susceptibility alleles among Caucasoid Northern Europeans and North Americans, and 84% of adult patients have either or both of these alleles [17, 18]. In contrast, the principal susceptibility allele for AIH in Japan is DR4 [15, 16]; DR3 is detected in none of them, however. DR4 is also frequent in adult patients in Argentina and Mexico [19, 20], while DRB13 prevails in Argentine and Brazilian children with AIH [21, 22]. DR4 is associated with better response and fewer relapse than DR3 in AIH patients from Western countries [1]. However, there have been no reports on the association of HLA types with treatment response in patients with AIH in Japan. In the present study, DR4 was detected in 32 of the 48 (67%) Japanese patients with AIH, with a frequency comparable to those in previous reports [15, 16]; DR4 was more common in AIH patients than in the general Japanese population (67 vs. 22%) [23]. In contrast, DR14 was comparably frequent in AIH patients and the general population of Japan (23 vs. 17%) [23]. Thus, DR4 would predispose the Japanese population to the development of AIH, while DR14 would not, albeit DR14 would increase the response to corticosteroids in AIH patients.

On the basis of DR4 that is more frequent in the individuals with than without DR3, these alleles have been regarded to behave independently and reciprocally toward the susceptibility for AIH. Such a possibility has been evaluated in peripheral blood mononuclear cells and lymphocytes infiltrating in the liver [24]. Liver lymphocytes are sensitized with hepatocytes or hepatic autoantigens. Even among inflammatory cells infiltrating the portal area, CD4+ lymphocytes predominate in the patients with than without AIH. These lines of evidence implicate the class-II MHC in the pathogenesis of AIH, of which DR4 and DR15 would play major roles in Japan. In the patients with AIH who are positive for LKM-1 antibodies, Th1 cells dominate in the cytokine production assay with a T-cell line specific for LKM-1 [25]. Combined, CD4+ lymphocytes would be crucially required in the manifestation of AIH by interacting with class-II MHC antigens.

In conclusion, the association of MHC class-II antigens with biochemical and histological responses to immunosuppressive treatment was evaluated in Japanese patients with AIH, for predicting their long-term outcomes. On the basis of the results obtained, DR14 would be associated with favorable treatment response in Japanese patients with AIH, which needs to be confirmed in an extended series of patients. The validity of such an assumption will be evaluated by in vitro studies, which are underway.

Acknowledgments This study was supported in part by grants from the Ministry of Health, Labour and Welfareof Japan.

References

1. Czaja AJ, Freese DK. Diagnosis and treatment of autoimmune hepatitis. *Hepatology*. 2002;36:479–497.
2. Krawitt EL. Autoimmune hepatitis. *N Engl J Med*. 2006;354:54–66.
3. Manns MP, Strassburg CP. Autoimmune hepatitis: Clinical challenges. *Gastroenterology*. 2001;120:1502–1517.
4. Czaja AJ, Doherty DG, Donaldson PT. Genetic bases of autoimmune hepatitis. *Dig Dis Sci*. 2002;47:2139–2150.
5. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology*. 1993;18:998–1005.
6. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31:929–938.
7. Toda G, Zeniya M, Watanabe F, et al. Present status of autoimmune hepatitis in Japan–correlating the characteristics with international criteria in an area with a high rate of HCV infection. *J Hepatol*. 1997;26:1207–1212.
8. Vergani D, Mieli-Vergani G. Autoimmune hepatitis. *Autoimmun Rev*. 2003;2:241–247.
9. Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. *J Hepatol*. 2004;40:646–652.
10. Czaja AJ, Carpenter HA. Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis. *Hepatology*. 2004;39:1631–1638.
11. Al-Chalabi T, Underhill JA, Portmann BC, McFarlane IG, Heneghan MA. Impact of gender on the long-term outcome and survival of patients with autoimmune hepatitis. *J Hepatol*. 2008;48:140–147.
12. Czaja AJ, Bianchi FB, Carpenter HA, et al. Treatment challenges and investigational opportunities in autoimmune hepatitis. *Hepatology*. 2005;41:207–215.
13. Czaja AJ, Menon KV, Carpenter HA. Sustained remission after corticosteroid therapy for type 1 autoimmune hepatitis: a retrospective analysis. *Hepatology*. 2002;35:890–897.
14. Ota M, Seki T, Fukushima H, Tsuji K, Inoko H. HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens*. 1992;39:187–202.
15. Miyake Y, Iwasaki Y, Takaki A, et al. Human leukocyte antigen DR status and clinical features in Japanese patients with type 1 autoimmune hepatitis. *Hepatol Res*. 2008;38:96–102.
16. Yoshizawa K, Ota M, Katsuyama Y, et al. Genetic analysis of the HLA region of Japanese patients with type 1 autoimmune hepatitis. *J Hepatol*. 2005;42:578–584.
17. Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1–B8-DR3 are independent risk factors. *Hepatology*. 1991;13:701–706.
18. Strettell MD, Donaldson PT, Thomson LJ, et al. Allelic basis for HLA-encoded susceptibility to type 1 autoimmune hepatitis. *Gastroenterology*. 1997;112:2028–2035.
19. Pando M, Larriba J, Fernandez GC, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology*. 1999;30:1374–1380.
20. Vazquez-Garcia MN, Alaez C, Olivo A, et al. MHC class II sequences of susceptibility and protection in Mexicans with autoimmune hepatitis. *J Hepatol*. 1998;28:985–990.
21. Fainboim L, Marcos Y, Pando M, et al. Chronic active autoimmune hepatitis in children. Strong association with a particular HLA-DR6 (DRB1*1301) haplotype. *Hum Immunol*. 1994;41:146–150.
22. Czaja AJ, Souto EO, Bittencourt PL, et al. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States. *J Hepatol*. 2002;37:302–308.

23. Maeda H, Hirata R, Tokunaga K. HLA-DNA typing and HLA in the Japanese: a review (*in Japanese*). *Nihon Ishokugakkai Zasshi*. 1999;34:55–64.
24. Ichiki Y, Aoki CA, Bowlus CL, Shimoda S, Ishibashi H, Gershwin ME. T cell immunity in autoimmune hepatitis. *Autoimmun Rev*. 2005;4:315–321.
25. Schlaak JF, Lohr H, Gallati H, Meyer zum Buschenfelde KH, Fleischer B. Analysis of the in vitro cytokine production by liver-infiltrating T cells of patients with autoimmune hepatitis. *Clin Exp Immunol*. 1993;94:168–173.

Hepatology Research

Hepatology Research 2010; 40: 347–368 doi: 10.1111/j.1872-034X.2010.00642.x

Special Report

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

Izumi Namiki,[1] Shuhei Nishiguchi,[2] Keisuke Hino,[3] Fumitaka Suzuki,[4] Hiromitsu Kumada,[4] Yoshihito Itoh,[5] Yusuhiro Asahina,[1] Akihiro Tamori,[6] Naoki Hiramatsu,[7] Norio Hayashi[7] and Masatoshi Kudo[8]

[1]Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashinoshi, Tokyo, [2]Department of Hepatobiliary and Pancreas Disease, Hyogo Medical University, Nishinomiya, [3]Department of Hepatobiliary and Pancreas Disease, Kawasaki Medical University, Kurashiki, [4]Department of Hepatology, Toranomon Hospital, Tokyo, [5]Molecular Gstroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, and [6]Department of Hepatology, Osaka City University Graduate School of Medicine, Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009), [7]Department of Gastroenterology, Osaka University and [8]Department of Gastroenterology, Kinki University, Osaka, Japan

The consensus meeting for the diagnosis, management and treatment for hepatitis C was held in 45th 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 course 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*.[1] 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

Correspondence: Mr Namiki Izumi, Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, 1-26-1 Kyonancho, Musashinoshi, Tokyo 180-8610, Japan. Email: nizumi@musashino.jrc.or.jp

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.

This article was previously published in Japanese in *Kanzo* 50:11, pp 665–677 (November 2009).

Received 24 September 2009; revision 1 December 2009; accepted 1 December 2009.

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 consensuses 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).[1,2]

PATHOGENESIS OF HEPATITIS C

HEPATITIS 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.[3] Next, two other essential proteins, scavenger receptor class B type I (SR-B1)[4] and claudin-1 (CLDN1),[5] and potentially additional accessory factors such as glycosaminoglycans and low-density protein receptors[6] were identified as receptors involved in HCV entry. Finally, the crucial factor was identified as the tight junction protein occludin (OCLN).[7] 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.[8] 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.[9–11] 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-α/β response that limits virus infection.[12,13] HCV NS3/4A protease cleaves IPS-1, releasing IPS-1 from the mitochondrial membrane.[14] 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 IFNβ.[15]

Secreted IFNβ engages the local tissue through the autocrine and paracrine processes of binding the IFN-α/β 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.[16] 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-α/β receptor signaling events.[17] 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.[18] The HCV NS5A and E2 proteins also bind double-strand RNA-activated protein kinase (PKR) and inhibit its catalytic activity,[19,20] 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.[21] HCV core protein also produces ROS in human hepatoma Huh-7 cells and HeLa cells.[22] 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.[23]

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)-α and disturbance of tyrosine phosphorylation of the insulin receptor substrate (IRS)1 by TNF-α has been demonstrated in HCV core transgenic mice.[24] Another possible mechanism is that HCV core-induced SOCS3 promotes proteosomal degradation of IRS1 and IRS2 through ubiquitination.[25] 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.[26] HCV core protein also upregulates the sterol regulatory element binding protein (SREBP)1c promoter activity through the enhanced binding of the LXRα/RXRα to LXR-response element,[27] 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 down-regulates the transcriptional activity of hepcidin, a negative regulator in iron homeostasis, in transgenic mice expressing the HCV polyprotein[28] and in HCV replicon cells[29] (Fig. 1).

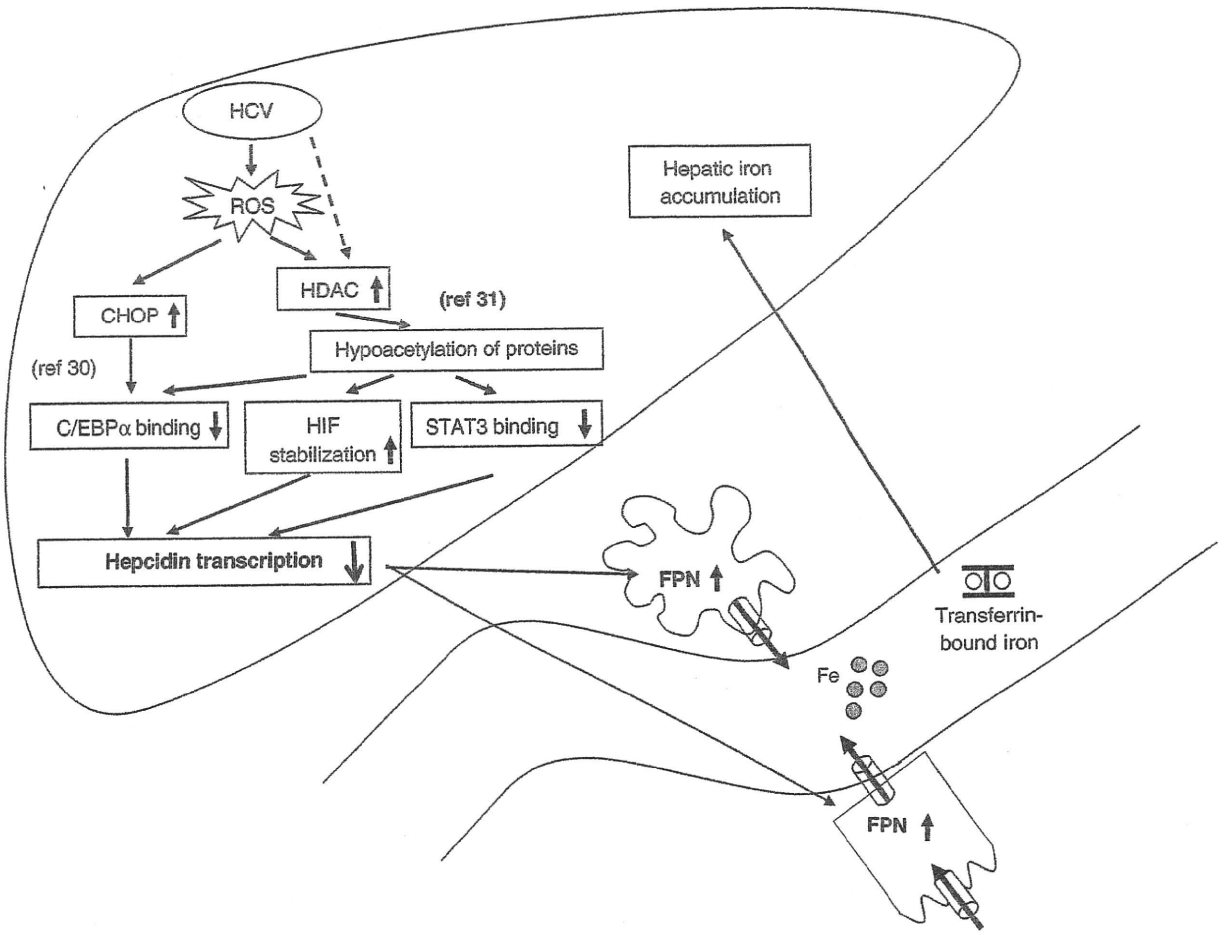

Figure 1 Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV. HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAt3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator. C/EBP, 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.[30–33] In addition, these metabolic disorders are related to the response to antiviral therapy. Insulin resistance[34] and hepatic steatosis[35] 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.[36] 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,[37] 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 monitoring 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.[38]

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.[39–41]

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.[42] 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.[43] 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.[44]

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.[45]

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 (IRRDR)

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.[46] 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.[47] 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[48] (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.[49] 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.[50] 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	
	2: non-double wild	0.102 (0.022–0.474)	0.004
Low-density lipoprotein cholesterol (mg/dL)	1: <86	1	
	2: ≥86	12.87 (2.177–76.09)	0.005
Sex	1: male	1	
	2: female	0.091 (0.017–0.486)	0.005
ICG R15 (%)	1: <10	1	
	2: ≥10	0.107 (0.017–0.678)	0.018
γ-Glutamyltransferase	1: <109	1	
	2: ≥109	0.096 (0.0011–0.819)	0.032
Ribavirin dose (mg/kg)	1: <11.0	1	
	2: ≥11.0	5.173 (1.152–23.22)	0.032

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).[51] 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.[52] 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.[55]

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.[54–57] 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 asymptomatically, 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.[58,59] 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.[60] 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.[61,62] 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%.[63–65]

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.*[66] calculated the average progression rate of hepatic fibrosis in CH-C to be 0.133 fibrosis units/year. In Japan, Shiratori *et al.*[67] 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.*[68] reported that median histological scores did not differ after 5 years of follow up in PNALT and Okanoue *et al.*[69] 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.[70] 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.*[71,72] 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.[73]

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.[74–76] Bruno *et al.*[75] 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.[1]

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-α-2A or -2b together with RBV has been evaluated in two nationwide phase III registration trials in Japan.[77,78] In one trial, which determined efficacy of PEG-IFNα-2b and RBV,[79] the SVR rate to 48-week combination therapy was 48% (121/254) in patients with HCV genotype 1b and a high viral load (≥100 KIU/mL). Another trial using PEG-IFN-α-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 (≥100 KIU/mL).[80] 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.[81,82] Other factors associated with virological response reported from Japan include the low-density lipoprotein cholesterol level,[83] α-fetoprotein (AFP) level,[83] whole-body insulin sensitivity index,[84] single nucleotide polymorphisms of MAP-KAPK3,[85] *RIG-I*/IPS-1 ratio,[86] Th1/Th2 ratio[45] and PKR response.[87] 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-

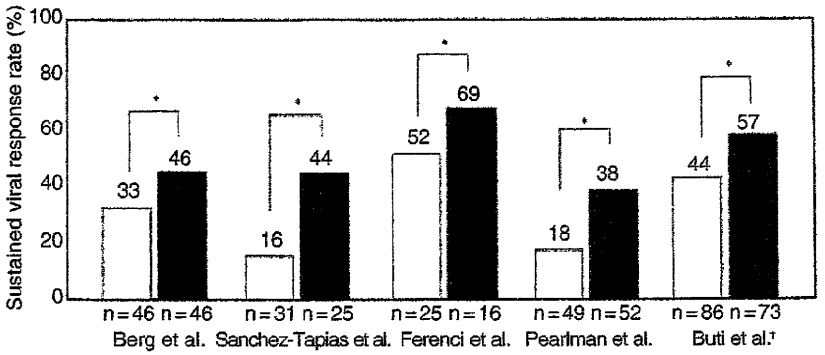

Figure 2 Comparison of sustained virological response rate between 48-week (open column) and 72-week (closed column) treatment with pegylated interferon and ribavirin in patients with partial early virological responder, which is defined as ≥2 log reduction in hepatitis C virus (HCV) RNA level compared to baseline HCV RNA level but detectable HCV RNA at treatment week 12. *Statistical significance between two treatment groups. †Comparison in patients with ≥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,[77,78] 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 Ampricor 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).[88] 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,[1] 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.[89–93] 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).[89–93]

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%, $P = 0.005$).[94] 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 a with wild type of ISDR sequence.[79] 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.[80,95–99] 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 week,[80,95,99] the benefit of a shortening the duration of therapy remains controversial.[96] 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 140×10^9/L, and body mass index over 30 kg/m^2,[100] 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.[101–103] Subsequently, there was one treatment study that focused exclusively on patients with compensated cirrhosis.[104] 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 150×10^9/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.[105–111] 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-IFNα-2b and RBV at two different doses (1.5 μg/kg per week of PEG-IFNα-2b together with 800 mg/day of RBV or 1.0 μg/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.[109] In a phase III clinical trial in Japan, the SVR rate was also low in non-responders but sufficiently high in relapsers.[77] 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.[112,113] In a randomized controlled trial that used two different doses of PEG-IFN-α-2a (360 or 180 μg/week) with two different durations of therapy (72- or 48-week),[112] 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.[112] In the other trial that used PEG-IFN-α-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.[113] 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.[112,113] 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

IN 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.[114] 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 2×10^6 copies of HCV.[115–118] Monotherapy with 180 μg/week of PEG-IFN-α-2a or 1.5 μg/kg per week of PEG-IFN-α-2b produced SVR rates of 16–46% in patients with fewer than 2×10^6 copies.[119–121] In Japanese patients with fewer than 1×10^5 copies of HCV, 6 MIU of IFN treatment for 24 weeks achieved an SVR rate of 86% (127/148).[122] 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 1×10^5 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.[123]

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.[124] Hemodialysis patients infected with HCV have a higher mortality rate than uninfected hemodialysis patients.[125] 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.[126]

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.[127] The overall SVR rate was reported to be 33–37% in hemodialysis patients.[128] 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-α-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.[129] 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.[130] 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-α-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.[131] 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.[132] 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).[133,134] 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.[134] 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.[135] 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.[136]

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.[137,138]

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.[64,139,140] In Japanese studies, IFN was effective not only in SVR patients, but also in non-SVR patients.[139,141] 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.[75]

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.[142,143] 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 *et al.*	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo *et al.*	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Suou *et al.*	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 *et al.*	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin *et al.*	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong *et al.*	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 *et al.*	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 *et al.*	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu *et al.*	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo *et al.*	Case–control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% s 71% $P = 0.04$	86% vs 56% $P = 0.004$

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

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 μg/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 μg/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.)

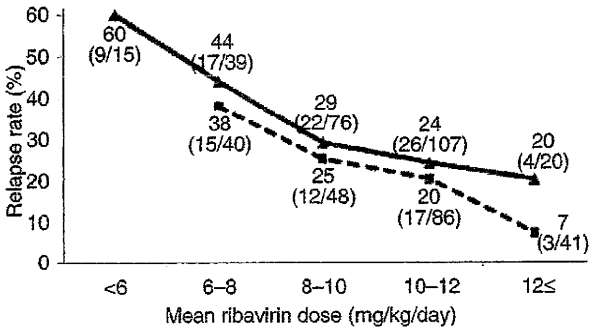

Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)-α-2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses (-▲-). Group with the mean PEG-IFN dose <1.4 μg kg/week (-■-). Group with the mean PEG-IFN dose ≥1.4 μg kg/week. 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-

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.[114] 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 ($100–140 \times 10^9$/L) and low platelet ($<100 \times 10^9$/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 ($\geq 150 \times 10^9$/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

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

1 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.
2 Shiffman RN, Shekelle P, Overhage J *et al.* Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standadization. *Ann Intern Med* 2003; **139**: 493–98.
3 Pileri P, Uematsu Y, Campagnoli S *et al.* Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938–41.
4 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.
5 Evans MJ, von Hahn T, Tscherne DM *et al.* Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; **446**: 801–5.
6 Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. *Cell Microbiol* 2008; **10**: 821–7.
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.
8 Flint M, von Hahn T, Zhang J *et al.* Diverse CD81 proteins support hepatitis C virus infection. *J Virol* 2006; **80**: 11331–42.
9 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.
10 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.
11 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.
12 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.
13 Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; **55**: 255–81.
14 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.
15 Gale M Jr, Foy EM. Evasion of intracellular host defense by hepatitis C virus. *Nature* 2005; **436**: 939–45.
16 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.
17 Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; **2**: 410–6.
18 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.
19 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.
20 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.
21 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.
22 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 Viloria 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

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 Ib 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, Bjøro 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.