

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

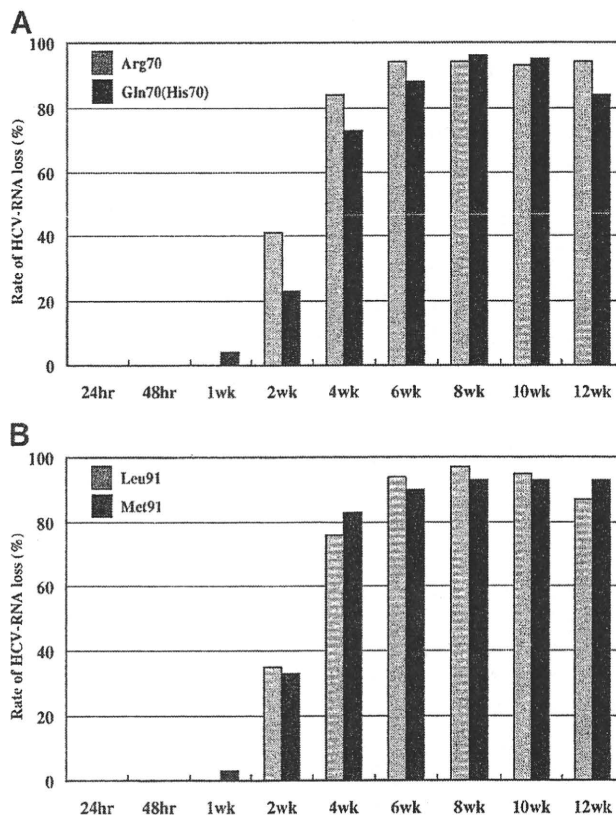


Fig. 1. Rates of HCV RNA loss according to substitutions of the core aa 70 and 91 at different time points after commencement of the triple therapy. At each time point, the rate of HCV RNA loss was not significantly different between Arg70 and Gln70(His70) (A) or between Leu91 and Met91 (B).

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>
(A) Fall after 24 hr of triple therapy							
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) Fall after 48 hr of triple therapy							
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 ( $\geq 7.0$  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  $\geq 7.0$  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-IFN $\alpha$ -2a, but that of the present study was a body weight-adjusted dose of PEG-IFN $\alpha$ -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

	70	80	90	100	110		70	80	90	100	110
Consensus	RRQIPKARR	PEGRTWAQPG	YPWPLYGNEG	LGWAGWLLSP	RGSRPSWGPT	Consensus	RRQIPKARR	PEGRTWAQPG	YPWPLYGNEG	LGWAGWLLSP	RGSRPSWGPT
<b>HCJ</b>			<b>M</b>			<b>HCJ</b>			<b>M</b>		
Case 1						Case 22		<b>A</b>		<b>M</b>	<b>N</b>
2					<b>S</b>	23	<b>Q</b>				
3		<b>A</b>	<b>A</b>			24	<b>Q</b>				<b>N</b>
4	<b>Q</b>	<b>A</b>		<b>M</b>		25					
5		<b>A</b>				26	<b>Q</b>	<b>A</b>		<b>M</b>	<b>N</b>
6	<b>Q</b>					27	<b>Q</b>	<b>A</b>			
7		<b>A</b>				28	<b>Q</b>	<b>A</b>		<b>M</b>	
8			<b>F</b>			29				<b>M</b>	
9		<b>Q</b>				30	<b>Q</b>			<b>M</b>	
10					<b>N</b>	31	<b>Q</b>			<b>M</b>	
11	<b>Q</b>	<b>A</b>		<b>M</b>		32	<b>H</b>			<b>M</b>	<b>N</b>
12		<b>A</b>				33	<b>Q</b>			<b>M</b>	
13		<b>A</b>				34					
14						35	<b>V</b>	<b>A</b>			
15						36		<b>A</b>			
16				<b>M</b>		37	<b>Q</b>	<b>S</b>		<b>M</b>	<b>N</b>
17	<b>Q</b>	<b>R</b>	<b>A</b>		<b>R</b>	38	<b>Q</b>	<b>A</b>		<b>M</b>	
18						39					<b>N</b>
19						40					
20						41		<b>A</b>			
21		<b>A</b>				42				<b>M</b>	
						43	<b>Q</b>			<b>M</b>	<b>N</b>
						44		<b>A</b>			
						45		<b>A</b>		<b>M</b>	
						46				<b>M</b>	
						47					<b>N</b>
						48	<b>Q</b>			<b>M</b>	<b>N</b>
						49	<b>Q</b>			<b>M</b>	<b>N</b>
						50		<b>S</b>		<b>M</b>	
						51				<b>M</b>	<b>N</b>
						52				<b>M</b>	
						53				<b>M</b>	<b>N</b>
						54	<b>L</b>	<b>A</b>			<b>S</b>
						55		<b>A</b>			
						56					<b>N</b>
						57	<b>Q</b>	<b>A</b>		<b>M</b>	
						58	<b>Q</b>	<b>A</b>		<b>M</b>	
						59		<b>A</b>			
						60	<b>Q</b>			<b>M</b>	<b>N</b>
						61	<b>Q</b>	<b>A</b>		<b>M</b>	
						62	<b>Q</b>	<b>A</b>			
						63				<b>M</b>	
						64	<b>Q</b>	<b>A</b>			<b>N</b>
						65					
						66	<b>Q</b>	<b>A</b>		<b>M</b>	

(≥3.0 log fall in HCV RNA at 24 hours; n=21)

(<3.0 log drop in HCV RNA at 24 hours; n=45)

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.

## REFERENCES

- Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. 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* 48:372–380.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007a. 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* 46:403–410.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H. 2007b. Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphisms in the viral core protein and very early dynamics of viremia. *Intervirology* 50:361–368.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007c. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 46:1357–1364.
- Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2009a. 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* 81:452–458.
- Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2009b. Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. *J Med Virol* 81:1032–1039.
- Blindenbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, Moradpour D, Blum HE, Alonzi T, Tripodi M, La Monica N, Heim MH. 2003. Expression of hepatitis C virus proteins inhibits interferon signaling in the liver of transgenic mice. *Gastroenterology* 124:1465–1475.
- Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, Heinrich PC, Haussinger D. 2003. IFN- $\alpha$  antagonistic activity of HCV core protein involved induction of suppressor of cytokine signaling-3. *FASEB J* 17:488–490.
- de Lucas, Bartolome J, Carreno V. 2005. Hepatitis C virus core protein down-regulates transcription of interferon-induced antiviral genes. *J Infect Dis* 191:93–99.
- Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE. 2007. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. *J Virol* 81:8211–8224.
- Dusheiko GM. 1998. The natural course of chronic hepatitis C: Implications for clinical practice. *J Viral Hepatol* 5:9–12.
- El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. 2008. Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 48:38–47.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C. 1995. Comparison of full-length sequences of interferon sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *J Clin Invest* 96:224–230.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 334:77–81.
- Fishman SL, Factor SH, Balestrieri C, Fan X, DiBisceglie AM, Desai SM, Benson G, Branch AD. 2009. Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 15:3205–3213.
- Forestier N, Reesink HW, Weegink CJ, McNair L, Kieffer TL, Chu HM, Purdy S, Jansen PL, Zeuzem S. 2007. Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. *Hepatology* 46:640–648.
- Fried MW, Shiffman ML, Reddy R, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347:975–982.
- Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L,

- McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlowsky JM, Zeuzem S, PROVE2 Study Team. 2009. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 360:1839–1850.
- Iino S, Tomita E, Kumada H, Suzuki H, Toyota J, Kiyosawa K, Tanikawa K, Sata M, Hayashi N, Kakumu S, Matsushima T, Mizokami M. 2005. Impact of daily high-dose IFN $\alpha$ -2b plus ribavirin combination therapy on reduction of ALT levels in patients with chronic hepatitis C with genotype 1 and high HCV RNA levels. *Hepatol Res* 31:88–94.
- Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H. 1998. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: A prospective observation of 2215 patients. *J Hepatol* 28:930–938.
- Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 87:9524–9528.
- Kenny-Walsh E. 1999. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med* 340:1228–1233.
- Lawitz E, Rodriguez-Torres M, Muir AJ, Kieffer TL, McNair L, Khunvichai A, McHutchison JG. 2008. Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis C patients. *J Hepatol* 49:163–169.
- Lin C, Gates CA, Rao BG, Brennan DL, Fulghum JR, Luong YP, Frantz JD, Lin K, Ma S, Wei YY, Perni RB, Kwong AD. 2005. In vitro studies of cross-resistance mutations against two hepatitis C virus serine protease inhibitors, VX-950 and BILN2061. *J Biol Chem* 280:36784–36791.
- Lin C, Kwong AD, Perni RB. 2006. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect Disord Drug Targets* 6:3–16.
- Makiyama A, Itoh Y, Yasui K, Mori K, Okita M, Nakayama M, Yamaoka J, Minami M, Nakajima T, Okanoue T. 2006. First phase viral kinetic parameters and prediction of response to interferon alpha-2b/ribavirin combination therapy in patients with chronic hepatitis C. *Hepatol Res* 36:94–99.
- Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling MH, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomized trial. *Lancet* 358:958–965.
- McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ, PROVEi Study Team. 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 360:1827–1838.
- Melen K, Fagerlund R, Nyqvist M, Keskinen P, Julkunen I. 2004. Expression of hepatitis C virus core protein inhibits interferon-induced nuclear import of STATs. *J Med Virol* 73:536–547.
- Modi AA, Hoofnagle JH. 2007. New therapies for hepatitis C. *Hepatology* 46:615–617.
- Muñoz de Rueda P, Casado J, Patón R, Quintero D, Palacios A, Gila A, Quiles R, León J, Ruiz-Extremera A, Salmerón J. 2008. Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. *J Virol* 82:6644–6653.
- Nakamoto S, Imazeki F, Fukai K, Fujiwara K, Arai M, Kanda T, Yonemitsu Y, Yokosuka O. 2009. Association between mutations in the core region of hepatitis C virus genotype 1 and hepatocellular carcinoma development. *J Hepatol* [Epub ahead of print].
- Niederer C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. 1998. Progress of chronic hepatitis C: Results of a large, prospective cohort study. *Hepatology* 28:1687–1695.
- Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H. 2009. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: A Japanese multi-center study. *J Gastroenterol* 44: 952–963.
- Suzuki F, Akuta N, Suzuki Y, Sezaki H, Yatsuji H, Kawamura Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Mineta R, Iwasaki S, Watahiki S, Kobayashi M, Miyakawa Y, Kumada H. 2009. Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks. *Hepatol Res* 39:1056–1063.
- Tsubota A, Arase Y, Someya T, Suzuki Y, Suzuki F, Saitoh S, Ikeda K, Akuta N, Hosaka T, Kobayashi M, Kumada H. 2005. Early viral kinetics and treatment outcome in combination of high-dose interferon induction vs. pegylated interferon plus ribavirin for naive patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 75:27–34.
- Vlotides G, Sorenson AS, Kopp F, Zitzmann K, Cengic N, Brand S, Zachoval R, Auernhammer CJ. 2004. SOCS-1 and SOCS-3 inhibit IFN- $\alpha$ -induced expression of the antiviral proteins 2,5-OAS and MxA. *Biochem Biophys Res Commun* 30:1007–1014.
- Zeuzem S. 2008. Telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in chronic hepatitis C patients. *J Hepatol* 49:157–159.
- Zhou Y, Müh U, Hanzelka BL, Bartels DJ, Wei Y, Rao BG, Brennan DL, Tigges AM, Swenson L, Kwong AD, Lin C. 2007. Phenotypic and structural analyses of hepatitis C virus NS3 protease Arg155 variants: Sensitivity to telaprevir (VX-950) and interferon alpha. *J Biol Chem* 282:22619–22628.

## 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.

Copyright © 2010 S. Karger AG, Basel

### 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,

KARGER

Fax +41 61 306 12 34  
E-Mail karger@karger.ch  
www.karger.com

© 2010 S. Karger AG, Basel  
0300–5526/10/0533–0188\$26.00/0

Accessible online at:  
www.karger.com/int

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 laparoscopy 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.

#### Acknowledgement

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

#### References

- 1 Simmonds P: Clinical relevance of hepatitis C virus genotypes. *Gut* 1997;40:291-293.
- 2 Haydon GH, Jarvis LM, Blair CS, Simmonds P, Harrison DJ, Simpson KJ, Hayes PC: Clinical significance of intrahepatic hepatitis C virus levels in patients with chronic HCV infection. *Gut* 1998;42:570-575.
- 3 Akuta N, Suzuki F, Tsubota A, Suzuki Y, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: 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;37:831-836.
- 4 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling MH, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. *Lancet* 2001;358:958-965.
- 5 Fried MW, Shiffman ML, Reddy R, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-982.
- 6 Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A: Peginterferon alfa-2b and ribavirin for 12 versus 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005;352:2609-2617.
- 7 Mangia A, Minerva N, Bacca D, Cozzolongo R, Agostinacchio E, Sogari F, Scotto G, Vinelli F, Ricci GL, Romano M, Carretta V, Petruzzellis D, Andriulli A: 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;49:358-363.
- 8 von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Häussinger D, Herrmann E, Zeuzem S: Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005;129:522-527.
- 9 Fujiwara K, Yokosuka O, Komine F, Moriyama M, Kato N, Yoshida H, Tanaka N, Imazeki F, Shiratori Y, Arakawa Y, Omata M; Tokyo Hepatitis Network: Twenty-four weeks of interferon alpha-2b in combination with ribavirin for Japanese hepatitis C patients: sufficient treatment period for patients with genotype 2 but not for patients with genotype 1. *Liver Int* 2006;26:520-528.
- 10 Akuta N, Suzuki F, Tsubota A, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in nonstructural protein 5A of hepatitis C virus genotype 2a low viral load and response to interferon monotherapy. *J Med Virol* 2003;69:376-383.
- 11 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 2a high viral load and virological response to interferon-ribavirin combination therapy. *Intervirology* 2009;52:301-309.
- 12 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL Jr, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-982.
- 13 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;38:645-652.
- 14 Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T, Kumada H: Genotypic subtyping of hepatitis C virus. *J Gastroenterol Hepatol* 1993;8:150-156.
- 15 Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ: Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994;19:1513-1520.
- 16 Buti M, Valdés A, Sánchez-Avila F, Esteban R, Lurie Y: Extending combination therapy with peginterferon alfa-2b plus ribavirin for genotype 1 chronic hepatitis C late responders: a report of 9 cases. *Hepatology* 2003;37:1226-1227.
- 17 Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S: Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006;130:1086-1097.
- 18 Sánchez-Tapias JM, Diago M, Escartín P, Enríquez J, Romero-Gómez M, Bárcena R, Crespo J, Andrade R, Martínez-Bauer E, Pérez R, Testillano M, Planas R, Solá R, García-Bengochea M, García-Samaniego J, Muñoz-Sánchez M, Moreno-Otero R; Ter-aViC-4 Study Group: Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006;131:451-460.
- 19 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;46:1688-1694.

- 20 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: 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;81:452-458.
- 21 Nousbaum JB, Cadranet JF, Savary O, Le-grand MC, Dumouchel P, Gou  rou H: Sustained virological response after a short course of treatment with interferon and ribavirin in two chronic hepatitis C patients. *J Hepatol* 2003;39:655-656.
- 22 Dalgard O, Bj  ro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H: Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* 2004;40:1260-1265.
- 23 Nagase Y, Yotsuyanagi H, Okuse C, Yasuda K, Kato T, Koike K, Suzuki M, Nishioka K, Iino S, Itoh F: Effect of treatment with interferon alpha-2b and ribavirin in patients infected with genotype 2 hepatitis C virus. *Hepatol Res* 2008;38:252-258.
- 24 Nomura H, Miyagi Y, Tanimoto H, Ishibashi H: Impact of early viral kinetics on pegylated interferon alpha 2b plus ribavirin therapy in Japanese patients with genotype 2 chronic hepatitis C. *J Viral Hepat* 2009;16:346-351.
- 25 Toyoda H, Kumada T, Kiriya S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Atsumi H, Nakano S, Arakawa T: Eight-week regimen of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C with hepatitis C virus genotype 2 and a rapid virological response. *Liver Int* 2009;29:120-125.
- 26 Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C: Mutations in non-structural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. *Hepatology* 1999;30:1045-1053.
- 27 Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H: Hepatocyte steatosis is an important predictor of response to interferon (IFN) monotherapy in Japanese patients infected with HCV genotype 2a: virological features of IFN-resistant cases with hepatocyte steatosis. *J Med Virol* 2005;75:550-558.

## Efficacy and Safety of Combination Therapy of Natural Human Interferon $\beta$ and Ribavirin in Chronic Hepatitis C Patients with Genotype 2 and High Virus Load

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

### Abstract

**Objective** The aim of this study was to evaluate the efficacy of combination therapy of natural human interferon-beta and ribavirin in patients infected with hepatitis C virus (HCV) genotype 2 and high virus load.

**Methods** Inclusion criteria were HCV-genotype 2, serum HCV RNA level of  $\geq 100$  KIU/mL before combination therapy. A total of 24 were enrolled in this retrospective cohort study. The treatment period of combination therapy was 24 weeks.

**Results** Of the 24 study patients, no patient stopped the treatment due to treatment-related adverse events. The dose of drugs were reduced in 8 patients. Twenty-one of 24 patients (87.5%) had sustained virological response (SVR) by the intention to treat analysis. The rate of negative HCV RNA at 8 week after the initiation of treatment was 18/21 (86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78;  $p=0.021$ )

**Conclusion** The combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and high virus load.

**Key words:** chronic hepatitis C, natural interferon-beta, ribavirin, HCV genotype 2

(Inter Med 49: 965-970, 2010)

(DOI: 10.2169/internalmedicine.49.3299)

### Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-10). SVR in the patients with HCV genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in group of low virus load and about 40-45% in high virus load (11). However, it has been reported that the SVR rate was about 80-90% in patients with genotype 2 and high virus load treated

with peginterferon and ribavirin for 24 week (12-14). Hence, IFN-monotherapy has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and low virus-load in Japan. On the other hand, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and high virus-load. Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and high virus load who showed low rate of SVR.

However, the dropout rates in patients treated with combination therapy of peginterferon and ribavirin are higher than those treated with IFN monotherapy (15-17). In particular,

<sup>1</sup>Department of Hepatology, Toranomon Hospital, Tokyo and <sup>2</sup>Hepatic Research Unit, Toranomon Hospital, Tokyo

Received for publication December 22, 2009; Accepted for publication February 10, 2010

Correspondence to Dr. Yasuji Arase, es9y-ars@asahi-net.or.jp

the adverse events due to combination therapy of IFN and ribavirin have a tendency to occur in elderly patients. Therefore, in the case of elderly patients, the physician in charge often avoids combination therapy of IFN and ribavirin due to side effects. However, recently, the life-span has been long in Japan. Thus, there is an ongoing need to refine treatment strategies with a strong effect and safety in HCV patients.

Festi et al reported that IFN-beta has sufficient tolerability (15). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with a high virus load (11). Enomoto et al have reported that IFN-beta plus ribavirin therapy might seem to have a strong effect and mild side effects originating from treatment (18, 19). However, to date there is little information regarding IFN-beta plus ribavirin therapy for chronic hepatitis C.

Thus, in the present study, we performed a retrospective study to examine the efficacy of combination therapy of IFN-beta and ribavirin in patients with genotype 2 and high virus load.

## Materials and Methods

### Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 2a or 2b; 2) serum level of HCV RNA of  $\geq 100$  KIU/mL before combination therapy; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 5) leukocytes  $>2,000/\text{mm}^3$ , platelet count  $>80,000/\text{mm}^3$ , and bilirubin  $<2.0$  mg/mL; 6) follow up for  $>6$  months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions and informed consent was obtained from each patient.

From December 2004 to May 2008, 24 HCV patients were enrolled in this retrospective cohort study at the study hospital.

A SVR was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver. 2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (20).

Next, predictors of SVR in patients with undetectable HCV RNA in serum during treatment were assessed. Finally, SVR rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy were examined.

### Combination therapy of IFN-beta and ribavirin

The study protocol was approved by the Human Ethics

Review Committee of Toranomon Hospital and a signed consent form was obtained each patient. Treatment was provided for 24 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks initially, followed by three times a week for 16-22 weeks. Ribavirin (Rebetol, Schering-Plough, Osaka, Japan) were given at the dose described based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for  $\leq 60$  kg, 800 mg for  $>60$  kg and  $\leq 80$  kg, and 1000 mg for  $>80$  kg). The period of daily administration in IFN-beta treatment was determined by the physician. The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at  $-80^\circ\text{C}$  until analyzed. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (21). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (22). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (20). Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

### Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher's exact test, Kruskal Wallis test, and/or logistic regression analysis. The following variables were evaluated as prognostic factors: sex, age, body mass index, a history of interferon therapy, a HCV RNA level, biochemical factors (AST, ALT, triglyceride, HDL-cholesterol, LDL-cholesterol), platelet count, HCV RNA 4, 8, 12 weeks after the initiation of IFN therapy, continuous negative period of HCV RNA during IFN therapy and period of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of  $<0.05$  was considered to indicate a significant difference.

## Result

### Clinical characteristics of the patients

A total of 24 patients were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age=55.9 years, male/female=11/13, and median

**Table 1. Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients**

Character	value
Patients, n	24
Sex, male (%)	11(45.8%)
Age (yrs)	55.9±10.2
BMI	23.0±2.5
A history of IFN (+)	12 (50.0%)
HCV RNA(KIU/mL)	870 (43-5000)
HCV genotype (2a/2b)	14/10
AST (IU/L)	71±51
ALT (IU/L)	130±122
FPG (mg/dL)	96±13
Triglyceride (mg/dL)	111±73
HDL cholesterol (mg/dL)	52±19
LDL cholesterol (mg/dL)	117±31
Platelet (10 <sup>4</sup> /mm <sup>3</sup> )	16.6±4.5
A regimen of daily administration of IFN-beta* (2-week/4-week/8-week)	10/5/9

Data are number of patients (percentage) or mean ± standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

\*The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen of daily administration of IFN-beta, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

(range) HCV-RNA=870(103-5,000) KIU/mL.

### Safety and tolerance of IFN

Of the 24 patients included in this study, none of the patients discontinued combination therapy because of IFN-related adverse events. However, 7 out of 24 patients had dose reduction of interferon and/or ribavirin due to side effects. IFN-beta dose reduction was necessary in one case due to the development of neutropenia. RBV dose reduction was applied in 6 patients, due to anemia.

The leukocyte count was  $4,700 \pm 1,390/\text{mm}^3$  and the platelet count was  $166,000 \pm 45,000/\text{mm}^3$  before the initiation of IFN therapy, whereas the values were  $3,020 \pm 1,05/\text{mm}^3$  and  $134,000 \pm 39,000/\text{mm}^3$ , respectively, two weeks after the initiation of the therapy.

### Efficacy of treatment

Out of the 24 patients enrolled in the present study, 21

patients (87.5%) had SVR by the intention-to-treat analysis. Patients aged  $\geq 65$  years were five in total. Four out of five patients aged  $\geq 65$  years had SVR. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The rate of negative HCV RNA at 8 weeks after the initiation of treatment was 18/21(86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 weeks after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78;  $P=0.021$ ). Moreover, the SVR was not significantly different based on the difference of period of daily administration of IFN-beta at the initial stage of treatment.

### Background of non-SVR cases

Three patients had negative HCV RNA at the end stage of treatment, but showed reappearance of HCV RNA after

**Table 2. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR**

	SVR (n=21)	Non-SVR (n=3)	p value <sup>†</sup>
Age (years old)	56.1 ± 9.1	57.0 ± 8.0	0.827
Sex (male/female)	12/9	2/1	0.449
BMI	22.9 ± 2.5	22.8 ± 2.6	1.000
a history of IFN (+/-)	11/10	1/2	0.759
HCV-load (KIU/mL)	794 ± 786	1545 ± 1797	0.759
AST (IU/L)	69 ± 47	44 ± 12	0.540
ALT (IU/L)	83 ± 39	70 ± 55	0.359
FPG (mg/dL)	96 ± 13	92 ± 3	0.813
Triglyceride (mg/dL)	112 ± 74	107 ± 57	0.614
HDL cholesterol (mg/dL)	51 ± 20	65 ± 17	0.297
LDL cholesterol (mg/dL)	113 ± 31	126 ± 15	0.540
Platelet (10 <sup>4</sup> /mm <sup>3</sup> )	16.3 ± 4.7	17.7 ± 5.3	0.701
HCV RNA (+/-) 4W	9/12	2/1	0.576
HCV RNA (+/-) 8W	3/18	2/1	0.099, 0.021 <sup>‡</sup>
HCV RNA (+/-) 12W	0/21	0/3	1.000
Period of daily administration of IFN* (2-week/4-week/8-week)	9/4/8	1/1/1	0.925

Data are number of patients (percentage) or mean ± standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

\*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks, followed by three times a week for 16-22 weeks. Figure of 2, 4, and 8 represents the (week) of daily administration of IFN-beta at the initial stage.

<sup>†</sup>Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher's exact test, Kruskal wallis test.

<sup>‡</sup>Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval = 1.75-914.78; p = .021)

the termination of treatment. Clinical backgrounds of these three cases with relapse of HCV RNA after the termination of treatment are shown in Table 3. In case 1 and 2, the attainment time of negativity of serum HCV RNA was 12 weeks after the initiation of treatment. In case 3, the adherence of both drugs of IFN-beta and ribavirin was less than two-third compared to scheduled dose.

## Discussion

We have described the efficacy of combination therapy of

IFN-beta and ribavirin in patients infected with HCV genotype 2a or 2b. The present study was limited to small size with genotype 2 and HCV-load of ≥100 KIU/mL and high virus load before combination therapy. SVR in the patients with genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in the group with a low virus load and about 40-45% with high virus load (11). Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and a high virus load who showed low rate of SVR. Moreover, 7 of 24 patients did not have a histological examination of the liver within one year before combination

**Table 3. Clinical Backgrounds of Patients with Non-SVR**

Case	Age/Sex	genotype	HCV	AST/ALT	response*	Adherence (%)	
			RNA	(IU/L)		IFN	RBV
1	53/M	2a	220	51/104	12W	104%	100%
2	67/M	2b	5000	30/27	12W	82%	84%
3	51/F	2a	103	50/51	4W	62%	68%

Data are number of patients (percentage) or mean  $\pm$  standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis

C virus; IFN, interferon; RBV, ribavirin

\*Response of HCV RNA means attainment time of negativity of serum HCV

RNA after the initiation of combination therapy

therapy. Another limitation is that the present study was not a randomized controlled study.

However, several findings from the present study have direct implications for combination therapy for chronic hepatitis C in the future. First, the present results suggest that drop-out rate due to side effects in combination therapy of IFN-beta and ribavirin is low. In the previous study, we have reported that the drop-out rate due to side effects in combination study of peginterferon and ribavirin was 8.4% in 0.5 year after the initiation of treatment and 14.9% in one year (15). In the present study, none of the patients discontinued combination therapy because of IFN-related adverse events.

Secondly, out of 24 patients given the combination therapy, 21 patients had SVR. This SVR rate is similar to that of the 24-week combination therapy of peginterferon and ribavirin reported previously (11-13).

Third, the patients with genotype 2 have the possibility of non-SVR in a regimen for 24-weeks when the attainment time of negativity of serum HCV RNA is longer than 8 weeks after the initiation of combination therapy. This indi-

cates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period of >24 weeks to obtain a high rate of SVR.

IFN-beta should be given intravenously. The intravenous injection is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to combination therapy of IFN-alpha and ribavirin (18, 19). Moreover, IFN-beta-induced mental disorders are milder than those induced by IFN-alpha (23). Thus, IFN-beta could be given in elderly patients of  $\geq 65$  years because of mild side effects (24).

In conclusion, the combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and a high virus load.

#### Acknowledgement

The present work was supported in part by grants-in-aid from the Japanese Ministry of Health, Labour and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

#### References

- 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* 358: 958-965, 2001.
- Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347: 975-982, 2002.
- Hadziyannis SJ, Sette H Jr, Morgan TR, et al; PEGASYS International Study Group. Peginterferon-alpha 2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 140: 346-355, 2004.
- 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* 123: 1061-1069, 2002.
- Shiffman ML, Di Bisceglie AM, Lindsay KL, et al; Hepatitis C Antiviral Long-Term Treatment against Cirrhosis Trial Group. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 126: 1015-1023, 2004.
- 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* 132: 103-112, 2007.
- Schalm SW, Weiland O, Hansen BE, et al; Eurohep Study Group for Viral Hepatitis. Interferon-ribavirin for chronic hepatitis C with and without cirrhosis: analysis of individual patient data of six controlled trials. *Gastroenterology* 117: 408-413, 1999.
- Bronowicki JP, Ouzan D, Asselah T, et al. Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 131: 1040-1048,

- 2006.
9. Jensen DM, Morgan TR, Marcellin P, et al. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* **43**: 954-960, 2006.
  10. Bruno S, Cammà C, Di Marco V, et al. Peginterferon alfa-2b plus ribavirin for naïve patients with genotype 1 chronic hepatitis C: a randomized controlled trial. *J Hepatol* **41**: 474-481, 2004.
  11. Mamori S, Suzuki F, Hosaka T, et al. Interferon monotherapy for patients with chronic hepatitis C and normal serum aminotransferase levels at commencement of treatment. *J Gastroenterol* **39**: 776-782, 2004.
  12. Dalgard O, Bjørø K, Hellum KB, et al. Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* **40**: 1260-1265, 2004.
  13. 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* **352**: 2609-2617, 2005.
  14. von Wagner M, Huber M, Berg T, et al. Peginterferon-alpha-2a (40 KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* **129**: 522-527, 2005.
  15. Festi D, Sandri L, Mazzella G, et al. Safety of interferon beta treatment for chronic HCV hepatitis. *World J Gastroenterol* **10**: 12-16, 2004.
  16. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* **43**: 54-63, 2006.
  17. Arase Y, Suzuki F, Suzuki Y, et al. Side effects of combination therapy of peginterferon and ribavirin for chronic hepatitis-C. *Intern Med* **46**: 1827-1832, 2007.
  18. Kurowski M, Enomoto N, Murakami T, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NSSA region of hepatitis C virus in relation to the response to interferon-beta therapy. *Hepatology* **25**: 750-753, 1997.
  19. Enomoto M, Tamori A, Kawada N, et al. Interferon-beta plus ribavirin for patients with hepatitis C virus genotype 1: a randomized pilot trial. *Gut* **55**: 139-140, 2006.
  20. Doglio A, Laffont C, Caroli-Bosc FX, et al. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. *J Clin Microbiol* **37**: 1567-1569, 1999.
  21. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* **36**: 862-865, 1998.
  22. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* **19**: 13-18, 1994.
  23. Katamura Y, Suzuki F, Akuta N, et al. Natural human interferon beta plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus and a high viral load. *Intern Med* **47**: 1827-1834, 2008.
  24. Arase Y, Suzuki F, Sezaki H, et al. Suitable treatment period in patients with virological response during combination therapy of peginterferon and ribavirin for chronic hepatitis C. *Intern Med* **47**: 1301-1307, 2008.



## HEPATOLOGY

**Efficacy of switching to entecavir monotherapy in Japanese lamivudine-pretreated patients**

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

\*Department of Hepatology, Toranomon Hospital, Tokyo, and <sup>†</sup>Research Institute for Hepatology, Toranomon Branch Hospital, Kawasaki, Japan

**Key words**

entecavir, hepatitis B virus, lamivudine, viral resistance.

Accepted for publication 6 October 2009.

**Correspondence**

Dr Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan.  
Email: fumitakas@toranomon.gr.jp

**Abstract**

**Background and Aims:** To assess the efficacy of switching Japanese chronic hepatitis B patients from lamivudine monotherapy to entecavir 0.5 mg/day.

**Methods:** A retrospective analysis was conducted on 134 patients switched to entecavir between September 2006 and February 2008 for 6 months or more. Patients were divided into three groups based on viral load at entecavir switching point (baseline < 2.6, 2.6–5.0 and > 5.0 log<sub>10</sub> copies/mL).

**Results:** At baseline, detection of lamivudine-resistant virus was highest in patients with higher hepatitis B virus (HBV) DNA (76% vs 23% in ≥ 2.6 and < 2.6 log<sub>10</sub> copies/mL, respectively), and in patients with longest previous exposure to lamivudine (52%, 28% and 24% for > 3 years, 1–3 years and < 1 year, respectively). Two years after entecavir switching, HBV DNA suppression to less than 2.6 log<sub>10</sub> copies/mL was achieved in 100% (32/32), 92% (12/13) and 44% (4/9) of patients in the less than 2.6, 2.6–5.0 and more than 5.0 log<sub>10</sub> copies/mL baseline groups, respectively. Alanine aminotransferase (ALT) normalization occurred in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively. One patient (2.6–5.0 log<sub>10</sub> copies/mL) with lamivudine-resistant mutants at baseline developed entecavir resistance at week 48 during follow up.

**Conclusion:** Switching to entecavir 0.5 mg/day achieves or maintains undetectable HBV DNA levels and ALT normalization over 2 years, especially in patients with viral load less than 5.0 log<sub>10</sub> copies/mL.

**Introduction**

Hepatitis B virus (HBV) infection is a serious public health threat affecting 350–400 million people worldwide, the majority of whom live in the Asia-Pacific region.<sup>1,2</sup> Chronically-infected people are at risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Studies have suggested that high serum HBV DNA is a key risk predictor of chronic hepatitis B (CHB) complications.<sup>3,4</sup> Therefore, the main purpose of CHB therapies is to permanently suppress viral replication and sustain viral suppression to prevent long-term liver damage.<sup>2,5,6</sup>

Lamivudine was the first nucleoside analog to be widely prescribed for CHB patients, mainly due to its antiviral efficacy and safety profile.<sup>2</sup> However, lamivudine's long-term efficacy is diminished by the emergence of drug-resistant substitutions, generally in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of the reverse transcriptase (rt) polymerase gene.<sup>7–9</sup> Detection of lamivudine-resistant HBV substitutions occurs in 15–30% and 70% of patients after 1 and 5 years of treatment, respectively.<sup>8</sup> Continuing lamivudine monotherapy in the presence of

lamivudine resistance is not recommended because it is no longer effective in suppressing viral replication.<sup>2</sup> Furthermore, the initial improvement in histology and clinical benefits may be reversed or decreased due to the emergence of lamivudine-resistant substitutions.

Antiviral efficacy of entecavir (0.5 mg/day) as first-line therapy was superior to lamivudine in treatment-naïve patients on all virological, biochemical and histological end-points after 48 weeks of treatment,<sup>10–14</sup> with very low rates of emergence of viral resistance (1.2% after 5 years of entecavir treatment).<sup>15,16</sup> Entecavir has a high genetic barrier to resistance,<sup>17–19</sup> requiring multiple substitutions (including YMDD mutations) to express viral resistance.<sup>16–21</sup> In agreement with this, entecavir-resistant mutants emerge more frequently in lamivudine-refractory patients.<sup>22,23</sup> In a study of hepatitis B e antigen (HBeAg)-positive lamivudine-refractory patients with high HBV DNA levels at baseline (mean > 9 log<sub>10</sub> copies/mL), switching to entecavir 1 mg/day achieved HBV DNA suppression to undetectable levels (< 300 copies/mL; 40%, 96 weeks) and alanine aminotransferase (ALT) normalization (81%, 96 weeks) at higher proportions than continued lamivudine

monotherapy,<sup>22</sup> although response to therapy was less pronounced than in treatment-naïve patients with comparable baseline levels of HBV DNA.<sup>10,13,14</sup> The probability of achieving HBV DNA suppression to undetectable levels at 96 weeks with entecavir was 73% in patients whose baseline HBV DNA was less than 7 log<sub>10</sub> copies/mL ( $n = 11$ ), and none of these patients developed entecavir resistance.<sup>22</sup>

In a randomized controlled trial of lamivudine-refractory Japanese patients with mean HBV DNA at baseline of 7.6–7.7 log<sub>10</sub> copies/mL, switching to entecavir (0.5 or 1 mg/day) for 48 weeks achieved HBV DNA suppression to below detectable levels in 33% of patients in the entecavir dose groups, and ALT normalization in 78–86%.<sup>24</sup> Switching to entecavir in patients with evidence of lamivudine-resistant substitutions and low viral load at switching point has not been prospectively investigated in Japanese patients. There are limited data concerning the efficacy of entecavir in lamivudine-pretreated patients who have not developed lamivudine resistance.

The objective of this study was to assess the efficacy of switching to entecavir 0.5 mg/day in Japanese lamivudine-pretreated patients whose HBV DNA levels at switching point (baseline) ranged from less than 2.6 to 7.6 log<sub>10</sub> copies/mL, with or without lamivudine-resistant substitutions.

## Methods

### Design and setting

A retrospective analysis of a CHB patient population ( $n = 134$ ) at Toranomon Hospital (Tokyo, Japan) was performed to identify patients switched from lamivudine 100 mg/day monotherapy to entecavir 0.5 mg/day between September 2006 and February 2008, and who had received entecavir for at least 6 months. Among all patients selected, only one had a history of adefovir add-on therapy prior to switching to entecavir (case report). Conserved serum from all patients was analyzed to determine baseline characteristics and study end-points.

### Study end-points

Clinical efficacy of entecavir was assessed as the proportion of patients achieving HBV DNA suppression to undetectable levels (< 400 copies/mL or < 2.6 log<sub>10</sub> copies/mL), and patients achieving ALT normalization (normal ALT levels: men 8–42 IU/L, women 6–27 IU/L). HBV DNA was measured using the polymerase chain reaction (PCR)-based Amplicor HBV Monitor assay (Roche Diagnostics, Indianapolis, IN, USA; lower limit of detection of < 2.6 log<sub>10</sub> copies/mL).<sup>25</sup> HBeAg loss in patients who were HBeAg-positive at baseline was also analyzed. Measurements were made from conserved samples taken at baseline, and after 6 months, 1 and 2 years from entecavir treatment initiation.

### Assessment of viral resistance

Conserved serum was used to detect the presence of viral lamivudine-resistant rtM204V/I substitutions in all patients at baseline, and following the entecavir switch in patients treated with entecavir for at least 6 months. Lamivudine-resistant virus (rtM204V/I or YMDD motif substitutions) was analyzed using a

combination of the quantitative enzyme-linked immunosorbent assay standardized using a purified *Taenia solium* cysticerci fraction (PCR enzyme-linked immunosorbent assay) and the enriched PCR enzyme linked minisequence assay.<sup>26</sup> Direct sequencing of HBV DNA polymerase reverse transcriptase site was also performed.<sup>27</sup> Detection of entecavir-resistant virus was conducted using direct sequencing of HBV DNA polymerase reverse transcriptase site.<sup>27</sup>

## Data analyses

Statistical comparisons between treatment groups were assessed using  $\chi^2$ -test and Kruskal–Wallis test where appropriate. Calculations were performed using StatView software (ver. 4.5J; Abacus Concepts, Berkeley, CA, USA). A two-tailed  $P$ -value less than 0.05 was considered statistically significant.

To identify predictive factors of HBV DNA negativity (suppression to below detectable levels) after 6 months of the entecavir switch, univariate and multivariate logistic regression analyses were carried out. Potential predictive factors at baseline included: sex; age; levels of aspartate aminotransferase (AST), ALT, albumin,  $\gamma$ -glutamyl transpeptidase, total bilirubin and  $\alpha$ -fetoprotein; platelet count; viral load; liver disease stage (cirrhosis or other); family history; HBV genotype; lamivudine treatment duration prior to entecavir switch; HBeAg status; and lamivudine resistance. Each variable was transformed into categorical data consisting of two simple ordinal numbers. All factors that were at least marginally associated with HBV DNA negativity ( $P < 0.10$ ) were used in a multiple logistic regression analysis. To assess relative risk confidence, odds ratio (OR) and 95% confidence interval (CI) were calculated. All analyses were performed using SPSS II software ver. 11.0 (SPSS, Chicago, IL, USA).

## Results

### Patient characteristics before switching to entecavir

Lamivudine-pretreated patients switched to entecavir 0.5 mg/day ( $n = 134$ ) were divided into three groups based on their HBV DNA level at the switching point: HBV DNA of less than 2.6 log<sub>10</sub> copies/mL ( $n = 92$ ), 2.6–5.0 log<sub>10</sub> copies/mL ( $n = 25$ ) and more than 5.0 log<sub>10</sub> copies/mL ( $n = 17$ ) (Table 1). Patients with HBV DNA levels of more than 5.0 log<sub>10</sub> copies/mL had the highest AST/ALT levels and highest proportion of HBeAg-positive cases ( $P < 0.05$ ). These patients had been treated with lamivudine for the shortest time period compared to patients from the two other groups ( $P < 0.05$ ; Table 1).

### Viral resistance to lamivudine at baseline

At baseline, lamivudine-resistant rtM204V/I mutant virus was detected in 23% of patients with HBV DNA of less than 2.6 log<sub>10</sub> copies/mL, compared to 76% in each of the HBV DNA 2.6–5.0 log<sub>10</sub> copies/mL and more than 5.0 log<sub>10</sub> copies/mL groups (Table 2). In all treatment groups, a higher occurrence of resistant virus was observed with longer exposure to lamivudine, independent of viral DNA levels.

**Table 1** Patient characteristics at point of switching to entecavir (baseline) and entecavir treatment duration

	All patients	Serum HBV DNA levels by baseline treatment group, log <sub>10</sub> copies/mL			P*
		< 2.6	2.6–5.0	> 5.0	
Patients, <i>n</i>	134	92	25	17	
Sex, <i>n</i> male/female	94/40	67/25	19/6	8/9	0.08
Age, years <sup>†</sup>	53 (23–83)	53 (27–83)	50 (32–77)	37 (23–77)	0.036
Bilirubin, mg/dL <sup>†</sup>	0.6 (0.2–3.4)	0.6 (0.2–3.4)	0.6 (0.3–1.8)	0.7 (0.3–1.2)	0.53
AST, IU/L <sup>†</sup>	24 (13–451)	23 (13–53)	23 (14–50)	37 (14–451)	0.0083
ALT, IU/L <sup>†</sup>	21 (8–1382)	21 (8–56)	20 (10–111)	46 (9–1382)	0.0002
Albumin, g/dL <sup>†</sup>	3.9 (2.7–4.8)	3.9 (2.7–4.4)	4.0 (3.3–4.8)	3.9 (3.6–4.6)	0.94
Histology, <i>n</i> CH/LC	89/45	56/36	19/6	14/3	0.11
HBeAg, <i>n</i> ±	30/104	11/81	5/20	14/3	< 0.0001
HBV DNA, log <sub>10</sub> copies/mL <sup>†</sup>	< 2.6 (< 2.6–7.6)	< 2.6	3.9 (2.7–5.0)	6.5 (5.1–7.6)	–
Genotype, <i>n</i> A/B/C/unknown	3/9/115/7	2/6/78/6	1/2/22/0	0/1/15/1	0.87
Treatment duration, months <sup>‡</sup>					
Lamivudine	36 (0.5–103)	36 (3–103)	70 (2–89)	17 (0.5–89)	0.009
Entecavir <sup>‡</sup>	21 (6–33)	20 (6–33)	24 (6–32)	27 (6–33)	0.034

\*Comparison of the three patient subgroups using the Kruskal–Wallis test; *P* < 0.05 was considered statistically significant.

<sup>†</sup>Data are median (range).

<sup>‡</sup>Entecavir treatment duration is from point of switching.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; LC, liver cirrhosis.

**Table 2** rtM204V/I mutant occurrence at baseline of switching to entecavir

	Duration of previous lamivudine treatment, years			All patients
	< 1	1–3	≥ 3	
Baseline treatment group				
< 2.6 log <sub>10</sub> copies/mL	1/10 (10%)	4/35 (11%)	16/47 (34%)	23%
2.6–5.0 log <sub>10</sub> copies/mL	1/5 (20%)	3/4 (75%)	15/16 (94%)	76%
> 5.0 log <sub>10</sub> copies/mL	3/6 (50%)	6/7 (86%)	4/4 (100%)	76%
All patients	24%	28%	52%	–

### Clinical efficacy of entecavir 0.5 mg/day

Switching to entecavir 0.5 mg/day for 1 year resulted in HBV DNA suppression to undetectable levels in the majority of patients with HBV DNA below 5.0 log<sub>10</sub> copies/mL (100% and 96% for HBV DNA < 2.6 and 2.6–5.0 log<sub>10</sub> copies/mL, respectively) (Table 3). This proportion was slightly decreased when previous lamivudine treatment duration exceeded 3 years in the 2.6–5.0 log<sub>10</sub> copies/mL group. In the HBV DNA more than 5.0 log<sub>10</sub> copies/mL group, approximately half (41%) of the patients achieved viral suppression after 1 year (Table 3); entecavir's efficacy seemed to decrease with prolonged previous exposure to lamivudine, with only 25% of patients having more than 3-year lamivudine treatment achieving undetectable viral load. Similarly, after 2 years, HBV DNA suppression was achieved by 100% and 92% of patients in the HBV DNA less than 2.6 and 2.6–5.0 groups, respectively, and by 44% of patients in the HBV DNA more than 5.0 log<sub>10</sub> copies/mL group (Table 3).

Among those who failed to suppress viral load, only one case of virological breakthrough was found (2.6–5.0 log<sub>10</sub> copies/mL group; described under case report). This patient had been previously exposed to lamivudine for more than 3 years.

Alanine aminotransferase levels were normalized in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively (Table 3). HBeAg loss was observed in 27% (3/11), 20% (1/5) and 29% (4/14) of patients with HBV DNA of less than 2.6, 2.6–5.0 and more than 5.0 log<sub>10</sub> copies/mL, respectively, in the first year.

### Lamivudine-resistant substitutions in patients switched to entecavir

Of the 130 patients who received entecavir treatment for at least 1 year, 11 cases failed to suppress HBV DNA to below less than 2.6 log<sub>10</sub> copies/mL and remained HBV DNA-positive in the first year (1 and 10 in the HBV DNA 2.6–5.0 and > 5.0 log<sub>10</sub> copies/mL groups, respectively; Table 3). Serum HBV DNA analysis confirmed the presence of rtM204V/I substitutions in 10 of these patients, of which six were rtM204I and three were rtM204V substitutions (Table 4); the remaining patient (2.6–5.0 log<sub>10</sub> copies/mL group; previous lamivudine exposure 5 years) carried a mixed type substitution, rtM204I plus rtM204V. The only HBV DNA-positive patient who did not

**Table 3** Clinical efficacy of entecavir 0.5 mg/day in lamivudine-pretreated patients

End-point by baseline treatment group	Duration of entecavir treatment		
	6 months	1 year	2 years
HBV DNA suppression to undetectable levels, n/N (%)			
< 2.6 log <sub>10</sub> copies/mL	90/92 (98%)	89/89 (100%)	32/32 (100%)
Previous lamivudine < 1 year	10/10 (100)	9/9 (100)	5/5 (100)
Previous lamivudine 1–3 years	35/35 (100)	35/35 (100)	14/14 (100)
Previous lamivudine > 3 years	45/47 (96)	45/45 (100)	13/13 (100)
2.6–5.0 log <sub>10</sub> copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
Previous lamivudine < 1 year	5/5 (100)	5/5 (100)	3/3 (100)
Previous lamivudine 1–3 years	4/4 (100)	4/4 (100)	2/2 (100)
Previous lamivudine > 3 years	15/16 (94)	14/15 (93)	7/8 (88)
> 5.0 log <sub>10</sub> copies/mL	5/17 (29%)	7/17 (41%)	4/9 (44%)
Previous lamivudine < 1 year	2/6 (33)	3/6 (50)	2/4 (50)
Previous lamivudine 1–3 years	2/7 (29)	3/7 (43)	2/4 (50)
Previous lamivudine > 3 years	1/4 (25)	1/4 (25)	0/1 (0)
ALT normalization, n/n (%)			
< 2.6 log <sub>10</sub> copies/mL	88/92 (96%)	83/89 (93%)	32/32 (100%)
2.6–5.0 log <sub>10</sub> copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
> 5.0 log <sub>10</sub> copies/mL	14/17 (82%)	13/17 (76%)	9/10 (90%)

ALT, alanine aminotransferase; HBV, hepatitis B virus.

**Table 4** HBV DNA positive rates in patients switched to entecavir 0.5 mg/day for at least 1 year

Baseline treatment group	HBeAg status	YMDD motif substitution	HBV DNA positive rate, n/N (%)	Duration of previous lamivudine treatment, years per patient
< 2.6 log <sub>10</sub> copies/mL	Positive	Wild (or none)	0/10 (0%)	n/a
		YIDD	0/1 (0%)	n/a
		YVDD	0/1 (0%)	n/a
	Negative	Wild (or none)	0/58 (0%)	n/a
		YIDD	0/15 (0%)	n/a
		YVDD	0/4 (0%)	n/a
2.6–5.0 log <sub>10</sub> copies/mL	Positive	Wild (or none)	0/4 (0%)	n/a
		YIDD + YVDD	1/1 (100%) <sup>†</sup>	5.0
		YVDD	0/1 (0%)	n/a
	Negative	Wild (or none)	0/2 (0%)	n/a
		YIDD	0/10 (0%)	n/a
		YVDD	0/6 (0%)	n/a
> 5.0 log <sub>10</sub> copies/mL	Positive	Wild (or none)	1/4 (25%)	0.2
		YIDD	6/9 (67%)	0.5; 1.3; 1.5; 2.7; 3.9; 7.4
		YVDD	1/1 (100%)	0.7
	Negative	YIDD	0/1 (0%)	n/a
		YVDD	2/2 (100%)	1.8; 4.5
		All patients		11/130 (8%)

YMDD motif substitutions: wild, rt204M; YIDD, rt204I; YVDD, rt204V; YIDD + YVDD, rt204I + rt204V.

<sup>†</sup>Patient with lamivudine-resistant HBV who developed entecavir resistance.

HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; n/a, not available.

carry any detectable lamivudine-resistant substitution had the shortest previous lamivudine exposure (< 6 months; Table 4).

Of the 10 patients carrying rtM204V/I substitutions, eight were HBeAg-positive; the other two patients were HBeAg-negative and carried a lamivudine-resistant rtM204V type substitution.

### Emergence of entecavir-resistant mutant: case report

One patient (2.6–5.0 log<sub>10</sub> copies/mL group) carrying a mixed substitution YIDD + YVDD (rtM204I + rtM204V) developed entecavir resistance with a recognized rtS202G substitution