

pressing the development of HCC, branched chain amino acids (BCAA)<sup>11</sup> are implemented. Also, nutrient supplements are utilized for stabilizing liver function.

## DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HBV and HCV ranging from chronic hepatitis to cirrhosis of various severities annually, since the fiscal year 2002. The principal aim of these guidelines is to decrease the incidence of HCC due to hepatitis virus infections in Japan. In accordance with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic viral liver disease every fiscal year. This article summarizes guidelines for the treatment of liver disease due to HBV. Guidelines for the treatment of liver disease due to HCV for the fiscal year 2008 are reported in the accompanying paper. They are formulated on evidence-based data that have been accumulated by members and cooperators of the study group. It will be necessary to improve these guidelines in the next fiscal year and henceforth, in accordance with many pieces of new evidence that are expected to evolve through enduring efforts and keen insights of members and cooperators of the study group.

In the treatment of chronic hepatitis B, novel therapeutic strategies have continued to evolve in previous guidelines. In guidelines of the fiscal year 2008, diverse new treatment arms are introduced for gaining the eventual goal of the “drug-free state”.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has been drafted and displayed on the web site ([www.jsh.or.jp/medical/index.html](http://www.jsh.or.jp/medical/index.html) [in Japanese]) as well, guidelines for the treatment of a spectrum of liver diseases due to HBV, ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. In view of the eventual goal of decreasing the incidence of HCC due to HBV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been introduced to the treatment of cirrhosis due to HBV infection. As a general rule, antiviral treatments are the mainstay in guidelines for the treatment of chronic hepatitis B. In addition to them, it is necessary to always keep in mind the fundamental concepts of these guidelines. It is our sincere hope that, for the treatment of each patient, readers will conduct their

clinical practice on the basis of these concepts, and then refer to appropriate individual guidelines, when they make decisions regarding treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in making treatment choices.

We can foretell that there is no end to the treatment of patients with chronic hepatitis and cirrhosis due to HBV, as it will keep evolving and improving in future guidelines. The enduring efforts of doctors and scientists, in pursuit of this goal, will fill in wide social and economic gaps in medical practices being served to the nation, and produce substantial and efficient interest in the medical economy on a national basis. In conducting treatment of patients with liver disease due to HBV infection, according to these guidelines, many new and unforeseen facets may surface that will require further improvements. Hence, it will be necessary to evaluate the therapeutic efficacy of these guidelines, and revise or add necessary supplements to them as required in the future.

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## Review Article

# Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan

Hiromitsu Kumada,<sup>1</sup> Takeshi Okanoue,<sup>2</sup> Morikazu Onji,<sup>3</sup> Hisataka Moriwaki,<sup>4</sup> Namiki Izumi,<sup>5</sup> Eiji Tanaka,<sup>6</sup> Kazuaki Chayama,<sup>7</sup> Shotaro Sakisaka,<sup>8</sup> Tetsuo Takehara,<sup>9</sup> Makoto Oketani,<sup>10</sup> Fumitaka Suzuki,<sup>11</sup> Joji Toyota,<sup>12</sup> Hideyuki Nomura,<sup>13</sup> Kentaro Yoshioka,<sup>14</sup> Masataka Seike,<sup>15</sup> Hiroshi Yotsuyanagi,<sup>16</sup> Yoshiyuki Ueno<sup>17</sup> and The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour and Welfare of Japan

<sup>1</sup>Department of Hepatology, Toranomon Hospital, Tokyo, <sup>2</sup>Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita, <sup>3</sup>Department of Gastroenterology and Metabolism, Ehime University Graduate School of Medicine, Ehime, <sup>4</sup>Department of Internal Medicine, Gifu University, Gifu, <sup>5</sup>Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashino, <sup>6</sup>Department of Internal Medicine, Shinshu University, Matsumoto, <sup>7</sup>Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, <sup>8</sup>Department of Gastroenterology and Hepatology, Fukuoka University School of Medicine, Fukuoka, <sup>9</sup>Department of Gastroenterology and Hepatology, Osaka University, Osaka, <sup>10</sup>Department of Digestive and Lifestyle-related Disease, Health Research Human and Environmental Science, Kagoshima, <sup>11</sup>Department of Hepatology, Toranomon Hospital, Tokyo, <sup>12</sup>Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo, <sup>13</sup>The Center of Liver Disease, Shin-Kokura Hospital, Kitakyusyu City, <sup>14</sup>Division of Liver, Biliary Tract and Pancreas Disease, Department of Internal Medicine, Fujita Health University, Aichi, <sup>15</sup>Department of Internal Medicine, Faculty of Medicine, Oita University, Oita, <sup>16</sup>Department of Infectious Disease, University of Tokyo, Tokyo, and <sup>17</sup>Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

In the 2008 guidelines for the treatment of patients with chronic hepatitis C, pegylated interferon (Peg-IFN) combined with ribavirin for 48 weeks are indicated for treatment-naïve patients infected with hepatitis C virus (HCV) of genotype 1. Treatment is continued for an additional 24 weeks (72 weeks total) in the patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA during 13–36 weeks on treatment. Re-treatment is aimed to either eradicate HCV or normalize transaminase levels for preventing the development of hepatocellular carcinoma (HCC). For patients with compensated cirrhosis, the clearance of HCV RNA is aimed toward improving histological damages and decreasing the development of HCC. The recommended therapeutic regimen is the initial daily dose of 6 million international units (MIU) IFN continued for 2–8 weeks

that is extended to longer than 48 weeks, if possible. IFN dose is reduced to 3 MIU daily in patients who fail to clear HCV RNA by 12 weeks for preventing the development of HCC. Splenectomy or embolization of the splenic artery is recommended to patients with platelet counts of less than  $50 \times 10^3/\text{mm}^3$  prior to the commencement of IFN treatment. When the prevention of HCC is at issue, not only IFN, but also liver supportive therapy such as stronger neo-minophagen C, ursodeoxycholic acid, phlebotomy, branched chain amino acids (BCAA), either alone or in combination, are given. In patients with decompensated cirrhosis, by contrast, reversal to compensation is attempted.

**Key words:** chronic hepatitis, cirrhosis, hepatocellular carcinoma, hepatitis C virus, interferon, liver supportive therapy, pegylated interferon, ribavirin

Correspondence: Dr Hiromitsu Kumada, Department of Hepatology, Toranomon Hospital, 1-3-1 Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. Email: kumahiro@toranomon.gr.jp

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## INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health Labor and Welfare of Japan, recruiting many specialists from all over the nation. They have been improved every year with many supplementary issues that have evolved, as our understanding of various aspects of viral hepatitis deepens and treatment options widen with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to infection with hepatitis C virus (HCV) in Japan. It is hoped that these guidelines will be accepted widely and implemented for helping as many patients as possible who suffer from sequelae of persistent HCV infection.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with HCV-induced liver disease covering a wide range from those with normal aminotransferase levels to those with decompensated cirrhosis.

## GUIDELINES FOR THE PRIMARY TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

TABLE 1 SUMMARIZES the antiviral therapy of treatment-naïve patients with chronic hepatitis C. In comparison with previous guidelines, the duration of combined treatment with pegylated interferon (Peg-IFN) and ribavirin is extended to 48–72 weeks for patients infected with HCV of genotype 1 in high viral loads (HVL:  $\geq 5$  log IU/mL by the Japanese criteria).<sup>1,2</sup> For patients infected with HCV of genotype 2 in HVL, Peg-IFN- $\alpha 2b$  and ribavirin for 24 weeks are indicated.

To patients with HCV-1 in low viral loads (LVL:  $< 5$  log IU/mL), either the standard IFN (not conjugated with polyethylene glycol) for 24 weeks, or the weekly monotherapy with Peg-IFN- $\alpha 2a$  for 24–48 weeks, is given.<sup>3</sup> Patients with HCV-2 in LVL receive either the standard IFN for 8–24 weeks, or the weekly monotherapy with Peg-IFN- $\alpha 2a$  for 24–48 weeks.

## GUIDELINES FOR THE RE-TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

FOR PATIENTS WHO receive re-treatment, first, it is imperatively prerequisite to: (i) identify factors for non-response to previous treatments; and (ii) decide whether to aim for clearance of HCV or to prevent the progression of hepatitis that can accelerate the development of hepatocellular carcinoma (HCC), and this can be monitored by alanine aminotransferase (ALT) and  $\alpha$ -fetoprotein (AFP) levels toward normalizing or stabilizing their levels (Table 2).<sup>4</sup> Second, IFN combined with ribavirin is the mainstay of re-treatment of patients with chronic hepatitis C. Third, long-term IFN monotherapy is recommended to patients who are not indicated to IFN/ribavirin or who have failed to respond to the combination therapy. However, some patients do not tolerate IFN due to side-effects or their complicating morbidities. In addition, IFN monotherapy does not always improve ALT levels. Such patients need to receive liver supportive therapy including stronger neominophagen C (SNMC)<sup>5</sup> and ursodeoxycholic acid (UDCA),<sup>6</sup> as well as phlebotomy, either alone or in combination. Therapeutic target ALT levels are: (i) within  $\times 1.5$  the upper limit of normal (ULN) for patients in fibrosis stage 1 (F1); and (ii) less than 30 IU/L in those in fibrosis stages 2 or 3 (F2/F3), as far as possible.

Table 1 Guidelines for the primary treatment of patients with chronic hepatitis C

Genotypes	Genotype 1	Genotype 2
Viral loads		
High viral load		
$\geq 5.0$ log IU/mL	• Peg-IFN- $\alpha 2b$ (Peg-Intron) + ribavirin (Rebetol) for 48–72 weeks	• Peg-IFN- $\alpha 2b$ (Peg-Intron) + ribavirin (Rebetol) for 24 weeks
$\geq 300$ fmol/L	• Peg-IFN- $\alpha 2a$ (Pegasys) + ribavirin (Copegus) for 48–72 weeks	
$\geq 1$ Meq/mL		
Low viral load		
$< 5.0$ log IU/mL	• Standard IFN for 24 weeks	• Standard IFN for 8–24 weeks
$< 300$ fmol/L	• Peg-IFN- $\alpha 2a$ (Pegasys) for 24–48 weeks	• Peg-IFN- $\alpha 2a$ (Pegasys) for 24–48 weeks
$< 1$ Meq/mL		

Peg-IFN, pegylated interferon.

**Table 2** Guidelines for re-treatment of chronic hepatitis C**Principles**

Selection has to be made between termination of HCV infection and normalization/stabilization of ALT as well as AFP levels (toward preventing aggravation of liver disease and development of HCC), after evaluating factors for non-response in the primary IFN treatment.

- 1 "IFN plus ribavirin" is the mainstay of re-treatment of patients who have failed to respond to the primary IFN therapy.
- 2 Long-term IFN is recommended to patients in whom ribavirin is not indicated or who have failed to respond to IFN/ribavirin; self-injection at home is approved for IFN- $\alpha$  (not for Peg-IFN).
- 3 Patients who are not indicated to IFN or have failed to improve ALT and AFP levels, in response to IFN, receive liver supportive therapy (SNMC, UDCA) and phlebotomy, either alone or in combination.
- 4 For preventing aggravation of liver disease (and development of HCC), ALT levels need to be controlled within  $1.5 \times \text{ULN}$  in patients in stage 1 fibrosis (F1), and as far as possible, 30 IU/L or lower in those in fibrosis stages 2–3 (F2/F3).
- 5 In treatment combined with ribavirin, dose and mode need to be selected, taking into consideration factors contributing to the response, such as age, sex, progression of liver disease, mutations in the HCV genome (amino acid substitutions in the core protein [aa70/aa91] and ISDR) and HCV RNA titers determined by the real-time PCR.

AFP,  $\alpha$ -fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

## SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS C

FOR THE FISCAL year 2008, the following items were supplemented to the treatment of chronic hepatitis C (Table 3).

- 1 The treatment of patients infected with HCV-1 in HVL with Peg-IFN/ribavirin for 72 weeks is modified by the early virological response (EVR) within 12 weeks after the start. Patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.<sup>1,2</sup>
- 2 Patients with HCV-1 in HVL who fail to clear HCV RNA detectable by real-time PCR but in whom

ALT levels normalize are continued on Peg-IFN/ribavirin until 48 weeks, so that normalized ALT levels endure longer after the completion of therapy.<sup>7</sup>

- 3 Patients who are not indicated to Peg-IFN/ribavirin, or who have failed to respond to previous treatments, receive long-term IFN monotherapy. During the first 2 weeks, IFN in the conventional dose is given daily or three times a week. Patients who do not clear HCV RNA during the maximal treatment period of 8 weeks receive half the conventional dose of IFN indefinitely.<sup>8</sup>

## GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C IN NORMAL ALT LEVELS

AS IN PREVIOUS guidelines, patients with chronic hepatitis C having normal ALT levels are stratified into four groups by ALT levels and platelet counts (Table 4). Patients with chronic hepatitis C who have normal ALT levels are reported to gain the sustained virological response (SVR) to antiviral treatments comparably frequently as those having elevated ALT levels. Taking this into consideration, patients with ALT levels of 30 IU/L or less and platelet counts of  $150 \times 10^3/\text{mm}^3$  or more are followed for ALT every

**Table 3** Supplements to guidelines for chronic hepatitis C

- 1 Criteria for extending the duration of Peg-IFN/ribavirin (to 72 weeks) in patients infected with HCV-1b in HVL: patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.<sup>1,2</sup>
- 2 Patients with HCV-1b in HVL who fail to lose HCV RNA detectable by real-time PCR, but in whom ALT levels normalize by 36 weeks, Peg-IFN/ribavirin is given till 48 weeks for maintaining normalized ALT levels long after the completion of treatment.
- 3 Long-term IFN monotherapy in patients who are not indicated to Peg-IFN/ribavirin, or have failed to respond to it: the usual dose of IFN daily or three times in week is given for the first 2 weeks, and when HCV RNA does not disappear within the maximal duration of 8 weeks, long-term treatment with half the usual dose of IFN is continued indefinitely.

ALT, alanine aminotransferase; HCV, hepatitis C virus; HVL, high viral loads; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon.

**Table 4** Guidelines for the treatment of patients with normal ALT levels toward preventing the development of HCC

Platelets	$\geq 150 \times 10^3/\text{mm}^3$	$< 150 \times 10^3/\text{mm}^3$
ALT		
$\leq 30$ IU/L	<ul style="list-style-type: none"> <li>Follow for ALT every 2-4 months.</li> <li>If ALT levels elevate, start antiviral treatments taking into consideration the possibility of SVR and risk for HCC.</li> </ul>	<ul style="list-style-type: none"> <li>Liver biopsy, if possible, and consider antiviral treatments for patients in A2/F2.</li> <li>Follow for ALT every 2-4 months, and consider antiviral treatments when ALT levels elevate, for patients without biopsy.</li> </ul>
31-40 IU/L	<ul style="list-style-type: none"> <li>Consider antiviral treatments for patients younger than 65 years.</li> </ul>	<ul style="list-style-type: none"> <li>Start treatments for chronic hepatitis C.</li> <li>Select treatments according to genotypes, viral load, age of patients, etc.</li> </ul>

ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

2-4 months. If ALT levels increase in them, antiviral treatments are considered based on the possibility of resolving HCV infection and the risk for developing HCC. In view of significant fibrosis present in patients with platelet counts of less than  $150 \times 10^3/\text{mm}^3$ , they are recommended to receive liver biopsy, if this is possible. Patients in fibrosis stage F2 or higher are evaluated for the indication to antiviral treatments. Patients with ALT levels between 31 and 40 IU/L are classified by platelet counts. Antiviral treatments are considered in those aged younger than 65 years who have platelet counts of  $150 \times 10^3/\text{mm}^3$  or more, while guidelines for patients with chronic hepatitis are applied to those with platelet counts of less than  $150 \times 10^3/\text{mm}^3$ .<sup>9,10</sup>

#### GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HCV

**P**ATIENTS WITH COMPENSATED cirrhosis who are not infected with HCV-1 in HVL receive either IFN- $\beta$  or IFN- $\alpha$  (Table 5). Since the fiscal year 2008, IFN- $\alpha$  has been approved for the treatment of patients infected with HCV-1 in HVL, with the aim of resolving infection and normalizing ALT as well as AFP levels by long-term therapy. Treatment duration was set at 1 year or longer, and because the longer the treatment duration the higher the SVR rate, 36 weeks has been recommended as the optimal treatment duration. Because the normalization of ALT/AST is important, even in patients who fail to clear HCV infection by these therapeutic regimens, treatment is better conducted for maintaining normal ALT/AST levels. Guidelines for maintaining liver function for preventing the development of HCC include liver supportive therapy with glycyrrhizin<sup>5</sup> and UDCA,<sup>6</sup> either alone or in combination. For treatment toward suppressing the

development of HCC, branched chain amino acids (BCAA)<sup>11</sup> or phlebotomy are adopted. Also, nutrient supplements are applied for stabilizing liver function.

#### SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CIRRHOSIS DUE TO HCV

**T**HE FOLLOWING ITEMS have been appended to supplement guidelines for the treatment of type C cirrhosis (Table 6).

**Table 5** Guidelines for treatment of type C cirrhosis

Principles	Compensated: termination of HCV infection Decompensated: reversal to compensation and prevention of HCC
Methods	<ol style="list-style-type: none"> <li>Eradication of HCV and normalization of ALT/AST (for patients with compensated cirrhosis). <ol style="list-style-type: none"> <li>HCV-1b in HVL (<math>\geq 5</math> log IU/mL) <ul style="list-style-type: none"> <li>IFN-<math>\alpha</math> (Sumiferon)</li> <li>Others <ul style="list-style-type: none"> <li>IFN-<math>\alpha</math> (Sumiferon)</li> <li>IFN-<math>\beta</math> (Feron)</li> </ul> </li> </ul> </li> <li>Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC. <ol style="list-style-type: none"> <li>Liver supportive therapy <ul style="list-style-type: none"> <li>Stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA), etc.</li> </ul> </li> <li>Branched chain amino acids (BCAA {Livact})</li> <li>Phlebotomy</li> </ol> </li> <li>Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).</li> </ol> </li></ol>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HVL, high viral loads; IFN, interferon.

**Table 6** Supplements to guidelines for type C cirrhosis

- 1 To start with, IFN for compensated cirrhosis is desired at 6 MIU daily for 2–8 weeks, as far as possible, and to continue for 48 weeks or longer, as for chronic hepatitis C.
- 2 In patients with compensated cirrhosis who fail to clear HCV RNA within 12 weeks on IFN, long-term therapy at 3 MIU should be considered for preventing HCC.
- 3 In patients with platelet counts  $<50 \times 10^3/\text{mm}^3$ , splenectomy or embolization of splenic artery is recommended before re-treatment, and after thorough evaluation has been made on the response to IFN to be expected.
- 4 For the prevention of HCC, not only IFN, but also liver supportive therapy (SNMC, UDCA, etc.), phlebotomy and branched chain amino acids, either alone or in combination, are recommended for improving ALT/AST and AFP levels.

AFP,  $\alpha$ -fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MIU, million international units; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

- 1 For treatment of type C cirrhosis with IFN, the initial dose of 6 million international units (MIU) daily is continued as long as possible (2–8 weeks). Thereafter, long-term IFN for 48 weeks or longer is desired as in the treatment of chronic hepatitis C.
- 2 In the treatment of type C cirrhosis, patients who fail to achieve EVR with the clearance of HCV RNA from serum within 12 weeks should receive long-term IFN at a dose of 3 MIU.
- 3 For patients with type C cirrhosis who have platelet counts of less than  $50 \times 10^3/\text{mm}^3$ , splenectomy or embolization of the splenic artery is desirable before commencing IFN therapy, after the efficacy of IFN has been evaluated thoroughly.<sup>12</sup>
- 4 For preventing the development of HCC, improvement in ALT, AST and AFP levels are aimed. Toward this end, not only IFN, but also liver supportive therapy (SNMC and UDCA), phlebotomy and BCAA are used, either alone or in combination.

## DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HCV ranging from chronic hepatitis to cirrhosis of various severities for the fiscal

year 2008. The principal aim of these guidelines is to decrease the incidence of HCC due to HCV infection in Japan. In accord with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic hepatitis C. They are prepared on evidence-based data that have been accumulated by members and cooperators of the study group. It is necessary to improve these guidelines in the next fiscal year and thereafter, in accordance with many pieces of new evidence that are expected to emerge through enduring efforts of members and cooperators of the study group.

In the treatment of chronic hepatitis C, the duration of antiviral treatments is extended to 72 weeks, which has been approved as of the fiscal year 2008, and criteria for the eligibility of extended treatment duration are clearly defined. Long-term antiviral treatments, extended up to 72 weeks, are hoped to increase the SVR even further. In addition, comprehensive guidelines for the treatment of cirrhosis have been improved with substantial additions, and their criteria for the indication made explicit.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has drafted, and also displayed online ([www.jsh.or.jp/medical/index.html](http://www.jsh.or.jp/medical/index.html) [in Japanese]), guidelines for a spectrum of liver diseases due to HCV, from chronic hepatitis to cirrhosis of various severities. In view of the eventual goal of decreasing the incidence of HCC due to HCV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been constructed for the treatment of cirrhosis due to HCV infection. As a general rule, antiviral treatments constitute the main body of guidelines for the treatment of chronic hepatitis C. Furthermore, the fundamental concept of these guidelines would need to be kept in mind always. It is our sincere hope that, for the treatment of each patient, readers will base their clinical practice on these guidelines, and refer to appropriate individual guidelines, when they make a decision on the treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in treatment choice.

It is our sincere desire that treatment of patients with chronic hepatitis and cirrhosis due to HCV will proceed following these guidelines. Efforts along these lines will rectify a wide gap in medical treatment served to the nation and raise substantial and efficient interest in the medical economy on the national basis. In practicing treatment according to these guidelines, it will be nec-

essary to evaluate their therapeutic efficacy, and revise or add necessary supplements to them as required in the future.

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# Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Affect Very Early Viral Dynamics During Treatment With Telaprevir, Peginterferon, and Ribavirin

Norio Akuta,<sup>1\*</sup> Fumitaka Suzuki,<sup>1</sup> Miharuru Hirakawa,<sup>1</sup> Yusuke Kawamura,<sup>1</sup> Hiromi Yatsuji,<sup>1</sup> Hitomi Sezaki,<sup>1</sup> Yoshiyuki Suzuki,<sup>1</sup> Tetsuya Hosaka,<sup>1</sup> Masahiro Kobayashi,<sup>1</sup> Mariko Kobayashi,<sup>2</sup> Satoshi Saitoh,<sup>1</sup> Yasuji Arase,<sup>1</sup> Kenji Ikeda,<sup>1</sup> and Hiromitsu Kumada<sup>1</sup>

<sup>1</sup>Department of Hepatology, Toranomon Hospital, Tokyo, Japan

<sup>2</sup>Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the rate of HCV RNA loss following 12-week triple therapy, and determine the effect of aa substitutions on very early (within 48 hr) viral dynamics. Sixty-seven patients infected with HCV genotype 1b (HCV-1b) and high viral load who received 12-week triple therapy were studied. RNA loss could be achieved in 2%, 34%, 80%, 92%, 95%, 94%, and 90% of the patients after 1, 2, 4, 6, 8, 10, and 12 weeks of triple therapy, respectively. After 24-hr treatment, the proportion of patients with Arg70 and Leu91 substitutions with  $\geq 3.0$  log fall in HCV RNA was significantly higher than those with  $< 3.0$  log fall ( $P = 0.008$ ). However, the aa substitution patterns in the core region did not influence the fall in HCV RNA after 48-hr treatment. Multivariate analysis identified substitutions of aa 70 and 91 ( $P = 0.014$ ) and level of viremia at baseline ( $\geq 7.0$  log IU/ml;  $P = 0.085$ ) as independent parameters that determined the  $\geq 3.0$  log fall in HCV RNA level after 24-hr triple therapy. It is concluded that 12-week triple therapy achieved high rates of loss of HCV RNA in Japanese patients infected with HCV-1b and high viral load, and that the aa substitution pattern in the core region seems to influence very early viral dynamics. *J. Med. Virol.* 82:575–582, 2010. © 2010 Wiley-Liss, Inc.

**KEY WORDS:** HCV; core region; NS5A-ISDR; telaprevir; peginterferon; ribavirin; very early viral dynamics

## INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999]. At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) with high viral loads ( $> 100$  KIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Iino et al., 2005; Tsubota et al., 2005]. Such background calls for efficient treatment of patients with chronic HCV infection.

Even with pegylated interferon (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b with high viral loads [Manns et al., 2001; Fried et al., 2002]. Recently, a new strategy was introduced for the treatment of chronic HCV infection by inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these drugs, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [Lin et al., 2006]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, results in a robust antiviral activity [Modi and Hoofnagle, 2007; Zeuzem, 2008]. Specifically, HCV RNA disappears in

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\*Correspondence to: Norio Akuta, MD, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-0001, Japan. E-mail: akuta-g@umin.ac.jp

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almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin [Lawitz et al., 2008; Suzuki et al., 2009]. However, patients resistant to treatment who do not achieve sustained virological response by the triple therapy, have been reported [Lawitz et al., 2008; Hézode et al., 2009; McHutchison et al., 2009]. The underlying mechanism of the response to the treatment is still not clear.

It is useful to evaluate treatment efficacy based on viral dynamics as an early predictor of PEG-IFN plus ribavirin combination therapy. Previous reports showed that decreases in HCV RNA levels were significantly greater in patients with than without sustained virological response from 24 hr to 12 weeks after the start of PEG-IFN plus ribavirin combination therapy in patients infected with HCV-1b and high viral load. Very early dynamics within 48 hr of such treatment is particularly important for early prediction of response to therapy [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Accordingly, the pretreatment predictors of very early dynamics during triple therapy of telaprevir with PEG-IFN and ribavirin were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with genotype 1b and high viral load are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009], and also affect the clinical outcome, including insulin resistance and hepatocarcinogenesis [Akuta et al., 2007c, 2009b; Fishman et al., 2009; Nakamoto et al., 2009]. However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict the very early dynamics and response to triple therapy of telaprevir with PEG-IFN and ribavirin.

The present study included 67 patients with HCV-1b and high viral load, who received triple therapy of telaprevir with PEG-IFN plus ribavirin and followed-up for 12 weeks or more after the start of treatment. The aims of the study were to determine the rate of loss of HCV RNA during treatment, and to identify the pretreatment factors that could predict very early viral dynamics (within 48 hr) after the start of treatment, including aa substitutions in the HCV core, the NS3, and the NS5A regions.

## PATIENTS AND METHODS

### Study Patients

Between May 2008 and May 2009, 67 patients infected with HCV were recruited to the study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (30%) patients were allocated to a 12-

week regimen of triple therapy [telaprevir (MP-424), PEG-IFN, and ribavirin], and 47 patients (70%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks. All patients were followed-up for at least 12 weeks after the start of triple therapy.

All patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C; (2) HCV-1b confirmed by sequence analysis; (3) HCV RNA levels of  $\geq 5.0$  log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese (Mongoloid) ethnicity; (5) age at study entry of 20–65 years; (6) body weight  $\geq 35$  and  $\leq 120$  kg at the time of registration; (7) lack of decompensated cirrhosis; (8) absence of hepatitis B surface antigen (HBsAg) in serum; (9) no history of HCC; (10) no previous treatment for malignancy; (11) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C; (12) no history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of  $\leq 50$  ml/min at baseline, diabetes requiring treatment or fasting glucose level of  $\geq 110$  mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline; and (13) hemoglobin level of  $\geq 12$  g/dl, neutrophil count  $\geq 1,500/\text{mm}^3$ , and platelet count of  $\geq 100,000/\text{mm}^3$  at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study.

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at a dose of 750 or 500 mg three times a day at an 8-hr (q8) interval after the meal. PEG-IFN $\alpha$ -2b (PEG-Intron; Schering Plough, Kenilworth, NJ) was injected subcutaneously with a median dose 1.5  $\mu\text{g}/\text{kg}$  (range: 1.3–2.0  $\mu\text{g}/\text{kg}$ ) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1,000 mg). All participating patients received these three drugs in the initial 12 weeks of the study.

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below  $1,500/\text{mm}^3$ , neutrophil count below  $750/\text{mm}^3$ , or platelet count below  $80,000/\text{mm}^3$ ; PEG-IFN was discontinued when these counts decreased below  $1,000/\text{mm}^3$ ,  $500/\text{mm}^3$ , or  $50,000/\text{mm}^3$ , respectively. When hemoglobin decreased to  $< 10$  g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800–600, and 1,000–600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to  $< 8.5$  g/dl. However, the dose of telaprevir

(MP-424) remained the same throughout the 12-week protocol, though the drug was discontinued altogether following the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFN $\alpha$ -2b and ribavirin was also terminated.

### Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at nine time points: 24 hr, 48 hr, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 12 weeks after the commencement of treatment. HCV RNA levels during treatment was evaluated in 66 (99%), 66 (99%), 65 (97%), 67 (100%), 64 (96%), 60 (90%), 58 (87%), 50 (75%), and 58 (87%) of the 67 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative. Reduction in HCV RNA levels at 24 and 48 hr relative to the baseline were investigated as very early dynamics.

### Detection of Amino Acid Substitutions in Core, NS3, and NS5A Regions

In the present study, aa substitutions of the core, NS3, and NS5A-ISDR regions were analyzed by direct sequencing. AA sequences in the upstream site (1027–1318 aa) of the NS3 region, including aa positions reported as resistance for telaprevir [Lin et al., 2005; Forestier et al., 2007; Zhou et al., 2007], were determined. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by PCR using the following primers. (a) Nucleotide sequences of the core region: the first-round PCR was performed with CE1 (sense: 5'-GTC TGC GGA ACC GGT GAG TA-3'; nucleotides: 134–153) and CE2 (antisense: 5'-GAC GTG GCG TCG TAT TGT CG-3'; nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense: 5'-ACT GCT AGC CGA GTA GTG TT-3'; nucleotides: 234–253) and CE6 (antisense: 5'-GGA GCA GTC GTT CGT GAC AT-3'; nucleotides: 934–953) primers. (b) Nucleotide sequences of NS3 region: the first-round PCR was performed with NS33F (sense: 5'-ACT TCT AGG ACC GGC CGA TA-3'; nucleotides: 3359–3378) and NS34R (antisense: 5'-GCT CGT CAC ACT TCT TCT TG-3'; nucleotides: 4517–4536) primers, and the second-round PCR with NS33F (sense) and NS36R (antisense: 5'-GTC TGT GAA GAC CGG AGA CC-3'; nucleotides: 3946–3965) primers. (c) Nucleotide sequences of NS5A-ISDR: the first-round PCR was performed with ISDR1 (sense: 5'-ATG CCC ATG CCA GGT TCC AG-3'; nucleotides: 6662–6681) and ISDR2 (antisense: 5'-AGC TCC GCC AAG GCA GAA GA-3'; nucleotides: 7350–7369) primers, and the second-round PCR with ISDR3 (sense: 5'-ACC GGA TGT GGC AGT

GCT CA-3'; nucleotides: 6824–6843) and ISDR4 (antisense: 5'-GTA ATC CGG GCG TGC CCA TA-3'; nucleotides: 7189–7208) primers ([a,c]; nested PCR. [b]; hemi-nested PCR). All samples were denatured initially at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1  $\mu$ l of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed on 67 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region, ISDR) reported by Enomoto et al. [1995, 1996] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type ( $\leq 1$ ) or mutant-type ( $\geq 2$ ).

### Statistical Analysis

Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to very early viral dynamics. The odds ratios and 95% confidence intervals (95% CI) were also calculated. All *P*-value  $< 0.05$  by the two-tailed test were considered significant. Variables that achieved statistical significance ( $P < 0.05$ ) or marginal significance ( $P < 0.10$ ) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with very early dynamics included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase ( $\gamma$ GTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, and aa substitution in the core, NS3, and the NS5A-ISDR regions. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL).

TABLE I. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon, and Ribavirin Triple Therapy in Japanese Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	67
Sex (M/F)	36/31
Age (years)*	54 (23–65)
History of blood transfusion	19 (28.4%)
Family history of liver disease	11 (16.4%)
Body mass index (kg/m <sup>2</sup> )*	22.7 (16.0–32.4)
Laboratory data*	
Serum aspartate aminotransferase (IU/l)	34 (15–118)
Serum alanine aminotransferase (IU/l)	43 (12–175)
Serum albumin (g/dl)	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase (IU/l)	35 (9–194)
Leukocyte count (/mm <sup>3</sup> )	4,900 (3,000–8,100)
Hemoglobin (g/dl)	14.2 (12.1–16.8)
Platelet count ( $\times 10^4$ /mm <sup>3</sup> )	17.4 (10.4–33.8)
Level of viremia (log IU/ml)	6.8 (5.1–7.6)
Alpha-fetoprotein ( $\mu$ g/L)	4 (2–38)
Total cholesterol (mg/dl)	184 (112–276)
High-density lipoprotein cholesterol (mg/dl)	46 (20–79)
Low-density lipoprotein cholesterol (mg/dl)	106 (47–191)
Triglycerides (mg/dl)	99 (49–215)
Fasting plasma glucose (mg/dl)	92 (66–107)
Treatment	
PEG-IFN $\alpha$ -2b dose ( $\mu$ g/kg)*	1.5 (1.3–2.0)
Ribavirin dose (mg/kg)*	11.5 (7.2–15.8)
Telaprevir dose (1,500/2,250 mg/day)	10/57

Data are number and percentages of patients, except those denoted by \*, which represent the median (range) values.

## RESULTS

Table I summarizes the profiles and laboratory data of the 67 patients at the commencement of treatment. They included 36 males and 31 females, aged 23–65 years (median, 54 years). The frequencies of Arg70 and Gln70 (His70) in the core region were 61% (41/67) and 39% (26/67), respectively. The frequencies of Leu91 and Met91 were 55% (37/67) and 45% (30/67), respectively. However, frequencies of wild-type and mutant-type in NS5A-ISDR were 96% (64/67) and 5% (3/67), respectively.

### Rates of Loss of HCV RNA During Treatment

The disappearance rate of HCV RNA during treatment was 0% (0/66), 0% (0/66), 2% (1/65), 34% (23/67), 80% (51/64), 92% (55/60), 95% (55/58), 94% (47/50), and 90% (52/58) at 24 hr, 48 hr, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 12 weeks, respectively.

According to the substitution of core aa 70, the rate of HCV RNA loss at each time point was not significantly different between Arg70 and Gln70(His70) (Fig. 1A). According to the substitution of core aa 91, the rate at each time point was not significantly different between Leu91 and Met91 (Fig. 1B).

### Very Early Dynamics According to Amino Acid Substitutions in the Core, the NS3, and the NS5A Regions

After 24 hr of commencement of the triple therapy, the proportion of patients who showed  $\geq 2.0$  log fall in HCV

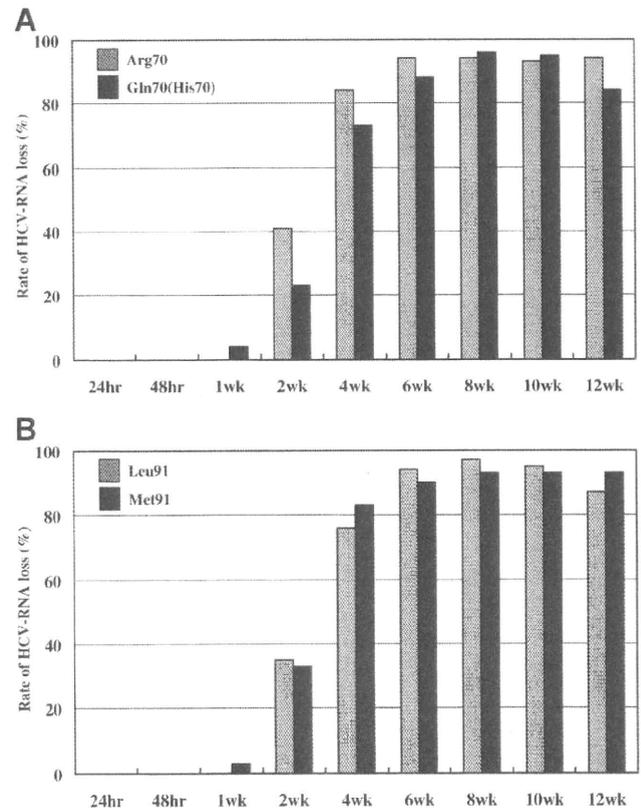


Fig. 1. Rates of HCV RNA loss according to substitutions of the core aa 70 and 91 at different time points after commencement of the triple therapy. At each time point, the rate of HCV RNA loss was not significantly different between Arg70 and Gln70(His70) (A) or between Leu91 and Met91 (B).

TABLE II. Falls in HCV RNA Levels From Baseline After 24 and 48 hr of Triple Therapy of Telaprevir, Peginterferon, and Ribavirin According to the Amino Acid Substitutions in the Core Region and NS5A Region in Patients Infected With HCV Genotype 1b

	Fall in HCV RNA <sup>a</sup> (log IU/ml)	≥2.0 log (n = 64)	<2.0 log (n = 2)	<i>P</i>	≥3.0 log (n = 21)	<3.0 log (n = 45)	<i>P</i>
<b>(A) Fall after 24 hr of triple therapy</b>							
Arg70 and Leu91	3.0 (1.8–4.0)	26 (40.6%)	2 (100%)	NS	14 (66.7%)	14 (31.1%)	0.008
Gln70(His70)	2.7 (2.3–3.5)	26 (40.6%)	0 (0%)	NS	5 (23.8%)	21 (46.7%)	NS
Met91	2.7 (2.0–3.3)	30 (46.9%)	0 (0%)	NS	4 (19.0%)	26 (57.8%)	0.004
Gln70(His70)andMet91	2.7 (2.3–3.3)	18 (28.1%)	0 (0%)	NS	2 (9.5%)	16 (35.6%)	0.037
ISDR wild-type	2.8 (1.8–4.0)	61 (95.3%)	2 (100%)	NS	2 (100%)	42 (93.3%)	NS
	Fall in HCV RNA <sup>a</sup> (log IU/ml)	≥3.0log (n = 62)	<3.0 log (n = 4)	<i>P</i>	≥4.0 log (n = 21)	<4.0 log (n = 45)	<i>P</i>
<b>(B) Fall after 48 hr of triple therapy</b>							
Arg70 and Leu91	3.8 (2.6–4.4)	27 (43.5%)	1 (25.0%)	NS	12 (57.1%)	16 (35.6%)	NS
Gln70(His70)	3.5 (2.8–4.3)	23 (37.1%)	3 (75.0%)	NS	6 (28.6%)	20 (44.4%)	NS
Met91	3.8 (2.8–4.5)	28 (45.2%)	2 (50.0%)	NS	8 (38.1%)	22 (48.9%)	NS
Gln70(His70) and Met91	3.5 (2.8–4.3)	16 (25.8%)	2 (50.0%)	NS	5 (23.8%)	13 (28.9%)	NS
ISDR wild-type	3.7 (2.6–4.5)	59 (95.2%)	4 (100%)	NS	20 (95.2%)	43 (95.6%)	NS

<sup>a</sup>Data are denoted by the median (range) values.

RNA level were not significantly different from that of patients who showed <2.0 log fall for all aa substitutions (Table II). However, a significantly higher proportion of patients with Arg70 and Leu91 substitutions showed ≥3.0 log drop in HCV RNA level than that of patients who showed a fall of <3.0 log (Table II, *P* = 0.008). In contrast, significantly fewer patients with Met91 showed ≥3.0 log drop in HCV RNA level than those who showed a fall of <3.0 log (*P* = 0.004). Likewise, significantly fewer patients with Gln70(His70) and Met91 showed a fall of ≥3.0 log in HCV RNA level than those who showed a fall of <3.0 log (Table II, *P* = 0.037). Thus, the fall in HCV RNA level at 24 hr was influenced by aa substitution patterns in the core region. Figure 2 shows the sequences of aa 61–110 of the core region in patients at the commencement of treatment.

At 48 hr, the proportion of patients who showed ≥3.0 log fall in HCV RNA was not significantly different from that of patients who showed <3.0 log drop for all aa substitutions (Table II). Similar results were noted in those patients who showed ≥ or <4.0 log fall in HCV RNA levels. Thus, the fall in HCV RNA level at 48 hr was independent of the aa substitution patterns in the core and NS5A regions.

Thus, the results did not identify aa substitution patterns in the upstream site of the NS3 region that influenced the fall in HCV RNA level from baseline after 24 and 48 hr of the commencement of the triple therapy. Furthermore, the frequency of the mutant-type in NS5A-ISDR was only 5%, and thus ISDR was not identified as a predictor of very early viral dynamics.

**Predictive Factors Associated With ≥3.0 Log Fall in HCV RNA Level at 24 hr**

Univariate analysis identified two parameters that correlated with a fall of ≥3.0 log in HCV RNA level after

24 hr of commencement of triple therapy either significantly or marginally: substitution of aa 70 and 91 (Arg70 and Leu91; *P* = 0.008) and level of viremia at baseline (≥7.0 log IU/ml; *P* = 0.054). Both these factors were also identified by multivariate analysis as independent parameters that either significantly or marginally influenced the ≥3.0 log fall in HCV RNA level after 24 hr of commencement of the triple therapy (Arg70 and Leu91; *P* = 0.014, HCV RNA ≥7.0 log IU/ml at baseline; *P* = 0.085, Table III).

**DISCUSSION**

Two previous studies (PROVE1 in USA, and PROVE2 in Europe) showed that 12-week triple therapy of telaprevir, PEG-IFN, and ribavirin could achieve undetectable HCV RNA levels in 70–80% of patients, and sustained virological response rates of 60–70% [Hézode et al., 2009; McHutchison et al., 2009]. In the present study, the rate of HCV RNA loss at 12 weeks were higher than those of the above two studies. The discrepancy between the present study and the above studies may be due to one or more factors. The first reason is probably the small number of Japanese patients infected with genotype 1b in the present study. The second could be the difference in body mass index. Body mass index of the patients studied (median; 23 kg/m<sup>2</sup>) was much lower than that of the participants of the previous study by McHutchison et al. (median; >25 kg/m<sup>2</sup>). The third reason is probably related to the type of PEG-IFN. PEG-IFN in the above reports was used at a fixed dose of PEG-IFNα-2a, but that of the present study was a body weight-adjusted dose of PEG-IFNα-2b. The present study was not designed to evaluate the sustained virological response and none of the patients was studied at 24 weeks after the end of the treatment protocol. Further studies of a larger number of patients matched for background are required to investigate the

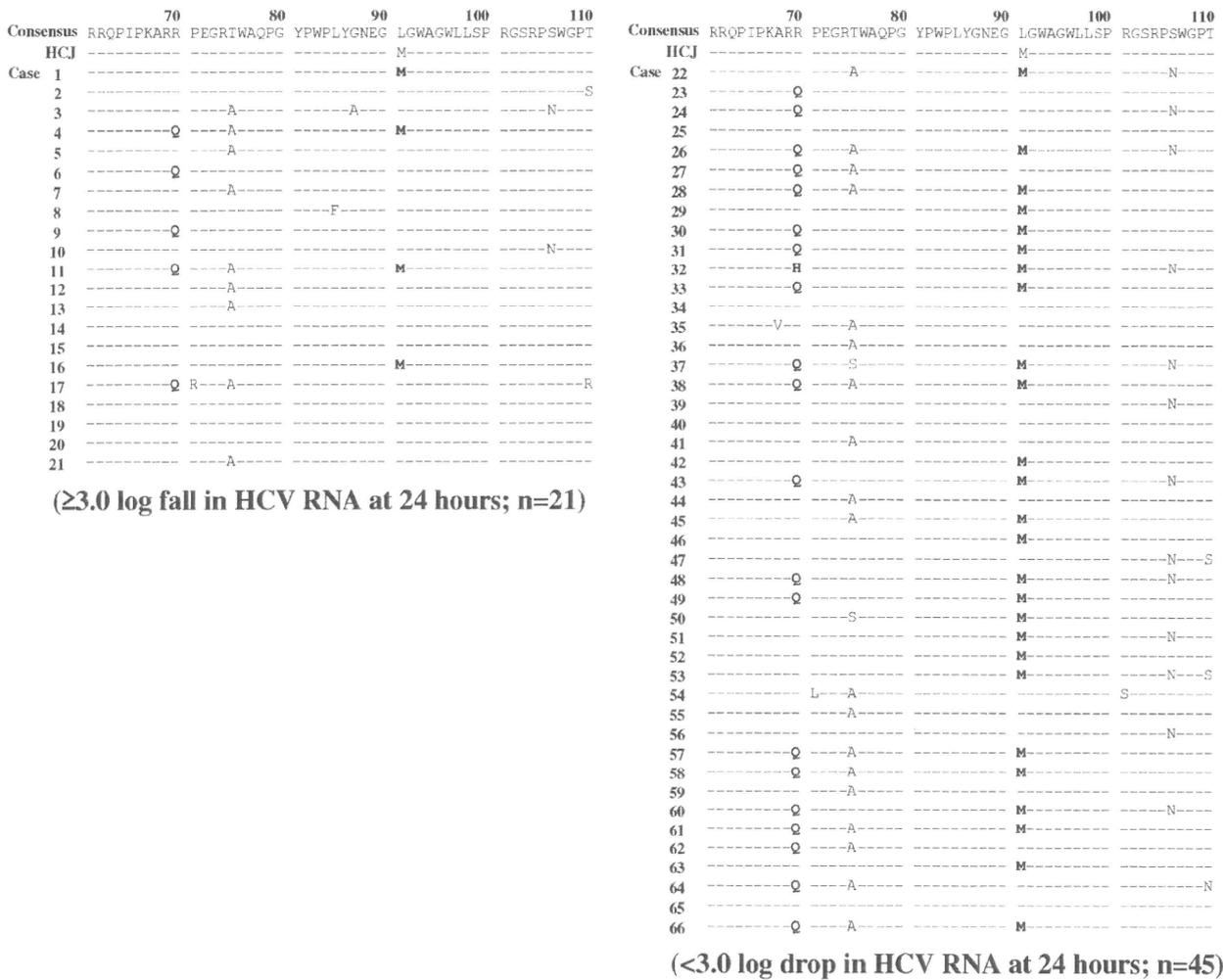


Fig. 2. Sequences of amino acids 61–110 in the core region at commencement of triple therapy in patients infected with HCV genotype 1b and high viral load. Dashes indicate amino acids identical to the consensus sequence of genotype 1b, and substituted amino acids are shown by standard single-letter codes. The amino acid patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters.

rate of HCV RNA loss during triple therapy and the sustained virological response rate.

A previous study based on a small number of 20 patients showed that the aa substitution pattern of the core region did not affect the virological response at 1 and 2 weeks after the start of triple therapy [Suzuki et al., 2009]. The present study is the first to report that the aa substitution pattern of the core region affect

significantly very early viral dynamics (within 48 hr) during triple therapy. Previous reports showed that very early dynamics (within 48 hr) after the start of IFN and ribavirin combination therapy was important for early prediction of treatment efficacy including sustained virological response [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Hence, the finding of the present study of aa substitution patterns

TABLE III. Multivariate Analysis of Factors Associated With ≥3.0 Log Fall in HCV RNA After 24-hr Triple Therapy of Telaprevir, Peginterferon, and Ribavirin Therapy in Japanese Patients Infected With HCV Genotype 1b

Factor	Category	Odds ratio (95% CI)	P
Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1	0.014
	2: Arg70 and Leu91	4.13 (1.33–12.8)	
Level of viremia (log IU/ml)	1: <7.0	1	0.085
	2: ≥7.0	2.73 (0.87–8.56)	

95% CI, 95% confidence interval. Only variables that achieved statistical significance ( $P < 0.05$ ) or marginal significance ( $P < 0.10$ ) on multivariate logistic regression analysis are shown.

of the core region as pretreatment predictor of very early viral dynamics could be also useful for early prediction of sustained virological response following triple therapy. Amino acid substitution patterns of the core region are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009]. Previous studies reported that the core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melén et al., 2004; de Lucas et al., 2005]. The present result could be also interpreted to mean that aa substitutions of the core region might be associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which is known to inhibit IFN- $\alpha$ -induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2',5'-OAS and MxA [Vlotides et al., 2004]. Furthermore, the result also indicates that aa substitutions of the core region might serve as a surrogate marker for other proteins associated with resistance to the antiviral actions of IFN. Further large-scale studies designed to examine the structural and functional impact of aa substitutions in the core region during each of monotherapy (PEG-IFN, ribavirin, and telaprevir), dual therapy (PEG-IFN/ribavirin and PEG-IFN/telaprevir), and triple therapy (PEG-IFN/ribavirin/telaprevir) should be conducted to confirm the above finding.

Another limitation of the present study was that aa substitutions in areas other than the core, the NS3, and the NS5A-ISDR regions of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRD, e.g., V3 of NS5A region) [El-Shamy et al., 2008; Muñoz de Rueda et al., 2008], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [Lin et al., 2005], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [Forestier et al., 2007; Zhou et al., 2007]. Thus, viral factors before and during triple therapy should be investigated in future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, a 12-week course of triple therapy of telaprevir, PEG-IFN, and ribavirin in patients infected with HCV-1b and high viral load achieved high rates of HCV RNA loss. The aa substitution pattern of the core region seems to affect the very early viral dynamics. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy.

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## Extending Combination Therapy with Peginterferon plus Ribavirin for Genotype 2 Chronic Hepatitis C Virological Responders: A Pilot Study of 7 Cases

Norio Akuta<sup>a</sup> Fumitaka Suzuki<sup>a</sup> Yasuji Arase<sup>a</sup> Miharuru Hirakawa<sup>a</sup>  
Yusuke Kawamura<sup>a</sup> Hiromi Yatsuji<sup>a</sup> Hitomi Sezaki<sup>a</sup> Yoshiyuki Suzuki<sup>a</sup>  
Tetsuya Hosaka<sup>a</sup> Masahiro Kobayashi<sup>a</sup> Mariko Kobayashi<sup>b</sup> Satoshi Saitoh<sup>a</sup>  
Kenji Ikeda<sup>a</sup> Hiromitsu Kumada<sup>a</sup>

<sup>a</sup>Department of Hepatology and <sup>b</sup>Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

### Key Words

Hepatitis C virus · Genotype 2 · Interferon · Ribavirin · Combination therapy, extended · Early virological response

### Abstract

**Objective:** In treatment-resistant patients with genotype 2 chronic hepatitis C the suitable treatment duration is still unclear. The aims were to investigate extending combination therapy with peginterferon plus ribavirin for genotype 2. **Methods:** 7 patients infected with genotype 2 at a high viral load and who did not achieve a sustained virological response (SVR) with the first course of 24-week IFN plus ribavirin were recruited into the study protocol with a total of 48 weeks of peginterferon plus ribavirin therapy. **Results:** SVR was achieved in 5 of 7 patients (71%). All 4 patients (100%) who were in relapse with the first course achieved SVR. Only 1 of 3 patients (33%) who had a non-virological response (NVR) with the first course achieved SVR. All 4 patients who had an early virological response (EVR) with the first course achieved EVR and SVR. Two of 3 patients who had no EVR with the first course also did not achieve EVR and SVR. One

patient who had no EVR or a NVR during the first course achieved EVR and SVR with the second course. **Conclusions:** Our results suggest that extending combination therapy for genotype 2 chronic hepatitis C might be useful for patients who relapse following 24-week combination therapy.

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### Introduction

The response to interferon (IFN)-related therapy varies according to hepatitis C virus (HCV) genotype [1, 2]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, and about 25% are genotype 2a [3]. The sustained virological response (SVR) to 48-week IFN plus ribavirin combination therapy is about 50% in genotype 1b infection, and the SVR to 24-week combination therapy is more than 80% in genotype 2 infection [4–9].

IFN plus ribavirin combination therapy carries potential serious side effects and is costly especially when used long enough to achieve a high SVR. For these reasons,

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Norio Akuta, MD  
Department of Hepatology  
Toranomon Hospital  
2-2-2 Toranomon, Minato-ku, Tokyo 105-0001 (Japan)  
Tel. +81 44 877 5111, Fax +81 44 860 1623, E-Mail akuta-gi@umin.ac.jp

especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (16 weeks or less) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. However, we also sometimes encounter treatment-resistant patients infected with genotype 2 [3, 10, 11]. Our recent report based on 24-week combination therapy showed that 17.5% of patients infected with genotype 2a were not able to achieve SVR, and especially that 81.5 and 18.5% of the non-SVR patients were in relapse or had a non-viral response (NVR), respectively [11]. Thus, the suitable treatment duration, based on the consideration of risk/benefit and cost/benefit, is still unclear in patients infected with genotype 2.

The present study included 7 Japanese adults with genotype 2 and a high viral load, who received a second course of combination therapy. The aims of the study were to investigate extending combination therapy with peginterferon (PEG)- $\alpha$ -2b plus ribavirin for genotype 2 chronic hepatitis C.

## Materials and Methods

### Study Population

A total of 292 HCV genotype 2-infected Japanese adult patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFN $\alpha$ -2b or IFN $\alpha$ -2b) plus ribavirin for 24 weeks between March 2002 and September 2008 at Toranomon Hospital, Tokyo, Japan. Among these, 7 of 52 patients who were not able to achieve a sustained virological response were recruited into the study protocol of 48-week combination therapy with PEG-IFN $\alpha$ -2b plus ribavirin. They fulfilled the following inclusion criteria: (1) no SVR with the first course of combination therapy regardless of completing the 24-week therapy; (2) combination therapy was stopped before completing the 24-week therapy due to a decrease in HCV RNA of  $<2.0$  log at 12 weeks after starting treatment based on qualitative PCR analysis [12, 13]; (3) negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emeryville, Calif., USA), and positive for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Pleasanton, Calif., USA); (4) infected with HCV genotype 2a or 2b alone; (5) high viral load ( $\geq 100$  KIU/ml) by quantitative analysis of HCV RNA with PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems Inc.) within the 2 months preceding enrolment; (6) no hepatocellular carcinoma; (7) body weight  $>40$  kg; (8) no co-infection with human immunodeficiency virus; (9) no treatment with antiviral or immunosuppressive agents within the 3 months preceding enrolment; (10) no alcoholics, lifetime cumulative alcohol intake  $<500$  kg (mild to moderate alcohol intake); (11) no other form of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease; (12) no

pregnant or lactating females; (13) all patients completed a 24-week follow-up program after cessation of treatment and SVR could be evaluated, and (14) each signed a form consenting to the study protocol that had been approved by the human ethics review committee.

Treatment efficacy was defined as: SVR = HCV-RNA-negative based on qualitative PCR analysis 24 weeks after the completion of treatment; relapse = HCV-RNA-negative at completion of treatment but HCV-RNA-positive 24 weeks after the completion, and NVR = HCV-RNA-positive at completion of treatment. Furthermore, an early virological response (EVR) was defined as patients who achieved a decrease in HCV-RNA of  $>2.0$  log within 12 weeks after starting treatment, based on quantitative PCR analysis.

### Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase and HCV-RNA levels. The serum samples were frozen at  $-80^{\circ}$  within 4 h of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [14]. HCV-RNA levels were measured by quantitative PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5–5,000 KIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA ( $<5$  KIU/ml) were also checked by qualitative PCR (Amplicor HCV v2.0, Roche Molecular Systems Inc.), which has a higher sensitivity than quantitative analysis, and the results are expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

### Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examinations contained 6 or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [15].

## Results

Table 1 summarizes the characteristics of the 7 patients at commencement of the second-course combination therapy with PEG-IFN plus ribavirin. There were 5 men and 2 women, aged 40–65 (median 55) years. Two cases were genotype 2a, and the other 5 cases were genotype 2b. They received PEG-IFN $\alpha$ -2b at a median dose of 1.4 (range 1.1–1.7)  $\mu$ g/kg subcutaneously each week. They also received oral ribavirin at a median dose of 10.6

**Table 1.** Baseline characteristics of patients infected with HCV genotype 2 at the commencement of the second-course combination therapy with peginterferon plus ribavirin, and treatment efficacy of the first and second course of combination therapy

Case No.	Genotype	Sex	Age years	Fibrosis	ALT IU/l	HCV RNA KIU/ml	1st EVR	1st Tx	2nd EVR	2nd Tx
1	2b	M	48	F1	41	5,000	+	relapse	+	SVR
2	2b	F	65	F1	35	1,200	+	relapse	+	SVR
3	2b	M	51	F3	71	310	+	relapse	+	SVR
4	2b	M	56	F1	78	720	+	relapse	+	SVR
5	2a	M	57	F1	240	1,500	-	NVR	+	SVR
6	2a	M	40	F2	434	650	-	NVR	-	NVR
7	2b	F	55	F3	132	1,300	-	NVR	-	NVR

EVR = Early virological response; NVR = non-virological response; SVR = sustained virological response; 1st EVR = EVR with the first course of combination therapy; 2nd EVR = EVR with the second course of combination therapy; Tx = treatment.

(range 7.0–12.6) mg/kg daily. In 3 patients (cases 1, 3, 7), the dose of ribavirin was reduced during treatment due to a fall in Hb concentration. Five patients (cases 1–5) achieved EVR and completed a total of 48 weeks. The other 2 patients did not achieve EVR, so they stopped combination therapy before completing the 48-week therapy (12 weeks for case 6, and 22 weeks for case 7).

#### *Virological Response Rates with the Second Course of Combination Therapy*

SVR was achieved by 5 of 7 patients (71.4%). All 4 patients (100%) who were in relapse with the first course of combination treatment achieved SVR with the second course. However, only 1 of 3 patients (33.3%) who had a NVR with the first course achieved SVR. All 4 patients (100%) who had an EVR with the first course achieved EVR and SVR with the second course. However, 2 of 3 patients (cases 6, 7) who had no EVR with the first course also did not have EVR and SVR with the second course. Thus, 2 patients (cases 6, 7) had no EVR and NVR with both the first and second courses, and could not achieve SVR. Interestingly, 1 patient (case 5) who had no EVR or NVR with the first course achieved EVR and SVR with the second course.

#### **Discussion**

In patients infected with genotype 1, previous studies have demonstrated that SVR rates of late virological responders (HCV-RNA-positive at 12 weeks and negative 24 weeks after the start of treatment) could be improved when treatment was extended to 72 weeks, compared

with a standard treatment duration of 48 weeks, largely as a result of reducing post-treatment relapse rates [16–20]. Thus, prolongation of therapy in genotype 1 may improve the virological response rate. However, it is not clear at present whether prolongation of treatment improves the SVR rate of treatment-resistant Japanese patients infected with genotype 2. This study of patients infected with genotype 2 showed that SVR rates of patients who were EVR and relapsed following the first course with a standard treatment duration of 24 weeks could be improved when treatment was extended to 48 weeks. Interestingly, 1 patient (case 5) who did not have EVR or NVR with the first course achieved EVR and SVR with the second course. This indicates that the SVR rates of patients who had an EVR with the second course might improve further by extending combination therapy regardless of NVR with the first course. To our knowledge, this is the first report to indicate that extending combination therapy to 48 weeks for genotype 2 might be useful.

In this study, 2 patients did not have an EVR or an NVR with both the first and second course and could not achieve SVR. The underlying mechanism(s) of the different virological responses to treatment in patients infected with genotype 2 is still unclear. Previous reports indicated that viral factors (e.g. viral load, aa substitutions in the NS5A region and core region, early viral kinetics, and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g. body mass index, fibrosis stage, and hepatocyte steatosis) might be important predictors of treatment response to IFN-related therapy in patients infected with HCV genotype 2a, in addition to treatment-related factors (e.g. treatment duration, and ribavirin dose) [6–11, 21–27]. One of the lim-

itations to this study is that due to the small number of patients we were not able to investigate treatment-resistant factors. Further studies should be performed to identify these viral and host factors before the start of combination therapy. Furthermore, more effective therapeutic regimens, including triple therapy with PEG-IFN plus ribavirin and telaprevir, should be developed for these patients who could not achieve SVR by extending dual therapy of PEG-IFN plus ribavirin.

In conclusion, our results suggest that extending combination therapy to 48 weeks for genotype 2 chronic hep-

atitis C might be useful for patients who had a relapse following the first course of 24-week combination therapy. In the future a large-scale prospective study based on intention-to-treat analysis should be conducted to confirm the above findings.

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