

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

**D**EFINITIONS 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 (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.<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>45</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-*

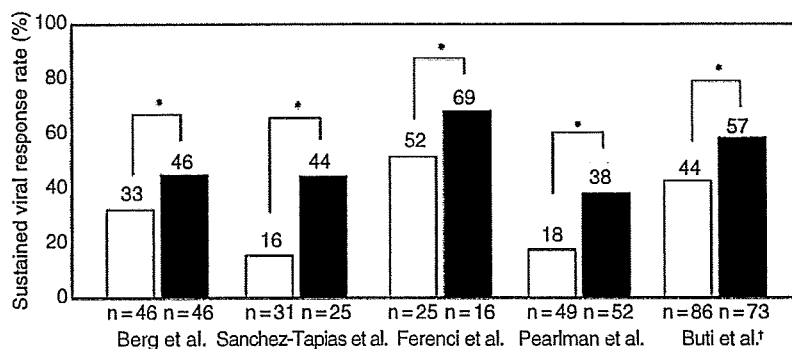


Figure 2 Comparison of sustained virological response rate between 48-week (open column) and 72-week (closed column) treatment with pegylated interferon and ribavirin in patients with partial early virological responder, which is defined as  $\geq 2$  log reduction in hepatitis C virus (HCV) RNA level 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>94</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 a 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 week,<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  $30 \text{ kg}/\text{m}^2$ ,<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; HCC, 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 ( $100\text{--}140 \times 10^9/\text{L}$ ) and low platelet ( $<100 \times 10^9/\text{L}$ ) groups were 10.9% and 21.6% in the IFN group ( $n = 217$ ) and 19.5% and 43.0% in the untreated group ( $n = 459$ ), respectively ( $P = 0.0005$ ). IFN independently decreased the risk of carcinogenesis risk with a hazard ratio of 0.56 ( $P = 0.035$ ). On the other hand, in the high platelet ( $\geq 150 \times 10^9/\text{L}$ ) group,

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

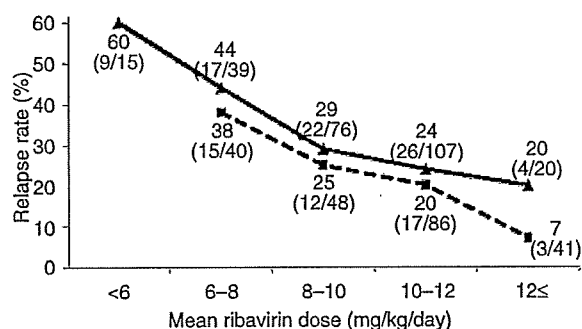
RBV dose was associated with a stepwise increase in relapse rate from 11% to 60% (Fig. 3).

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 (—▲—). Group with the mean PEG-IFN dose <1.4  $\mu$ g/kg/week (---■---). 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-

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.<sup>171,172</sup> 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- $\alpha$ -2a and RBV for 12 weeks.

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

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

### REFERENCES

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

- 23 Korenaga M, Wang T, Li Y *et al.* Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* 2005; 280: 37481–8.
- 24 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; 126: 840–8.
- 25 Kawaguchi T, Yoshida T, Harada M *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; 165: 1499–508.
- 26 Perlemuter G, Sabile A, Letteron P *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; 16: 185–94.
- 27 Moriishi K, Mochizuki R, Moriya K *et al.* Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2007; 104: 1661–6.
- 28 Nishina S, Hino K, Korenaga M *et al.* Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* 2008; 134: 226–38.
- 29 Miura K, Taura K, Kodama Y, Schnabl B, Brenner DA. Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* 2008; 48: 1420–9.
- 30 Veldt BJ, Chen W, Heathcote EJ *et al.* Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; 47: 1856–62.
- 31 Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; 97: 3036–43.
- 32 Kato J, Kobune M, Nakamura T *et al.* Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* 2001; 61: 8697–702.
- 33 Furutani T, Hino K, Okuda M *et al.* Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. *Gastroenterology* 2006; 130: 2087–98.
- 34 Romero-Gomez M, Del Mar 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- $\alpha$  therapy in hepatitis C virus infection. *Hepatology* 1994; 19: 1088–94.
- 40 Lau JY, Davis GL, Kniffen J *et al.* Significance of serum hepatitis C virus RNA in chronic hepatitis C. *Lancet* 1993; 341: 1501–4.
- 41 Yamada G, Takatani M, Kishi F *et al.* Efficacy of interferon alfa therapy in chronic hepatitis C patients depends primarily on hepatitis C virus RNA level. *Hepatology* 1995; 22: 1351–4.
- 42 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. *J Clin Invest* 1995; 96: 224–30.
- 43 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; 334: 77–81.
- 44 Pascu M, Martus P, Hohne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; 53: 1345–54.
- 45 Shirakawa H, Matsumoto A, Joshita S *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; 48: 1753–60.
- 46 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; 48: 38–47.
- 47 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; 48: 372–80.
- 48 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; 46: 403–10.
- 49 Donlin MJ, Cannon NA, Yao E *et al.* Pretreatment sequence diversity differences in the full-length hepatitis

- C virus open reading frame correlate with early response to therapy. *J Virol* 2007; 81: 8211–24.
- 50 Akuta N, Suzuki F, Kawamura Y *et al.* Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; 46: 1357–64.
  - 51 Fishman SL, Factor SH, Balestrieri C *et al.* Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 2009; 15: 3205–13.
  - 52 Ogata S, Florese RH, Nagano-Fujii M *et al.* Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. *J Clin Microbiol* 2003; 41: 2835–41.
  - 53 Nishise Y, Saito T, Sugahara K *et al.* Risk of hepatocellular carcinoma and secondary structure of hepatitis C virus (HCV) NS3 protein amino-terminus, in patients infected with HCV subtype 1b. *J Infect Dis* 2007; 196: 1006–9.
  - 54 Mattsson L, Sonnerborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-years follow-up study of hepatitis C virus markers. *Liver* 1993; 13: 274–8.
  - 55 Barrera JM, Bruguera M, Ercilla MG *et al.* Persistent hepatitis C viremia after self-limiting posttransfusion hepatitis C. *Hepatology* 1995; 21: 639–44.
  - 56 Amoroso P, Rapicetta M, Tosti ME *et al.* Correlation between virus genotype and chronicity rate in acute hepatitis C. *J Hepatol* 1998; 28: 939–44.
  - 57 Tanaka E, Kiyosawa K. Natural history of acute hepatitis C. *J Gastroenterol Hepatol* 2000; 15: E97–104.
  - 58 Seeff LB, Buskell-Bales Z, Wright EC *et al.* Long-term mortality after transfusion-associated non-A, Non B hepatitis. *N Engl J Med* 1992; 327: 1906–11.
  - 59 Seeff LB, Miller RN, Rabkin CS *et al.* 45-year follow-up of hepatitis C virus infection in healthy young adults. *Ann Intern Med* 2000; 132: 105–11.
  - 60 Kenny-Walsh E, the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Eng J Med* 1999; 340: 1228–33.
  - 61 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; 12: 671–5.
  - 62 Tong MJ, El-Farra NS, Reikes AR *et al.* Clinical outcomes after transfusion-associated hepatitis C. *N Eng J Med* 1995; 332: 1463–6.
  - 63 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
  - 64 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IJIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999; 131: 174–81.
  - 65 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. *J Hepatol* 1999; 30: 653–9.
  - 66 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METEVIR, CLINIVIR and DOSVIRC groups. *Lancet* 1997; 349: 825–32.
  - 67 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; 132: 517–24.
  - 68 Persico M, Persico E, Suozzo R *et al.* Natural history of hepatitis C virus carriers with persistently normal aminotransferase. *Gastroenterology* 2000; 118: 760–4.
  - 69 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; 43: 599–605.
  - 70 Tarao K, Rino Y, Ohkawa S *et al.* Association between high serum ALT and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. *Cancer* 1999; 86: 589–94.
  - 71 Kumada T, Toyoda H, Kiriya S *et al.* Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. *Gut* 2007; 56: 738–9.
  - 72 Kumada T, Toyoda H, Kiriya S *et al.* Long-term follow-up of patients with hepatitis C with a normal alanine aminotransferase. *J Med Virol* 2009; 81: 446–51.
  - 73 Suruki R, Hayashi K, Kusumoto K *et al.* Alanine aminotransferase level as a predictor of hepatitis C virus-associated hepatocellular carcinoma incidence in a community-based population in Japan. *Int J Cancer* 2006; 119: 192–5.
  - 74 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; 101: 1616–22.
  - 75 Bruno S, Stroffolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; 45: 579–87.
  - 76 George S, Bacon BR, Brunt EM *et al.* Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; 49: 729–38.

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



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

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

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

## Add-on combination therapy with adefovir dipivoxil induces renal impairment in patients with lamivudine-refractory hepatitis B virus

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Received January 2009; accepted for publication March 2009

**SUMMARY.** Combination therapy with adefovir dipivoxil (ADV) and lamivudine (LAM) is recommended for patients infected with LAM-refractory hepatitis B virus (HBV). However, the effects of such therapy on renal function and serum phosphorus levels have not been fully evaluated. Combination therapy with ADV and LAM was given to 37 patients infected with LAM-refractory HBV, including 17 with hepatic cirrhosis. Serum HBV DNA levels decreased to below 2.6 log<sub>10</sub> copies/mL in 23 (62%) of 37 patients at 12 months, 25 (78%) of 32 patients at 24 months, and 16 (84%) of 19 patients at 36 months. Except for one cirrhotic patient, serum alanine aminotransferase levels were below 50 IU/L in all patients during combination therapy. Serum creatinine levels increased in 14 (38%) of 37 patients, and

serum phosphate levels decreased to below 2.5 mg/mL in 6 (16%) of 37 patients during combination therapy. Patients who received combination therapy for 36 months or longer had a significantly incidence of elevated serum creatinine levels. Fanconi syndrome occurred in a 57-year-old woman with cirrhosis after ADV was added to LAM. Combination therapy with ADV and LAM can maintain biochemical remission in patients with LAM-refractory HBV. However, the dosing interval of ADV should be adjusted according to renal function and serum phosphate levels in patients receiving long-term treatment.

**Keywords:** adefovir dipivoxil, Fanconi syndrome, hepatitis B virus, lamivudine.

### INTRODUCTION

Hepatitis B virus (HBV) is a widely prevalent pathogen that causes chronic hepatitis, hepatic cirrhosis, and hepatocellular carcinoma (HCC) [1]. Interferon and nucleos(t)ide analogues are used for antiviral therapy in patients infected with chronic hepatitis B infection [2,3]. Lamivudine (LAM) is the first nucleoside analogue approved for the treatment of HBV-infected liver disease. LAM suppresses HBV replication

in most patients and improves hepatic inflammation [4]. However, more than 60% of patients with HBV who receive long-term LAM therapy become resistant to the drug within 4 years after starting treatment [5]. For LAM-resistant HBV, a switching-to adefovir dipivoxil (ADV) or entecavir (ETV) treatment is a choice of treatment [6,7]. However, in ADV monotherapy for LAM-refractory chronic hepatitis B, virological breakthrough due to development of ADV resistant mutations occurred in three (21%) of 14 patients within 18 months [8]. In another study, ETV-resistant mutations developed in 12 (8%) of 151 patients with LAM-refractory chronic hepatitis B who received 1 mg ETV once daily for 2 years [7]. In contrast, combination therapy with ADV and LAM decreased HBV DNA levels in patients with LAM-resistant HBV and maintained the effect without virological and biochemical breakthroughs for 3 years [8,9]. In one study performed in Japan, ADV-resistant mutations occurred in only 2 of 129 patients with LAM-refractory chronic hepatitis B who received ADV plus LAM for 2 years [10].

Abbreviations: AD, adefovir dipivoxil; ALT, alanine aminotransferase; anti-HBe, antibodies to HBeAg; ETV, entecavir; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; LAM, lamivudine; TDF, tenofovir disoproxil fumarate.

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