

**Fig. 3** Proportions of patients with normal ALT ( $ALT \leq 1.0 \times ULN$ ) over time in the lamivudine-refractory, long-term treatment cohort. Seventy-eight patients had abnormal ALT ( $ALT > 1.0 \times ULN$ ) at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

this proportion increased to 55% (36/65) by week 96 of ETV-060 (148 weeks total entecavir treatment time). Of the 17 patients who discontinued treatment during ETV-060, one patient had HBV DNA of  $>400$  copies/mL at the last on-treatment measurement.

#### Biochemical response

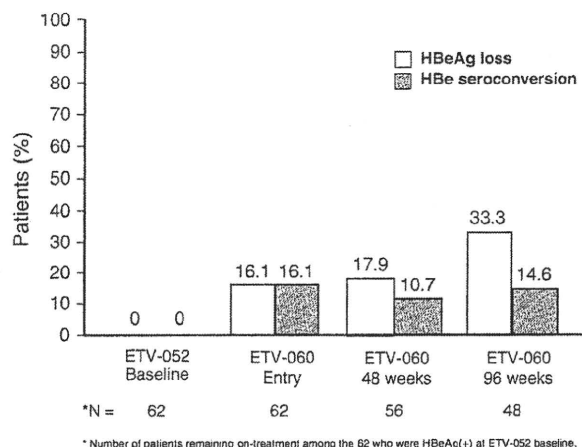
At pretreatment baseline, 95.1% (78/82) of patients had abnormal ALT ( $ALT > 1.0 \times ULN$ ; Table 1; Fig. 3). After 52 weeks of treatment in ETV-052, 79.5% (62/78) of patients had normalized ALT. After 96 weeks of further treatment in ETV-060 (148 weeks total entecavir treatment time), ALT had normalized in 85.2% (52/61) of patients.

#### Serologic response

Sixty-two patients (76%) were HBeAg-positive at pretreatment baseline (Table 1; Fig. 4). At ETV-060 entry, 16.1% (10/62) of these patients had achieved HBe seroconversion and the same number had lost HBeAg (Fig. 4). After 96 weeks in ETV-060 (148 weeks total entecavir treatment time), 33.3% of patients (16/48) had lost HBeAg and 14.6% (7/48) had undergone HBe seroconversion.

#### Resistance analysis

No substitutions associated with entecavir resistance emerged during study ETV-052 [32]. Eighty-one of 82 patients were monitored for resistance from ETV-052 baseline through to the end of treatment in ETV-060 (1 patient refused consent for resistance testing). Thirty-one patients developed genotypic resistance to entecavir during



**Fig. 4** Proportions of patients with HBeAg loss and HBe seroconversion over time in the lamivudine-refractory, long-term treatment cohort. Sixty-two patients were HBeAg positive at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

the second or third year of treatment, of whom 21 experienced virologic breakthrough. The 3-year cumulative probability of resistance was 35.9% [35].

#### Safety

Mean exposure to entecavir during study ETV-060 was 101.3 weeks (range 7.1–148). All patients experienced at least one adverse event, and 11% (9/82) experienced serious adverse events (Table 2). One patient was diagnosed with HCC at week 57 of ETV-060. Eight patients (9.8%) discontinued treatment during ETV-060 because of adverse events, such as increased ALT, virologic breakthrough, and genotypic resistance emergence. Five of these eight patients had received entecavir 0.5 mg daily during phase II study ETV-052, and three received entecavir 1 mg from phase II baseline. There were no ALT flares during ETV-060, and no deaths were reported during the study.

#### Entecavir 1-mg cohort

A subset of 42 patients (42/82) received the recommended 1-mg dose of entecavir for lamivudine-refractory CHB from phase II baseline through to the end of treatment in study ETV-060. In this subset, among patients with available samples, 54% (19/35) had HBV DNA of  $>400$  copies/mL, 84% (27/32) had ALT of  $\geq 1 \times ULN$ , and 15% (4/27) achieved HBe seroconversion after 3 years of continuous treatment with entecavir 1 mg daily. Genotypic resistance emerged in 13 patients in this cohort, and 9 of 13 patients experienced virologic breakthrough. The cumulative 3-year probability of resistance was 30.4%.

**Table 2** Summary of safety during ETV-060 in the lamivudine-refractory long-term treatment cohort

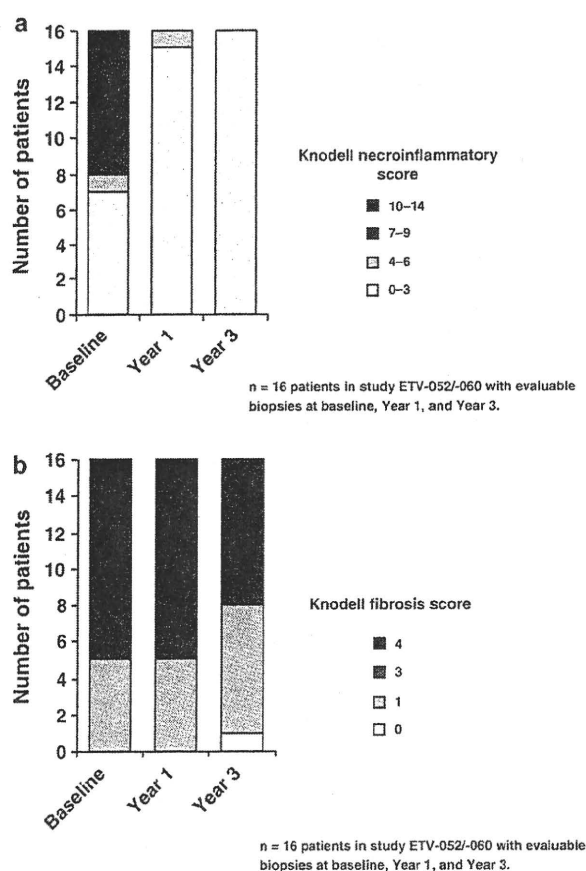
	n (%)
ETV-060	
Entecavir 1.0 mg	
n = 82 (%)	
Any adverse event	82 (100)
Clinical adverse events	78 (95.1)
Clinical serious adverse events	6 (7.3)
Grade 3–4 clinical adverse events	2 (2.4)
Most frequent clinical adverse events	
Nasopharyngitis	57 (69.5)
Headache	21 (25.6)
Diarrhea	12 (14.6)
Back pain	8 (9.8)
Laboratory adverse events	77 (93.9)
Laboratory serious adverse events	3 (3.7)
Grade 3–4 laboratory adverse events	15 (18.3)
ALT increased	24 (29.3)
ALT flare <sup>a</sup>	0
Discontinuations due to adverse events	8 (9.8)
Deaths	0

<sup>a</sup> ALT > 2 × baseline and >10 × ULN

Sixteen (16/42) patients in the 1-mg cohort had paired evaluable liver biopsies from three time points: pretreatment (phase II) baseline, week 48, and week 148 total entecavir treatment time (ETV-060, week 96). Of these, 81% (13/16) demonstrated histologic improvement from baseline through week 148. The mean Knodell necroinflammatory score improved from 6.06 at baseline to 1.44 at week 148, and all patients (16/16) exhibited minimal necroinflammation (a Knodell necroinflammatory score of ≤3 points) at week 148 (Fig. 5a). Knodell fibrosis scores improved in 38% (6/16) of patients from baseline through week 148, and the mean Knodell fibrosis score decreased from 2.44 at baseline to 1.94 at week 148 (Fig. 5b). Liver biopsy assessments using the New Inuyama classification system confirmed the results obtained using the Knodell classification system (data not shown).

## Discussion

This report describes the results of 3 years of continuous entecavir therapy in a lamivudine-refractory patient population. All patients in the lamivudine-refractory, long-term treatment cohort had highly elevated levels of HBV DNA with documented lamivudine-resistance mutations at baseline, and 94% were infected with HBV genotype C. This represents a population with potentially poor long-term outcomes. Patients with lamivudine-resistant HBV may



**Fig. 5** Distribution of Knodell necroinflammatory scores (a) and Knodell fibrosis scores (b) at baseline, year 1 (48 weeks), and year 3 (148 weeks) for the 16 patients who had evaluable liver biopsies at all 3 time points

have cross-resistance to other antivirals, and genotype C infection is associated with low rates of HBe seroconversion and high rates of liver disease progression [7, 25, 36]. These results show that entecavir therapy for up to 3 years for this population resulted in durable HBV DNA suppression and ALT normalization. More than 50% of patients in the cohort achieved undetectable HBV DNA and almost 90% normalized ALT by year 3. Similar levels of HBV DNA suppression and ALT normalization were observed for the subset of patients who received entecavir 1 mg daily throughout the treatment period. Among patients with liver biopsies from three time points (all of whom received the recommended 1-mg dose of entecavir from phase II baseline), substantial improvements in liver histology were observed: more than 80% of patients demonstrated histologic improvement at year ++3 and slow improvements in fibrosis were observed in 38% of patients.

In previously published results of a multinational clinical trial, entecavir demonstrated potent inhibition of viral

replication in HBeAg-positive, lamivudine-refractory patients infected with a variety of HBV genotypes (A–D) [28, 30]. In that trial, after 48 weeks of treatment with entecavir 1 mg daily, the mean change from baseline in HBV DNA was  $-5.11 \log_{10}$  copies/mL, and 19% of patients achieved HBV DNA of  $>300$  copies/mL. Among patients who continued to a second year of entecavir therapy, the mean change from baseline in HBV DNA increased to  $-5.9 \log_{10}$  copies/mL, and 40% of patients achieved HBV DNA of  $>300$  copies/mL. In the current study in Japanese patients, 54% achieved HBV DNA of  $>400$  copies/mL. The higher proportion of Japanese patients suppressing HBV DNA to below the PCR limit of quantification in the current study likely reflects the effect of an additional year of entecavir therapy, as well as the lower baseline HBV DNA ( $7.69 \log_{10}$  vs.  $9.59 \log_{10}$  copies/mL in the multinational study). The relatively low rate of HBe seroconversion observed in this study (15%) may be related to infection with genotype C virus. In studies in Japan and elsewhere in Asia, HBV genotype C has been associated with lower seroconversion rates than with other HBV genotypes [7, 36–38].

Achieving and maintaining HBV DNA suppression is a principal goal of CHB therapy [25, 39]. Data from prospective long-term studies have shown that elevated HBV DNA levels are associated with the development of long-term complications including cirrhosis and HCC [12–14]. Other research has correlated durable HBV DNA suppression with improved liver histology among antiviral-treated patients [19, 40]. Liaw et al. [15] showed that lamivudine therapy benefits CHB patients with advanced liver disease by reducing the risk of liver disease progression, including the development of HCC. In the present study, the reduction in hepatic necroinflammation and fibrosis observed in a subset of patients through 3 years, along with the durable virologic suppression observed in the larger cohort, suggests that entecavir helps halt or reverse liver disease progression that can lead to poor long-term outcomes.

The emergence of lamivudine resistance can lead to serious clinical consequences, including elevated levels of HBV DNA, exacerbations of hepatitis, and hepatic decompensation [18, 22, 23, 41]. While early studies of patients with lamivudine-resistant HBV suggested that switching to adefovir was efficacious, subsequent work demonstrated the rapid emergence of adefovir resistance in this patient population [42–44]. The emergence of adefovir resistance in this setting can be associated with viral rebound and hepatic decompensation [45]. Adding adefovir to ongoing lamivudine for patients who have developed lamivudine resistance has been recommended as a strategy to reduce the subsequent emergence of adefovir resistance [25, 46]. This strategy is most efficacious in patients with

low HBV DNA levels and requires continued resistance surveillance [47, 48]. Studies evaluating the combination of entecavir with adefovir in lamivudine-resistant patients are currently in progress.

The rate of genotypic resistance to entecavir reported here is consistent with the rate that has been observed in multinational populations of lamivudine-refractory patients [49]. In nucleoside-naïve patients, emergence of entecavir resistance is rare because of entecavir's potent viral load reduction and high genetic barrier to resistance [49, 50]. Substitutions at M204  $\pm$  L180 were detected at baseline for all patients described in this report and have been shown in previous studies to reduce *in vitro* susceptibility to entecavir by approximately eightfold [51]. Resistance to entecavir requires the presence of the rtM204V/I lamivudine-resistance substitution plus at least one additional amino acid substitution at rtT184, rtS202, or rtM250. In the current study, for the subset of patients who received entecavir 1 mg daily throughout the treatment period, the cumulative rate of entecavir resistance was 30% through 3 years. This is consistent with the rate observed in the entire lamivudine-refractory, long-term treatment cohort and in multinational studies of lamivudine-refractory patients through 3 years (36%) [49]. Combining entecavir with an antiviral with a different resistance profile, such as tenofovir or adefovir, may result in less frequent resistance emergence.

Entecavir was well tolerated during treatment in study ETV-052, with no discontinuations due to adverse events and three early on-treatment flares that were transient and associated with declining levels of HBV DNA [32]. Throughout the extended treatment period during ETV-060, entecavir continued to be well tolerated with relatively few discontinuations and no ALT flares observed. There were no deaths during the study, and one patient was diagnosed with HCC at week 57 of ETV-060. The extent to which long-term treatment with entecavir may reduce development of HCC in CHB patients remains under investigation.

In summary, these results show that treatment with entecavir for up to 3 years in lamivudine-refractory CHB results in continued benefit beyond the first year, including durable HBV DNA suppression and progressive improvements in liver histology, with a resistance profile consistent with that observed in other studies. Entecavir at the recommended dose of 1 mg daily is an option for patients with lamivudine-refractory CHB. Additional research evaluating the combination of entecavir plus adefovir or tenofovir in this patient population is ongoing.

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## 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
- Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997;337:1733–1745
- World Health Organization. Hepatitis B Fact Sheet WHO/204. Geneva: World Health Organization; 2000 [cited 2008 June 30]. <http://www.who.int/mediacentre/factsheets/fs204/en/>.
- Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, et al. Chronic hepatitis B virus infection in Asian countries. *J Gastroenterol Hepatol* 2000;15:1356–1361
- Usuda S, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, et al. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the pre S2-region product. *J Virol Methods* 1999;80:97–112
- Hou J, Liu X, Gu F. Epidemiology and prevention of hepatitis B virus infection. *Int J Med Sci* 2005;2:50–57
- Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, et al. A case-control study of clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. *Hepatology* 2001;33:218–223
- Jang JW, Lee YC, Kim MS, Lee SY, Bae SH, Choi JY, et al. A 13-year longitudinal study of the impact of double mutations in the core promoter region of hepatitis B virus on HBeAg seroconversion and disease progression in patients with genotype C chronic active hepatitis. *J Viral Hepat* 2007;14:169–175
- Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005;97:265–272
- Orito E, Mizokami M. Differences of HBV genotypes and hepatocellular carcinoma in Asian countries. *Hepatol Res* 2007;37(Suppl 1):S33–S35
- Chan HLY, Hui AY, Wong ML, Am Tse, Hung LC, Wong VW, et al. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004;53:1494–1498
- Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, et al. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006;130:678–686
- Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006;295:65–73
- Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, et al. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005;54:1610–1614
- Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004;351:1521–1531
- Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999;341:1256–1263
- Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998;339:61–68
- Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003;125:1714–1722
- Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, et al. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003;124:105–117
- Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004;19:1276–1282
- Lai CL, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003;36:687–696
- Papathodoridis GV, Dimou E, Laras A, Papadimitropoulos V, Hadziyannis SJ. Course of virologic breakthrough under long-term lamivudine in HBeAg-negative precore mutant HBV liver disease. *Hepatology* 2002;36:219–226
- Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. *Hepatology* 2004;40:883–891
- Andreone P, Gramenzi A, Cursaro C, Biselli M, Cammà C, Trevisani F, et al. High risk of hepatocellular carcinoma in anti-HBe positive liver cirrhosis patients developing lamivudine resistance. *J Viral Hepat* 2004;11:439–442
- Lok ASF, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007;45:507–539
- Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006;354:1001–1010
- Lai CL, Shouval D, Lok AS, Lai CL, Shouval D, Lok AS, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006;354:1011–1020
- Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J, et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006;130:2039–2049.
- Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizetto M, Schiff ER, et al. A dose-ranging study of the efficacy and

- tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 2005;129:1198–1209
30. Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical and serology outcomes through 96 weeks. *Hepatology* 2008;48:99–108.
  31. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007;133:1437–1444.
  32. Suzuki F, Toyoda J, Katano Y, Sata M, Moriyama M, Imazeki F, et al. Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 2008;23(9):1320–1326.
  33. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1:431–435.
  34. Ichida F, Tsuji T, Omata M, Ichida T, Inoue K, Kamimura T, et al. New Inuyama classification: new criteria for histological assessment of chronic hepatitis. *Int Hepatol Commun* 1996;6:112–119
  35. Yokosuka O, Kumada H, Toyota J, Takaguchi K, Kobashi H, Shindo M, et al. Three-year assessment of entecavir (ETV) resistance in nucleoside-naïve and lamivudine (LVD) refractory Japanese patients with chronic hepatitis B (CHB). *Hepatol Int* 2008;2:A161. Abstract No.: FP067
  36. Nakayoshi T, Maeshiro T, Nakayoshi T, Nakasone H, Sakugawa H, Kinjo F, et al. Difference in prognosis between patients infected with hepatitis B virus with genotype B and those with genotype C in the Okinawa Islands: a prospective study. *J Med Virol* 2003;70:350–354
  37. Duong TN, Horiike N, Michitaka K, Yan C, Mizokami M, Tanaka Y, et al. Comparison of genotypes C and D of the hepatitis B virus in Japan: a clinical and molecular biological study. *J Med Virol* 2004;72:551–557
  38. 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
  39. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008;2:263–283
  40. Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for CHB infection: analysis and review of the literature. *Hepatology* 2003;37:1309–1319.
  41. Liaw YF, Chien RN, Yeh CT. No benefit to continue lamivudine therapy after emergence of YMDD mutations. *Antivir Ther* 2004;9:257–62
  42. Peters MG, Hann HW, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2004;126:91–101
  43. Fung SK, Chae HB, Fontana RJ, Conjeevaram H, Marrero J, Oberhelman K, et al. Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006;44:283–290
  44. Lee YS, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, et al. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006;43:1385–1391
  45. Fung SK, Andreone P, Han SH, Rajender Reddy K, Regev A, Keeffe EB, et al. Adefovir-resistant hepatitis B can be associated with viral rebound and hepatic decompensation. *J Hepatol* 2005;43:937–943
  46. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* 2007;45:307–313
  47. Lampertico P, Vigano M, Manenti E, Iavarone M, Lunghi G, Colombo M. Adefovir rapidly suppresses hepatitis B in HBeAg-negative patients developing genotypic resistance to lamivudine. *Hepatology* 2005;42:1414–1419
  48. Lampertico P, Marzano A, Levrero M, Santantonio T, Di Marco V, Brunetto M, et al. Adefovir and lamivudine combination therapy is superior to adefovir monotherapy for lamivudine-resistant patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2007;46(Suppl 1):S191
  49. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009;49:1503–1514
  50. Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside-naïve patients with hepatitis B. *Hepatology* 2006;44:1656–1665
  51. Baldick CJ, Tenney DJ, Mazzucco CE, Eggers BJ, Rose RE, Pokornowski KA, et al. Comprehensive evaluation of hepatitis B reverse transcriptase substitutions associated with entecavir resistance. *Hepatology* 2008;47:1473–1482

## Special Report

## Management of hepatitis C; Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology (2009)

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The consensus meeting for the diagnosis, management and treatment for hepatitis C was held in 45<sup>th</sup> annual meeting for the Japan Society of Hepatology (JSH) in June 2009 where the recommendations and informative statements were discussed including organizers and presenters. The several important informative statements and recommendations have been shown. This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. Thus, this is the first report of JSH consensus of hepatitis C. The rate of development of hepatocellular carcinoma (HCC) in HCV-infected patients in Japan is higher than in the USA, because the average age

of the HCV-infected patients is greater and there are more patients with severe fibrosis of the liver than in the USA. In Japan, more than 60% of HCV-infected patients are genotype 1b infection, and they show lower response to perinterferon and ribavirin combination treatment. To improve the response rate is also an important issue in our country. To establish the original recommendations and informative statements to prevent the development of HCC is a very important issue in Japan.

**Key words:** chronic hepatitis C, peginterferon, ribavirin, fibrosis of the liver, hepatocellular carcinoma, HCV mutation

## INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major public health problem and a leading cause of death from liver disease in Japan. Two million people are infected, and more than 30 000 patients die from hepatocellular carcinoma (HCC) and/or liver cirrhosis every

year. HCC is the fourth leading cause of death from malignant neoplastic disease, and prevention of the development of HCC is an urgent issue in Japan. The purpose of this consensus is to provide clinicians with consensus-based approaches to diagnosis and treatment of HCV infection.

The consensus meeting for the diagnosis, management and treatment for hepatitis C 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 and compared with AASLD practice guidelines which has been published in *Hepatology*.<sup>1</sup> This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. This is the first report of the JSH consensus of hepatitis C. Established information regarding the pathogenesis and contributing factors for disease

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Disclaimer Statement: The view expressed in these consensus do not necessarily represent the view of the National Health Insurance of Japan, or the Japanese Government.

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Table 1 Grading system for recommendations

	Description
Classification	
Class I	Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation procedure or treatment is beneficial, useful and effective
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a diagnostic evaluation, procedure or treatment
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Conditions for which there is no evidence and/or general agreement that a diagnostic evaluation, procedure/treatment is not useful/effective and in some cases may be harmful
Level of evidence	
Level A	Data derived from multiple randomized clinical trials or meta-analysis
Level B	Data derived from a single randomized trial, or non-randomized studies
Level C	Only consensus opinion of experts, case studies or standard of care

progression which were agreed by the organizers and presenters are shown as informative statements, and clinically useful consensus are shown as "Recommendations". The rate of development of HCC in HCV-infected patients in Japan is higher than that in the USA, because the average age of the patient is greater and there are more patients with severe fibrosis of the liver than in the USA. To establish original recommendations and informative statements to prevent the development of HCC is a very important issue in our country. The quality of recommendations or informative statements is required to show a "class" (reflecting benefit vs risk) and "level" (assessing strength or certainty) of evidence according to AASLD practice guidelines (Table 1).<sup>1,2</sup>

## PATHOGENESIS OF HEPATITIS C

**H**EPATITIS C VIRUS infection causes acute and chronic hepatitis (CH), cirrhosis and HCC. The severity and rate of progression of the disease are highly variable and may reflect both host and viral factors, but

the mechanisms of pathogenesis are incompletely understood. Thus, understanding the mechanisms of HCV pathogenesis is an important goal of HCV research.

### Entry pathway of HCV

For the virus, the first step in propagation is enter into hepatocytes. A decade ago, the HCV envelop protein E2 was shown to bind human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes.<sup>3</sup> Next, two other essential proteins, scavenger receptor class B type I (SR-B1)<sup>4</sup> and claudin-1 (CLDN1),<sup>5</sup> and potentially additional accessory factors such as glycosaminoglycans and low-density protein receptors<sup>6</sup> were identified as receptors involved in HCV entry. Finally, the crucial factor was identified as the tight junction protein occludin (OCLN).<sup>7</sup> Interestingly, both CLDN1 and OCLN are components of tight junctions which are structures forming firm seals between adjacent cells. The initial adhesion of HCV to hepatocytes may be mediated by accessory factors and/or direct interaction with SR-B1 and CD81 proteins. On transfer to a tight junction complex, HCV may interact directly with CLDN1 and/or OCLN, allowing viral uptake into the cell.

Hepatitis C virus infects only humans and chimpanzees. Once these HCV entry factors were identified, the next concern was to determine which factors dictate species-specific tropism. CD81 proteins from other mammals, such as the mouse, are used inefficiently by HCV.<sup>8</sup> Although HCV does not discriminate between human and mouse SR-B1 and CLDN1, mouse OCLN like CD81 cannot substitute for the related human protein in aiding viral entry. These findings indicate that CD81 and OCLN represent minimal human-specific entry factors.

*Informative statement: CLDN1 and OCLN in addition to CD81 and SR-B1 are required for entering of HCV into hepatocytes, and especially CD81 and OCLN represent minimal human-specific entry factors. (Grade A.)*

### Evasion of intracellular host defense by HCV

One of the mechanisms by which HCV infection is likely to lead to be persistent is evasion of intracellular host defense through a complex combination of processes that include interference of interferon (IFN) signaling, modulation of its effectors and continual viral genetic variation. The HCV genome contains pathogen-associated molecular pattern (PAMP) signatures which

are recognized by the retinoic-inducible gene I (*RIG-I*) and specific Toll-like receptors when introduced into naïve cells.<sup>9–11</sup> Viral signaling through *RIG-I* and its adaptor protein, IFN promoter-stimulator 1 (IPS-1), activates IFN regulatory factor-3 (IRF-3) and the host IFN- $\alpha/\beta$  response that limits virus infection.<sup>12,13</sup> HCV NS3/4A protease cleaves IPS-1, releasing IPS-1 from the mitochondrial membrane.<sup>14</sup> Cleavage results in subcellular redistribution of IPS-1 and loss of interaction with *RIG-I*, thereby preventing downstream activation of IRF-3 and induction of IFN $\beta$ .<sup>15</sup>

Secreted IFN $\beta$  engages the local tissue through the autocrine and paracrine processes of binding the IFN- $\alpha/\beta$  receptors. This results in activation of the Jak-signal transducer and activator of transcription (STAT) pathway, in which the receptor-associated Jak and Tyk1 protein kinases catalyze the phosphorylation of STAT proteins. The resulting IFN-stimulated gene factor-3 (ISGF3) transcription factor complex localizes in the cell nucleus, where it binds to the IFN-stimulated response element (ISRE) within the promoter/enhancer region of IFN-stimulated genes (ISG). Jak-STAT signaling leads to a second wave of transcriptional activity stimulating ISG expression in the infected cells. Expression of the HCV core protein has been associated with increased expression levels of suppressor of cytokine signaling (SOCS)-3.<sup>16</sup> The SOCS proteins are known for their role as negative regulators and inhibitors of Jak-STAT signaling, where they mediate a classic negative feedback loop on IFN- $\alpha/\beta$  receptor signaling events.<sup>17</sup> The HCV NS5A protein has been shown to induce interleukin (IL)-8 production leading to partial inhibition of the IFN-induced antiviral response, probably through the alteration of ISG expression.<sup>18</sup> The HCV NS5A and E2 proteins also bind double-strand RNA-activated protein kinase (PKR) and inhibit its catalytic activity,<sup>19,20</sup> which allows HCV to evade in part the translational-suppressive actions of IFN. Thus, HCV evasion of the host response includes various strategies directed by viral proteins to control IFN signaling, ISG expression or function.

*Informative statement: HCV evades intracellular host defenses through a complex combination of processes that include IFN signaling, modulation of its effectors and continual viral genetic variation. These mechanisms include cleavage of IPS-1 by the NS3/4A protease, inhibition of Jak-STAT signaling by HCV-induced SOCS3, inhibition of the IFN-induced antiviral response by NS5A-induced IL-8, and/or inhibition of catalytic activity of PKR by the NS5A and E2 proteins. (Grade A.)*

### Oxidative stress induced by HCV

Oxidative stress is well known to be present in CH-C to a greater degree than in other inflammatory liver diseases. Although the mechanisms underlying oxidative stress induced by HCV have not been elucidated fully, there are several lines of evidence suggesting that HCV directly generates reactive oxygen species (ROS) *in vitro* and *in vivo*. Hepatic ROS production is significantly higher in HCV core transgenic mice than in normal control mice in the absence of hepatic inflammation.<sup>21</sup> HCV core protein also produces ROS in human hepatoma Huh-7 cells and HeLa cells.<sup>22</sup> Analysis of the interaction of HCV core protein with mitochondria in transgenic mice and direct interaction of recombinant core protein and isolated mitochondria indicated oxidation of the mitochondrial glutathione pool and an increase in ROS production by the mitochondrial electron transport complex I, suggesting that direct interaction of core protein with mitochondria is an important cause of the oxidative stress seen in CH-C.<sup>23</sup>

*Informative statement: Mitochondrial dysfunction induced by HCV leads to ROS generation that causes the oxidative stress seen in CH-C. (Grade A.)*

### Metabolic disorders caused by HCV

Epidemiological studies have suggested a link between type 2 diabetes and chronic HCV infection, which implies HCV-induced insulin resistance. A high level of tumor necrosis factor (TNF)- $\alpha$  and disturbance of tyrosine phosphorylation of the insulin receptor substrate (IRS)1 by TNF- $\alpha$  has been demonstrated in HCV core transgenic mice.<sup>24</sup> Another possible mechanism is that HCV core-induced SOCS3 promotes proteosomal degradation of IRS1 and IRS2 through ubiquitination.<sup>25</sup> Hepatic steatosis is one of the histopathological features in CH-C. HCV core protein inhibits microsomal triglyceride transfer protein activity and secretion of very low density lipoprotein.<sup>26</sup> HCV core protein also upregulates the sterol regulatory element binding protein (SREBP)1c promoter activity through the enhanced binding of the LXR $\alpha$ /RXR $\alpha$  to LXR-response element,<sup>27</sup> which leads to an increase in transcription of genes involved in hepatic fatty acid synthesis. Hepatic iron accumulation is also a histopathological feature of CH-C, even though its levels are not extremely high. HCV-induced ROS downregulates the transcriptional activity of hepcidin, a negative regulator in iron homeostasis, in transgenic mice expressing the HCV polyprotein<sup>28</sup> and in HCV replicon cells<sup>29</sup> (Fig. 1).



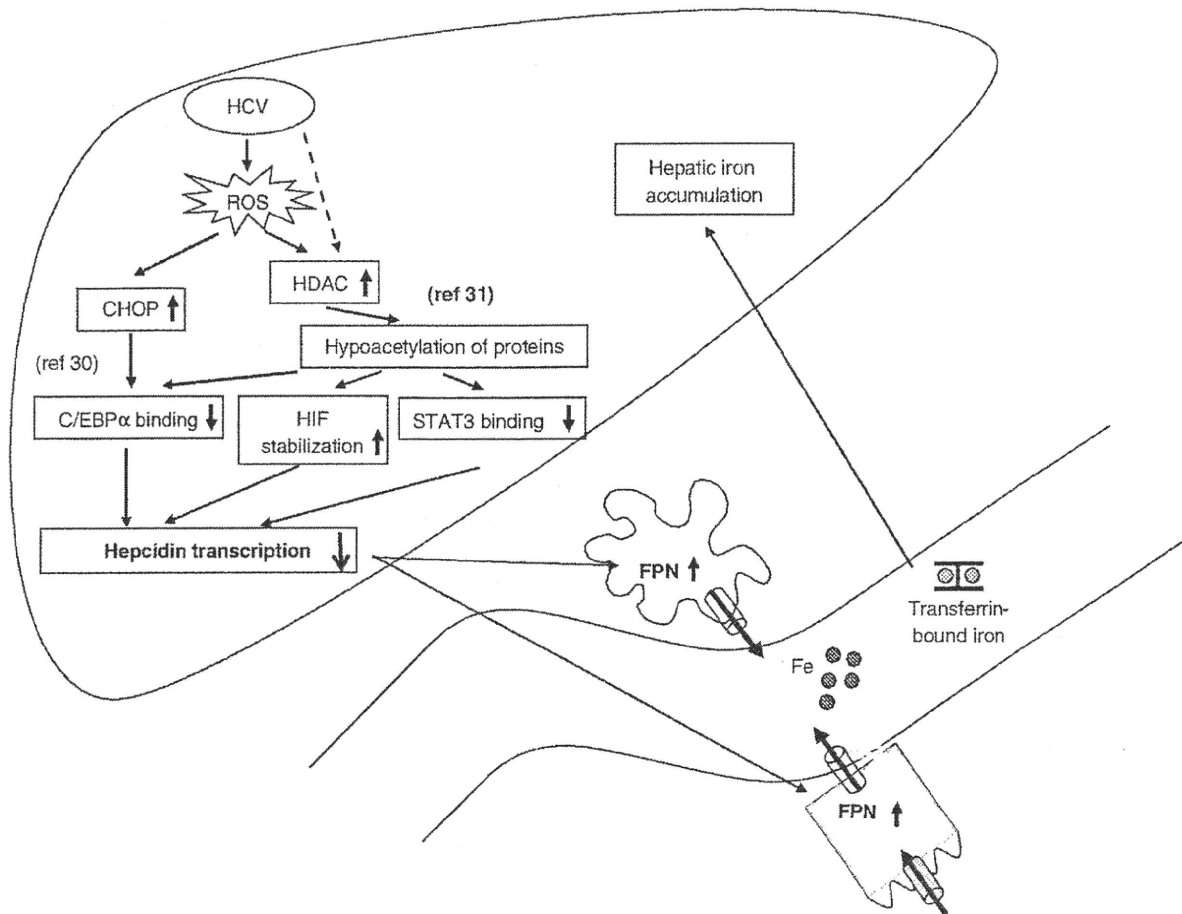


Figure 1 Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV. HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAT3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator. C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP homology protein; FPN, ferroportin; HCV, hepatitis C virus; HDAC, histone deacetylase; HIF, hypoxia inducible factor; ROS, reactive oxygen species; STAT, signal transducer and activation of transcription.

Metabolic disorders caused by HCV such as insulin resistance, hepatic steatosis and iron accumulation are clinically important in terms of amplification of oxidative stress and involvement in hepatocarcinogenesis in CH-C.<sup>30–33</sup> In addition, these metabolic disorders are related to the response to antiviral therapy. Insulin resistance<sup>34</sup> and hepatic steatosis<sup>35</sup> seem to be negatively correlated with response to antiviral therapy in CH-C.

*Informative statement: HCV induces insulin resistance, hepatic steatosis, and/or hepatic iron accumulation, which is associated with hepatocarcinogenesis in CH-C. (Grade A.)*

*Recommendation 1: Insulin resistance and hepatic steatosis seem to be negatively correlated with response to*

*antiviral therapy in CH-C, whereas it remains controversial whether hepatic iron accumulation is related to a poor response to therapy. (Level 2a, Grade C.)*

### Liver biopsy for evaluating pathogenesis of hepatitis C

Assessment of the extent of liver fibrosis is still of great importance in terms of predicting the response to antiviral therapy and hepatocarcinogenesis in CH-C. It is also apparent that as many as a quarter of CH-C patients with persistently normal aminotransferase values have significant fibrosis.<sup>36</sup> The recently developed transient elastography that uses ultrasound and low-frequency elastic waves to measure liver elasticity has

Table 2 Definitions of virological responses to interferon therapy

RVR (rapid virological response)	Undetectable HCV RNA at week 4
cEVR (complete early virological response)	Undetectable HCV RNA at week 12
pEVR (partial early virological response)	Two log drop of HCV RNA without undetectable level at week 12
LVR (late virological response)	Undetectable HCV RNA between week 13 and 24 week
NVR (null virological response)	Positive HCV RNA during treatment
Relapse	Undetectable HCV RNA at end of treatment followed by detectable level after treatment
SVR (sustained virological response)	Undetectable HCV RNA at 24 weeks after treatment

improved the ability to define the extent of fibrosis without a liver biopsy, particularly when combined with other non-invasive markers,<sup>37</sup> but it is not yet ready to replace liver biopsy. Among the pathological features, steatosis and excess hepatocellular iron that affect disease progression and possibly impede treatment response are difficult to diagnose without liver biopsy. Thus, a liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment.

*Recommendation 2: A liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment. (Level 1, Grade C.)*

## VIRAL LOAD, GENOTYPE, VIRAL MUTATIONS

DEFINITIONS OF VIROLOGICAL responses to IFN therapy are summarized in Table 2.

### HCV RNA assay and genotype

In clinical practice, the usual approach is to test initially for antibodies to HCV (anti-HCV), then to use HCV RNA to document viremia. The quantity of HCV RNA is useful to know before providing and monitor-

ing HCV treatment. For HCV RNA determination, quantitative tests based on target amplification (reverse transcriptase polymerase chain reaction [RT-PCR]) and signal amplification (branched DNA [bDNA]) techniques with differing sensitivity and linear measuring ranges are commercially available. The COBAS AmpliCor HCV Monitor Test v2.0 (Roche Molecular Systems, Branchburg, NJ, USA), however, requires sample dilutions for accurate quantification of high-titer specimens. In addition, the assay displays relatively low sensitivities of approximately 600 IU/mL. Recently, the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems) and AccuGene m-HCV (Abbott Molecular, Des Plaines, IL, USA) have become available. These meet the requirements for highly sensitive detection and reliable quantification of HCV in clinical samples.

There are six major HCV genotypes. Genotype specificity predicts the likelihood of treatment response and determines the duration of treatment. Therefore, HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response.<sup>38</sup>

Many reports showed that sustained virological response (SVR) rates in IFN monotherapy and IFN plus ribavirin (RBV) combination therapy were higher in patients who had lower pretreatment RNA levels and genotype 2 infections.<sup>39-41</sup>

*Recommendation 3: HCV RNA level and genotype should be determined in all HCV-infected persons prior to treatment in order to predict the efficacy of response of therapy. SVR rate in IFN therapy are higher in patients who had lower pretreatment RNA levels and genotype 2 HCV infections in IFN therapy. (Level 1, Grade A.)*

### HCV mutation

#### IFN sensitivity determining region (ISDR)

Enomoto *et al.* were able to demonstrate a strong correlation between the number of mutations within the carboxy terminal region of the NS5A gene, the ISDR spanning codons 2209-2248, and response to IFN therapy.<sup>42</sup> Thus, no patient infected with HCV with a wild-type ISDR sequence (identical to the prototype Japanese HCV strain [HCV-J]) responded to IFN therapy whereas all patients infected with the "mutant type", defined by four or more amino acid substitutions in this region, showed an SVR.<sup>43</sup> These initial findings have been confirmed by other Japanese studies but controversial data were reported from other parts of the world, particularly from Europe and the

USA. This may indicate that geographical factors account for different sensitivities of HCV genotype 1b infection to antiviral therapy. Pascu *et al.* reported that the distribution of wild-, intermediate- and mutant-type ISDR sequences differed significantly between Japanese ( $n = 655$ ) (44.1%, 37.6% and 18.3%, respectively) and European patients ( $n = 525$ ) (24.8%, 63.4% and 11.8%, respectively;  $P = 0.001$ ). However, there was a significant positive correlation between the number of ISDR mutations and SVR rate, irrespective of geographical region.<sup>44</sup>

Moreover, Shirakawa *et al.* reported that a logistic regression model that includes the sequence of ISDR of HCV, and other factors (T-helper cell [Th]1/Th2 ratio, bodyweight and neutrophil count) can be useful for accurately predicting accurately the SVR rate before pegylated (PEG)-IFN and RBV combination therapy.<sup>45</sup>

*Recommendation 4: The ISDR should be evaluated before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)*

#### IFN/RBV resistance-determining region (IRRD)

El-Shamy *et al.* have reported recently that a high degree of sequence variation in the V3 and the pre-V3 regions (amino acid [aa]2334-2355) of NS5A, which they refer to collectively as the IRRDR (aa2334-2379), was closely correlated with virological response in HCV-1b-infected patients treated with PEG-IFN and RBV.<sup>46</sup> A high degree (>6 aa substitutions) of sequence variation in the IRRDR

should be a useful marker for predicting SVR, whereas a less diverse (<5) IRRDR sequence predicts non-SVR.

#### Amino acid substitutions in the HCV core region

Akuta *et al.* identified pretreatment substitutions of aa70 and aa91 in the core region as independent and significant pretreatment factors associated with virological non-response, based on 48-week combination therapy of IFN plus RBV.<sup>47</sup> Moreover, they identified aa70 and aa91 substitutions in the core region as predictors of response to PEG-IFN-RBV therapy in Japanese patients infected with HCV genotype 1b<sup>48</sup> (Table 3). Donlin *et al.* reported sequencing the complete pretreatment genotype 1 HCV open reading frame using samples from 94 participants in the Virahep-C study to assess the effects of viral diversity on response to therapy.<sup>49</sup> Genotype 1b sequences from patients with more than 3.5 log declines in viral RNA levels by day 28 (marked responders) were more variable than those from patients with declines of less than 1.4 log (poor responders) in core and NS3. Moreover, arginine (R) at aa70 in the core region was related to a marked response.

Recently evaluations were made of the impact of aa substitutions in HCV core region on hepatocarcinogenesis. Akuta *et al.* reported that cumulative hepatocarcinogenesis rates in double wild-type (arginine at aa70/leucine at aa91) of the HCV core region were significantly lower than those in the non-double wild type in CH-C patients.<sup>50</sup> Moreover, another report showed that a logistic regression model developed

Table 3 Factors associated with sustained virological response to 48-week pegylated interferon plus ribavirin combination therapy in patients infected with hepatitis C virus genotype 1b, identified by multivariate analysis ( $n = 114$ ) 52

Factor	Category	Risk ratio (95% confidence interval)	P
Amino acid substitution in core region	1: double wild	1	0.004
	2: non-double wild	0.102 (0.022-0.474)	
Low-density lipoprotein cholesterol (mg/dL)	1: <86	1	0.005
	2: ≥86	12.87 (2.177-76.09)	
Sex	1: male	1	0.005
	2: female	0.091 (0.017-0.486)	
ICG R15 (%)	1: <10	1	0.018
	2: ≥10	0.107 (0.017-0.678)	
γ-Glutamyltransferase	1: <109	1	0.032
	2: ≥109	0.096 (0.0011-0.819)	
Ribavirin dose (mg/kg)	1: <11.0	1	0.032
	2: ≥11.0	5.173 (1.152-23.22)	

through analysis of full-length core gene sequences identified seven polymorphisms significantly associated with increased HCC risk (36G/C [aaK12N], 209A [aaR70Q], 271U/C [aaL91M], 309A/C, 435A/C, 481A and 546A/C).<sup>51</sup> HCV core gene sequence data might provide useful information about HCC risk.

*Recommendation 5: Amino acid substitutions in the HCV core region (aa70 and aa91) should be determined before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)*

### NS3 protein secondary structure

Recently, Ogata *et al.* reported that HCV-1b strains can be classified into different groups based on the secondary structure of an amino-terminal portion of the NS3 protein and that specific strains are more prevalent among patients with HCC.<sup>52</sup> Moreover, the cumulative incidence of HCC was highest among patients infected with specific group HCV-1b, in whom the risk of HCC significantly increased compared with that among patients infected with another group (hazard ratio = 4.95 [95% confidence interval = 1.43–17.11]) after adjustment for age and histological stage.<sup>53</sup>

*Informative statement: NS3 protein secondary structure may be related to hepatocarcinogenesis. (Grade B.)*

## NATURAL HISTORY OF CH-C

### Progression to cirrhosis and HCC

PREVIOUS PUBLICATIONS REPORTED that approximately 60–80% of patients with acute hepatitis C develop chronic infection in the natural course.<sup>54–57</sup> Because it is difficult to ascertain precisely when the HCV infection occurred except for patients who had blood transfusions, and because chronic infection progresses slowly and asymptotically, the natural entity of the disease has not been elucidated fully. Seeff *et al.* compared the long-term prognosis of HCV antibody-positive and -negative young men and reported that liver disease-related death was very rare in HCV antibody-positive patients.<sup>58,59</sup> Kenny-Walsh studied the liver histology of 363 young women 17 years after HCV infection and showed that 83% had no or mild hepatic fibrosis whilst 2% had liver cirrhosis.<sup>60</sup> These results demonstrate that progression to serious liver disease is a rare event two decades after infection of young people with HCV.

On the other hand, in blood transfusion-associated CH-C patients the mean interval to liver cirrhosis is

estimated to be approximately 20–30 years and that to HCC approximately 30–40 years.<sup>61,62</sup> Because HCC is the most serious complication of HCV-infected people, it is desirable to predict the overall incidence of HCC in each patient. Up to now, many investigators have reported a close relationship between the stage of hepatic fibrosis and incidence of HCC. According to reports from Japan, the annual incidence of new HCC in liver cirrhosis is estimated to be approximately 5–8%.<sup>63–65</sup>

*Informative statement: The natural history of CH-C is highly variable. HCV infection does not have much impact on the overall mortality of all the infected people, whereas progression to liver cirrhosis is observed 20–30 years and to HCC 30–40 years after infection. In Japan, the annual incidence of HCC in liver cirrhosis is estimated to be 5–8%. (Level 2b, Grade B.)*

*Recommendation 6: Treatment of HCV-infected people should be determined in consideration of the higher annual incidence of HCC in patients with liver cirrhosis in Japan as compared to Western countries. (Level 2b/3, Grade B.)*

### Progression of fibrosis

The rate of progression of fibrosis varies among patients with CH-C. Poynard *et al.*<sup>66</sup> calculated the average progression rate of hepatic fibrosis in CH-C to be 0.133 fibrosis units/year. In Japan, Shiratori *et al.*<sup>67</sup> reported this to be 0.10 fibrosis units/year. In HCV carriers with persistently normal aminotransferase levels (PNALT), progression of hepatic fibrosis is slower. Persico *et al.*<sup>68</sup> reported that median histological scores did not differ after 5 years of follow up in PNALT and Okanoue *et al.*<sup>69</sup> calculated the average progression rate of hepatic fibrosis in PNALT to be 0.05 fibrosis units/year.

*Informative statement: On average, progression of hepatic fibrosis in CH-C is 0.10–0.13 fibrosis score units/year. The hepatic stage/grade score of HCV carriers with PNALT are generally low and the progression of hepatic fibrosis is slow. Excessive alcohol intake, insulin resistance and hepatic steatosis are the major factors which induce the progression of hepatic fibrosis. (Level 2b, Grade B.)*

### Alanine aminotransferase (ALT) levels

Alanine aminotransferase is an easy tool to evaluate hepatocellular damage in liver diseases. In the past, a higher incidence of HCC was reported in liver cirrhotic patients with elevated ALT levels.<sup>70</sup> The normal range of serum ALT level varies according to the institutions or hospitals, but it is likely to be located between 30 IU/L

and 40 IU/L. Recently, Kumada *et al.*<sup>71,72</sup> demonstrated that the cumulative incidence of hepatocarcinogenesis increased in parallel with the increase in ALT average integration value in CH-C even in patients with normal ALT levels. In a community-based study, an elevated ALT level (>35 IU/l) was shown to be a significant risk factor of HCC development.<sup>73</sup>

*Recommendation 7: To prevent the occurrence of HCC, levels of serum ALT should be controlled at below 30 IU/l. (Level 3, Grade A.)*

### IFN administration

More than two decades have passed since IFN began to be used to treat CH-C patients. Nowadays, more than 70% of HCV-infected people can be cured by the combination therapy of PEG-IFN plus RBV. However, even in patients who were cured of HCV infection and attained an SVR, the occurrence of HCC may be reported long after completion of IFN therapy. The risk factor of HCC occurrence after IFN therapy is a combination of advanced hepatic fibrosis score before therapy, older age and male sex.<sup>74–76</sup> Bruno *et al.*<sup>75</sup> reported that annual incidence of HCC occurrence in liver cirrhosis after attaining SVR was 0.66%, which was one-third of the incidence of HCC in liver cirrhosis without a virological response (non-SVR).

*Recommendation 8: Surveillance is required for the occurrence of HCC in patients with CH-C and liver cirrhosis. Even if IFN-based therapy is successful in attaining SVR, screening for the detection of HCC by computed tomography (CT), magnetic resonance imaging or ultrasonography and measurement of the serum tumor markers should be carried out routinely, especially for patients with advanced hepatic fibrosis, older age and male sex, because they are at high risk for the occurrence of HCC. (Level 2b, Grade A.)*

### Indication of IFN therapy for CH-C

Interferon-based therapy is used to treat chronic HCV-infected patients worldwide and PEG-IFN plus RBV is the first choice indication for CH-C patients. Because IFN and RBV have a variety of adverse effects including depression and thyroid dysfunction, “who and how” to treat should be determined with caution. The AALSD practice guideline advocates that treatment decision should be individualized based on the severity of liver disease, the potential for serious side-effects, the likelihood of treatment response, the presence of comorbid condition and the patient’s readiness for treatment.<sup>1</sup>

*Recommendation 9: Treatment decision of IFN therapy for CH-C should be individualized based on the body/*

*mental condition, probability of successful therapy and prolonged survival, and likelihood of provoking serious adverse effects. Scores of hepatic stage/grade should be considered as well. For aged patients, in whom HCV infection is regarded as the major determinant of survival, IFN-based therapy should be considered with caution. (Level 3, Grade A.)*

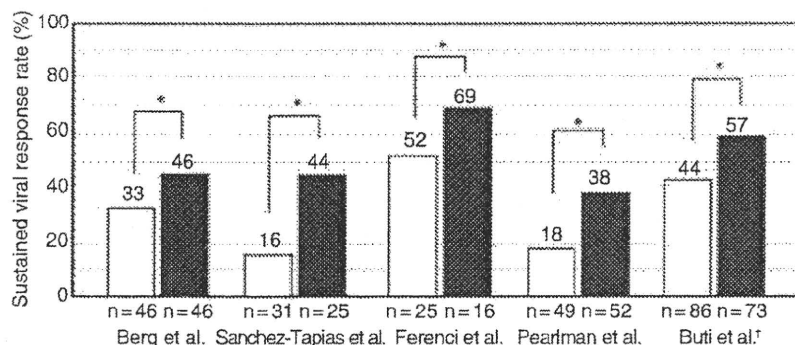
### PEG-IFN AND RBV COMBINATION THERAPY

#### Factors associated with virological response to PEG-IFN and RBV combination therapy

TREATMENT WITH PEG-IFN- $\alpha$ -2A or -2b together with RBV has been evaluated in two nationwide phase III registration trials in Japan.<sup>77,78</sup> In one trial, which determined efficacy of PEG-IFN $\alpha$ -2b and RBV,<sup>79</sup> the SVR rate to 48-week combination therapy was 48% (121/254) in patients with HCV genotype 1b and a high viral load ( $\geq 100$  KIU/mL). Another trial using PEG-IFN- $\alpha$ -2a and RBV demonstrated an SVR rate to 48-week combination therapy of 59% (57/96) in patients with HCV genotype 1b and a high viral load ( $\geq 100$  KIU/mL).<sup>80</sup> Based on these results, the currently recommended standard therapy for the patients with CH-C in Japan is the combination of a PEG-IFN together with RBV, except for the treatment naïve patients with a low viral load for whom a PEG-IFN monotherapy is recommended.

These clinical trials identified the following factors that are associated with non-SVR in patients with HCV genotype 1b and a high viral load: (i) older patients; (ii) non-responders to previous IFN therapy; (iii) advanced fibrosis; (iv) female sex; and (v) poor adherence below 80%. In marked contrast to the data from Europe and the USA, the SVR rate in Japanese female patients is lower than that in the male patients. Several community-based retrospective studies in Japan also demonstrated that female patients, especially older female patients, are more difficult to treat compared with other patients.<sup>81,82</sup> Other factors associated with virological response reported from Japan include the low-density lipoprotein cholesterol level,<sup>83</sup>  $\alpha$ -fetoprotein (AFP) level,<sup>83</sup> whole-body insulin sensitivity index,<sup>84</sup> single nucleotide polymorphisms of MAPKAPK3,<sup>85</sup> RIG-I/IPS-1 ratio,<sup>86</sup> Th1/Th2 ratio<sup>85</sup> and PKR response.<sup>87</sup> Association between viral mutations and treatment response is discussed in depth above.

*Recommendation 10: Predictors associated with a non-SVR to PEG-IFN and RBV include: (i) age older than 60 years, particularly older women; (ii) advanced fibro-*



**Figure 2** Comparison of sustained virological response rate between 48-week (open column) and 72-week (closed column) treatment with pegylated interferon and ribavirin in patients with partial early virological responder, which is defined as  $\geq 2$  log reduction in hepatitis C virus (HCV) RNA level compared to baseline HCV RNA level but detectable HCV RNA at treatment week 12. \*Statistical significance between two treatment groups. †Comparison in patients with  $\geq 80\%$  adherence is shown.

sis; (iii) non-responder to previous IFN therapy; and (iv) poor adherence below 80%. (Level 2a, Grade B.)

### Response-guided therapy for patients with HCV genotype 1

Measuring the rate of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV, and useful for determining the optimal duration of therapy. In two nationwide registration trials conducted in Japan,<sup>77,78</sup> the SVR rate was high, from 76–100% in patients whose HCV RNA was cleared rapidly from serum by week 4, and 71–73% in patients who achieved undetectable HCV RNA from week 5 to week 12. In contrast, the SVR rate in patients with late clearance of HCV RNA from week 13 to week 24 was low at 29–36%. No patients without clearance of HCV RNA by week 24 achieved SVR. It should be noted that time point of HCV clearance was determined by measurement of serum HCV RNA utilizing the Amplicor HCV method in these trials.

*Recommendation 11: Measuring the time of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV. Measurement of HCV RNA is recommended at weeks 4, 12 and 24. (Level 1, Grade A.)*

As mentioned above, patients whose HCV RNA measured by Amplicor HCV had not cleared by week 24 were unable to achieve SVR with 48-week standard PEG-IFN and RBV therapy. However, in a retrospective study conducted in 52 patients without HCV RNA clearance from serum by week 24, the rate of ALT normalization 6 months after the completion of therapy (so-called biochemical response) was 56% (5/9) and 62% (8/13) of

patients achieved ALT normalization up to 2 years after the completion of therapy (sustained biochemical response).<sup>88</sup> Therefore, the proposal that recommends a continuation of PEG-IFN and RBV therapy for 48 weeks in biochemical responders at week 24 even without HCV clearance has been accepted widely in Japan. This proposal is in marked contrast to the AASLD practice guideline,<sup>1</sup> in which treatment discontinuation is strongly recommended in patients whose HCV RNA remains positive at week 24.

*Recommendation 12: It is impossible to achieve SVR in patients without HCV RNA clearance by week 24 measured by Amplicor HCV. (Level 1, Grade A.) However, it is recommended to continue the therapy for 48 weeks even in patients without HCV RNA clearance by week 24 if ALT normalizes at week 24, because a sustained biochemical response can be obtained in these patients. (Level 4, Grade C.)*

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV RNA between weeks 12 and 24, was evaluated in five studies.<sup>89–93</sup> These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response (Fig. 2).<sup>89–93</sup>

In Japan, a randomized controlled trial was conducted in 113 patients with HCV genotype 1b and a high viral load, comparing a 48-week treatment group and extended treatment group where patients were treated for an additional 44 weeks after clearance of

HCV RNA from serum. In this trial, the SVR rate was 36% in the 48-week treatment group and 53% in the extended treatment group, and the SVR rate was significantly higher in patients in the extended treatment group who became HCV RNA-negative during the period week 16–24 (9% vs 78%,  $P=0.005$ ).<sup>96</sup> In addition, in a case-control study matched for age, sex and the timing of HCV RNA clearance from serum, the SVR rate was high at 62% in the 72-week treatment group ( $n=65$ ) compared to 33% in the 48-week treatment group ( $n=130$ ), and the extended treatment was particularly effective in patients with HCV core mutations at aa70 and aa91 as well as patients with wild type of ISDR sequence.<sup>79</sup> Accordingly, 72-week extended treatment is recommended for patients who are slow to clear of HCV RNA between weeks 12 and 24.

Currently, HCV RNA clearance from serum is determined by real-time PCR detection, although most of former studies utilized the Amplicor HCV method for this purpose. Because real-time PCR is highly sensitive, it should be reevaluated in terms of who gains benefit from extended therapy. Currently, there is no sufficient evidence to determine this. Nevertheless, substantial number of community-based Japanese study using real-time PCR detection suggested that SVR could be obtained by 72-week treatment if HCV RNA became undetectable by week 36. Accordingly, when determining the timing of HCV RNA clearance using real-time PCR detection, 72-week treatment could be recommended for patients who achieve HCV RNA clearance between weeks 12 and 36.

*Recommendation 13: 72-week extended therapy should be considered for patients with HCV genotype 1 who have delayed HCV RNA clearance from serum between weeks 12 and 24. (Level 2a, Grade B.)*

*Recommendation 14: When using a real-time detection PCR method for measurement of HCV RNA, SVR can be obtained by 72-week extended treatment in patients who have achieved HCV RNA clearance by week 36. (Level 2b, Grade C.)*

### Response-guided therapy for patients with HCV genotype 2

Six trials have evaluated a shortening of the duration of therapy from 24 weeks to 12–16 weeks for patients with chronic HCV genotype 2 and 3.<sup>80,95–99</sup> Although the data from some of these trials suggest that patients with genotype 2 and 3 infection who achieve viral clearance from serum by week 4 can shorten their treatment duration to 12–16 weeks,<sup>80,95,99</sup> the benefit of a shortening the duration of therapy remains controversial.<sup>96</sup> In a recent

study by Mangia *et al.*, the factors associated with relapse after shorter duration of therapy are identified as age over 45 years, pre-treatment platelet count of less than  $140 \times 10^9/L$ , and body mass index over  $30 \text{ kg/m}^2$ ,<sup>100</sup> suggesting shortening the duration of therapy can be considered only in particular patients without predictors associated with relapse. Because most Japanese patients have risk factors for relapse such as older age and advanced fibrosis, shortening the duration of the therapy is not generally recommended for Japanese patients with genotype 2, even if they achieve viral clearance by week 4.

### PEG-IFN and RBV combination therapy in patients with compensated cirrhosis

In the early Western registration trials, patients with HCV-related compensated cirrhosis did achieve SVR but at lower rates than did those without cirrhosis.<sup>101–103</sup> Subsequently, there was one treatment study that focused exclusively on patients with compensated cirrhosis.<sup>104</sup> In this study, 124 patients with compensated cirrhosis were assigned randomly to an RBV 1000/1200-mg (standard dose) group and 600/800-mg (low dose) group to determine the efficacy of PEG-IFN and RBV combination therapy. The SVR was achieved in 52% of patients who received the standard RBV dose and in 38% of those treated with the low dose. Serious adverse events developed in 14% and 18% of recipients of the standard and low RBV doses, respectively, while dose reduction was necessary in 78% and 57% of the two groups, respectively. HCV genotype 2/3 and platelet count over  $150 \times 10^9/L$  were identified as factors contributing to SVR. Thus, patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. Although PEG-IFN and RBV for patients with compensated cirrhosis has not been approved yet in Japan, the following recommendation is reasonable.

*Recommendation 15: Patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. (Level 3, Grade B.)*

### Retreatment with PEG-IFN and RBV combination therapy for patients who failed to respond to previous IFN treatment

Seven randomized controlled trials have been reported so far that examine the efficacy of PEG-IFN and RBV

combination therapy in patients who failed to respond to previous standard IFN therapy with or without RBV.<sup>105–111</sup> The SVR rate varies among these trials ranging 6–45%, and was lower among non-responders to previous IFN therapy compared with relapsers. In a study using PEG-IFN $\alpha$ -2b and RBV at two different doses (1.5  $\mu$ g/kg per week of PEG-IFN $\alpha$ -2b together with 800 mg/day of RBV or 1.0  $\mu$ g/kg per week of PEG-IFN together with 1000–1200 mg/day of RBV), the SVR rate was low at 10% and 6% in non-responders to previous treatment, but was high at 50% and 32% in relapsers, respectively.<sup>109</sup> In a phase III clinical trial in Japan, the SVR rate was also low in non-responders but sufficiently high in relapsers.<sup>77</sup> Accordingly, PEG-IFN and RBV combination therapy is well indicated for patients who relapse after standard IFN therapy with or without RBV.

Data on retreatment of patients who failed to respond to previous PEG-IFN plus RBV therapy have been evaluated in two trials.<sup>112,113</sup> In a randomized controlled trial that used two different doses of PEG-IFN- $\alpha$ -2a (360 or 180  $\mu$ g/week) with two different durations of therapy (72- or 48-week),<sup>112</sup> an SVR was achieved in 7–14% of patients. It should be noted, however, that the SVR was favorable at 52% in patients who achieved HCV RNA clearance from serum by week 12 in the 72-week treatment arm.<sup>112</sup> In the other trial that used PEG-IFN- $\alpha$ -2b and RBV in 2333 patients who failed to respond to previous PEG or standard IFN together with RBV, an SVR was achieved in 56% of patients whose HCV RNA was cleared from serum by week 12 and in 48% of those with genotype 1.<sup>113</sup> Accordingly, it is reasonable to propose that SVR could be obtained by retreatment with PEG-IFN and RBV in patients who achieve HCV RNA clearance by week 12 of retreatment, even if they failed to respond to previous PEG-IFN and RBV combination therapy.<sup>112,113</sup> In contrast, in the AASLD practice guideline, retreatment with PEG-IFN and RBV is not recommended for patients who did not achieve an SVR after a prior full course of PEG-IFN and RBV. Because it is still unclear who is more likely to respond to retreatment with PEG-IFN and RBV, and new drugs such as protease inhibitors may be indicated in the near future for patients who failed to respond to previous PEG-IFN and RBV therapy, data with retreatment of PEG-IFN and RBV should be accumulated to enable a conclusive recommendation.

*Recommendation 16: Retreatment with PEG-IFN and RBV can be considered for non-responders and relapsers who were treated previously with IFN-based therapy with or without RBV. An SVR could be obtained in these*

*patients whose HCV RNA is cleared from serum by week 12 of retreatment with PEG-IFN and RBV. (Level 2b, Grade B.)*

## MONOTHERAPY WITH IFN OR PEG-IFN

**I**N JAPAN, IFN monotherapy has been used to treat HCV infection since 1992. Today, IFN monotherapy is used only in patients with specific characteristics because combination therapy with PEG-IFN and RBV has achieved a high rate of SVR. Recently, a large randomized control trial (RCT) of maintenance therapy with a low dose of PEG-IFN was reported.<sup>114</sup> There were no differences in progression of liver disease between a PEG-IFN group and a control group. However, Japanese studies of elderly patients or patients who received maintenance therapy for longer periods showed that IFN can improve outcomes in advanced hepatic fibrosis.

### Naïve patients with low viral loads

Previous studies showed that 3 MIU of IFN monotherapy achieved SVR rates of 15–45% in patients with fewer than  $2 \times 10^6$  copies of HCV.<sup>115–118</sup> Monotherapy with 180  $\mu$ g/week of PEG-IFN- $\alpha$ -2a or 1.5  $\mu$ g/kg per week of PEG-IFN- $\alpha$ -2b produced SVR rates of 16–46% in patients with fewer than  $2 \times 10^6$  copies.<sup>119–121</sup> In Japanese patients with fewer than  $1 \times 10^5$  copies of HCV, 6 MIU of IFN treatment for 24 weeks achieved an SVR rate of 86% (127/148).<sup>122</sup> PEG-IFN monotherapy for 48 weeks similarly achieved an SVR rate of 86% (106/123). A recent RCT showed that PEG-IFN monotherapy for 24 weeks produced the same SVR rate as similar treatment for 48 weeks in patients with fewer than  $1 \times 10^5$  copies of HCV. On the basis of these results, monotherapy with IFN or PEG-IFN is considered to be an effective treatment for naïve patients with fewer than 5.0 log copies/mL of HCV.<sup>123</sup>

*Recommendation 17: Monotherapy with IFN or PEG-IFN can be considered for naïve patients with low viral loads (<5.0 log copies/mL). (Level 2a, Grade B.)*

### Patients with chronic kidney disease

Patients with chronic kidney disease (CKD) who undergo hemodialysis have a high prevalence of HCV infection. In Japan, one study reported that HCV RNA was detected in 117 (22%) of 543 patients who underwent maintenance hemodialysis.<sup>124</sup> Hemodialysis patients infected with HCV have a higher mortality rate than uninfected hemodialysis patients.<sup>125</sup> This higher



mortality is attributed to the frequent progression to cirrhosis and/or HCC in HCV-infected patients who receive hemodialysis.

Because RBV is excreted renally, it is currently contraindicated in patients with CKD who have a creatinine clearance of less than 50 mL/min. In addition, pharmacokinetic studies have shown that the clearance of IFN is lower in patients who undergo hemodialysis than in patients who have normal renal function.<sup>126</sup>

Studies of antiviral therapy in patients who undergo hemodialysis suggest that IFN monotherapy is generally well tolerated and that SVR rates are higher than those in patients with normal renal function.<sup>127</sup> The overall SVR rate was reported to be 33–37% in hemodialysis patients.<sup>128</sup> However, the number of subjects in these trials was too low to support confident conclusions. Adverse events are common in this population, and many patients discontinue therapy prematurely because of such events. A recent RCT showed in EASL 2008 that 135 µg/week of PEG-IFN- $\alpha$ -2a for 48 weeks achieved an SVR rate of 39% (23/38), whereas a dose of 90 µg/week produced an SVR rate of 35% (16/43). In 74% of the patients, treatment was completed as scheduled.

Another important point is when to initiate antiviral therapy in hemodialysis patients. IFN might induce allograft rejection and renal failure.<sup>129</sup> Therefore, IFN therapy should be considered before renal transplantation. The next issue to be resolved is the efficacy and safety of low-dose RBV combination therapy in hemodialysis patients.

In 2008, KDIGO proposed guidelines for the treatment of patients with CKD.<sup>130</sup> In Japan, a committee including hepatologists and specialists for CKD is planning a clinical trial for HCV-infected patients with CKD.

*Recommendation 18: 3 MIU of IFN thrice weekly or 90 or 135 µg of PEG-IFN- $\alpha$ -2a weekly is recommended for patients with CKD. (Level 2a, Grade B.)*

### Patients with acute HCV infection

Acute HCV infection progresses to chronic infection in approximately 70% of patients.<sup>131</sup> Antiviral treatment should therefore be considered for this group of patients. On the other hand, it is difficult to identify patients with self-limited disease not requiring therapy. The results of previous studies indicate that anti-HCV treatment should be initiated if HCV RNA is detected continuously for more than 12–16 weeks. If treatment is initiated within this period, monotherapy with IFN or PEG-IFN achieves an SVR rate of more than 80% in patients with acute HCV infection.<sup>132</sup> Reliable evidence

showing that additional treatment with RBV improves the SVR rate in such patients is not available.

*Recommendation 19: Patients with acute HCV infection should be considered as candidates for antiviral therapy. If HCV RNA is detected continuously for 12 or 16 weeks from the onset, treatment with 6 MIU of IFN or 180 µg of PEG-IFN monotherapy should be initiated. (Level 2a, Grade B.)*

### Patients who receive curative treatment for HCC

Hepatocellular carcinoma frequently recurs in HCV-infected patients, even after curative therapy for HCC. Prevention of the recurrence of HCC is essential in such patients. Several RCT showed that the incidence of HCC was low in an IFN-treated group, compared to a control group (Table 4).<sup>133,134</sup> For example, Kubo *et al.* reported that 3 MIU IFN monotherapy thrice weekly for 96 weeks inhibited the recurrence of HCC in patients who had undergone a curative resection.<sup>134</sup> Furthermore, Shiratori *et al.* performed an RCT in 74 patients who had received curative percutaneous ethanol injection therapy for HCC. They reported that second and third recurrences of HCC were less frequent in patients who received IFN.<sup>135</sup> In an Italian study of 150 patients who had undergone curative resection, the recurrence rate of HCC 2 years after operation was significantly lower among patients who received IFN.<sup>136</sup>

Japanese studies showed that the survival rate was also improved by IFN treatment owing to the suppression of HCC and/or the progression of hepatic failure.<sup>137,138</sup>

*Recommendation 20: IFN therapy should be considered for patients after curative treatment for HCC. (Level 1, Grade A.)*

### Maintenance therapy for patients with advanced hepatic fibrosis

Previous studies of patients with advanced hepatic fibrosis, defined as a fibrosis score 3 or 4, showed that IFN monotherapy inhibited the occurrence of HCC, compared to patients who did not receive IFN.<sup>64,139,140</sup> In Japanese studies, IFN was effective not only in SVR patients, but also in non-SVR patients.<sup>139,141</sup> On the other hand, an Italian study showed that the incidence of HCC decreased only in cirrhotic patients in whom HCV was eradicated by IFN therapy.<sup>75</sup>

Case-control studies in patients older than 60 years showed that a low dose of IFN reduced ALT and AFP levels and decreased the incidence of HCC, compared to a control group.<sup>142,143</sup> RCT for IFN monotherapy non-

Table 4 Interferon monotherapy for patients after curative treatment for hepatocellular carcinoma

Author	Study design	No. of patients (IFN group vs non-IFN group)	Age (IFN group vs non-IFN group)	Interferon	Sustained virological response	Follow-up duration (months)	HCC recurrence (IFN group vs non-IFN group)	Survival (IFN group vs non-IFN group)
Ikeda <i>et al.</i>	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo <i>et al.</i>	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Suou <i>et al.</i>	Pilot study	18 vs 22	61 vs 62	alpha	6 (33%)	60	28% vs 82% $P < 0.001$	100% vs 73% $P < 0.05$
Shiratori <i>et al.</i>	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin <i>et al.</i>	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong <i>et al.</i>	Prospective case-control study	16 vs 16	69 vs 68	alpha	2 (13%)	36	69% vs 80% $P = 0.157$	100% vs 88% $P = 0.45$
Sakaguchi <i>et al.</i>	Case-control study	24 vs 33	69 vs 67	alpha	1 (4%)	36	14% vs 73% $P = 0.011$	100% vs 94% $P = 0.25$
Mazzaferro <i>et al.</i>	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu <i>et al.</i>	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo <i>et al.</i>	Case-control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% vs 71% $P = 0.04$	86% vs 56% $P = 0.004$

IFN, interferon; ICC, hepatocellular carcinoma; RCT, randomized control study.

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.<sup>144</sup> In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.<sup>145–147</sup> 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.<sup>114</sup> 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.<sup>138</sup>

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

**I**KEDA *ET AL.* elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.<sup>132</sup> At 5 and 10 years, hepatocarcinogenesis rates in the intermediate ( $100\text{--}140 \times 10^3/\text{L}$ ) and low platelet ( $<100 \times 10^3/\text{L}$ ) 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 ( $\geq 150 \times 10^3/\text{L}$ ) 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.<sup>148–150</sup>

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.<sup>101,151–155</sup> McHutchison *et al.* revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN- $\alpha$ -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.<sup>152</sup>

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.<sup>156</sup> As a result, the mean dose of PEG-IFN- $\alpha$ -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.<sup>157</sup> In the analysis of 472 patients responding to PEG-IFN- $\alpha$ -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- $\alpha$ -2a (135  $\mu$ g/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.<sup>158</sup> Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.<sup>159</sup>

**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- $\alpha$ -2a or over 1.2  $\mu$ g/kg per week of PEG-IFN- $\alpha$ -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- $\alpha$ -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.)

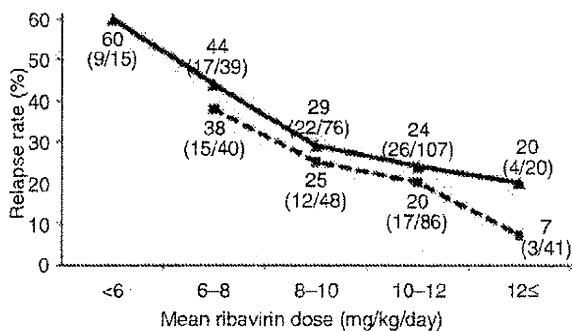


Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)- $\alpha$ -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses ( $\blacktriangle$ ). Group with the mean PEG-IFN dose <1.4  $\mu$ g/kg/week ( $\blacksquare$ ). Group with the mean PEG-IFN dose  $\geq$ 1.4  $\mu$ g/kg/week. There was no significant difference between the two PEG-IFN- $\alpha$ -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 ( $\geq$ 80 IU/mL) than in those with a lower ALT value (<80 IU/mL).<sup>70</sup> 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.<sup>160</sup>

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.<sup>161,162</sup> 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.<sup>163,164</sup>

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

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

**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-