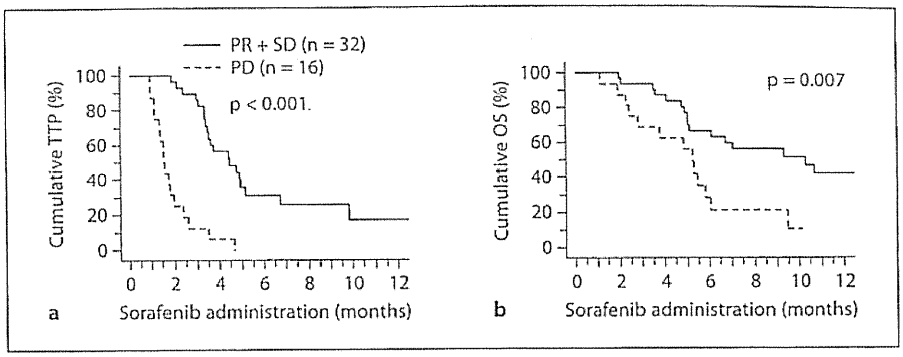

Fig. 1. Comparison of cumulative TTP (a) and OS (b) in the PR + SD and PD groups according to RECIST.

(n = 8); (b) body weight ≤40 kg (n = 2), and (c) history of treatment for varices or ascites (n = 12). The median baseline AFP level was 572 ng/ml (range, 2.3–148,000), and the median baseline DCP level was 424 mAU/ml (range, 15–305,000). The mean observation period was 7.2 ± 4.5 months.

Antitumor Responses 4 Weeks after the Start of Sorafenib Therapy

According to RECIST, 4 weeks after the start of sorafenib therapy, there were no complete responses, 2 PR, 30 SD, and 16 PD. The response rate was 4.2%, and the disease control rate was 66.7%.

Cumulative TTP and OS in the PR + SD and PD Groups

Cumulative TTP in the two groups according to RECIST is shown in figure 1a. The median observation period was 3.2 months. The median TTP was significantly longer in the PR + SD group than in the PD group (4.4 vs. 1.5 months; hazard ratio, 0.14; 95% CI, 0.06–0.29; $p < 0.001$).

Cumulative OS in the two groups according to RECIST is shown in figure 1b. The median observation period was 5.7 months. The median OS was significantly longer in the PR + SD group than in the PD group (10.3 vs. 5.2 months; hazard ratio, 0.36; 95% CI, 0.17–0.78; $p = 0.007$).

Comparison of Actual and Relative Levels of AFP at Baseline, and 2 and 4 Weeks after the Start of Sorafenib Therapy (Stratified by Antitumor Response)

AFP was not measured in 9 and 1 patients 2 and 4 weeks after starting sorafenib administration, respectively. Accordingly, AFP was analyzed in 39 and 47 patients 2 and 4 weeks after starting sorafenib administration, respectively.

Table 1. Baseline characteristics of the 48 HCC patients enrolled in this study

Mean age, years	69.9 ± 10.0
Male/female	38/10
HBV/HCV/NBNC	6/30/12
ECOG PS (0/1)	29/19
Child-Pugh score (5/6/7)	24/21/3
HCC stage (III/IVA/IVB)	11/18/19
Initial therapy/therapy for recurrence	6/42
Sorafenib starting dosage (800/400 mg)	26/22
Median serum AFP level, ng/ml	572
Range	2.3–148,000
Median serum DCP level, mAU/ml	424
Range	15–305,000
Mean observation period, months	7.2 ± 4.5

Numbers of patients are shown unless indicated otherwise. HBV/HCV = Hepatitis B/C virus; NBNC = non-HBV, non-HCV; ECOG = Eastern Cooperative Oncology Group; PS = performance status.

Data comparing actual AFP levels at baseline, and 2 and 4 weeks after starting sorafenib administration, both for the total patients and when stratified by antitumor response according to RECIST, are shown in table 2. Among the total number of patients, AFP showed no statistically significant differences between baseline and 2-week treatment levels, but in the PD group, AFP levels after 2 weeks of treatment were significantly elevated versus baseline levels ($p = 0.013$). Similarly, in the total number of patients, AFP showed no statistically significant differences between baseline and 4-week treatment levels, but in the PD group, AFP was significantly higher after 4 weeks of treatment compared with baseline levels ($p = 0.002$). In the PR + SD group, the median actual AFP level 4 weeks after starting sorafenib administration was higher than that at 2 weeks; however, there were no sig-

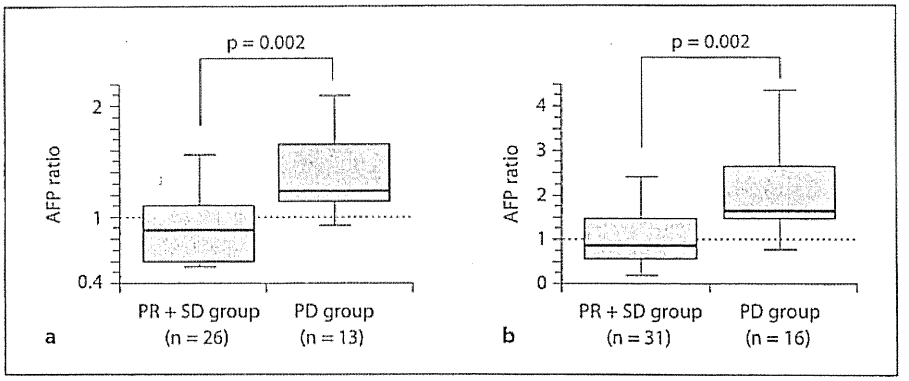

Fig. 2. AFP ratios 2 (a) and 4 weeks (b) after the start of sorafenib treatment in the PR + SD and PD groups according to RECIST.

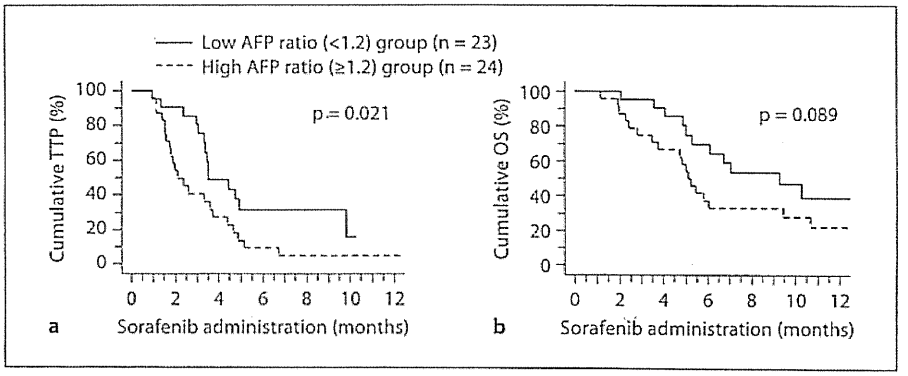

Fig. 3. Comparison of cumulative TTP (a) and OS (b) in the groups with low (<1.2) and high AFP ratio (≥1.2) 4 weeks after starting sorafenib therapy.

Table 2. Comparison of actual AFP levels (ng/ml) at baseline, and 2 and 4 weeks after the start of sorafenib therapy (stratified by antitumor response)

Groups	Baseline	After 2 weeks	p value	After 4 weeks	p value
Total	572 (2.3–148,000)	481 (2.2–163,300)	0.155	676 (1.1–281,700)	0.077
PR + SD	245.5 (2.3–148,000)	198 (2.2–163,300)	0.657	311 (1.1–281,700)	0.518
PD	2,321 (8.6–62,400)	3,303 (6.4–52,840)	0.013	6,258.5 (6.4–237,000)	0.002

nificant differences between AFP levels after 2 and 4 weeks (p = 0.423). On the other hand, in the PD group, the median actual AFP level 4 weeks after starting sorafenib administration was significantly higher than that after 2 weeks (p = 0.003).

Figure 2 compares the AFP ratios stratified by antitumor effects according to RECIST after 2 and 4 weeks of sorafenib treatment. AFP ratios 2 and 4 weeks after the start of sorafenib administration were 0.88 (range, 0.28–1.79) and 0.88 (range, 0.07–3.17) in the PR + SD group, and 1.24 (range, 0.74–2.12) and 1.63 (range, 0.64–7.35) in the PD group. At both time points, the ratio in the PR + SD group was significantly lower than in the PD group (p = 0.002, p = 0.002).

Cumulative TTP and OS in the Groups with Low and High AFP Ratio 4 Weeks after the Start of Sorafenib Therapy

The median AFP ratio 4 weeks after the start of sorafenib therapy was 1.2 (0.1–7.4).

Cumulative TTP (according to RECIST) in the groups with low (<1.2) and high AFP ratio (≥1.2) 4 weeks after the start of sorafenib therapy is shown in figure 3a. The median TTP was significantly longer in the low AFP (n = 23) ratio group than in the high AFP ratio group (n = 24; 3.5 vs. 2.1 months; hazard ratio, 0.46; 95% CI, 0.23–0.91; p = 0.021).

Cumulative OS in the low (n = 23) and high AFP ratio groups (n = 24) 4 weeks after the start of sorafenib therapy

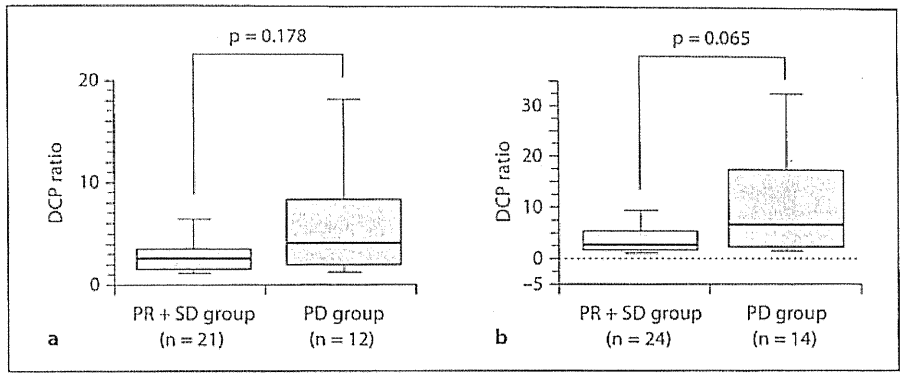

Fig. 4. DCP ratios 2 (a) and 4 weeks (b) after the start of sorafenib treatment in the PR + SD and PD groups according to RECIST.

Table 3. Comparison of actual DCP levels (mAU/ml) at baseline, and 2 and 4 weeks after the start of sorafenib therapy (stratified by antitumor response)

Patients	Baseline	After 2 weeks	p value	After 4 weeks	p value
Total	424.5 (15–305,000)	741 (26–798,000)	<0.001	2,025 (78–1,020,000)	<0.001
PR + SD	425.5 (15–216,000)	741 (30–323,000)	<0.001	1,715 (81–524,000)	<0.001
PD	575.5 (19–305,000)	6,186.5 (26–798,000)	0.002	20,550 (78–1,020,000)	0.001

is shown in figure 3b. The median OS tended to be higher in the low than in the high AFP ratio group (9.3 vs. 5.1 months; hazard ratio, 0.53; 95% CI, 0.25–1.12; p = 0.089).

Comparison of Actual and Relative Levels of DCP at Baseline, and 2 and 4 Weeks after the Start of Sorafenib Therapy (Stratified by Antitumor Response)

In the analysis of DCP, 7 patients who were taking vitamin K and 1 patient who was on warfarin were excluded. In addition, DCP was not determined in 7 and 2 patients 2 and 4 weeks after starting sorafenib administration, respectively. Accordingly, DCP was analyzed in 33 patients 2 weeks and in 38 patients 4 weeks after starting sorafenib administration.

Data comparing actual DCP levels at baseline, and 2 and 4 weeks after starting sorafenib administration, both for the total number of patients and patients stratified by antitumor response according to RECIST, are shown in table 3. Actual levels of DCP after 2 weeks of treatment were significantly higher than baseline levels in the total number of patients, the PR + SD group and the PD group. After 2 weeks of treatment, DCP was elevated in 97.0% (32/33) of the patients. Similarly, actual levels of DCP after 4 weeks of treatment were also significantly elevated from baseline levels in all patient groups; the total number of patients, the PR + SD group, and the PD group. After 4 weeks of treatment, DCP was elevated in 92.1% (35/38) of the patients.

Figure 4 compares the DCP ratios between the PR + SD and PD groups according to RECIST after 2 and 4 weeks of sorafenib therapy. The DCP ratios 2 and 4 weeks after the start of sorafenib administration were 2.57 (range, 0.87–10.02) and 2.72 (range, 0.30–13.46) in the PR + SD group, and 4.02 (range, 1.12–35.03) and 6.73 (range, 1.25–45.08) in the PD group. There were no significant differences between the PR + SD and PD groups at either time point (p = 0.178, p = 0.065).

Cumulative TTP and OS in the Low and High DCP Ratio Groups 4 Weeks after the Start of Sorafenib Therapy

The median DCP ratio 4 weeks after the start of sorafenib therapy was 4.0 (0.3–45.1).

Cumulative TTP (according to RECIST) in the low (<4) and the high DCP ratio (≥4) groups 4 weeks after the start of sorafenib therapy is shown in figure 5a. There were no significant differences in the median DCP ratio between the low (n = 19) and the high DCP ratio group (n = 19; 3.5 vs. 2.4 months; hazard ratio, 1.06; 95% CI, 0.51–2.19; p = 0.874).

Cumulative OS in the low (n = 19) and high DCP ratio groups (n = 19) 4 weeks after the start of sorafenib thera-

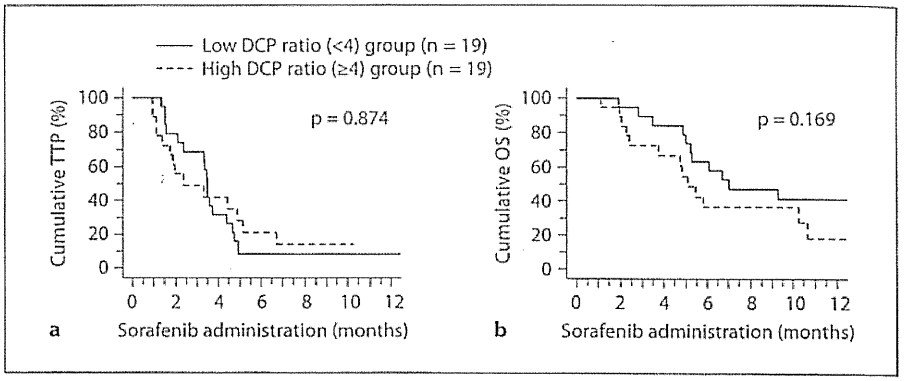

Fig. 5. Comparison of cumulative TTP (**a**) and OS (**b**) in the groups with low (<4) and high DCP ratio (≥4) 4 weeks after starting sorafenib therapy.

py is shown in figure 5b. There were no significant differences in median DCP ratio between the low and high DCP ratio groups (7.2 vs. 5.1 months; hazard ratio, 0.57; 95% CI, 0.25–2.55; p = 0.169).

Discussion

In the present study, we investigated the relationships between the changes in tumor markers, AFP and DCP, and antitumor responses in the early period following administration of sorafenib to patients with advanced HCC, and found that the relationship for AFP was different from that for DCP. With regard to AFP, both 2 and 4 weeks after starting sorafenib therapy, the ratio in the PR + SD group was significantly lower than in the PD group. On the other hand, DCP was elevated in both the PD group and the PR + SD group, and there were no statistically significant differences between the two groups either 2 or 4 weeks after starting sorafenib therapy. These results suggest that the time course changes for AFP in the early period after starting sorafenib administration are useful for predicting antitumor response assessed by image analysis.

Several studies have reported that the primary effect of sorafenib is inhibition of tumor growth rather than tumor shrinkage [2, 3, 23, 24]. In the SHARP Study, it was reported that the response rate in the sorafenib group based on RECIST criteria was only 2.3%, but both the cumulative TTP and cumulative survival duration were prolonged [2]. This can be considered an example of the limitations of using RECIST criteria, which focus on changes in the size of the entire tumor for evaluation of the therapeutic efficacy of molecularly targeted drugs. In the present study, cumulative TTP and OS were significantly better in the PR + SD group than in the PD group. In view of these findings, the primary clinical benefit of sorafenib is disease stabilization. Accordingly, it is important to evaluate treatment response in patients treated with sorafenib. In the present study, we analyzed the tumor marker response according to radiological response using the RECIST criteria. On the other hand, modified RECIST criteria were recently proposed as a method to assess arterial involvement [25]. Further investigation using these modified RECIST criteria is thus necessary.

In order to evaluate tumor responses, the formal recommendation of the panel of experts in HCC-Design Clinical Trials was to conduct imaging surveillance every 6–8 weeks using CT or MRI [4]. In our hospital, dynamic MDCT was obtained after 4 weeks of sorafenib treatment in order to assess early therapeutic effects. We found that antitumor responses 4 weeks after sorafenib administration correlated with both TTP and OS. Therefore, the present results indicate that it may be beneficial to evaluate the time point response 4 weeks after sorafenib administration in patients receiving sorafenib.

In the present study, AFP was significantly elevated in the PD group both 2 and 4 weeks after the start of administration compared with baseline. There has only been one report on AFP response after sorafenib therapy [26]. Shao et al. [26] reported the AFP responder group as patients whose AFP levels decreased to less than 0.8-fold of baseline levels within 1 month following sorafenib administration, while the non-responder group did not show this decrease. Consistent with our results, both the cumulative survival and TTP rates were significantly better in the AFP responder group than in the non-responder group. Hence, in the case of sorafenib therapy, changes in AFP levels may be correlated with the antitumor effects evaluated by image analysis, similarly to the course following other therapies for HCC, such as hepatic resection, radiofrequency ablation therapy, and transarterial chemoembolization. A comparison of the actual AFP levels 2 and 4

weeks after starting sorafenib administration in the PD group revealed that the median value after 4 weeks was significantly higher than that after 2 weeks. Even in the PR + SD group, the median value after 4 weeks was higher than that after 2 weeks. There were no significant differences between AFP levels after 2 and 4 weeks; thus, one of the reasons for this phenomenon was unevenness of AFP levels owing to the small sample size in this study.

With regard to DCP, there have been numerous reports that the time course change in DCP following treatment for HCC reflects therapeutic efficacy [17–19]. However, in the present study, we found that both the actual and relative levels of DCP were elevated in >90% of the patients, not only in the PD group but also in the PR + SD group, both 2 and 4 weeks after starting sorafenib therapy. To our knowledge, there have been no comprehensive clinical reports regarding the time course changes in DCP following sorafenib treatment. In a case report by Nakazawa et al. [27], DCP levels were markedly increased following treatment, even in patients who achieved a complete response on the basis of image analysis. From basic research, Murata et al. [28] reported that culturing a liver cancer cell line (HepG2) under hypoxic conditions resulted in increased DCP production by the cells. One possible mechanism for the increased DCP levels following sorafenib administration is that sorafenib-mediated inhibition of angiogenesis places tumor cells under hypoxic conditions, subsequently leading to increased DCP production. Thus, the increase in DCP levels following sorafenib administration may reflect HCC cell ischemia. Based on our results, increases in DCP soon after the start of sorafenib administration, regardless of antitumor effect, are not useful for assessing the antitumor responses, as DCP may increase in response to the ischemia caused by sorafenib.

Assessment by image analysis is the gold standard for evaluating antitumor responses of anticancer drugs [4, 22, 23]. However, such image analysis can be difficult in patients with multiple HCC lesions, vascular invasion, extrahepatic metastases, or ischemic tumors. In particular, patients in whom therapy using sorafenib is indicated are often in advanced stages of disease. There are limitations in using only radiological criteria to evaluate sorafenib treatment.

Our results suggest that the determination of early changes in AFP is useful for evaluating both antitumor response and prognostic efficacy of sorafenib, as assessed by TTP and OS, in patients with advanced HCC. In patients with advanced HCC treated with sorafenib, it is important to evaluate therapeutic efficacy as early as possible, as appropriate and early evaluation of sorafenib therapy can avoid unnecessary adverse events and allow second-line therapy when sorafenib therapy is not effective. In addition, determination of early changes in AFP is useful for evaluating the efficacy of new molecularly targeted agents currently under development. At present, there is no effective second-line treatment and we could not confirm whether continuing sorafenib administration would prolong the survival of patients with elevated AFP. Therefore, we cannot conclude that sorafenib therapy should be stopped in the case of elevated AFP ratio after 2 or 4 weeks of treatment. However, when an effective second-line treatment becomes available, an elevated AFP ratio may be a good indicator for switching to second-line therapy.

On the other hand, with regard to early changes in DCP, caution is required when assessing the antitumor response of sorafenib, as DCP elevation can occur irrespective of therapeutic effects.

In conclusion, our results suggest that early evaluation of AFP after starting sorafenib therapy is useful for predicting antitumor response. In contrast, early elevation of DCP does not necessarily suggest treatment failure of sorafenib. Appropriate and early evaluation of efficacy of sorafenib by AFP determination can provide valuable information that may influence subsequent decisions regarding patient management, thus avoiding unnecessary adverse events and allowing the opportunity for second-line therapy.

Acknowledgment

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Disclosure Statement

The authors declare that they have no financial conflicts of interest.

References

1 Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–7109.

2 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J, SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
3 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34.
4 Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ, Panel of Experts in HCC-Design Clinical Trials: Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
5 Arii S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, Kudo M: Management of hepatocellular carcinoma: report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). Hepatol Res 2010;40:667–685.
6 Kudo M, Ueshima K: Positioning of a molecular-targeted agent, sorafenib, in the treatment algorithm for hepatocellular carcinoma and implication of many complete remission cases in Japan. Oncology 2010;78:154–166.
7 Taketa K: Alpha-fetoprotein: reevaluation in hepatology. Hepatology 1990;12:1420–1432.
8 Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S: Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. Hepatology 1994;19:61–66.
9 Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B: Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med 1984;310:1427–1431.
10 Fujiyama S, Morishita T, Hashiguchi O, Sato T: Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. Cancer 1988;61:1621–1628.
11 Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H: The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer 1998;82:1643–1648.
12 Toyoda H, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, Oka H, Yamazaki O, Manabe T, Urano F, Chung H, Kudo M, Matsunaga T: Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. J Hepatol 2008;49:223–232.
13 Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, Ibrahim SM, Sato KT, Baker T, Miller FH, Newman S, Omary R, Abecassis M, Benson AB 3rd, Salem R: Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. J Clin Oncol 2009;27:5734–5742.
14 Chan SL, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, Lam KC, Chan AT, Mok TS, Yeo W: New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Clin Oncol 2009;27:446–452.
15 Kim BK, Ahn SH, Seong JS, Park JY, Kim do Y, Kim JK, Lee do Y, Lee KH, Han KH: Early α-fetoprotein response as a predictor for clinical outcome after localized concurrent chemoradiotherapy for advanced hepatocellular carcinoma. Liver Int 2011;31:369–376.
16 Vora SR, Zheng H, Stadler ZK, Fuchs CS, Zhu AX: Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. Oncologist 2009;14:717–725.
17 Shimada M, Takenaka K, Fujiwara Y, Gion T, Kajiyama K, Maeda T, Shirabe K, Sugimachi K: Des-gamma-carboxy prothrombin and alpha-fetoprotein positive status as a new prognostic indicator after hepatic resection for hepatocellular carcinoma. Cancer 1996;78:2094–2100.
18 Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Yoshida H, Shiina S, Omata M: Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. Cancer 2001;91:561–569.
19 Iwadou S, Nouso K, Kuwaki K, Kobayashi Y, Nakamura S, Tanaka H, Miyoshi K, Ohnishi H, Miyake Y, Shiraha H, Iwasaki Y, Shiratori Y, Yamamoto K: Time-dependent analysis of predisposing factors for the recurrence of hepatocellular carcinoma. Liver Int 2010;30:1027–1032.
20 Bruix J, Sherman M: American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology 2011;53:1020–1022.
21 Liver Cancer Study Group of Japan: General Rules for the Clinical and Pathological Study of Primary Liver Cancer, ed 2. Tokyo, Kanehara, 2003.
22 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–247.
23 Furuse J, Ishii H, Nakachi K, Suzuki E, Shimizu S, Nakajima K: Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. Cancer Sci 2008;99:159–165.
24 Prete SD, Montella L, Caraglia M, Maiorino L, Cennamo G, Montesarchio V, Piai G, Febbraro A, Tarantino L, Capasso E, Palmieri G, Guarrasi R, Bianco M, Mamone R, Savastano C, Pisano A, Vincenzi B, Sabia A, D'Agostino A, Faiola V, Addeo R: Sorafenib plus octreotide is an effective and safe treatment in advanced hepatocellular carcinoma: multicenter phase II So.LAR. study. Cancer Chemother Pharmacol 2010;66:837–844.
25 Lencioni R, Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52–60.
26 Shao YY, Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL: Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. Cancer 2010;116:4590–4596.
27 Nakazawa T, Hidaka H, Shibuya A, Koizumi W: Rapid regression of advanced hepatocellular carcinoma associated with elevation of des-gamma-carboxy prothrombin after short-term treatment with sorafenib – a report of two cases. Case Rep Oncol 2010;3:298–303.
28 Murata K, Suzuki H, Okano H, Oyamada T, Yasuda Y, Sakamoto A: Cytoskeletal changes during epithelial-to-fibroblastoid conversion as a crucial mechanism of des-gamma-carboxy prothrombin production in hepatocellular carcinoma. Int J Oncol 2009;35:1005–1014.

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Original Article

Changes in hepatitis C viral load during first 14 days can predict the undetectable time point of serum viral load by pegylated interferon and ribavirin therapy

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Aim: In the treatment of chronic hepatitis C, pegylated interferon (PEG-IFN) and ribavirin combination therapy must be continued for an adequate duration to improve the rate of sustained virological response. We attempted to predict the time point at which serum hepatitis C virus (HCV) RNA are undetectable during combination therapy.

Methods: Patients with HCV genotype 1b were enrolled in a model preparation ($n = 35$) and a validation group ($n = 70$). All patients received PEG-IFN-α-2b/ribavirin combination therapy for at least 48 weeks, and serological samples were screened a minimum of 17 times during the therapy. Serum HCV RNA were measured by the Abbott RealTime HCV assay. Using the HCV dynamics model described by Neumann *et al.*, we used multiple linear regression analysis to select factors that affected the undetectable time point.

Results: Difference in viral load between weeks 1 and 2 was the only predictive factor for the undetectable time point of serum HCV RNA ($r^2 = 0.67$, $P < 0.0005$), and we derived the following prediction equation: undetectable time point (week) = 13.495 × (viral load at day 14 [log IU/mL] – viral load at day 7 [log IU/mL]) + 25.456. The equation was applicable to the validation group.

Conclusion: We created a formula for predicting the undetectable time point from viral load measurements early in PEG-IFN-α-2b/ribavirin combination therapy. An early response reflects sensitivity to therapy, and the estimation of an undetectable time point would be useful for determining the optimal duration of treatment for chronic hepatitis C patients.

Key words: hepatitis C, interferon, kinetics, real-time polymerase chain reaction, undetectable time point

INTRODUCTION

INTERFERON (IFN)-BASED therapy is the main form of therapy for chronic hepatitis C, but it requires a long-term period to complete, typically lasting at least 48 weeks for hepatitis C virus (HCV) genotypes 1 and 4. The final therapeutic effect is eradication of HCV, which is referred to as a sustained virological response (SVR). Although combination therapy with pegylated (PEG)-IFN-α and ribavirin is now established as the standard treatment for chronic HCV infection genotype 1b, the SVR rate in these patients is still approximately 50%.[1–3] Moreover, it is difficult to know the treatment outcomes during treatment and follow-up period.

Various factors have been investigated to predict the treatment efficacy before initiation of therapy, including pretreatment viral load,[4] viral genotype,[5] and gene sequences, such as IFN sensitivity determining region,[6] and host factors, including sex, age, fibrosis stage and race.[7,8] These factors cannot be modified by therapy and are unfortunately not completely reliable for predicting therapeutic response. However, other studies have documented the importance of the period when HCV is cleared from the serum (we define this as the

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"undetectable time point").[9–13] When an undetectable time point is achieved within 4 weeks of therapy initiation, the SVR rate is high. In contrast, the later the undetectable time point, the lower the SVR rate. One disadvantage with this prediction method during therapy is that SVR cannot be predicted until serum viral clearance. If one can predict the undetectable time point early during the treatment, physicians can modify and optimize the ongoing treatment.

There are various patterns of patient response to IFN therapy. In clinical settings, the following three response patterns are observed: (i) SVR; (ii) non-virological response (NVR), in which viral loads continue to be detected during therapy; and (iii) relapse, in which viral loads transiently drop below the detection limit but become detectable again after the end of therapy.[8] Mathematical models have been developed for analyzing therapy-induced changes in HCV viral load. Neumann *et al.*[14] introduced a model for IFN monotherapy in 1998, and a pharmacokinetic model for PEG-IFN has been developed by Powers *et al.*[15] These models are very useful for understanding the therapeutic effects of IFN on HCV.

In recent years, techniques to quantify serum viral RNA levels have advanced. The detection limit and the dynamic range of the quantitative real-time polymerase chain reaction (PCR) assay are lower and wider than those of Amplicor PCR assay.[16,17] As a result, the real-time PCR assay can show us the more accurate viral dynamics. In the present study, we used the model of Powers *et al.*[15] and real-time PCR to measure serum viral loads. Our aim was to ascertain whether it is possible to predict the undetectable time point during the early stage of PEG-IFN-α-2b/ribavirin combination therapy for genotype 1b patients with a high viral load, which is the most difficult-to-treat phenotype of HCV.

METHODS

Patients

THE MODEL PREPARATION group comprised 35 patients with biopsy-proven chronic hepatitis C who were treated at the Musashino Red Cross Hospital from 2000–2001. All patients had HCV genotype 1b and a high viral load (>100 000 IU/mL) as determined by the Amplicor-HCV Monitor Assay (Roche Diagnostics, Tokyo, Japan). Patients with other liver disease, such as liver cirrhosis, autoimmune hepatitis or alcoholic liver injury, were excluded. None of the patients had hepatitis B virus-related antigens, antibodies or anti-HIV antibodies. At the time of enrollment, it was confirmed that none of the patients were taking drugs that could affect their immune system. The dosage of ursodeoxycholic acid and glycyrrhizin was not changed during therapy.

The model validation group comprised 70 patients with biopsy-proven chronic hepatitis C who were treated at the Musashino Red Cross Hospital from 2004–2006. As with the model preparation group, all patients had HCV genotype 1b and a high viral load, and patients with liver cirrhosis or alcoholic liver injury were excluded. None of the patients had hepatitis B virus-related antigens, antibodies or anti-HIV antibodies.

Informed consent was obtained from all patients in writing. The present study was approved by the Ethics Review Board of Musashino Red Cross Hospital in accordance with the Declaration of Helsinki.

Treatment protocol

All patients received at least 48 weeks of PEG-IFN-α-2b (PegIntron; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough) combination therapy. In the model validation group, if viral clearance was not achieved by week 12, combination therapy was prolonged to 72 weeks. PEG-IFN-α-2b (1.5 μg/kg per week) was administrated s.c. Ribavirin was administrated p.o. at 600 mg/day twice daily to patients weighing less than 60 kg, and 800 mg/day was given to patients weighing between 60 and 80 kg. The dosage of PEG-IFN-α-2b was reduced to 0.75 μg/kg per week when white blood cells, neutrophils or platelets dropped below 1500, 750 or $80 \times 10^3/mm^3$, respectively. When hemoglobin concentration dropped below 10 g/dL, the dosage of ribavirin was reduced from 600 to 400 mg/day for patients weighing less than 60 kg, and from 800 to 600 mg/day for patients weighing between 60 and 80 kg. Both drugs were discontinued when white blood cells, neutrophils, platelets or hemoglobin levels dropped below $1000/mm^3$, $500/mm^3$, $50 \times 10^3/mm^3$ or 8.5 g/dL, respectively.

HCV dynamics in serum

To analyze viral dynamics, serum samples were collected from each patient according to the following schedule with respect to the start of PEG-IFN-α2b/ribavirin combination therapy: immediately before and at 4, 8 h, and 1, 2, 4, 7, 8, 14 and 28 days after the therapy was started; and then at 4-week intervals until completion of the therapy. HCV viral loads were measured in all serum samples using the Abbott RealTime HCV assay (Abbott Molecular, Des Plaines, IL, USA) at an Abbott laboratory in the USA.[16] The dynamic range

was 1.08–8 $\log_{10}$ IU/mL. The assay is standardized to the 2nd World Health Organization (WHO) International Standard for HCV RNA (National Institute for Biological Standards and Control code 96/798). Nucleic acid extraction was performed on 0.5-mL samples using an Abbott *m2000sp* (Abbott Molecular). The Abbott *m2000rt* (Abbott Molecular) was used for reverse transcription, PCR amplification and detection/quantification. A single-stranded linear probe was used as the HCV probe.

Definitions of response to therapy

The undetectable time point was defined as the first time the viral load dropped below the detection limit (1.08 $\log_{10}$ IU/mL) during therapy. Patients with SVR had no detectable viral load 6 months after the end of PEG-IFN-α-2b/ribavirin combination therapy. Patients in relapse had no detectable viral load at the end of therapy but had a detectable viral load 6 months after the end of therapy. Patients with NVR had a detectable viral load throughout the treatment period.

Calculation of the HCV dynamic parameters

Hepatitis C virus dynamic parameters (c, δ, ε, T_0 and V_0) were calculated from viral loads with equations for HCV dynamics.[15] The parameter c is the constant viral death rate, δ is the death rate of infected cells, ε is the effect of PEG-IFN on blocking production of virus from infected cells, and T_0 and V_0 are the numbers of uninfected cells and virus at the start of therapy, respectively.

Statistical analysis

SAS ver. 9.13 was used for the statistical analysis. *P*-values of less than 0.05 were considered significant.

RESULTS

Baseline patient characteristics

TABLE 1 SHOWS the baseline characteristics of the patients. The SVR rate was 60% and 27 patients accomplished undetectable serum HCV until 24 weeks after the therapy was started. The therapy was discontinued in three of the 35 patients because of a reduction in

Table 1 Patient characteristics at baseline

	Model preparation group ($n = 35$)	Model verification group ($n = 70$)
Age (years)	52.1 ± 9.9	57.8 ± 11
Sex (male/female)	24/11	36/34
BMI	23.7 ± 2.9	23.9 ± 3.7
Hemoglobin (g/dL)	14.7 ± 1.2	14.2 ± 1.6
Platelet count ($\times 10^3/\mu L$)	17.9 ± 4.8	15.5 ± 5.2
Albumin (g/dL)	4.2 ± 0.33	3.92 ± 0.048
ALT (U/L)	91.7 ± 64	80.0 ± 7.4
Liver histology (Metavir score)		
A (0/1/2/3/4/not measured)	0/17/13/5/0/0	0/40/26/2/0/2
F (0/1/2/3/4/not measured)	0/17/15/3/0/0	2/23/25/18/0/2
Viral load (log IU/mL)		
At pretreatment	5.49 ± 0.52	5.54 ± 0.92
At 7th day of treatment	4.05 ± 0.98	4.75 ± 1.05
at 14th day of treatment	3.23 ± 1.41	4.23 ± 1.29
Durations of therapy		
(48 weeks/72 weeks/dropout)	32/0/3	45/7/18
Drug adherence†		
(PEG-IFN/ribavirin/both/non-)	7/5/2/21	6/21/30/13
Outcome (SVR/relapse/NVR)	21/6/8	20/26/24
Actual undetectable time point‡		
(14/28 days/8/12/16/20/24/28/32 weeks/therapy end)	3/7/8/4/1/2/2/0/0	2/2/12/14/4/4/2/2/4

†Patients numbers with dose reduction during the therapy.
‡NVR cases were excluded.
BMI, body mass index; ALT, alanine aminotransferase; PEG-IFN, pegylated interferon; SVR, sustained virological response; NVR, non-virological response.

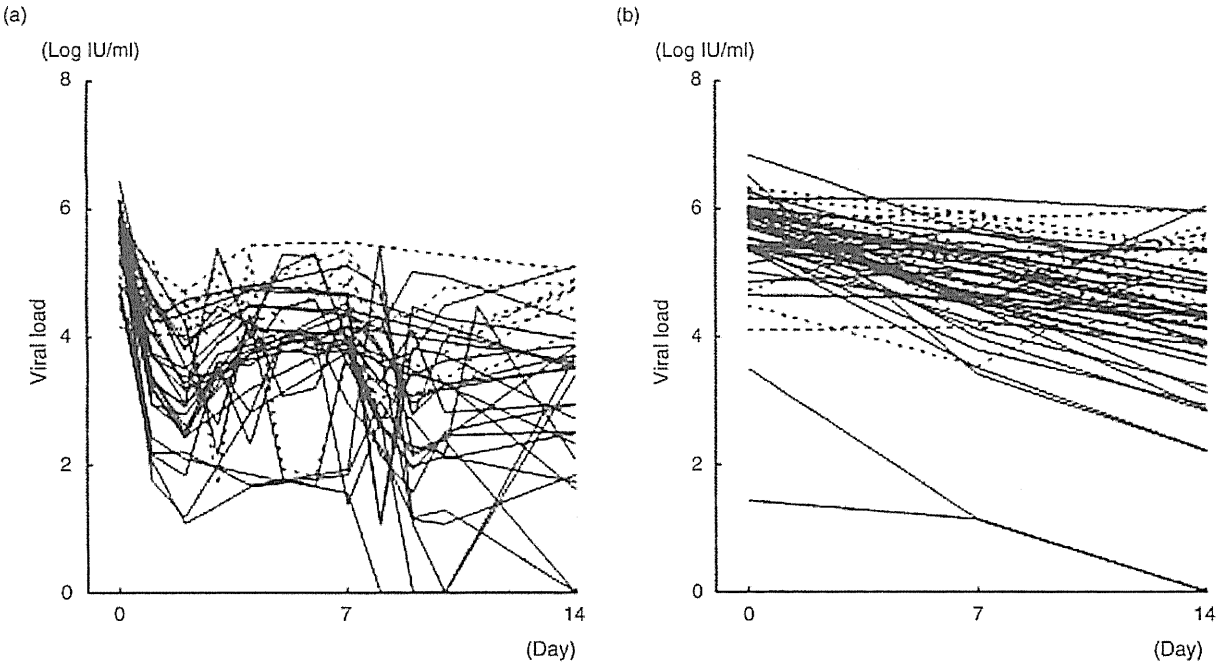

Figure 1 Early hepatitis C virus (HCV) dynamics of model preparation group (a) and of model validation group (b). The patients with incomplete blood collection were excluded from the figure of the model validation group. Solid line, dynamics of those who accomplished undetectable serum HCV until the therapy ended; dotted line, of those in whom serum HCV was detected through the whole therapy.

the hemoglobin concentration, a reduction in the neutrophil count and a worsening of depressive symptoms. In comparison to the model preparation group, there were more NVR patients, and the SVR rate was 29% in the model validation group. There were six patients who accomplished undetectable serum HCV after 24 weeks, and the latest patients achieved it 40 weeks after the therapy started. More patients had advanced hepatic fibrosis in the model validation group than in the model preparation group. Eighteen patients discontinued the combination therapy for various reasons, for example, decreased neutrophil count. The early HCV dynamics of both group are shown in Figure 1.

Undetectable time point prediction

From the model preparation group, 29 patients were analyzed and six patients were excluded for the following reasons: therapy was discontinued before viral clearance in one patient, PEG-IFN dosage was decreased before viral clearance in three patients, viral load increased during therapy in one patient, and an incomplete series of samples were obtained from one patient.

First, we hypothesized that the HCV dynamic parameters have a possibility to predict the undetectable time point. HCV dynamic parameters were calculated with three dataset patterns of viral loads, as follows: (i) immediately before and at 4, 8 h, and 1, 2, 4, 7 and 8 days; (ii) before and at 8 h, and 1, 2, 4 and 7 days; and (iii) before and at 4, 8 h, and 1, 2, 4 and 7 days after the therapy was started. Unfortunately, no significant factors for prediction of the undetectable time points were detected in these HCV dynamic parameters (Table 2), even when adding parameters of age and sex.

Next, we investigated the possibility using early-stage treatment dynamics. Multiple linear regression analysis was conducted for viral load, and changes in viral load up to day 14 as the explanatory variables and undetectable time points as the objective variables. Among various factors which became significant alone, the decrease in viral load from day 7 to 14 was found to be the best predictor for the undetectable time points by multiple linear regression analysis ($r^2 = 0.67$, Table 3). Then, whole datasets were analyzed again including HCV dynamic parameters, sex, age, viral loads and viral

Table 2 Calculated HCV-dynamic parameters of model preparation group

Dataset	Dataset 1† median (range)	*P*	Dataset 2‡ median (range)	*P*	Dataset 3§ median (range)	*P*
c	0.77 (0.032–5.21)	0.73	1.54 (0.0515–7.58)	0.37	2.75 (0.040–6.19)	0.85
δ	0.0033 (0–0.69)	0.76	0.013 (0–0.99)	0.094	0.053 (0–0.70)	0.91
ε	0.28 (0.023–0.84)	0.30	0.067 (0.0083–0.72)	0.038	0.28 (0.023–0.71)	0.18
T_0	0.36 (0.0001–0.95)	0.63	0.415 (0.0049–0.98)	0.23	0.36 (0.007–0.90)	0.21
V_0	5.49 (4.40–6.69)	0.53	4.99 (4.10–6.48)	0.090	5.29 (4.30–6.69)	0.29
R^2	0.012		0.090		0.056	

†Dataset 1: serum hepatitis C virus (HCV) load immediately before and at 4, 8 h, and 1, 2, 4, 7, 8 days after the therapy was started.
‡Dataset 2: serum HCV load before and at 8 h, and 1, 2, 4, 7 days after the therapy was started.
§Dataset 3: serum HCV load before and at 4, 8 h, and 1, 2, 4, 7 days after the therapy was started.

load changes. The results showed that only the change in viral load from day 7 to 14 was associated with the prediction of the undetectable time point ($r^2 = 0.67$). Finally, prediction in each patient was valid (Cook's D = 0.046, mean, data not shown), and we derived the following prediction formula:

Undetectable time point (week) = 13.495 × (viral load at day 14 [log IU/mL] − viral load at day 7 [log IU/mL]) + 25.456.

The degree of decrease in viral load from day 7 to 14 for the model preparation group and the actual

Table 3 Early viral dynamics of model preparation group, correlation to undetectable time point and the result of multiple linear regression analysis

	Viral load (log IU/mL)	Spearman's rank correlation test coefficient (*P*-value)	Multiple linear regression analysis r^2 (*P*-value)
Pretreatment (0 days)	5.48 ± 0.30	0.27 (0.28)	Excluded
4 h	5.66 ± 0.22	0.045 (0.82)	Excluded
8 h	5.55 ± 0.19	0.026 (0.89)	Excluded
1 day	3.74 ± 0.75	0.68 (<0.001)	Excluded
2 days	3.20 ± 0.76	0.66 (<0.001)	Excluded
4 days	4.01 ± 0.74	0.56 (0.002)	Excluded
7 days	4.05 ± 0.75	0.77 (<0.001)	Excluded
8 days	3.34 ± 0.80	0.67 (<0.001)	Excluded
14 days	3.52 ± 0.95	0.87 (<0.001)	Excluded
Subtracted values of viral load (log scale)			
1 day – 0 days	−1.78 ± 0.88	0.59 (0.001)	Excluded
2 days – 0 days	−2.18 ± 0.79	0.53 (0.003)	Excluded
4 days – 0 days	−1.46 ± 0.65	0.72 (0.000)	Excluded
7 day – 0 days	−1.38 ± 0.80	0.38 (0.049)	Excluded
14 days – 0 days	−2.24 ± 1.17	0.83 (0.000)	Excluded
2 days – 1 day	−0.55 ± 0.13	0.085 (0.67)	Excluded
4 days – 1 day	0.17 ± 0.25	0.22 (0.27)	Excluded
7 days – 1 day	0.44 ± 0.46	0.27 (0.19)	Excluded
14 days – 1 day	−0.42 ± 0.46	0.76 (<0.001)	Excluded
4 days – 2 days	0.61 ± 0.23	0.12 (0.54)	Excluded
7 days – 2 days	0.86 ± 0.50	0.12 (0.56)	Excluded
14 days – 2 days	0.11 ± 0.44	0.76 (<0.001)	Excluded
7 days – 4 days	−0.11 ± 0.17	0.047 (0.82)	Excluded
14 days – 4 days	−0.7 ± 0.37	0.78 (<0.001)	Excluded
14 days – 7 days	−0.86 ± 0.50	0.76 (<0.001)	0.667 (<0.0005)

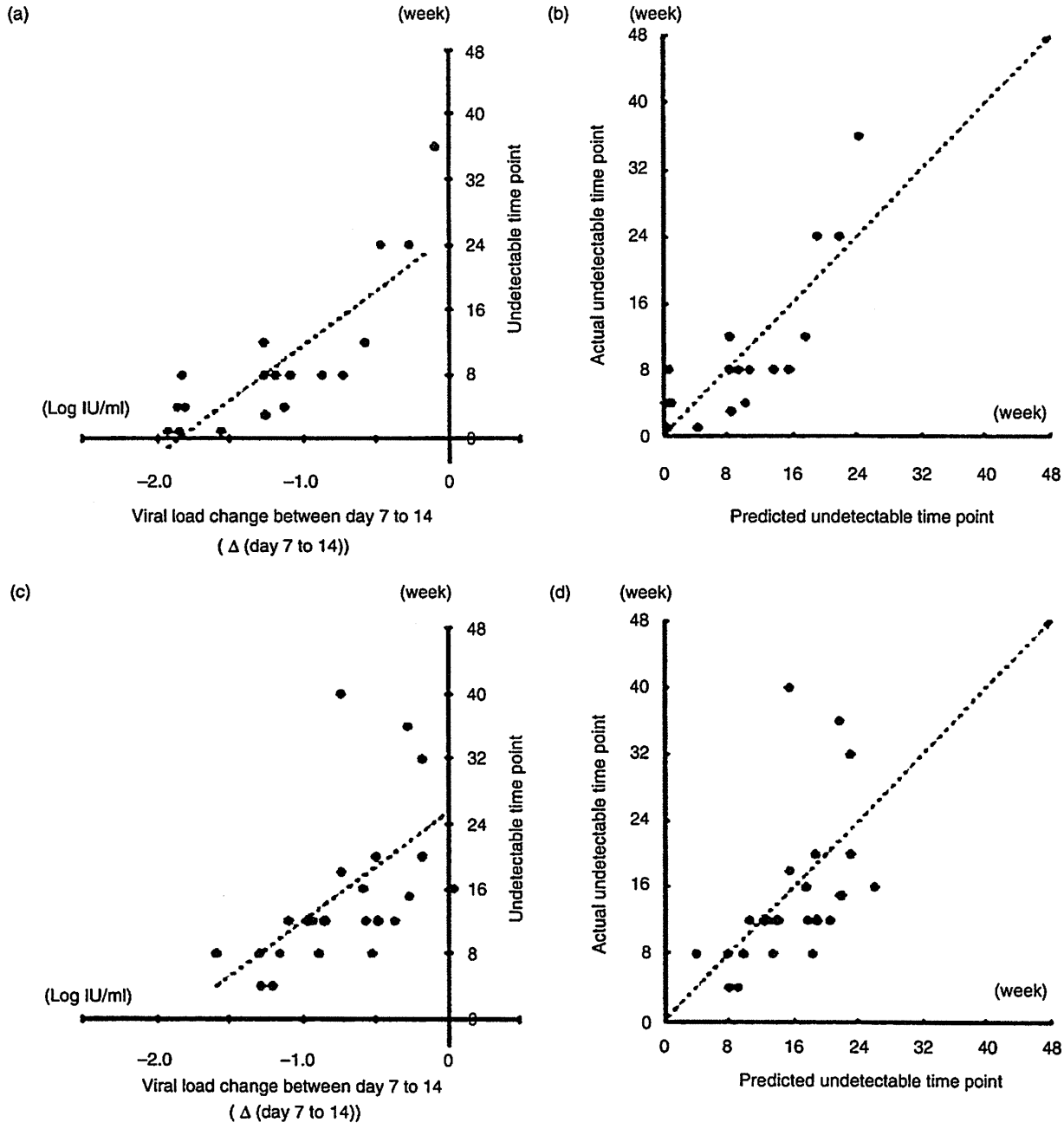

Figure 2 Correlation between the undetectable time point and the decrease in viral load from day 7 to 14 (a,b) and correlation between the actual and predicted undetectable time points (c,d). (a,c) Results of analyses for the model preparation group; and (b,d) analyses for the model validation group. Black circles, actual cases; dotted line, (a,c) estimate obtained from the prediction formula; (b,d) equal values of actual and predicted undetectable time points.

undetectable time point are plotted in Figure 2(a), which shows a very strong and a significant correlation ($r^2 = 0.67$, $P < 0.0005$).

The validity of the prediction formula was investigated in the validation group. Analysis was possible in 32 patients, as the other patients were excluded from the analysis due to the following reasons: therapy was discontinued before viral clearance in eight patients, PEG-IFN dosage was reduced before viral clearance in nine patients and viral clearance was achieved before day 14 in two patients. There were six cases of NVR, and incomplete blood collections from 13 patients on day 7 and/or 14. A strong and a significant correlation was demonstrated between the undetectable time points that were predicted using this formula and the actual undetectable time points (Fig. 2c, $r = 0.53$, $P = 0.005$).

Although only one case was predicted to achieve a rapid virological response (undetectable viral load at week 4)[13] in the model validation group, the actual undetectable time point of this patient was week 8 (Fig. 2d). In contrast, all nine cases who were predicted to achieve a complete early virological response (undetected viral load until week 12),[13] the actual undetectable time points of these patients were within week 12. Because the prediction formula was derived by the least squares method, half of the patients, who were predicted not to achieve the complete early virological response, actually achieved it.

DISCUSSION

NUMEROUS STUDIES HAVE documented that the undetectable time point is related to therapeutic responses, and its usefulness in predicting therapeutic efficacy is clear.[9–13] In the present study, we were able to derive a formula for predicting the undetectable time point for patients with HCV genotype 1b and high serum viral loads during PEG-IFN-α-2b/ribavirin combination therapy. Though the various parameters for the HCV dynamics were investigated, the change in viral load from day 7 to 14 was the only parameter that was useful for predicting the undetectable time point.

The standard length of PEG-IFN/ribavirin combination therapy is 48 weeks for patients with HCV genotype 1b and high serum viral loads; however, a 72-week administration is recommended to improve therapeutic response.[3,13,18] Therefore, when undetectable time points are predicted as from weeks 13–24 by our formula, the SVR rates could be improved by continuing the IFN therapy for longer periods. By prediction of the undetectable time point early during the treatment using our formula, the physician can make early modification and optimization of currently ongoing therapy.

Another important issue of PEG-IFN/ribavirin treatment is adherence to treatment. Because dose reductions may delay the time until serum viral clearance, patients in whom the dosage of IFN and ribavirin was reduced during therapy were excluded in the present study. However, there are many patients in whom the dosage of drugs has to be reduced during therapy for a wide variety of clinical reasons. If reducing dosage before the predicted undetectable time point, administration of IFN for longer periods should be considered.

In conclusion, we created a formula for predicting the undetectable time point in patients treated with PEG-IFN-α-2b/ribavirin combination therapy. Viral eradication is the ultimate objective of IFN-based therapy, but many patients failed to achieve viral eradication for some reason. Because our prediction formula for the undetectable time point was made with a small population, it is necessary to correct it by further analysis with a larger population. However, an early viral response reflects efficacy of the therapy, and the estimation of an undetectable time point by our formula would be useful for determining the optimal duration of treatment in the early period of the therapy for each chronic hepatitis C patient.

REFERENCES

1 Glue P, Rouzier-Panis R, Raffanel C *et al.*; The Hepatitis C Intervention Therapy Group. A dose-ranging study of pegylated interferon alfa-2b and ribavirin in chronic hepatitis C. *Hepatology* 2000; 32: 647–53.
2 Reddy KR, Wright TL, Pockros PJ *et al.* Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. *Hepatology* 2001; 33: 433–8.
3 Sánchez-Tapias JM, Diago M *et al.*; TeraViC-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: 451–60.
4 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon- therapy in hepatitis C virus infection. *Hepatology* 1994; 19: 1088–94.
5 Chayama K, Tsubota A, Kobayashi M *et al.* Pretreatment virus load and multiple amino acid substitutions in the interferon sensitivity-determining region predict the outcome of interferon treatment in patients with chronic genotype 1b hepatitis C virus infection. *Hepatology* 1997; 25: 745–9.
6 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon

in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; 334: 77–81.
7 Davis GL. Prediction of response to interferon treatment of chronic hepatitis C. *J Hepatol* 1994; 21: 1–3.
8 Asahina Y, Izumi N, Hirayama I *et al.* Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. *Gastroenterology* 2008; 134: 1396–405.
9 Tong MJ, Blatt LM, McHutchison JG, Co RL, Conrad A. Prediction of response during interferon alfa 2b therapy in chronic hepatitis C patients using viral and biochemical characteristics: a comparison. *Hepatology* 1997; 26: 1640–5.
10 Lee WM, Reddy KR, Tong MJ *et al.* Early hepatitis C virus-RNA responses predict interferon treatment outcomes in chronic hepatitis C. The Consensus Interferon Study Group. *Hepatology* 1998; 28: 1411–5.
11 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645–52.
12 Poordad F, Reddy KR, Martin P. Rapid virologic response: a new milestone in the management of chronic hepatitis C. *Clin Infect Dis* 2008; 46: 78–84.
13 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335–74.
14 Neumann AU, Lam NP, Dahari H *et al.* Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998; 282: 103–7.
15 Powers KA, Dixit NM, Ribeiro RM, Golia P, Talal AH, Perelson AS. Modeling viral and drug kinetics: hepatitis C virus treatment with pegylated interferon alfa-2b. *Semin Liver Dis* 2003; 23 (Suppl 1): 13–8.
16 Halfon P, Bourlière M, Pénaranda G, Khiri H, Ouzan D. Real-time PCR assays for hepatitis C virus (HCV) RNA quantitation are adequate for clinical management of patients with chronic HCV infection. *J Clin Microbiol* 2006; 44: 2507–11.
17 Gelderblom HC, Menting S, Beld MG. Clinical performance of the new rRoche COBAS TaqMan HCV Test and High Pure System for extraction, detection and quantitation of HCV RNA in plasma and serum. *Antivir Ther* 2006; 11: 95–103.
18 Berg T, Wagner MV, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; 130: 1086–97.

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C型肝炎ウイルス感染の病態と治療法 3
ペグインターフェロンとリバビリン併用療法の治療成績と寄与因子

朝比奈靖浩

あさひな やすひろ：武蔵野赤十字病院 消化器科

● はじめに

わが国では，年間約3万5千人が肝癌で死亡しており，その多くはC型肝炎ウイルス(HCV)が原因である。わが国のC型肝炎患者は高齢化しており，他の先進国とは異なり，その発がんリスクが高い[1]。したがって，C型肝炎対策による肝癌撲滅は国の重要課題のひとつであり，肝臓専門医のみならず臨床医においては，インターフェロン（IFN）を中心としたC型肝炎の治療を積極的に行い，肝炎対策の効果を上げることが強く求められている。

わが国のC型慢性肝炎の約70%を占めるHCV genotype 1b型かつ高ウイルス量症例，いわゆる難治例に対して，2004年12月よりペグインターフェロン（Peg-IFN)-α＋リバビリン併用療法が導入され，約50%の症例でウイルス学的著効（SVR）が得られるようになった。しかし，再燃例や無効例もいまだ多く，実際の臨床現場では治療効果を予測しそれに応じた対策が求められている。本稿ではPeg-IFN-α＋リバビリン併用療法の実際の臨床における治療成績を概説するとともに，治療効果に寄与する因子および問題点について述べる。

● Peg-IFN-α＋リバビリン併用療法の治療成績

Peg-IFN-α＋リバビリン併用療法が導入されて以来，実臨床における治療成績が明らかとなってきた。そこで，当院においてPeg-IFN-α2b＋リバビリン併用療法を施行した614例のうちgenotype 1b型かつ高ウイルス量の488例を対象とし，治療成績と治療効果に寄与する因子について検討した。

すでに最終治療効果を判定しえた症例について検討すると，48週投与例のSVR率は薬剤減量・中止例も含めたITT解析では40%であったが，予定投与量の80%以上を投与しえた症例（PP解析）では61%と高率であった（図1)。一方，予定投与量の80%以上を投与しえた症例においても，治療終了後のHCV-RNAの再燃を26%に認め，さらに48週の治療中にHCV-RNAが陰性化しないウイルス学的不応（NVR）例を13%に認めた（図1)。これらのうち，48週投与でいったんはHCV-RNAが血中から消失する再燃例では，いわゆるresponse-guided therapyを行うなどの治療の工夫で，治療成績の向上が期待される。しかし，NVR例に対しては現行の治療法の工夫ではSVRを得ることは困難なことが多く，難治要因の解明と新薬を含め

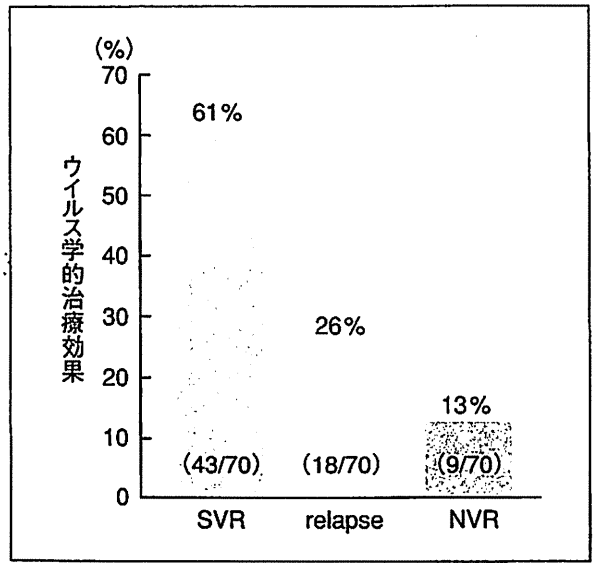

図 1　ペグインターフェロン α2b＋リバビリン 48 週併用療法におけるウイルス学的治療効果

予定投与量の 80%以上が投与された症例での検討 (n=292)。SVR：ウイルス学的著効，relapse：再燃，NVR：ウイルス学的不応

た新たな対策が必要と考えられる。

● Peg-IFN-α＋リバビリン併用療法の治療効果に影響を与える治療前因子

genotype 1b 型かつ高ウイルス量症例における Peg-IFN-α＋リバビリン併用療法の SVR に関与する治療前因子を単変量解析を用いて解析すると，年齢，性別，過去の IFN 治療効果のほか，ヘモグロビン濃度，血小板数，血清クレアチニン濃度，AST 値，γ-GTP 値，LDL コレステロール値，血糖値，さらに肝組織における脂肪化および線維化の程度などが有意な因子としてあげられた。また，HCV NS5A 領域のインターフェロン感受性決定領域(ISDR)の変異数[2]や，HCV コア 70 番・91 番のアミノ酸変異といったウイルス学的因子も有意であった。これらの因子をもとに多変量解析を用いて検討すると，①年齢 60 歳未満，②男性，③F2 以下の線維化非進行例，④LDL コレステロール非低値例，⑤ISDR 変異数が 2 個以上の症例，⑥HCV コア 70 番・91 番が野生型の症例は，それぞれ SVR が得られやすい症例と考えられた。

一方，NVR に関与する治療抵抗因子としては，①HCV コア変異，②架橋形成を伴う高度線維化，③血清γ-GTP 高値，④血清クレアチニン低値が多変量解析により抽出され，これらの治療抵抗因子を有する症例は，現行の治療では難治性で不応性となることが多い。特に，HCV コア変異は NVR に強く関与しコア 70 番・91 番両方に変異が認められると，NVR となるオッズ比は 8.0 ときわめて高かった。しかし，現在までのところ HCV コア変異の治療抵抗性に関連する詳細な機序は明らかとなっていない。

● ウイルス変異と治療効果

これまで述べてきたように，ISDR や HCV コア遺伝子のウイルス変異は Peg-IFN-α＋リバビリン併用療法の治療効果と密接な関連がある。そこで当院で Peg-IFN-α2b＋リバビリン併用療法を施行した症例において，ISDR とコア変異別のウイルス学的治療効果を検討した。それによると，ISDR 野生型の症例では，48 週治療中にいったんは HCV-RNA が陰性化するが，治療後に再燃する症例が ISDR 非野生型の症例に比し多いことがわかった。一方，コア 70 番・91 番変異を有する症例では，48 週治療中に HCV-RNA が陰性化しない NVR の症例がコア野生型の症例に比し多かった。したがって，ISDR は再燃に，コア変異は NVR に関与していると考えられ，これら 2 つの遺伝子変異のウイルス学的治療効果に及ぼす特徴を考慮して，個々の症例において治療方針を決定することが有用と考えられた。

● 治療中の抗ウイルス効果と治療成績

治療中においては，そのウイルス学的反応をモニターすることが，最終治療効果を予測するうえできわめて重要である。すなわち，IFN 療法中における HCV-RNA の消失時期と SVR 率との間には密接な関連があり，種々の治療ガイドラインでは，HCV-RNA 陰性化時期により最適な治療期間を設定するなどの，いわゆる response-guided therapy が推奨されている。

当院の症例において，リアルタイム PCR 法でみた HCV-RNA の陰性化時期と治療完遂例に

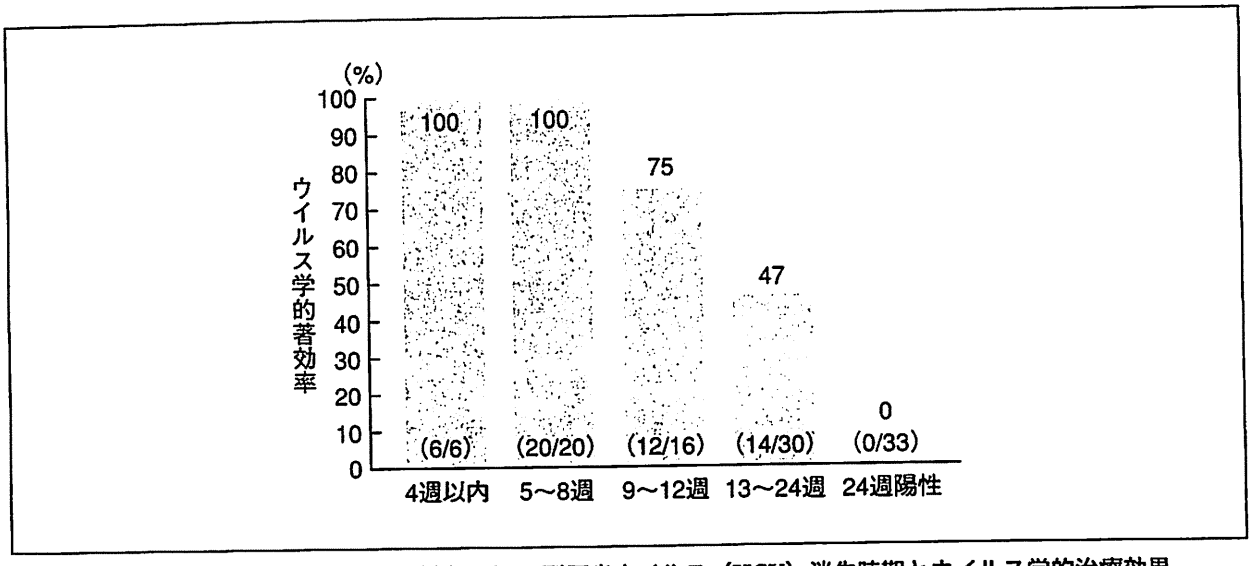

図 2　リアルタイム PCR 法で判定した C 型肝炎ウイルス（HCV）消失時期とウイルス学的治療効果
ペグインターフェロン α2b＋リバビリン併用 48 週療法での検討。中止例を除く。

おける SVR の関係を検討すると，治療開始 8 週以内に HCV-RNA が陰性化していれば，全例 SVR となり，SVR 率は 100％であった。また，9〜12 週の陰性化例でも 75％の症例で SVR が得られた。それに対して，13〜24 週に陰性化した症例では再燃率が高く，48 週投与での SVR 率は 47％で，さらに 24 週以降に陰性化した症例からは 1 例も SVR が得られなかった（図 2）。したがって，HCV-RNA 陰性化時期の遅れた症例における治療後の再燃をいかに減らすかが，治療成績を向上させるためには大変重要な課題である。

● 再燃に関与する因子の検討

そこで当院において Peg-IFN-α2b＋リバビリン併用 48 週療法を行った症例について，再燃にかかわる治療前因子を解析した。その結果，単変量解析では，年齢，過去の IFN 治療効果，ヘモグロビン値，血小板数，肝組織の脂肪化，ISDR 変異が有意であった。さらに，多変量解析では①年齢，②ISDR 変異が独立因子として抽出され，すなわち高齢者や ISDR 変異型が野生型の症例は 48 週治療後の再燃が多いことがわかった。

先に述べたように，治療後の再燃には治療中の HCV-RNA 消失時期が強く関連するため，HCV-RNA 消失時期別に年齢と ISDR で層別化してその再燃率を検討した。それによると，リアルタイム PCR 法で 9〜12 週に HCV-RNA が陰性化した症例において，60 歳未満の症例や ISDR 非野生型の症例では治療後再燃を認めなかったのに対し，60 歳以上かつ ISDR 野生型の症例では 48 週治療で再燃が認められた。一方，リアルタイム PCR 法で 13〜24 週に HCV-RNA が陰性化したいわゆる late virological response（LVR）の症例における再燃率は総じて高率だが，60 歳未満かつ ISDR 非野生型症例の再燃率は低率であった。

したがって，再燃率の低下のためには，HCV-RNA の陰性化時期に加えて，年齢やウイルス変異といった再燃要因を考慮して最適な治療期間を設定するなど，治療スケジュールを個別に決定する必要があると考えられた。

● 自然免疫系遺伝子の肝内発現プロファイルと抗ウイルス効果

一方，NVR 例に対しては現行の治療法の工夫では SVR を得ることは困難なことが多く，難治要因の解明と新たな対策が必要である。前述のようにわれわれの臨床的解析では，Peg-IFN-α＋リバビリン併用療法における難治要因にかかわるものとして，ウイルス因子のほかに血清 γ-GTP 高値などのなんらかの宿主因子の関与も示唆されている。そこで，われわれは生体防

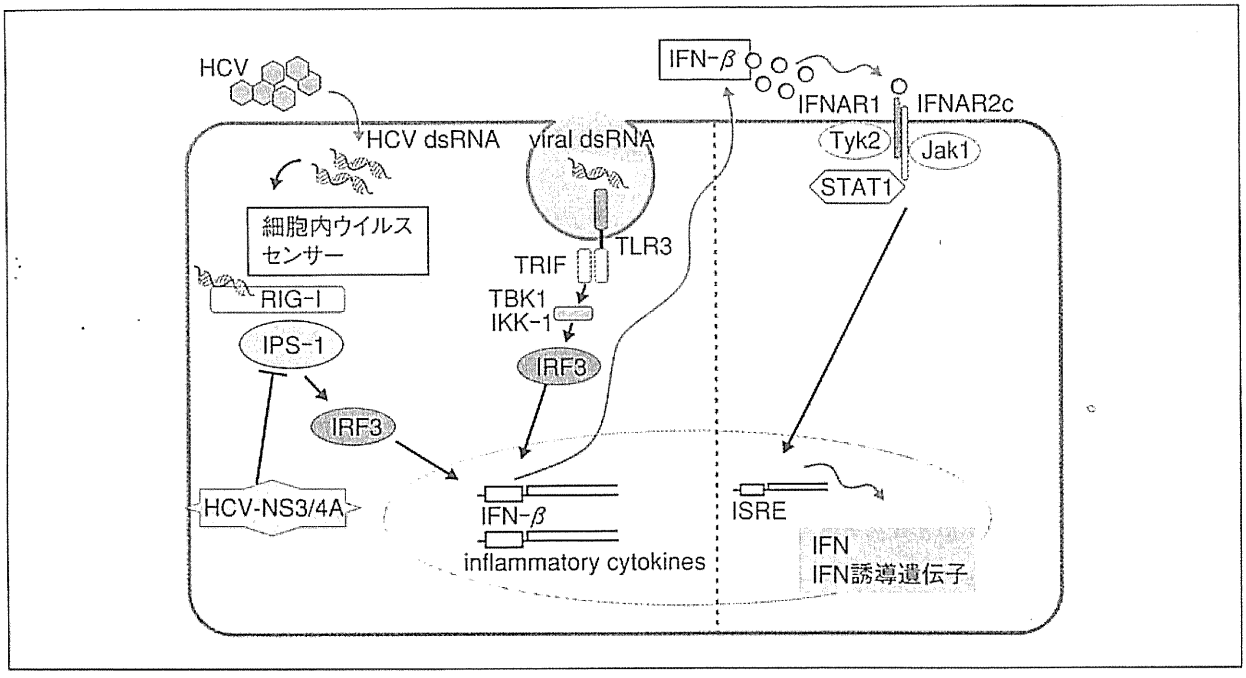

図 3　RIG-I/IPS-1 系を中心とした宿主自然免疫機構
RIG-I：retinoic acid inducible gene I，IPS：インターフェロン-β promoter stimulator

御にかかわる宿主因子である自然免疫機構と治療効果について注目した。

近年の *in vitro* の研究によると，HCV に対する生体防御には宿主自然免疫機構が重要とされる。すなわち，HCV が細胞に感染すると，まず HCV 由来の RNA が細胞内のウイルスセンサーである RIG-I(retinoic acid inducible gene I)によって探知され，そのシグナルがアダプター分子である IPS-1(IFN-β promoter stimulator-1：別名 MAVS，Cardif，VISA）を介して核に伝達され，IFN-β が産生されるとされる。この RIG-I/IPS-1 系による自然免疫の作動が HCV 感染に際して生体側で起こる最初の防御機構であるとされ，IFN-β は I 型の IFN 受容体に結合し，Jak-STAT 系を介し大量の IFN の産生や IFN 誘導遺伝子（ISG：interferon stimulated gene）の誘導を引き起こし，宿主を抗ウイルス状態にすると考えられている（図 3)。

一方，HCV の NS3/4 A セリンプロテアーゼは IPS-1 を分解することが示されており，HCV は RIG-I/IPS-1 系を標的とすることで，巧みに宿主の自然免疫系から逃れているとされる（図 3)。したがって，RIG-I/IPS-1 系は宿主による HCV の排除，およびそれに対する HCV の抵抗性に重要な鍵を握っていることは間違いないと考えられるが，ヒトにおける臨床的意義はほとんど解明されていなかった。

そこで，われわれは治療により大量に外因性 IFN を投与してもウイルス排除が起こらない NVR の症例では，この宿主自然免疫機構になんらかの特徴があると考え，Peg-IFN-α2b＋リバビリン併用療法を施行した 1b 高ウイルス量の C 型慢性肝炎 74 例を対象として，細胞内ウイルスセンサーである RIG-I およびアダプター分子である IPS-1，さらに IFN 誘導遺伝子である ISG15 などの mRNA の治療前肝生検組織における肝内発現量を定量した[3]。その結果，RIG-I や ISG15 の肝内遺伝子発現は，治療中 HCV が減衰しない NVR 群で SVR 群に比し有意に高発現していたのに対して，IPS-1 の治療前肝内遺伝子発現は NVR 群で有意に低値で，RIG-I/IPS-1 比は NVR 群で有意に高かった（NVR：SVR＝1.3：0.4）（図 4)。ROC 解析では ISG15，USP18 発現および RIG-I/IPS-1 比の area under the curve は 0.9 以上で，これらの遺伝子の治療前における肝内発現を定量することは，これまで困難とされてきた Peg-IFN-α＋リバビリン併用療法の最終治療効果を治療前に予測

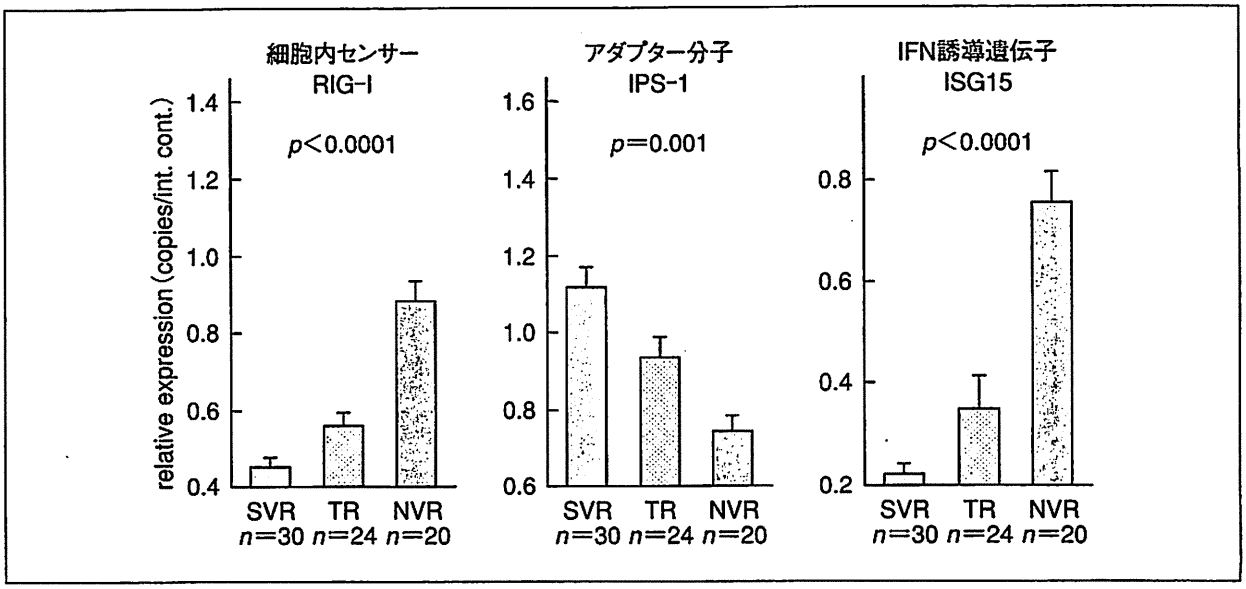

図 4　自然免疫系分子の肝内遺伝子発現とウイルス学的治療効果（文献 3 を引用，改変）

するのにきわめて有用と考えられた。

一方，治療による自然免疫系遺伝子の発現誘導の反応性を検討すると，治療前に高発現状態となっていた RIG-I や ISG15 は，Peg-IFN-α＋リバビリン投与による発現誘導が NVR 例では有意に低いことがわかった[3]。したがって，NVR 例では治療前に内因性 IFN により自然免疫系がすでに up regulation されているため，治療である外因性 IFN に対する反応性が減弱していることが示唆され，IFN に対する不応性のメカニズムを探る糸口となると考えられた。

● IL28B 近傍の一遺伝子多型（SNP）と治療効果および自然免疫系遺伝子発現との関連

さらに，最近 IL28B 近傍の SNP と Peg-IFN-α＋リバビリン併用 48 週投与における治療効果との関連が報告されている[4~6]。これについては他稿に詳しいが，当院においても IL28B 近傍の SNP と Peg-IFN-α2b＋リバビリン併用療法中の血中 HCV 動態と治療成績を検討した。それによると IL28B の minor allele の症例では，治療中に HCV 減衰がほとんど得られない，いわゆる null responder が多いことがわかり，最終的治療効果も IL28B の major allele の症例における NVR 率が 10% であったのに対し，minor allele の症例では約 70% が NVR であった。したがって，IL28B の minor allele は，NVR ときわめて強い関連があることが示唆された。また，IL28B minor allele にかかわる臨床的背景を単変量解析で検討すると，血清 γ-GTP 高値や LDL コレステロール低値，肝脂肪化などの宿主因子と関連していることがわかった。そこで，前述の宿主自然免疫系遺伝子発現と IL28B 近傍の SNP との関連を検討すると，IL28B の minor allele の症例では，有意に RIG-I や ISG15 の治療前の肝内発現が major allele の症例に比し高く，反対に IPS-1 の発現は低い傾向を認めた。多変量解析では NVR にかかわる独立因子として，RIG-I/IPS-1 比と IL28B minor allele が抽出されたことから，RIG-I/IPS-1 系を中心とした宿主自然免疫機構は IL28B 近傍の SNP とともに NVR と関連していることが示唆された。

● おわりに

Peg-IFN-α＋リバビリン併用療法が臨床応用され広く施行されるようになり，その効果規定因子や難治要因も次第に明らかとなってきた。したがって，今後はこれらの要因を個々の症例において検討し，的確な治療効果予測を行い，より有効な対策を講じることで治療成績の向上をはかる必要がある。