of treatment, first; safety of treatment, second; and efficacy of treatment for a patient, last. Antiviral therapy should not be given up because the expected SVR rate is low.

Recommendation 22: Antiviral therapy should be offered even to CH-C patients whose SVR rates are expected to be low if type C chronic liver disease is the prognostic determinant (prognosis is improved by HCV elimination) for the individual patient, and the expected adverse effects are tolerable to the patients. (Level 6, Grade B/C.)

Effect of drug adherence of PEG-IFN and RBV on virological response

The relationship between drug exposure and antiviral effect of PEG-IFN plus RBV combination therapy has been reported in several papers.^{101,151–155} McHutchison *et al.* revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN- α -2b and RBV for 80% or more of the scheduled duration of therapy was significantly higher than that of patients who received less than 80% of one or both drugs (51% vs 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment.¹⁵²

Recently, Oze *et al.* evaluated how reducing drug doses affects complete early virological response (c-EVR) defined as HCV RNA negativity at week 12, using 984 patients with CH-C genotype 1.¹⁵⁶ As a result, the mean dose of PEG-IFN- α -2b, and not RBV, during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), not RBV.

Hiramatsu *et al.* reported on whether dose reduction of RBV (or PEG-IFN) has an effect on virological relapse in PEG-IFN plus RBV treatment for patients with CH-C genotype 1.¹⁵⁷ In the analysis of 472 patients responding to PEG-IFN- α -2b plus RBV, stepwise reduction of the

RBV dose was associated with a stepwise increase in relapse rate from 11% to 60% (Fig. 3).

Improving the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* examined low-dose PEG-IFN- α -2a (135 μ g/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.¹⁵⁸ Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.¹⁵⁹

Recommendation 23: In genotype 1 patients, PEG-IFN is dose-dependently correlated with c-EVR, independent of RBV dose. The administration over 80% of the scheduled dose of PEG-IFN- α -2a or over 1.2 μ g/kg per week of PEG-IFN- α -2b should be chosen as a starting dose: a marked dose reduction of PEG-IFN should not be risked at the start even for patients with disadvantage (e.g. aged patients). (Level 2b/3, Grade B.)

Recommendation 24: In genotype 1 patients, RBV shows a dose-dependent correlation with the relapse after treatment. Maintaining the RBV dose over 80% of the scheduled dose or over 10 mg/kg per day (12 mg/kg per day, if possible) during the complete treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to PEG-IFN- α -2b plus RBV, especially in c-EVR patients. (Level 2b/3, Grade B.)

Recommendation 25: In genotype 2/3 patients, reducing drug doses of PEG-IFN and RBV (down to 400 mg/day) has no significant effect on virological responses. (Level 2a, Grade B.)

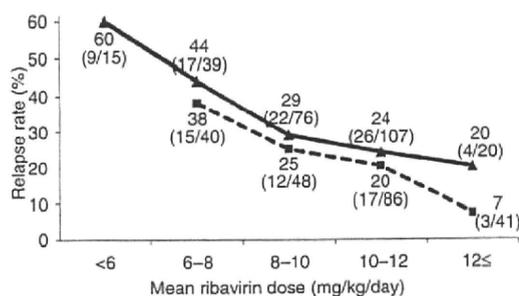


Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)- α -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses (\blacktriangle). Group with the mean PEG-IFN dose <1.4 μ g/kg/week (\blacksquare). Group with the mean PEG-IFN dose \geq 1.4 μ g/kg/week. There was no significant difference between the two PEG-IFN- α -2b-dose groups ($P = 0.17$).

Treatment for patients without elimination of HCV

Tarao *et al.* showed the rate of HCC appearance was significantly higher in HCV-related cirrhotic patients with a high ALT value (≥ 80 IU/mL) than in those with a lower ALT value (< 80 IU/mL).⁷⁰ This suggested that suppression of inflammation in the liver with HCV infection is very important to prevent the hepatocarcinogenesis in patients with HCV-related cirrhosis.

Omata *et al.* assessed the effects of oral ursodeoxycholic acid (UDCA) on serum biomarkers. CH-C patients with elevated ALT were assigned randomly to 150 ($n = 199$), 600 ($n = 200$) or 900 mg/day ($n = 197$) UDCA intake for 24 weeks. As a result, the median changes in serum ALT at the end of treatment were shown to be -15.3, -29.2 and -36.2%, respectively, although serum HCV RNA did not change in any group.¹⁶⁰

A glycyrrhizin product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical, Tokyo, Japan), is used widely in Japan and has been reported to improve ALT levels and liver inflammation.^{161,162} Furthermore, Ikeda *et al.* reported liver carcinogenesis was suppressed by long-term administration of glycyrrhizin, using a cohort of 1249 patients, and its favorable effect on hepatocellular carcinogenesis in those patients with IFN-resistant CH-C.^{163,164}

Repeated phlebotomy has been shown to be effective for the improvement of serum ALT as well as progression of fibrosis,³² however, it remains controversial whether the effects of IFN improve with extensive phlebotomy.¹⁶⁵⁻¹⁶⁹

In Japan, Yano *et al.* showed the iron removal by repeated phlebotomy improved serum ALT levels in patients with CH-C.¹⁷⁰

Recommendation 26: Patients whose HCV RNA was not eradicated by PEG-IFN plus RBV and whose ALT and/or AFP levels were not improved by IFN monotherapy or those without indication for IFN therapy should be treated with the liver-supporting therapy (SNMC, UDCA), and if the effect of this medication is inadequate, phlebotomy can be used in combination. (Level 3/6, Grade B/C.)

Treatment of patients with decompensated cirrhosis

The compensated patients who failed to eradicate HCV by antiviral therapy and decompensated patients should be referred for consideration of liver transplantation and liver supporting therapy should be performed. Long-

term nutritional supplementation with oral branched-chain amino acid (BCAA) has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers.^{171,172} Early interventional with oral BCAA was shown to prolong the liver transplant waiting period by preserving hepatic reserve in cirrhosis.

Recommendation 27: Patients with compensated cirrhosis for the prevention of hepatocellular carcinogenesis, should be treated by not only IFN but also with liver supporting therapy (SNMC, UDCA) and/or phlebotomy and/or BCAA in order to improve the liver inflammation and AFP levels. (Level 3, Grade C.)

Novel antiviral drugs

Telaprevir, a protease inhibitor specific to the HCV non-structural 3/4A serine protease, reduced HCV RNA levels rapidly in early studies. McHuthison *et al.* reported the improved SVR rate with triple therapy for 12 weeks followed by PEG-IFN- α -2a and RBV for 12 weeks.

Thus, the treatment for CH-C is progressing. Therefore, as a treatment strategy, PEG-IFN plus RBV combination therapy should be performed early for aged patients and the patients with the advanced fibrosis. However, the novel antiviral drugs, such as protease inhibitors and polymerase inhibitors, should be taken into account as a candidate of treatment for the patients who can wait for the oncoming drugs.

Recommendation 28: Novel antiviral drugs, such as a protease inhibitor or a polymerase inhibitor, in combination with PEG-IFN plus RBV, can improve the SVR rates in genotype 1 CH-C patients. (Level 2a, Grade A.)

REFERENCES

- Ghany MG, Strader DB, Thomas DL *et al.* AASLD practice guidelines. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335–74.
- Shiffman RN, Shekelle P, Overhage J *et al.* Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standardization. *Ann Intern Med* 2003; 139: 493–98.
- Pileri P, Uematsu Y, Campagnoli S *et al.* Binding of hepatitis C virus to CD81. *Science* 1998; 282: 938–41.
- Scarselli E, Ansuini H, Cerino R *et al.* The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002; 21: 5017–25.
- Evans MJ, von Hahn T, Tschernie DM *et al.* Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; 446: 801–5.
- Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. *Cell Microbiol* 2008; 10: 821–7.
- Ploss A, Evans MJ, Gaysinskaya VA *et al.* Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009; 457: 882–6.
- Flint M, von Hahn T, Zhang J *et al.* Diverse CD81 proteins support hepatitis C virus infection. *J Virol* 2006; 80: 11331–42.
- Sumpter R Jr, Loo YM, Foy E *et al.* Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. *J Virol* 2005; 79: 2689–99.
- Yoneyama M, Kikuchi M, Natsukawa T *et al.* The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; 5: 730–7.
- Li K, Chen Z, Kato N, Gale M Jr, Lemon SM. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. *J Biol Chem* 2005; 280: 16739–47.
- Kawai T, Takahashi K, Sato S *et al.* IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005; 6: 981–8.
- Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; 55: 255–81.
- Loo YM, Owen DM, Li K *et al.* Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. *Proc Natl Acad Sci USA* 2006; 103: 6001–6.
- Gale M Jr, Foy EM. Evasion of intracellular host defense by hepatitis C virus. *Nature* 2005; 436: 939–45.
- Bode JG, Ludwig S, Ehrhardt C *et al.* IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. *FASEB J* 2003; 17: 488–90.
- Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; 2: 410–6.
- Polyak SJ, Khabar KS, Paschal DM *et al.* Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *J Virol* 2001; 75: 6095–106.
- Taylor DR, Shi ST, Romano PR *et al.* Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999; 285: 107–10.
- Noguchi T, Satoh S, Noshi T *et al.* Effects of mutation in hepatitis C virus nonstructural protein 5A on interferon resistance mediated by inhibition of PKR kinase activity in mammalian cells. *Microbiol Immunol* 2001; 45: 829–40.
- Moriya K, Nakagawa K, Santa T *et al.* Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* 2001; 61: 4365–70.
- Okuda M, Li K, Beard MR *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; 122: 366–75.

- 23 Korenaga M, Wang T, Li Y *et al.* Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* 2005; 280: 37481-8.
- 24 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; 126: 840-8.
- 25 Kawaguchi T, Yoshida T, Harada M *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; 165: 1499-508.
- 26 Perlemuter G, Sabile A, Letteron P *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; 16: 185-94.
- 27 Moriishi K, Mochizuki R, Moriya K *et al.* Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2007; 104: 1661-6.
- 28 Nishina S, Hino K, Korenaga M *et al.* Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* 2008; 134: 226-38.
- 29 Miura K, Taura K, Kodama Y, Schnabl B, Brenner DA. Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* 2008; 48: 1420-9.
- 30 Veldt BJ, Chen W, Heathcote EJ *et al.* Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; 47: 1856-62.
- 31 Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; 97: 3036-43.
- 32 Kato J, Kobune M, Nakamura T *et al.* Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* 2001; 61: 8697-702.
- 33 Furutani T, Hino K, Okuda M *et al.* Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. *Gastroenterology* 2006; 130: 2087-98.
- 34 Romero-Gomez M, Del Mar Vilorio M, Andrade RJ *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; 128: 636-41.
- 35 Harrison SA, Brunt EM, Qazi RA *et al.* Effect of significant histologic steatosis or steatohepatitis on response to antiviral therapy in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2005; 3: 604-9.
- 36 Martinot-Peignoux M, Boyer N, Cazals-Hatem D *et al.* Prospective study on anti-hepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. *Hepatology* 2001; 34: 1000-5.
- 37 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343-50.
- 38 Strader DB, Wright T, Thomas DI, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 38: 1147-71.
- 39 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon- α therapy in hepatitis C virus infection. *Hepatology* 1994; 19: 1088-94.
- 40 Lau JY, Davis GL, Kniffen J *et al.* Significance of serum hepatitis C virus RNA in chronic hepatitis C. *Lancet* 1993; 341: 1501-4.
- 41 Yamada G, Takatani M, Kishi F *et al.* Efficacy of interferon alfa therapy in chronic hepatitis C patients depends primarily on hepatitis C virus RNA level. *Hepatology* 1995; 22: 1351-4.
- 42 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. *J Clin Invest* 1995; 96: 224-30.
- 43 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; 334: 77-81.
- 44 Pascu M, Martus P, Hohne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; 53: 1345-54.
- 45 Shirakawa H, Matsumoto A, Joshita S *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; 48: 1753-60.
- 46 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; 48: 38-47.
- 47 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48: 372-80.
- 48 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; 46: 403-10.
- 49 Donlin MJ, Cannon NA, Yao E *et al.* Pretreatment sequence diversity differences in the full-length hepatitis

- C virus open reading frame correlate with early response to therapy. *J Virol* 2007; 81: 8211–24.
- 50 Akuta N, Suzuki F, Kawamura Y *et al.* Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; 46: 1357–64.
 - 51 Fishman SL, Factor SH, Balestrieri C *et al.* Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 2009; 15: 3205–13.
 - 52 Ogata S, Florese RH, Nagano-Fujii M *et al.* Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. *J Clin Microbiol* 2003; 41: 2835–41.
 - 53 Nishise Y, Saito T, Sugahara K *et al.* Risk of hepatocellular carcinoma and secondary structure of hepatitis C virus (HCV) NS3 protein amino-terminus, in patients infected with HCV subtype 1b. *J Infect Dis* 2007; 196: 1006–9.
 - 54 Mattsson L, Sonnerborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-years follow-up study of hepatitis C virus markers. *Liver* 1993; 13: 274–8.
 - 55 Barrera JM, Bruguera M, Ercilla MG *et al.* Persistent hepatitis C viremia after self-limiting posttransfusion hepatitis C. *Hepatology* 1995; 21: 639–44.
 - 56 Amoroso P, Rapicetta M, Tosti ME *et al.* Correlation between virus genotype and chronicity rate in acute hepatitis C. *J Hepatol* 1998; 28: 939–44.
 - 57 Tanaka E, Kiyosawa K. Natural history of acute hepatitis C. *J Gastroenterol Hepatol* 2000; 15: E97–104.
 - 58 Seeff LB, Buskell-Bales Z, Wright EC *et al.* Long-term mortality after transfusion-associated non-A, Non B hepatitis. *N Engl J Med* 1992; 327: 1906–11.
 - 59 Seeff LB, Miller RN, Rabkin CS *et al.* 45-year follow-up of hepatitis C virus infection in healthy young adults. *Ann Intern Med* 2000; 132: 105–11.
 - 60 Kenny-Walsh E, the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999; 340: 1228–33.
 - 61 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; 12: 671–5.
 - 62 Tong MJ, El-Farra NS, Reikes AR *et al.* Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; 332: 1463–6.
 - 63 Ikeda K, Saitoh S, Koide 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.
 - 64 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999; 131: 174–81.
 - 65 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. *J Hepatol* 1999; 30: 653–9.
 - 66 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METEVIR, CLINIVIR and DOSVIRC groups. *Lancet* 1997; 349: 825–32.
 - 67 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; 132: 517–24.
 - 68 Persico M, Persico E, Suozzo R *et al.* Natural history of hepatitis C virus carriers with persistently normal aminotransferase. *Gastroenterology* 2000; 118: 760–4.
 - 69 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; 43: 599–605.
 - 70 Terao K, Rino Y, Ohkawa S *et al.* Association between high serum ALT and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* 1999; 86: 589–94.
 - 71 Kumada T, Toyoda H, Kiriya S *et al.* Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. *Gut* 2007; 56: 738–9.
 - 72 Kumada T, Toyoda H, Kiriya S *et al.* Long-term follow-up of patients with hepatitis C with a normal alanine aminotransferase. *J Med Virol* 2009; 81: 446–51.
 - 73 Suruki R, Hayashi K, Kusumoto K *et al.* Alanine aminotransferase level as a predictor of hepatitis C virus-associated hepatocellular carcinoma incidence in a community-based population in Japan. *Int J Cancer* 2006; 119: 192–5.
 - 74 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; 101: 1616–22.
 - 75 Bruno S, Stroppolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; 45: 579–87.
 - 76 George S, Bacon BR, Brunt EM *et al.* Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; 49: 729–38.

- 77 Iino S, Okita K, Omata M *et al.* Clinical efficacy of PEG-Interferon alfa-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load –retrospective comparison with Interferon alfa-2b and ribavirin combination therapy for 24 weeks. *Kantansui* 2004; 49: 1099–121.
- 78 Yamada G, Iino S, Okuno T *et al.* Virological response in patients with hepatitis C virus genotype 1b and a high viral load: impact of peginterferon-alpha-2a plus ribavirin dose reductions and host-related factors. *Clin Drug Investig* 2008; 28: 9–16.
- 79 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; 81: 452–8.
- 80 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; 352: 2609–17.
- 81 Sezaki H, Suzuki F, Kawamura Y *et al.* Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. *Dig Dis Sci* 2009; 54: 1317–24.
- 82 Kogure T, Ueno Y, Fukushima K *et al.* Pegylated interferon plus ribavirin for genotype 1b chronic hepatitis C in Japan. *World J Gastroenterol* 2008; 14: 7225–4230.
- 83 Akuta N, Suzuki F, Kawamura Y *et al.* Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2007; 79: 1686–95.
- 84 Mizuta T, Kawaguchi Y, Eguchi Y *et al.* Whole-body insulin sensitivity index is a highly specific predictive marker for virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients with genotype 1b and high viral load. *Dig Dis Sci* 2010; 55: 183–9.
- 85 Tsukada H, Ochi H, Maekawa T *et al.* A Polymorphism in MAPKAPK3 Affects Response to Interferon Therapy for Chronic Hepatitis C. *Gastroenterology* 2009; 136: 1796–805.
- 86 Asahina Y, Izumi N, Hirayama I *et al.* Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. *Gastroenterology* 2008; 134: 1396–405.
- 87 Asahina Y, Izumi N, Umeda N *et al.* Pharmacokinetics and enhanced PKR response in patients with chronic hepatitis C treated with pegylated interferon alpha-2b and ribavirin. *J Viral Hepat* 2007; 14: 396–403.
- 88 Sezaki H, Suzuki F, Kawamura Y *et al.* Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res* 2007; 37: 787–92.
- 89 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-Alfa-2a plus ribavirin. *Gastroenterology* 2006; 130: 1086–109.
- 90 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; 131: 451–60.
- 91 Ferenci P, Laferl H, Scherzer TM *et al.* Customizing treatment with peginterferon alfa-2a (40kD) (PEGASYS®) plus ribavirin (COPEGUS®) in patient with HCV genotype 1 or 4 infection: interim results of a prospective randomized trial. *Hepatology* 2006; 44: 336a.
- 92 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; 46: 1688–94.
- 93 Buti M, Lurie Y, Zakharova NG *et al.* Extended treatment duration in chronic hepatitis C genotype 1-infected slow responders: final results of the SUCCESS study (abstract #141). *J Hepatol* 2009; 50 (Suppl 1): S58.
- 94 Ide T, Hino T, Ogata K *et al.* A randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative-status in patients with genotype 1b chronic hepatitis C. *Am J Gastroenterol* 2009; 104: 70–5.
- 95 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; 129: 522–7.
- 96 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; 357: 124–34.
- 97 Dalgard O, Bjørø K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; 47: 35–42.
- 98 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; 47: 1837–45.
- 99 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; 56: 553–9.
- 100 Mangia A, Minerva N, Bacca D *et al.* Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. *Hepatology* 2009; 49: 358–63.
- 101 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–65.

- 102 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–82.
- 103 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140: 346–55.
- 104 Helbling B, Jochum W, Stamenic I *et al.* HCV-related advanced fibrosis/cirrhosis: randomized controlled trial of pegylated interferon alpha-2a and ribavirin. *J Viral Hepat* 2006; 13: 762–9.
- 105 Bergmann JF, Vrolijk JM, van der Schaar P *et al.* Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007; 27: 1217–25.
- 106 Diago M, Crespo J, Oliveira A *et al.* Clinical trial: pharmacodynamics and pharmacokinetics of re-treatment with fixed-dose induction of peginterferon alpha-2a in hepatitis C virus genotype 1 true non-responder patients. *Aliment Pharmacol Ther* 2007; 26: 1131–8.
- 107 Carr C, Hollinger FB, Yoffe B *et al.* Efficacy of interferon alpha-2b induction therapy before retreatment for chronic hepatitis C. *Liver Int* 2007; 27: 1111–8.
- 108 Mathew A, Peiffer LP, Rhoades K *et al.* Sustained viral response to pegylated interferon alpha-2b and ribavirin in chronic hepatitis C refractory to prior treatment. *Dig Dis Sci* 2006; 51: 1956–61.
- 109 Jacobson IM, Gonzalez SA, Ahmed F *et al.* A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. *Am J Gastroenterol* 2005; 100: 2453–62.
- 110 Herrine SK, Brown RS Jr, Bernstein DE *et al.* Peginterferon alpha-2a combination therapies in chronic hepatitis C patients who relapsed after or had a viral breakthrough on therapy with standard interferon alpha-2b plus ribavirin: a pilot study of efficacy and safety. *Dig Dis Sci* 2005; 50: 719–26.
- 111 Shiffman ML, Di Bisceglie AM, Lindsay KL *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; 126: 1015–23.
- 112 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009; 150: 528–40.
- 113 Poynard T, Colombo M, Bruix J *et al.* Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009; 136: 1618–28.
- 114 Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; 359: 2429–41.
- 115 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1485–92.
- 116 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; 352: 1426–32.
- 117 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1493–9.
- 118 Reichard O, Norkrans G, Frydén A *et al.* Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. *Lancet* 1998; 351: 83–7.
- 119 Zeuzem S, Feinman SV, Rasenack J *et al.* Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; 343: 1666–72.
- 120 Lindsay KL, Trepo C, Heintges T *et al.* A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; 34: 395–403.
- 121 Pockros PJ, Carithers R, Desmond P *et al.* Efficacy and safety of two-dose regimens of peginterferon alpha-2a compared with interferon alpha-2a in chronic hepatitis C: a multicenter, randomized controlled trial. *Am J Gastroenterol* 2004; 99: 1298–305.
- 122 Akuta N, Suzuki F, Tsubota A *et al.* Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. *J Hepatol* 2002; 37: 831–6.
- 123 Iwasaki Y, Shiratori Y, Hige S, Nishiguchi S *et al.* A randomized trial of 24 versus 48 weeks of peginterferon a-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatol Int* 2009; 3: 468–79.
- 124 Masuko K, Okuda K, Meguro T *et al.* Hepatitis C virus antibodies, viral RNA and genotypes in sera from patients on maintenance haemodialysis. *J Viral Hepat* 1994; 1: 65–71.
- 125 Fabrizi F, Martin P, Dixit V, Bunnapradist S, Dulai G. Meta-analysis: effect of hepatitis C virus infection on mortality in dialysis. *Aliment Pharmacol Ther* 2004; 20: 1271–7.
- 126 Rostaing L, Chatelut E, Payen JL *et al.* Pharmacokinetics of alphaIFN-2b in chronic hepatitis C virus patients undergoing chronic hemodialysis or with normal renal function: clinical implications. *J Am Soc Nephrol* 1998; 9: 2344–8.

- 127 Russo MW, Goldsweig CD, Jacobson IM, Brown RS Jr. Interferon monotherapy for dialysis patients with chronic hepatitis C: an analysis of the literature on efficacy and safety. *Am J Gastroenterol* 2003; 98: 1610-5.
- 128 Fabrizi F, Dixit V, Messa P, Martin P. Interferon monotherapy of chronic hepatitis C in dialysis patients: meta-analysis of clinical trials. *J Viral Hepat* 2008; 15: 79-88.
- 129 Kamar N, Ribes D, Izopet J, Rostaing L. Treatment of hepatitis C virus infection (HCV) after renal transplantation: implications for HCV-positive dialysis patients awaiting a kidney transplant. *Transplantation* 2006; 82: 853-6.
- 130 KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int Suppl* 2008; 109: S1-99.
- 131 Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; 13: 34-41.
- 132 Ikeda K, Arase Y, Kawamura Y *et al.* Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C. *Am J Med* 2009; 122: 479-86.
- 133 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; 32: 228-32.
- 134 Kubo S, Nishiguchi S, Hirohashi K *et al.* Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001; 134: 963-7.
- 135 Shiratori Y, Shiina S, Teratani T *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; 138: 299-306.
- 136 Mazzaferro V, Romito R, Schiavo M *et al.* Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; 44: 1543-54.
- 137 Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002; 89: 418-22.
- 138 Shiratori Y, Ito Y, Yokosuka O *et al.* Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; 142: 105-14.
- 139 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; 346: 1051-5.
- 140 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124-30.
- 141 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; 129: 94-9.
- 142 Arase Y, Ikeda K, Suzuki F *et al.* Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. *J Med Virol* 2007; 79: 1095-102.
- 143 Nomura H, Kashiwagi Y, Hirano R *et al.* Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: A pilot study. *Hepatol Res* 2007; 37: 490-7.
- 144 Shiffman ML, Hofmann CM, Contos MJ *et al.* A randomized, controlled trial of maintenance interferon therapy for patients with chronic hepatitis C virus and persistent viremia. *Gastroenterology* 1999; 117: 1164-72.
- 145 Saito Y, Saito H, Tada S *et al.* Effect of long-term interferon therapy for refractory chronic hepatitis c: preventive effect on hepatocarcinogenesis. *Hepatogastroenterology* 2005; 52: 1491-6.
- 146 Arase Y, Ikeda K, Suzuki F *et al.* Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. *J Med Virol* 2007; 79: 1485-90.
- 147 Akuta N, Suzuki F, Kawamura Y *et al.* Efficacy of low-dose intermittent interferon-alpha monotherapy in patients infected with hepatitis C virus genotype 1b who were predicted or failed to respond to pegylated interferon plus ribavirin combination therapy. *J Med Virol* 2008; 80: 1363-9.
- 148 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; 39: 1069-77.
- 149 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; 43: 54-63.
- 150 Hiramatsu N, Oze T, Tsuda N *et al.* Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? *Hepatol Res* 2006; 35: 185-9.
- 151 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645-52.
- 152 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123: 1061-9.

- 153 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; 132: 103–12.
- 154 Reddy KR, Shiffman ML, Morgan TR *et al.* Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007; 5: 124–9.
- 155 Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007; 46: 371–9.
- 156 Oze T *et al.* Pegylated interferon alpha-2b affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN alpha-2b plus ribavirin. *J Viral Hepat* 2009; 16: 578–85.
- 157 Hiramatsu N *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; 16: 586–94.
- 158 Weiland O, Hollander A, Mattsson L *et al.* Lower-than standard dose peg-IFN alfa-2a for chronic hepatitis C caused by genotype 2 and 3 is sufficient when given in combination with weight-based ribavirin. *J Viral Hepat* 2008; 15: 641–5.
- 159 Inoue Y, Hiramatsu N, Oze T *et al.* Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses. *J Viral Hepat* (in press).
- 160 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; 56: 1747–53.
- 161 Suzuki H, Ohta Y, Takino T *et al.* Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Double blind trial. *Asian Med J* 1983; 26: 423–38.
- 162 Wildhirt E. Experience in Germany with glycyrrhizic acid for the treatment of chronic viral hepatitis. In: Nishioka K, Suzuki H, Mishiro S, Oda T, eds. *Viral Hepatitis and Liver Disease*. Tokyo, Springer-Verlag, 1994; 658–61.
- 163 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494–500.
- 164 Ikeda K, Arase Y, Kobayashi M *et al.* A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1249 patients. *Dig Dis Sci* 2006; 51: 603–9.
- 165 Piperno A, Sampietro M, D'Alba R *et al.* Iron stores, response to alpha-interferon therapy and effects of iron depletion in chronic hepatitis C. *Liver* 1996; 16: 248–54.
- 166 Fong TL, Han SH, Tsai NC *et al.* A pilot randomized, controlled trial of the effect of iron depletion on long-term response to alpha-interferon in patients with chronic hepatitis C. *J Hepatol* 1998; 28: 369–74.
- 167 Herrera JL. Iron depletion is not effective in inducing a virologic response in patients with chronic hepatitis C who failed to respond to interferon therapy. *Am J Gastroenterol* 1999; 94: 3571–5.
- 168 Fontana RJ, Israel J, LeClair P *et al.* Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. *Hepatology* 2000; 31: 730–6.
- 169 Di Bisceglie AM, Bonkovsky HL, Chopra S *et al.* Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who previously not responded to interferon: a multicenter, prospective randomized, controlled trial. *Hepatology* 2000; 32: 135–8.
- 170 Yano M, Hayashi H, Yoshioka K *et al.* A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. *J Gastroenterol* 2004; 39: 570–4.
- 171 Marchesini G, Bianchi G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; 124: 1792–801.
- 172 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; 360: 1827–38.



ISSN 1386-6346

Hepatology Research



Volume 41 Issue 1 January 2011

The Official Journal of the Japan Society of Hepatology

A large, abstract graphic in shades of gray dominates the lower half of the cover. It consists of several overlapping, curved shapes that resemble stylized leaves or petals, arranged in a circular pattern around a central point.

wileyonlinelibrary.com/journal/hepr



WILEY-
BLACKWELL

Review Articles

- 1 Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009
O Yokosuka, M Kurosaki, F Imazeki, Y Arase, Y Tanaka, K Chayama, E Tanaka, H Kumada, N Izumi, M Mizokami and M Kudo
- 22 T-helper 17 cell: A distinctive cell in liver diseases
C Ye, W Li, M Zheng and Y Chen

Original Articles

Clinical hepatology

- 30 Hepatitis C virus-specific CD8+ T cell frequencies are associated with the responses of pegylated interferon- α and ribavirin combination therapy in patients with chronic hepatitis C virus infection
T Tatsuji, T Takehara, T Miyagi, S Nakazuru, E Mito, T Kanto, N Hiramatsu and N Hayashi
- 39 Acute hepatitis B in Japan: Incidence, clinical practices and health policy
A Sako, H Yasunaga, H Horiguchi, H Hashimoto, N Masaki and S Matsuda
- 46 Efficacy of short-term dexamethasone therapy in acute-on-chronic pre-liver failure
X-Q Zhang, L Jiang, J-P You, Y-Y Liu, J Peng, H-Y Zhang, B-Y Xu and Q Mao

Basic hepatology

Human study

- 54 Combined pre-S deletion and core promoter mutations related to hepatocellular carcinoma: A nested case-control study in China
L-S Qu, T-T Liu, F Jin, Y-M Guo, T-Y Chen, Z-P Ni and X-Z Shen
- 64 Role of dihydropyrimidine dehydrogenase and thymidylate synthase expression in immunohistochemistry of intrahepatic cholangiocarcinoma
M Nishi, M Shimada, T Utsunomiya, Y Morine, S Imura, T Ikemoto, H Mori, J Hanaoka and Y Bando
- 71 Organic anion transporter 1B1 (SLC01B1) polymorphism and gallstone formation: High incidence of Exon4 CA genotype in female patients in North India
A Srivastava, A Srivastava, N Srivastava, G Choudhuri and B Mittal

Experimental study

- 79 Mesalamine-induced B7-H1 expression on hepatic stellate cells attenuates autoimmune liver injury
J-H Lee, J-H Yoon, Y-J Lee, JI Yang, M-S Kwak, E1 Cho, ES Jang, SJ Yu, DH Choi, EU Jung, HY Kim, BH Kim, GE Chung, YJ Kim, J-J Jang, CY Kim and H-S Lee

Short Communication

- 87 Carnosic acid prevents obesity and hepatic steatosis in *ob/ob* mice
T Wang, Y Takikawa, T Satoh, Y Yoshioka, K Kosaka, Y Totemichi and K Suzuki

Case Report

- 93 Hepatic nodular hamartoma containing liver cysts, ductal plate malformations and peribiliary glands
T Terada
- 99 Erratum

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 HBsAg 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 10¹⁰ 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,⁹⁴ 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

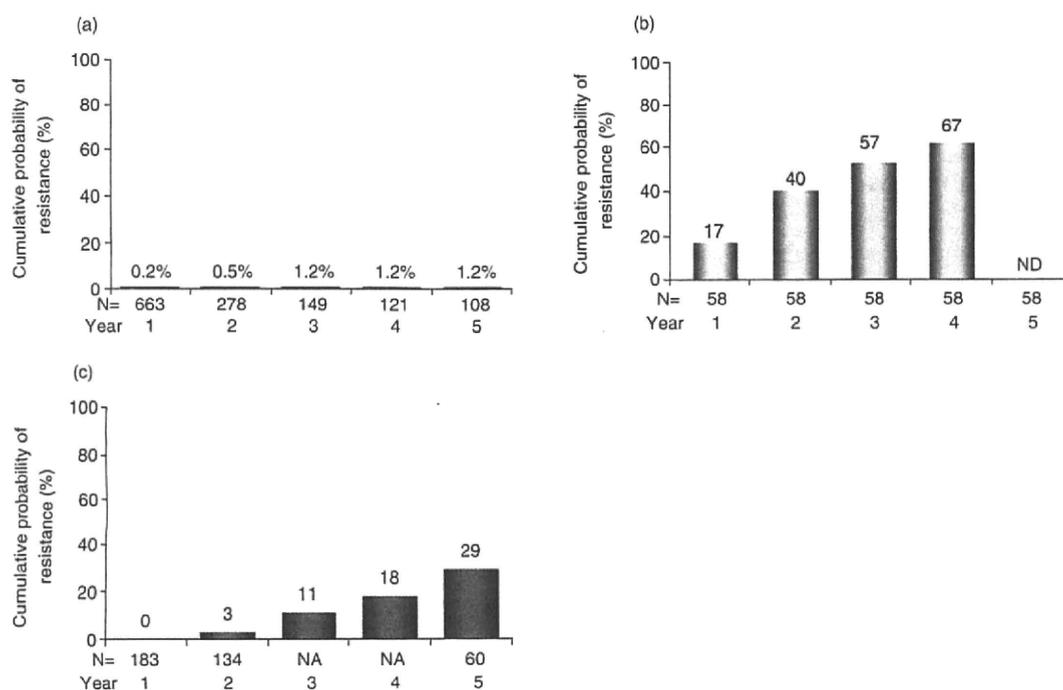


Figure 1 Cumulative probability of resistance after the initiation of entecavir (ETV), lamivudine (LVD) and adefovir (ADV) for patients with hepatitis B e-antigen. (a) Cumulative probability of resistance after the initiation of ETV.¹⁵⁹ (b) Cumulative probability of resistance after the initiation of LVD.¹⁵⁶ (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 rtT128N 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 10^3 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 10^3 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 10^3 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