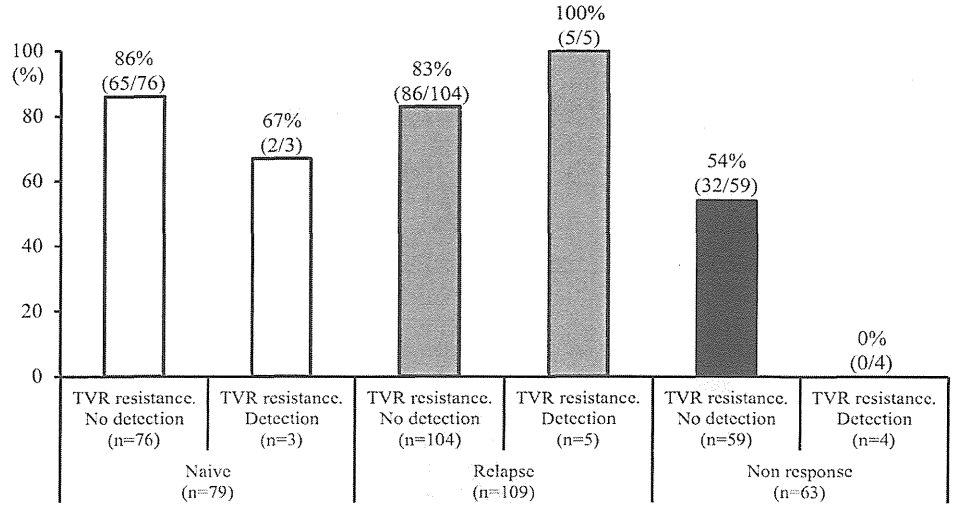

FIG 1 The rates of sustained virological response by the combination of response to prior treatment and presence of telaprevir (TVR)-resistant variants by direct sequencing at baseline are shown. Of those who showed nonresponse to prior treatment, a higher proportion of patients with undetected TVR-resistant variants (54%) achieved a sustained virological response than patients with detected TVR-resistant variants (0%) ($P = 0.053$).

Table 4 summarizes the profiles of 4 patients with nonresponse to prior treatment and in whom telaprevir-resistant variants were detected by direct sequencing at baseline. All of these 4 patients did not achieve an SVR with triple therapy. Interestingly, both T54S as a telaprevir-resistant variant and Q80L as a TMC435-resistant variant (19) were detected by direct sequencing at baseline.

Evolution of telaprevir-resistant variants over time as investigated by ultradeep sequencing in patients who received the second course of triple therapy. Two of 60 patients who did not achieve an SVR with the first course of triple therapy with telaprevir received the second course of triple therapy with telaprevir. They were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral loads.

Figure 2A shows the clinical course of case 1. In the first course of triple therapy with telaprevir (T12PR24) in a 57-year-old, V36C (0% of 32,413× coverage) was not detected by ultradeep sequencing at baseline of the first course, but very-high-frequency variants of V36C (97.2% of 36,757× coverage) were detected at the time of reelevation of viral loads. In the second course of triple therapy with telaprevir (T12PR54) when the patient was 59 years old, very-high-frequency variants of V36C (98.1% of 94,547× coverage) persisted at baseline of the second course, despite the passing of 2 years after cessation of the first therapy course. Case 1 achieved HCV RNA-negative status at 20 weeks after the start of the second course (late virological response), so PEG-IFN and ribavirin therapy was extended to 54 weeks. In conclusion, case 1 achieved an SVR after the second course of triple therapy with telaprevir, despite the persistence of very-high-frequency variants.

Figure 2B shows the clinical course of case 2. In the first course of triple therapy with telaprevir (T12PR24) in a 61-year-old patient, R155Q (0% of 23,751× coverage) and A156T (0% of 16,040× coverage) were not detected by ultradeep sequencing at baseline of the first course, but very-low-frequency variants of R155Q (0.2% of 11,572× coverage) and A156T (0.2% of 16,040× coverage) were detected at the time of reelevation of viral loads. In the second course of triple therapy with telaprevir (T12PR20) when the patient was 64 years old, R155Q (0% of 80,572× coverage) and A156T (0% of 87,686× coverage) were not detected by ultradeep sequencing at baseline of the second course, which was 2 years after cessation of the first course. In conclusion, case 2 achieved an SVR by the second course of triple therapy with telaprevir, despite the history of the emergence of variants.

TABLE 4 Profiles of 4 patients with nonresponse to prior treatment and detection of telaprevir-resistant variants by direct sequencing at baseline

Case no.	Sex	Age (yr)	Response to prior treatment[a]	Amino acid detected at aa position: 36	54	80	155	156	168	170	Time of HCV RNA-negative result during treatment (wks)	Efficacy of triple therapy
1	Male	70	Nonresponse to IFN monotherapy	V	S	L	R	A	D	I	2	Non-SVR
2	Male	47	Nonresponse to IFN monotherapy	V	S	L	R	A	D	I	4	Non-SVR
3	Male	61	Nonresponse to RBV combination therapy	V	S	L	R	A	D	I	3	Non-SVR
4	Female	60	Nonresponse to RBV combination therapy	V	S	L	R	A	D	I	4	Non-SVR

[a] RBV, ribavirin.

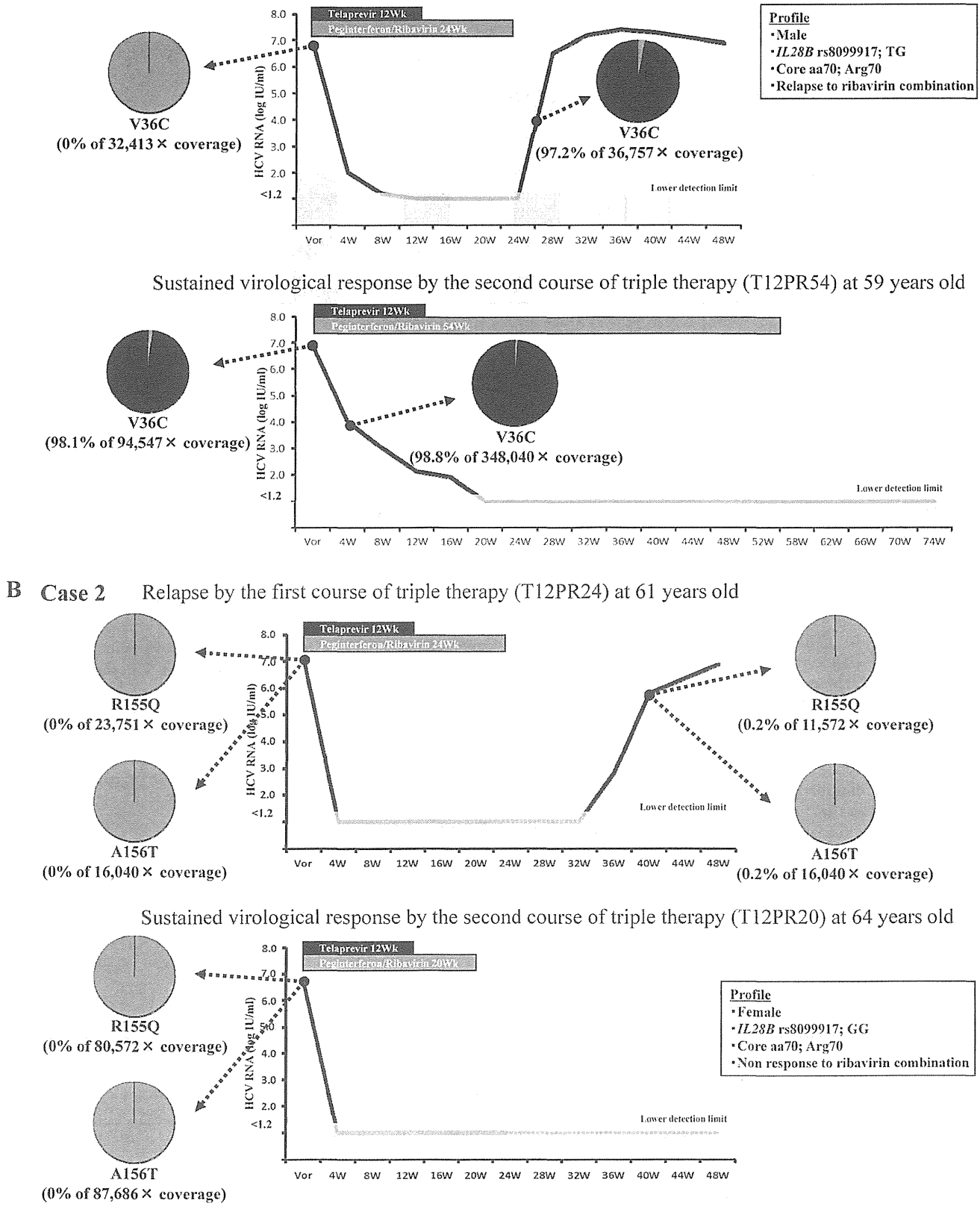

FIG 2 Two patients who did not achieve a sustained virological response with the first course of triple therapy with telaprevir received the second course of the triple therapy with telaprevir. They were analyzed for telaprevir-resistant variants by ultradeep sequencing at baseline and at the time of reelevation of viral loads. (A) Case 1 achieved a sustained virological response with the second course of therapy despite the persistence of very-high-frequency variants. (B) Case 2 achieved a sustained virological response with the second course of therapy despite the history of the emergence of variants.

DISCUSSION

Patients who fail to achieve an SVR to triple therapy need to be identified to avoid unnecessary side effects, high costs, and the emergence of telaprevir-resistant variants. Host genetic factors (e.g., *IL28B* genotype), and viral factors (e.g., amino acid substitutions in the core/NS5A region) have often been used as pretreatment predictors of poor virological response to PEG-IFN–ribavirin dual therapy (9–11, 15, 17) and telaprevir–PEG-IFN–ribavirin triple therapy (24–26). However, the pretreatment factors associated with the detection of telaprevir-resistant variants at the time of reelevation of viral load are still unknown. The present study identified that the detection of telaprevir-resistant variants at the time of reelevation of viral load can be predicted by a combination of host (*IL28B* rs8099917 genotype and leukocyte count), viral (variants of aa 54 at baseline), and treatment factors (PEG-IFN dose). All of the 4 patients with nonresponse to prior treatment and in whom telaprevir-resistant variants were detected at baseline did not achieve an SVR with triple therapy, and the use of the combination of nonresponse to prior treatment and the detection of telaprevir-resistant variants at baseline had high specificity and PPV for the prediction of a non-SVR. This finding suggests that there is a complex relationship between host susceptibility to IFN and viral sensitivity to NS3/4A protease inhibitors in determining treatment efficacy. Interestingly, in all of the 4 patients, both T54S as a telaprevir-resistant variant and Q80L as a TMC435-resistant variant (19) were detected by direct sequencing at baseline. This result suggests that patients with the above two factors should be carefully introduced to NS3/4A protease inhibitors besides telaprevir because of the high risk of the emergence of resistant variants. However, the present study was performed with a small number of patients, so further studies based on a larger number of patients should be performed.

In the present study employing ultradeep sequencing technology, 2 patients who did not achieve an SVR with the first course of triple therapy with telaprevir received the second course of the triple therapy with telaprevir. They achieved an SVR with the second course, despite the persistence of very-high-frequency variants (case 1, 98.1% for V36C) or a history of the emergence of variants (case 2, 0.2% for R155Q and 0.2% for A156T) as determined by ultradeep sequencing. This finding may be due to one or more reasons. One reason is probably related to the high susceptibility of telaprevir-resistant variants to IFN. One previous study indicated that mice infected with the resistant strain (A156F [99.9%]) developed only low-level viremia, and the virus was successfully eliminated with IFN therapy (27). In the other clinical report, telaprevir-resistant variants that emerged during 24-week telaprevir monotherapy were eliminated by the combination therapy of PEG-IFN plus ribavirin (28). Furthermore, this finding probably suggests that a small number of mutant-type viral RNAs may be incomplete or defective, since a large proportion of viral genomes are thought to be defective due to their high replication and mutation rates (29). Further studies employing ultradeep sequencing should be performed to evaluate whether a history of the emergence of NS3/4A protease inhibitor-resistant variants, besides telaprevir-resistant variants, affects the efficacy of a second course of NS3/4A protease inhibitor-based treatment.

The results of the present study should be interpreted with caution, since the study was performed with a small number of Japanese patients infected with HCV-1b. Any generalization of the results should await confirmation by a multicenter randomized trial based on a larger number of patients, including patients of other races and those infected with HCV-1a. Furthermore, the other limitation of the present study is that the loss of telaprevir-resistant variants was not investigated long after the cessation of therapy. Further large-scale studies should be performed to investigate the impacts of telaprevir-resistant variants on the response to treatment using new drugs, including direct-acting antiviral agents.

In conclusion, this study based on Japanese patients infected with HCV-1b indicates that telaprevir-resistant variants at the time of reelevation of viral load can be predicted by a combination of host, viral, and treatment factors. In those patients with no response to prior treatment, the present results suggest that telaprevir-resistant variants at baseline might partly affect the efficacy of triple therapy treatment. This finding indicates the clinical utility of detecting telaprevir-resistant variants to predict treatment efficacy, and it suggests a complex relationship between host susceptibility to IFN and viral sensitivity to NS3/4A protease inhibitors in determining treatment efficacy. Further large-scale prospective studies are needed to investigate the clinical usefulness of telaprevir-resistant variants and to develop more effective therapeutic regimens in patients infected with HCV-1.

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Effect of Type 2 Diabetes on Risk for Malignancies Includes Hepatocellular Carcinoma in Chronic Hepatitis C

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The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for malignancies after the termination of interferon (IFN) therapy in Japanese patients for hepatitis C virus (HCV). A total of 4,302 HCV-positive patients treated with IFN were enrolled. The mean observation period was 8.1 years. The primary outcome was the first onset of malignancies. Evaluation was performed using the Kaplan-Meier method and Cox proportional hazard analysis. A total of 606 patients developed malignancies: 393 developed hepatocellular carcinoma (HCC) and 213 developed malignancies other than HCC. The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, and 19.7% at 15 years. HCC occurred significantly ($P < 0.05$) when the following characteristics were present: advanced histological staging, sustained virological response not achieved, male sex, advanced age of ≥50 years, total alcohol intake of ≥200 kg, and presence of type 2 diabetes (T2DM). T2DM caused a 1.73-fold enhancement in HCC development. In patients with T2DM, HCC decreased when patients had a mean hemoglobin A1c (HbA1c) level of <7.0% during follow-up (hazard ratio, 0.56; 95% confidence interval, 0.33-0.89; $P = 0.015$). The cumulative development rate of malignancy other than HCC was 2.4% at 5 years, 5.1% at 10 years, and 9.8% at 15 years. Malignancies other than HCC occurred significantly when patients were of advanced age of ≤50 years, smoking index (package per day × year) was ≥ 20, and T2DM was present. T2DM caused a 1.70-fold enhancement in the development of malignancies other than HCC. *Conclusion:* T2DM causes an approximately 1.7-fold enhancement in the development of HCC and malignancies other than HCC in HCV-positive patients treated with IFN. In T2DM patients, maintaining a mean HbA1c level of <7.0% reduces the development of HCC. (HEPATOLOGY 2013;57:964-973)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease worldwide. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20%-50% of cases over a period of 10-30 years.[1,2] In addition, HCV is a major risk factor for hepatocellular carcinoma (HCC).[3-7]

On the other hand, the prevalence of patients with type 2 diabetes mellitus (T2DM) is increasing in many nations, including Japan.[8] Thus, the management of T2DM patients who are chronically infected with HCV is one of the most important issues confronted by physicians. Few studies have reported relationships between T2DM and total malignancies, including HCC in HCV patients. In addition, it is not clear whether the stringent control of T2DM is necessary for protecting the development of malignancies in HCV patients. This issue needs to be confirmed via long-term follow-up of a large cohort of patients at high risk of developing malignancy.

Abbreviations: CH, chronic hepatitis; CI, confidence interval; HbA1c, hemoglobin A1c; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; IFN, interferon; LC, liver cirrhosis; SVR, sustained virological response; T2DM, type 2 diabetes mellitus; TAI, total alcohol intake.

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With this background in mind, the present study was initiated to investigate the cumulative incidence and risk factors of malignancies, including HCC after prolonged follow-up in HCV patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and March 2009 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan, was 7,205. Of these, 4,302 patients met the following enrollment criteria: (1) no evidence of malignancies by physical examination, biochemical tests, abdominal ultrasonography, gastrofiberscope (or gastrography), or chest X-ray (or computed tomography); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (3) positivity for serum HCV-RNA before the initiation of IFN therapy; (4) period of $\geq$1 month to $\leq$1 year of IFN therapy; (5) negativity for hepatitis B surface antigens, antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay, or indirect immunofluorescence assay; (6) age of $\geq$30 years to $\leq$80 years; (7) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatic arthritis; and (8) repeated annual examinations during follow-up. Annual examinations included biochemical tests, tumor marker (carcinoembryonic antigen, alpha-fetoprotein, and prostate-specific antigen [only in men]), and abdominal ultrasonography. Patients with were excluded from the study if they had illnesses that could seriously reduce their life expectancy or if they had a history of carcinogenesis.

The primary outcome was the first development of malignancy. The development of malignancies was diagnosed by clinical symptoms, tumor marker, imaging (ultrasonography, computed tomography, or magnetic resonance imaging), and/or histological examination.[9-15] All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effects of IFN therapy to each patient and/or the patient's family. In addition, the physicians in charge received permission for the use of serum stores and future use of stored serum. Informed consent for IFN therapy and future use of stored serum was obtained from all patients. The study was approved by the Institutional Review Board of our hospital.

Medical Evaluation. Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and body mass index was calculated as kg/m^2. All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits, including questions on alcohol intake and smoking history.

The value for hemoglobin A_{1C} (HbA_{1C}) was estimated as a National Glycohemoglobin Standardization Program equivalent value (%). Patients were defined as having T2DM when they had a fasting plasma glucose level of $\geq$126 mg/dL and/or HbA_{1C} level of $\geq$6.5%.[16]

Patients were regarded as hypertensive when systolic blood pressure was $\geq$140 mm Hg and/or diastolic blood pressure was $\geq$90 mm Hg for at least three visits. Smoking index (packs per day $\times$ year) and total alcohol intake (TAI) were evaluated by the sum of before, during, and after the IFN therapy.

Laboratory Investigation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported.[17] HCV-RNA was determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at -80°C before IFN therapy were used. The linear dynamic range of the assay was 1.2-7.8 log IU/

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Table 1. Clinical Backgrounds at Initiation of Follow-up in Enrolled Patients

Variable	Total	HCC Group	Non-HCC Malignancy Group	Without Events Group	P
No. of patients	4,302	393	213	3,696	
Age, years	52.0 ± 11.8	55.8 ± 7.9	57.9 ± 9.1	51.3 ± 12.1	<0.001
Sex, male/female	2528/1774	272/121	129/84	2127/1569	<0.001
Height, cm	163.0 ± 9.2	162.8 ± 8.3	163.3 ± 9.1	163.0 ± 9.3	0.772
Weight, kg	61.4 ± 13.0	62.3 ± 10.6	60.8 ± 10.1	61.3 ± 13.4	0.142
BMI	23.0 ± 4.0	23.4 ± 3.0	22.8 ± 2.8	23.0 ± 4.1	0.012
Blood pressure, mm Hg					
Systolic	128 ± 18	132 ± 19	133 ± 20	127 ± 17	<0.001
Diastolic	77 ± 13	80 ± 12	80 ± 13	77 ± 13	<0.001
TAI, kg*	95 ± 92	151 ± 101	135 ± 81	85 ± 89	<0.001
Smoking index*	6.4 ± 9.4	10.8 ± 11.1	12.5 ± 11.8	5.5 ± 8.7	<0.001
AST, IU/L	42 ± 44	64 ± 55	42 ± 31	40 ± 42	<0.001
ALT, IU/L	44 ± 53	72 ± 63	43 ± 43	42 ± 52	<0.001
GGT, IU/L	54 ± 61	63 ± 65	56 ± 45	53 ± 38	0.007
Albumin, g/dL	4.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.2	4.1 ± 0.2	0.310
Triglyceride, mg/dL	101 ± 53	104 ± 54	105 ± 50	100 ± 52	0.329
Cholesterol, mg/dL	170 ± 32	165 ± 31	169 ± 33	171 ± 32	0.025
FPG, mg/dL	100 ± 22	110 ± 26	104 ± 22	98 ± 21	<0.001
HbA1c, %, NSPG	5.6 ± 1.2	5.9 ± 1.4	5.7 ± 1.4	5.5 ± 1.1	<0.001
T2DM, +/−	267/4,035	63/330	34/179	170/3,526	<0.001
Platelet count, $\times 10^4/mm^3$	17.1 ± 5.1	13.7 ± 4.9	16.5 ± 5.4	17.5 ± 5.4	<0.001
Staging, LC/non-LC	433/3,869	113/285	27/189	293/3,395	<0.001
HCV genotype, 1b/2a/2b/other	2,721/995/458 /128	283/52/20/38	121/62/18/12	2,317/881/420/78	<0.001
HCV RNA, log IU/mL	6.06 ± 1.05	6.22 ± 0.52	6.05 ± 0.86	6.04 ± 1.05	0.003
IFN monotherapy†/combination therapy‡	2,861/1,441	358/35	175/38	2,328/1,368	<0.001
Efficacy, SVR/non-SVR	1,900/2,402	44/349	88/125	1,768/1,928	<0.001

Data are presented as no. of patients or mean ± SD.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; M, male; NGSP, National Glycohemoglobin Standardization Program.

*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

†Outbreak of IFN monotherapy: recombinant IFN-α2a, n = 220, recombinant IFN-α2b, n = 183, natural IFN-α, n = 1,678, natural IFN-α, n = 691, total dose of IFN = 560 ± 164 megaunit. Outbreak of pegylated IFN monotherapy: pegylated IFN-α2a, n = 89, total dose of pegylated IFN = 7.52 ± 2.24 mg.

‡Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, n = 335, total dose of IFN = 508 ± 184 megaunit, total dose of ribavirin = 160 ± 68 g; natural IFN-β + ribavirin, n = 101, total dose of IFN = 502 ± 176 megaunit, total dose of ribavirin = 156 ± 67 g; pegylated IFN-α2b+ribavirin, n = 1,005 cases, total dose of pegylated IFN = 4.14 ± 1.10 mg, total dose of ribavirin = 206 ± 58 g.

mL, and the undetectable samples were defined as negative. A sustained virological response (SVR) was defined as clearance of HCV-RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

Evaluation of Liver Cirrhosis. Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained 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. The size of specimens for examination was more than six portal areas.[18]

Follow-up. The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital. Physical examination and biochemical tests were conducted at each examination together with a regular checkup. In addition, annual examinations during follow-up were undertaken. When a patient had complaints during follow-up, the physician in charge performed additional examinations based on symptoms. Four hundred eighteen patients were lost to follow-up. The final date of follow-up in 418 patients with loss of follow-up was regarded as the last consulting day. In addition, 881 patients were retreated with IFN. The final date of follow-up in 881 patients re-treated with IFN were regarded as the time of the initiation of IFN retreatment. Thus, 418 patients with loss of follow-up and 881 patients retreated with IFN were counted censored data in statistical analysis.[19] The mean follow-up period was 6.8 (SD 4.3) years in 418 patients with loss of follow-up and 7.5 (SD 4.8) years in 881 patients retreated with IFN. Censored patients were counted in the analysis.

Statistical Analysis. Clinical differences among three groups of patients with HCC with malignancies other than HCC without events were evaluated using the Kruskal-Wallis test. The cumulative development rates of malignancies were calculated using the Kaplan-Meier technique, and differences in the curves were

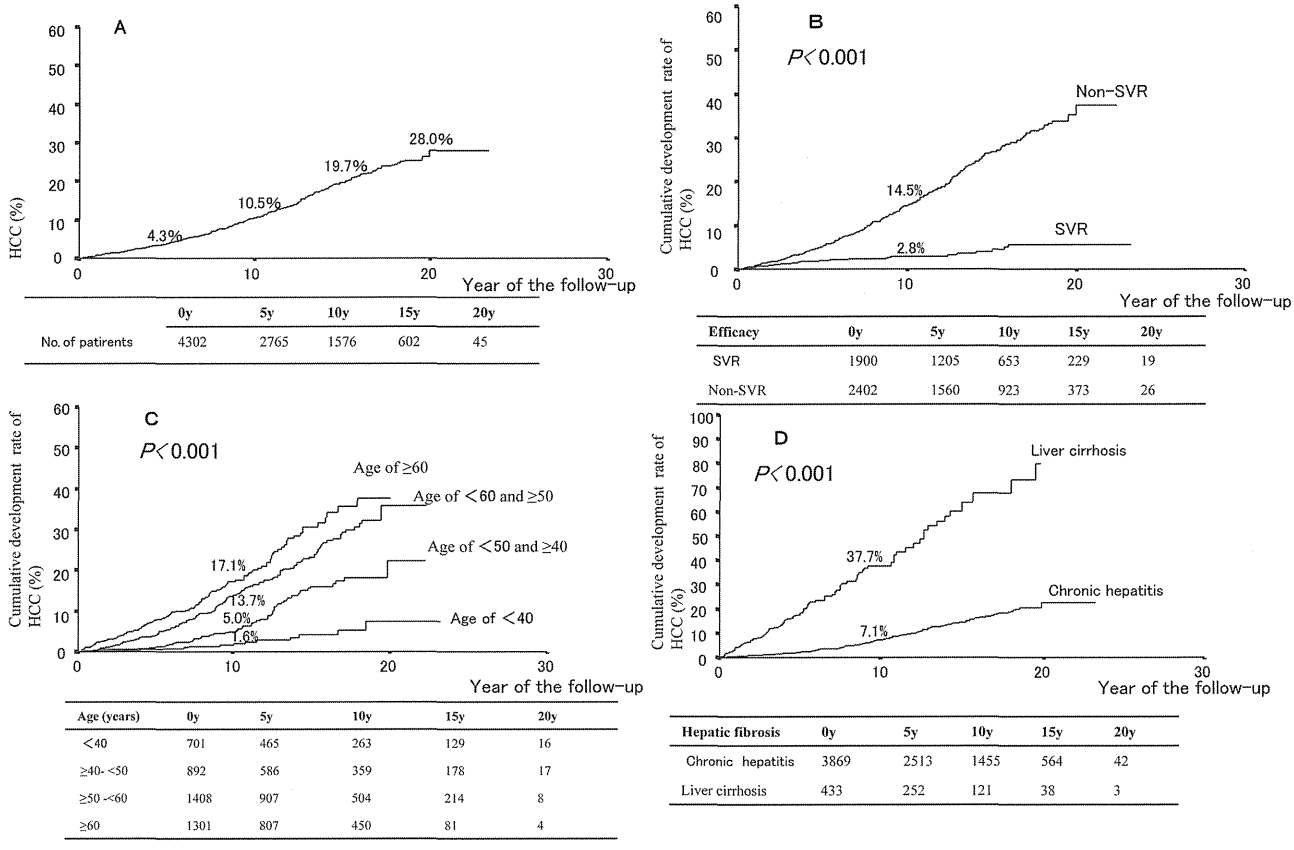


Fig. 1. Cumulative development rate of HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) efficacy, (C) age, and (D) hepatic fibrosis.

tested using the log-rank test.[20,21] Independent risk factors associated with malignancies were studied using the stepwise Cox regression analysis.[22] The following variables were analyzed for potential covariates for incidence of primary outcome: (1) age, sex, T2DM, and hypertension at the initiation time of follow-up; (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy; (3) average value of body mass index, aspartate aminotransferase, alanine aminotransferase, triglyceride, total cholesterol, and platelet count during follow-up; (4) sum value of smoking and alcohol before, during, and after the IFN therapy; and (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A $P < 0.05$ was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1shows the baseline characteristics of the 4,302 enrolled patients at initiation of follow-up. The patients were divided into three groups: with HCC, with malignancies other than HCC, and without events. There were significant differences in several baseline characteristics among the three groups. The SVR rate was 34.4% (985/2,861) in IFN monotherapy and 63.5% (915/1,441) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 1,900. The mean follow-up was 8.1 (SD 5.0) years.

Development and Breakdown of Malignancies. As shown in Table 1, 606 of 4,302 patients developed malignancies: 393 developed HCC and 213 developed malignancies other than HCC. HCC accounted for 33.3% (44/132) of malignancies in patients with SVR and 73.6% (349/474) in patients without SVR. The breakdown of malignancies other than HCC was as follows: stomach cancer, n = 36; colon cancer, n = 35; lung cancer, n = 20; malignant lymphoma, n = 19; pancreatic cancer, n = 12; prostatic cancer, n = 16; breast cancer, n = 15; other cancers, n = 60.

Predictive Factors for the Development of HCC. The cumulative development rate of HCC was 4.3% at 5 years, 10.5% at 10 years, 19.7% at 15 years, and 28.0% at 20 years (Fig. 1A). The factors associated with the development of HCC are shown in Table 2. Multivariate Cox proportional hazards analysis

Table 2. Predictive Factors for Development of HCC in Enrolled Patients

	Univariate Analysis		Cox Regression Analysis	
Variable	HR (95% CI)	P	HR (95% CI)	P
Age, years (per 10)	1.84 (1.64-2.06)	<0.001	1.97 (1.71-2.28)	<0.001
Sex, male/female	1.47 (1.18-1.83)	<0.001	1.67 (1.24-2.23)	0.001
BMI, ≥22/<22	1.37 (1.12-1.66)	0.002		
T2DM, +/−	2.77 (2.13-3.60)	<0.001	1.73 (1.30-2.30)	<0.001
Hypertension, +/−	1.32 (1.02-1.71)	0.036		
Smoking index, ≥20/<20*	1.43 (1.14-1.79)	0.002		
TAI, kg, ≥200/<200*	2.13 (1.74-2.61)	<0.001	1.45 (1.11-1.88)	0.007
AST, IU/L, ≥34/<34	3.00 (2.40-3.89)	<0.001		
ALT, IU/L, ≥36/<36	2.74 (2.16-3.42)	<0.001		
GGT, IU/L, ≥109/<109	1.79 (1.19-2.46)	0.039		
Albumin, g/dL, <3.9/≥3.9	1.92 (1.37-2.55)	0.015		
Triglyceride, mg/dL, ≥100/<100	1.14 (0.94-1.37)	0.179		
Cholesterol, mg/dL, <150/≥150\	1.38 (1.10-1.72)	0.004		
Platelet count, $\times 10^4/mm^3$, <15/≥15)	3.27 (2.56-4.17)	<0.001		
Histological diagnosis, LC/non-LC	7.09 (5.59-9.01)	<0.001	5.01 (3.92-6.40)	<0.001
Combination of ribavirin, +/−	0.66 (0.45-0.97)	0.033		
Type of IFN, α/β	1.10 (0.85-1.41)	0.474		
Total dose of IFN, MU, ≥500/<500	1.12 (0.91-1.38)	0.291		
HCV genotype, ½	1.67 (1.30-2.14)	<0.001		
HCV-RNA, log IU/mL, ≥5/<5	1.02 (0.98-1.05)	0.315		
Efficacy, non-SVR/SVR	4.78 (3.47-6.59)	<0.001	4.93 (3.53-6.89)	<0.001

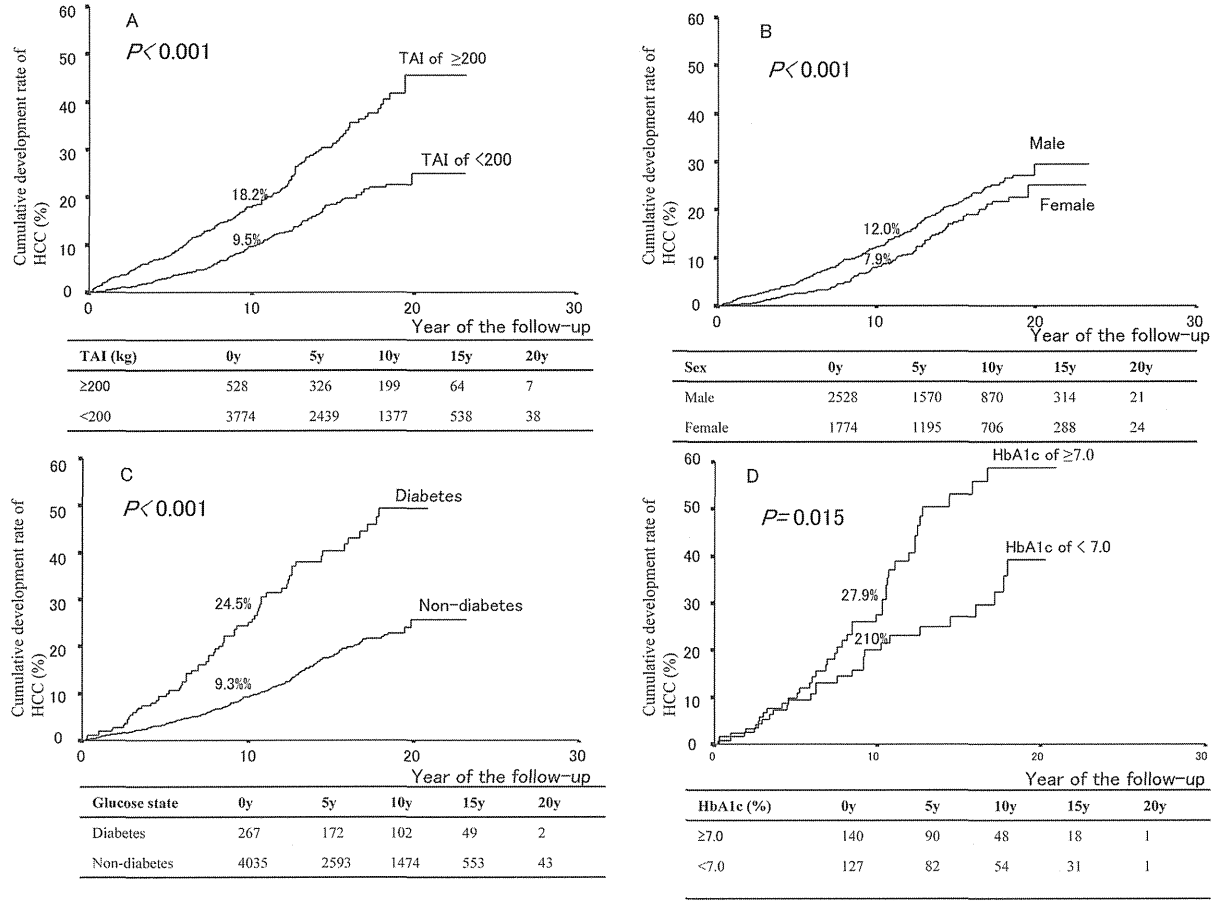
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein.

*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

showed that HCC occurred when patients had liver cirrhosis (hazard ratio [HR], 5.01; 95% confidence interval [CI], 3.92-6.40; $P < 0.001$), non-SVR (HR, 4.93; 95% CI, 3.53-6.89; $P < 0.001$), age increments of 10 years (HR, 1.97; 95% CI, 1.71-2.28; $P < 0.001$), T2DM (HR, 1.73; 95% CI, 1.30-2.30; $P < 0.001$), male sex (HR, 1.67; 95% CI, 1.24-2.23; $P = 0.001$), and TAI of ≥ 200 kg (HR, 1.45; 95% CI, 1.11-1.88; $P = 0.007$). Fig. 1B-D and Fig. 2A-C show the cumulative development rates of HCC based on difference of IFN efficacy, age, hepatic fibrosis, TAI, sex, and T2DM. The 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis by using the Kaplan-Meier Method (Fig. 1D). Fig. 2D shows the development rates of HCC in T2DM patients according to difference of mean hemoglobin A1c (HbA1c) level during follow-up. HCC decreased when T2DM patients had a mean HbA1c level of <7.0% during follow-up (HR, 0.56; 95% CI, 0.33-0.89; $P = 0.015$). The development of HCC was reduced by 44% in T2DM patients with a mean HbA1c level of <7.0% compared with those with a mean HbA1c level of ≥7.0%.

Table 3 shows the development rate of HCC and risk factors in four groups classified by the difference of hepatic fibrosis and efficacy of IFN therapy. The development rate of HCC per 1,000 person years was 1.55 in patients with chronic hepatitis (CH) at baseline and SVR (CH+SVR), 18.23 in patients with liver cirrhosis (LC) at baseline and SVR (LC+SVR), 13.53 in patients with chronic hepatitis at baseline and non-SVR (CH+non-SVR), and 50.43 in patients with LC at baseline and non-SVR (LC+non-SVR). The risk of HCC development in the CH+SVR group was advanced age, male sex, TAI of ≥200 kg, and T2DM. T2DM enhanced the development of HCC with statistical significance in three groups of CH+SVR, CH+non-SVR, and LC+non-SVR.

Predictive Factors for Development of Malignancies Other than HCC. The cumulative development rate of malignancies other than HCC was 2.4% at 5 years, 5.1% at 10 years, 9.8% at 15 years, and 18.0% at 20 years (Fig. 3A). The factors associated with the development of malignancies other than HCC are shown in Table 4. Malignancies other than HCC occurred when patients had age increments of 10 years (HR, 2.19; 95% CI, 1.84-2.62; $P < 0.001$), smoking index of ≥20 (HR, 1.89; 95% CI, 1.41-2.53; $P < 0.001$), and T2DM (HR, 1.70; 95% CI, 1.14-2.53; $P = 0.008$). Fig. 3B-D shows the cumulative development rates of malignancies other than HCC based on difference of age, smoking index, and T2DM. Fig. 3E shows the risk of malignancies other than HCC in T2DM patients according to mean HbA1c level during follow-up. The HR of HCC development in



TAI (kg)	0y	5y	10y	15y	20y
≥200	528	326	199	64	7
<200	3774	2439	1377	538	38

Sex	0y	5y	10y	15y	20y
Male	2528	1570	870	314	21
Female	1774	1195	706	288	24

Glucose state	0y	5y	10y	15y	20y
Diabetes	267	172	102	49	2
Non-diabetes	4035	2593	1474	553	43

HbA1c (%)	0y	5y	10y	15y	20y
≥7.0	140	90	48	18	1
<7.0	127	82	54	31	1

Fig. 2. Cumulative development rate of HCC based on the difference of (A) TAI, (B) sex, (C) diabetic state, and (D) mean HbA1c level during follow-up in T2DM patients.

patients with mean HbA1c level of <7.0% versus those with mean HbA1c level of ≥7.0% was 0.62 (95% CI, 0.31-1.23; $P = 0.170$). There was no significant difference in development of malignancies other than HCC based on the difference of mean HbA1c level during follow-up. Table 5 shows the impact based

Table 3. Development Rate of HCC Based on Hepatic Fibrosis and Efficacy of IFN Therapy

Variable	CH + SVR	LC + SVR	CH + Non-SVR	LC + Non-SVR
No. of patients	1,751	149	2,118	284
Age, years	51.7 ± 12.1	56.9 ± 9.8	51.5 ± 11.7	57.2 ± 9.9
Sex, male/female	1,082/669	91/58	1,190/928	165/119
HbA1c (%, NSPG)	5.5 ± 0.7	5.8 ± 0.8	5.7 ± 0.7	6.1 ± 0.8
TAI, kg	86 ± 91	104 ± 99	97 ± 90	129 ± 102
Patients with T2DM	74	13	133	47
Patients with HCC	22	22	233	116
1,000 person years of HCC	1.55	18.23	13.53	50.43
Age, years (per 10)*	2.60 (1.48-4.58)	1.83 (0.95-3.55)	2.07 (1.75-2.46)	1.09 (0.87-1.37)
P value	0.001	0.070	<0.001	0.477
Sex, male/female*	3.42 (1.01-11.63)	3.41 (1.00-11.63)	1.34 (0.99-1.81)	1.93 (1.25-3.00)
P value	0.049	0.050	0.058	0.003
TAI, kg, ≥200/<200*	2.68 (1.14-6.34)	3.84 (1.83-9.85)	2.21 (1.65-2.95)	1.54 (1.03-2.31)
P value	0.024	0.004	<0.001	0.038
T2DM, +/−*	4.76 (1.60-14.10)	2.48 (0.57-10.86)	2.53 (1.76-3.65)	1.87 (1.16-3.01)
P value	0.005	0.228	<0.001	0.010

Abbreviations: CH + Non-SVR, patients with CH at baseline and non-SVR 6 months after IFN therapy; CH + SVR, patients with CH at baseline and SVR 6 months after IFN therapy; LC + Non-SVR, patients with LC at baseline and non-SVR 6 months after IFN therapy; LC + SVR, patients with LC at baseline and SVR 6 months after IFN therapy.

*Hazard ratio (95% confidence interval) and *P* value by Cox proportional hazards analysis.

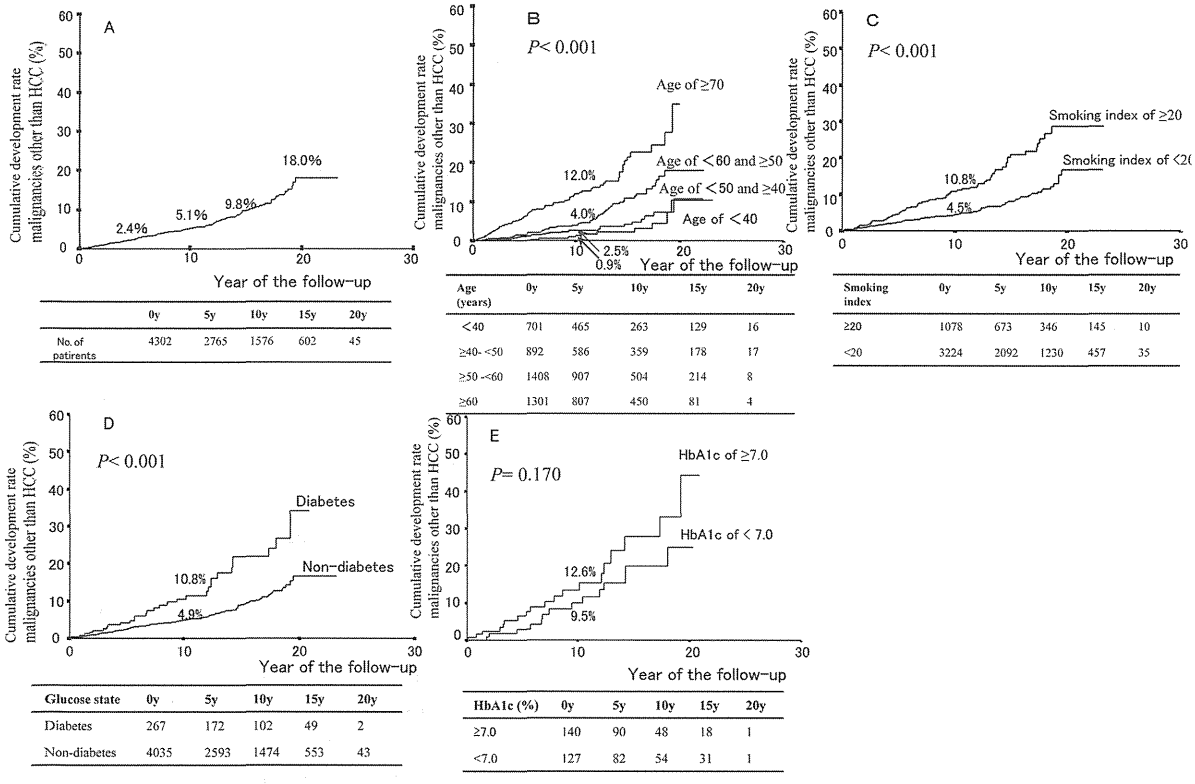


Fig. 3. Cumulative development rate of malignancies other than HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) age, (C) smoking index, (D) diabetic state, and (E) mean HbA1c level during follow-up in T2DM patients.

on three factors of age, smoking index, and T2DM for the development of each malignancy other than HCC by using Cox regression analysis. Aging enhanced carcinogenesis of stomach, colon, lung, prostate, breast, and pancreas with statistical significance. Smoking enhanced lung cancer and colorectal cancer

Table 4. Predictive Factors for Development of Malignancies Other than HCC

	Univariate Analysis		Cox-Regression Analysis	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age, years (per 10)	2.23 (1.88-2.65)	< 0.001	2.19 (1.84-2.62)	<0.001
Sex, male/female	1.06 (0.79-1.40)	0.759		
BMI, ≥22/<22	0.97 (0.75-1.24)	0.767		
T2DM, +/−	2.56 (1.76-3.72)	<0.001	1.70 (1.14-2.53)	0.008
Hypertension, +/−	2.33 (1.70-3.18)	<0.001		
Smoking index, ≥20/<20*	2.74 (2.06-3.65)	<0.001	1.89 (1.41-2.53)	<0.001
TAI, kg, ≥200/<200*	1.77 (1.33-2.37)	<0.001		
AST, IU/L, ≥34/<34	0.89 (0.65-1.20)	0.412		
ALT, IU/L, ≥36/<36	0.98 (0.72-1.34)	0.891		
GGT, IU/L, ≥109/<109	1.26 (0.79-2.01)	0.350		
Albumin, g/dL, <3.9/≥3.9	1.41 (0.90-2.04)	0.145		
Triglyceride, mg/dL, ≥100/<100	1.28 (1.03-1.60)	0.030		
Total cholesterol, mg/dL, <150/≥150	1.10 (0.82-1.46)	0.548		
Platelet count, × 10^4/mm^3, <15/≥15	1.39 (1.02-1.91)	0.038		
Histological diagnosis, LC/non-LC	1.77 (1.13-2.75)	0.012		
Combination of ribavirin, +/−	0.66 (0.44-0.97)	0.034		
Type of IFN, α/β	1.05 (0.75-1.47)	0.789		
Total dose of IFN, MU, ≥500/<500	1.31 (0.96-1.77)	0.084		
HCV genotype, ½	1.30 (0.80-2.93)	0.432		
HCV RNA, log IU/mL, ≥5/<5	0.89 (0.50-1.23)	0.612		
Efficacy, non-SVR/SVR	0.85 (0.64-1.12)	0.232		

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase.
*Smoking index is defined as packs per day × year. TAI and smoking index indicate the sum before and after first consultation.

Table 5. Impact Based on Age, Smoking Index, and Diabetes for Development of Malignancies Other than HCC

	Age, Years (per 10)		Smoking Index, ≥20/<20		Diabetes, +/−	
Malignancy	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Gastric cancer (n = 36)	2.48 (1.62-3.78)	<0.001	1.69 (0.83-3.43)	0.146	2.29 (0.95-5.52)	0.065
Colorectal cancer (n = 35)	1.91 (1.28-2.86)	0.002	2.27 (1.13-4.58)	0.022	1.78 (0.68-4.66)	0.240
Lung cancer (n = 20)	2.33 (1.35-4.01)	0.002	2.90 (1.25-6.74)	0.013	1.53 (0.45-5.24)	0.496
Prostatic cancer (n = 16)	2.84 (1.32-6.13)	0.008	1.89 (0.88-3.15)	0.266	0.71 (0.09-5.47)	0.735
Breast cancer (n = 15)	2.86 (1.30-6.29)	0.009	1.29 (0.17-10.19)	0.808	1.20 (0.16-9.39)	0.859
Malignant lymphoma (n = 19)	2.21 (1.26-3.88)	0.006	1.25 (0.44-3.56)	0.671	1.39 (0.32-6.12)	0.663
Pancreatic cancer (n = 12)	3.32 (1.44-7.65)	0.005	1.41 (0.45-4.82)	0.578	3.75 (1.02-13.88)	0.046

with statistical significance. In addition, T2DM enhanced the pancreatic cancer with statistical significance and tended to enhance the gastric cancer.

Discussion

This study describes the development incidence of HCC or malignancies other than HCC after the termination of IFN therapy in HCV patients. Patients at Toranomon Hospital comprised mainly government employees, office workers, and business persons. Most patients were regularly recommended to undergo annual multiphasic health screening examinations. In the present study, patients who had undergone annual multiphasic health screening examinations were enrolled. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the development incidence and predictive factors for total malignancies after IFN therapy for HCV patients. First, the 10-year cumulative rates of HCC after IFN therapy was determined to be 7.1% in 3,869 patients with chronic hepatitis and 37.7% in 433 patients with cirrhosis using the Kaplan-Meier method. Our previous studies showed via retrospective analysis that the 10-year cumulative rates of HCC were 12.4% for 456 patients with chronic hepatitis and 53.2% for 349 patients with cirrhosis.[7,23] Although patient selection bias for IFN treatment versus no treatment had been noted in the previous studies, the results suggest the possibility that IFN therapy reduces the development of HCC in HCV patients. Several historical data in Japan suggest that IFN therapy reduces the development of HCC in HCV patients.[24-26]

Second, HCC occurred with statistical significance when the following characteristics were present: non-SVR, advanced age, cirrhosis, TAI of ≥200 kg, male sex, and T2DM. T2DM caused a 1.73-fold enhancement in HCC development. Several authors have reported an increased risk of HCC among patients with the following characteristics: non-SVR, cirrhosis, male sex, advanced age, and T2DM.[24-28] Our results show that physicians in charge of aged male patients with non-SVR, advanced fibrosis, TAI of ≥200 kg, and T2DM should pay attention to the development of HCC after IFN therapy. In addition, maintaining a mean HbA1c level of <7.0% during follow-up reduced the development of HCC. This result indicates that stringent control of T2DM is important for protecting the development of HCC.

Third, the development rate of HCC per 1,000 person years was about 1.55 in 1,751 patients with chronic hepatitis at baseline and SVR. In these patients, the risk factors associated with HCC were advanced age, male sex, TAI, and T2DM. We compared the HCC development rate in patients with chronic hepatitis at baseline and SVR to the general population. A total of 5,253 individuals without HCV antibody and hepatitis B surface antigen, who underwent annual multiphasic health screening examinations in our hospital were evaluated as controls. Individuals with either of the following criteria were excluded: (1) illness that could seriously reduce their life expectancy or (2) history of carcinogenesis. They were selected by matching 3:1 with patients who had chronic hepatitis at baseline and SVR for age, sex, T2DM, and follow-up periods. In control individuals, the mean age was 51.7 years; the prevalence (number) of male patients was 61.8% (3,246); the prevalence (number) of T2DM patients was 4.2% (222); the mean follow-up period was 8.0 years. The number of development of HCC in control individuals was only five. This result suggests that the development rate of HCC in patients with chronic hepatitis at baseline and SVR is higher than that in the general population.

Fourth, HCC accounted for 33.3% in SVR patients and 73.6% in non-SVR patients. According to Matsuda et al.,[29] the outbreak of malignancies in the Japanese male population was observed in the following order in 2005: gastric cancer 20.4% > colon

cancer 16.0% > lung cancer 15.4% > prostate cancer 10.9% > HCC 7.4%. On the other hand, the outbreak of malignancies in the Japanese female population was observed in the following order in 2005: breast cancer 18.0% > colon cancer 16.2% > gastric cancer 13.6% > lung cancer 9.3% > uterine cancer 6.8%. Our results show that HCC is the most common cause of malignancy, not only in the non-SVR group but also in the SVR group.

Finally, malignancies other than HCC occurred with statistical significance when patients were of advanced age, were smokers, and had T2DM. Our result indicates that smoking enhances lung cancer and colorectal cancer. Many authors have reported that smoking is a direct cause of cancers of the oral cavity, esophagus, stomach, pancreas, larynx, lung, bladder, kidney, and colon.[30,31] In addition, the present study indicates that T2DM enhances pancreatic cancer with statistical significance and tends to enhance gastric cancer. T2DM showed up to about 1.7-fold increase in development of malignancies other than HCC. A recent meta-analysis of cohort studies have revealed that diabetic patients increase risk of pancreatic cancer, HCC, bladder cancer, non-Hodgkin's lymphoma, colorectal cancer, and breast cancer.[32-39]

Although the role of T2DM in carcinogenesis remains speculative, the following possible mechanisms have been reported: (1) hyperglycemia increases malignancy risk via increasing oxidative stress and/or activating the rennin-angiotensin system[40]; (2) insulin resistance increases malignancy risk via down-regulation of serine/threonine kinase II to adenosine monophosphate–activated protein kinase pathway[41]; (3) reduced insulin secretion increases malignancy risk via down-regulation of sterol regulatory element-binding protein-1c with consequent up-regulation of insulin-like growth factor.[42]

T2DM is increasing dramatically worldwide over the past decades.[8] It is estimated that about 7 million people are affected by diabetes mellitus in Japan. Approximately 8%-10% of adults in Japan have T2DM. The risk factors associated with T2DM include family history, age, sex, obesity, smoking, physical activity, and HCV.[43-46] In the near future, T2DM will be increasing in HCV-positive patients.

This study is limited in that it was a retrospective cohort trial. Another limitation is that patients were treated with different types of antivirus therapy for different durations. In addition, T2DM patients were treated with different types of drugs during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, T2DM causes an approximately 1.7-fold enhancement in the development of HCC and malignancies other than HCC after IFN therapy. Additionally, in T2DM patients, maintaining a mean HbA1c level of <7.0% during follow-up reduced the development of HCC.

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Original Article

Exploratory study on telaprevir given every 8 h at 500 mg or 750 mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients

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Aim: The aims of this study are to assess the antiviral effects, safety and telaprevir (TVR) pharmacokinetics in two cohorts given TVR every 8 h (q8h) at doses of 500 mg and 750 mg with peginterferon-α-2b and ribavirin in chronic hepatitis C patients.

Methods: Twenty chronic hepatitis C (HCV) patients with genotype 1b in high viral loads were randomly assigned to two TVR-based regimens of 750 mg q8h (group A) and 500 mg q8h (group B) in combination with peginterferon-α-2b and ribavirin for 12 weeks.

Results: Although the difference was not statistically significant other than trough concentration (C_{trough}) at week 4, the parameters of maximum concentration (C_{max}), the area under the concentration time curve ($AUC_{0-\infty}$) and C_{trough} tended to be higher in group A than those in group B. The antiviral effects were similar in the two groups (sustained virological response rates [SVR], 40% in group A, 50% in group B). The discontinuation rates by anemia were 30% in group A and 20% in group B. Serum creatinine concentrations were lower in group B than those in group A.

Conclusion: Although the exposure to TVR tended to be lower in 500 mg q8h than that in 750 mg q8h, the SVR rates in both groups were similar. The result suggests that the 500 mg q8h dose may be one option for treatment. In addition, the present findings indicate that the development of adverse events which increase with a TVR-based regimen, specifically anemia and creatinine, could be avoided by dose adjustment of TVR.

Key words: anemia, chronic hepatitis C, creatinine increase, pharmacokinetics, telaprevir

INTRODUCTION

THE WORLD HEALTH organization (WHO) estimates that approximately 170 million people are infected with hepatitis C virus (HCV).[1] Decompensated cirrhosis and hepatocellular carcinoma (HCC) develop in approximately 30% of individuals infected with HCV and result in a fatal outcome.[2,3] In Japan, it is estimated that more than 1.5 million people are chronically infected with hepatitis C. Telaprevir (TVR), a potent HCV protease inhibitor, has recently been approved for the treatment of people suffering from chronic genotype 1 HCV infection in the USA, European Union (EU) and Japan. The overseas phase 3 studies demonstrate that patients who received TVR in combination with peginterferon (PEG IFN)-α-2b and ribavirin (RBV) achieved significantly higher rates of sustained virological response (SVR) than those who received only PEG IFN and RBV, regardless of their prior treatment experience with the anti-HCV agents.[4–6] The high SVR rates were also observed in the Japanese phase 3 studies of the TVR-based triple regimen.[7,8] In Japanese patients, anemia was the most common side-effect in the TVR-based triple regimen. The epidemiology of chronic hepatitis C (CHC) in Japan takes on a different aspect

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from that of the USA and EU; thus, the age of the majority of Japanese patients is high and their bodyweights are low in comparison with those in Caucasians.[4–8] As a result, the RBV dose-reduction rates and the discontinuation rates of TVR treatment due to adverse events are higher in Japan than those in the USA and EU,[4–8] though the addition of RBV increased the SVR rates in patients receiving TVR-based regimens.[9] These backgrounds call for more efficient treatment of the aged and/or lower bodyweight in patients with CHC in Japan.

The antiviral activity at different doses of TVR was examined after administration of TVR alone for 14 days at 450 mg every 8 h (q8h), 750 mg q8h or 1250 mg q12h,[10] and the greatest HCV RNA reduction and the highest plasma trough concentrations (C_{trough}) were achieved in the 750 mg q8h cohort. On the basis of this result, the TVR 750 mg q8h regimen was selected in the TVR-based triple therapy thereafter. Indeed, TVR 750 mg q8h co-administrated with PEG IFN or PEG IFN/RBV resulted in greater HCV RNA reduction than that after the administration of TVR alone. The Advisory Committee Briefing Document for NDA prepared by the TVR Review Team reports that the higher exposure to TVR was significantly associated with the increased risk of anemia and grade 2 or higher hemoglobin toxicity, defined as hemoglobin of less than 10 g/dL or any decrease from baseline of more than 3.5 g/dL.[11] In addition, the comparison of individual exposure estimated from population pharmacokinetic analysis demonstrated that age, race, sex or weight/body mass index (BMI) of subjects had no clinically relevant effects on TVR exposure.[12]

We previously reported the dynamics of HCV RNA during 12 weeks of triple therapy of TVR (q8h at two doses of 500 mg and 750 mg) with PEG IFN and RBV in Japanese CHC patients.[13] From this perspective, in this study, we explored the antiviral effects, safety and TVR pharmacokinetics in the above Japanese CHC patients.

METHODS

Study design and organization

THIS DOUBLE-ARM, RANDOMIZED, open-label study was conducted between April 2008 and March 2009 at the Department of Hepatology in the Toranomon Hospital in compliance with Good Clinical Practice Guidelines and the Declaration of Helsinki. Before the study, the protocol and informed consent forms were approved by the Institutional Review Board. All patients had given informed consent in writing after sufficient explanation before they participated in this trial.

Patients

This study was conducted using 20 CHC patients who were selected according to the following inclusion and exclusion criteria.[13] Inclusion criteria: (i) diagnosed with CHC; (ii) infected with HCV-1b confirmed by the sequence analysis in the NS5B region; (iii) HCV RNA levels of 5.0 $\log_{10}$ IU/mL or higher determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) Japanese race (Mongoloid), aged 20–65 years at the entry; and (v) bodyweight of 35 kg or more but 120 kg or less at the time of registration. Exclusion criteria were the same as previously described.[13]

Study design

The 20 patients were randomly allocated to two groups with different doses of TVR by a third party institute, Bellsystem24 (Tokyo, Japan). TVR was administrated at a dose of 750 mg (group A) or 500 mg (group B) q8h intervals after meal. PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) was injected s.c. to them at a median dose of 1.50 μg/kg (range, 1.250–1.739 μg/kg) once a week. RBV (Rebetol; MSD) was administrated at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1000 mg). These three drugs were administrated for 12 weeks. After completion or discontinuation of the triple therapy, a follow-up observation was performed for 24 weeks. Doses of PEG IFN and RBV were reduced or their administration was discontinued, as required, based on the reduction of hemoglobin levels, white blood cell count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG IFN was reduced to half, when either leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm^3 or platelet count below 80×10^3/mm^3. PEG IFN was withdrawn when they decreased below 1000/mm^3, 500/mm^3 or 50×10^3/mm^3, respectively. When hemoglobin decreased below 10 g/dL, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when hemoglobin decreased below 8.5 g/dL. The decrease of TVR dose was not permitted, and its administration was stopped when the discontinuation was appropriate due to the development of adverse events.

In cases where the administration of TVR stopped, the administration of PEG IFN-α-2b and RBV was terminated also.

This study was registered at Clinical Trials (no. NCT00630058).

NS5A interferon-sensitivity determining region (ISDR) and core amino acid (a.a.) substitutions

Amino acid substitutions in the HCV core and NS5A ISDR regions were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. A core a.a. substitution at positions 70 and 91 (core 70 and core 91, respectively) was determined according to the procedure of Akuta *et al.*,[14,15] and the number of ISDR substitutions was determined using the methods of Enomoto *et al.*[16,17]

Single-nucleotide polymorphism (SNP) genotyping

Interleukin (IL)-28B (rs8099917 and rs12979860) and inosine triphosphate pyrophosphatase (rs1127354) were genotyped by the Invader assay, TaqMan assay or direct sequencing, as described elsewhere.[18–20]

HCV RNA measurements

Antiviral effects of TVR on HCV were assessed by measuring plasma HCV RNA levels. Blood samples were obtained on day 1 before dosing and at 2.5, 4, 8, 16 and 24 h after the first dose (the 8- and 16-h samples were collected before administration of the second and third administration, respectively). Pre-dose samples were obtained on days 2, 3, 8, 14, 29, 43, 57, 86, 92, 99, 113, 141, 169, 197, 225 and 253. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL.

Pharmacokinetic assessments

Blood samples were collected immediately before the first dose in the morning, and at 1, 2.5, 4, 6, 8, 12, 16 and 24 h after the first dose on days 1, 14 and 85 to determine the concentrations of TVR in the plasma. Samples were also taken before the first dose in the morning on days 3, 8, 29, 43, 57 and 99 for evaluation of trough concentrations of TVR.

Plasma concentrations of TVR were determined using a high-performance liquid chromatographic apparatus fitted with a mass spectrometer. Plasma concentrations and actual plasma-sampling times were used to calculate the area under the plasma concentration time curve from 0–8 h (AUC_{0-8h}) and terminal half-life ($t_{1/2}$) by the non-compartmental method using WinNonlin software Version 5.2.1. The maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were directly determined from the observed values on days 1, 14 and 85.

Safety assessments

During the on-study period, patients were monitored for safety at regular intervals from the start of dosing through every hospital visit. Safety assessments included physical examinations, clinical laboratory tests and check of adverse events. After the treatment was completed or aborted, patients were monitored for safety by the standard practice of investigators.

Statistical analysis

Hepatitis C virus RNA values in $\log_{10}$ IU/mL were summarized using descriptive statistics for each treatment group and at scheduled time points. From the plasma concentrations of TVR and clinical laboratory data, the descriptive statistics were calculated. Continuous variables between groups were compared by Student's *t*-test or Mann–Whitney *U*-test. The number of patients with adverse events was summarized by MedDRA (ver. 12.0) system organ class, preferred term and relationship to study drug. All statistical analyses were performed using the validated ver. 9.1.3 of the SAS System (SAS Institute, Cary, NC, USA) or SPSS software (ver. 19.0.0; IBM, Armonk, NY, USA).

RESULTS

Baseline demographic and virological characteristics of the 20 patients with CHC who received the triple treatment

TABLE 1 LISTS the baseline demographic and virological characteristics of the 20 patients who received the triple therapy with TVR, PEG IFN and RBV for 12 weeks. All of them were infected with HCV-1b in high viral loads with a median of 6.48 $\log_{10}$ IU/mL in group A and 6.80 $\log_{10}$ IU/mL in group B. Of the 20 patients in the study, 12 (60%) were older than 50 years. The bodyweights of 10 (50%) patients were lower than 60 kg. Of the 20 patients, 10 (50%) did not receive antiviral treatments previously, six (30%) did not respond to previous monotherapy with the standard IFN and four (20%) failed to respond to PEG IFN and RBV (non-responder) previously.

Table 1 Baseline characteristics of patients with chronic hepatitis C who received a telaprevir-based triple therapy

No. of patients	Group A (750 mg q8h) $n = 10$	Group B (500 mg q8h) $n = 10$	Total $n = 20$
Sex (male/female)	6/4	4/6	10/10
Age (years) (median [range])	47.0 (42–62)	55.0 (36–65)	53.5 (36–65)
Height (cm) (median [range])	163.00 (147.3–178.5)	160.25 (148.7–175.8)	160.75 (147.3–178.5)
Weight (kg) (median [range])	61.95 (38.0–72.6)	61.00 (44.3–79.0)	61.95 (38.0–79.0)
HCV RNA (log_{10} IU/mL) (median [range])	6.48 (5.6–7.2)	6.80 (5.5–7.2)	6.78 (5.5–7.2)
rs8099917 (TT/TG/GG)	8/2/0	5/5/0	13/7/0
rs12979860 (CC/CT/TT)	8/2/0	5/5/0	13/7/0
rs1127354 (CC/CA/AA)	8/2/0	9/1/0	17/3/0
Core a.a. 70 (W/M)	6/4	6/4	12/8
Core a.a. 91 (W/M)	9/1	6/4	15/5
ISDR (0–1/≥2)	10/0	9/1	19/1
WBC (/mm^3) (median [range])	4900 (3600–6300)	5200 (4100–7800)	4900 (3600–7800)
Plt ($\times 10^4/mm^3$) (median [range])	164 (95–248)	160 (129–243)	163 (95–248)
Hb (g/dL) (median [range])	14.20 (12.8–16.0)	14.00 (11.7–16.8)	14.20 (11.7–16.8)
ALT (IU/L) (median [range])	57.0 (36–94)	43.0 (26–167)	49.5 (26–167)
GGT (IU/L) (median [range])	45.0 (15–85)	35.0 (7–142)	38.5 (7–142)
Creatinine (g/dL) (median [range])	0.765 (0.49-0.93)	0.725 (0.45-0.89)	0.755 (0.45-0.93)
History of IFN-based therapy			
Treatment naïve	6 (60.0)	4 (40.0)	10 (50.0)
IFN monotherapy	3 (30.0)	3 (30.0)	6 (60.0)
PEG IFN/RBV	1 (10.0)	3 (30.0)	4 (40.0)

ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; Hb, hemoglobin; IFN, interferon; ISDR, interferon sensitivity-determining region; M, mutant; PEG, pegylated; Plt, platelets; RBV, ribavirin; W, wild type; WBC, white blood cell.

Pharmacokinetics

The pharmacokinetic parameters of TVR in group A (750 mg q8h) and group B (500 mg q8h) on days 1, 14 and 85 are given in Table 2. The TVR C_{trough} on days 1 and 3, and weeks 1, 2, 4, 6, 8 and 12 in both groups are shown in Figure 1(a). Because the C_{trough} did not reach the steady state until day 2 in group A and group B as shown in Figure 1(a), the parameters relating to exposure (C_{max}, AUC_{0-8h} and C_{trough}) on day 1 were lower than those on days 14 and 85 in both groups (Table 2). The mean value of $t_{1/2}$ on day 1 (4.87 and 4.03 h in groups A and B, respectively) was shorter than those on the other days (6.22 to 10.00 h), while mean t_{max} were approximately the same on these 3 days. The values of $t_{1/2}$ and t_{max} were not different between the two groups. Although the difference was not statistically significant other than the C_{trough} at week 4, the parameters of C_{max}, $AUC_{0-\infty}$ and C_{trough} tended to be higher in group A than those in group B.

Virological response and SVR

Figure 1(b) illustrates a comparison of the serum HCV RNA levels (mean ± standard deviation [SD]) in patients between group A and group B during the TVR triple therapy. Similar decreases were observed in both groups. Characteristics and clinical outcomes of the individual patients are shown in Table 3. The SVR rates were 40% (4/10 patients) in group A and 50% (5/10) in group B. The SVR rates in the naïve patients were 67% (4/6) in group A and 75% (3/4) in group B, while the SVR rates in non-responders to the IFN monotherapy were 0% (0/3) in group A and 67% (2/3) in group B, and those in non-responders to the PEG IFN and RBV therapy were 0% in both groups (0/1 vs 0/3). At week 2, the percentage of subjects with undetectable HCV RNA was 40% in group A and 60% in group B. The percentage of subjects with undetectable HCV RNA at week 4 (rapid viral response: RVR) in group A was similar to that in group B (80% vs 70%). Eight (80%) of the 10 patients with undetectable HCV RNA at week 2 achieved SVR. One patient (undetectable HCV RNA at week 2) who stopped the treatment at week 4 achieved transient response (TR).

Four of five naïve patients with IL-28B rs8099917 TT and wild-type core a.a. 70 achieved SVR. Two of four naïve patients with rs8099917 TT and mutant-type core a.a. 70 achieved SVR, and the other naïve patient with

Table 2 Pharmacokinetic parameters of plasma telaprevir

	n	C_{max} (μg/mL)	t_{max}† (h)	AUC_{0-8h} (μg·h/mL)	C_{trough}‡ (μg/mL)	$t_{1/2}$ (h)
(a) Group A (750 mg q8h)						
Day 1	10	1.62 ± 0.43	2.51 (2.25–6.00)	7.53 ± 1.93	0.846 ± 0.500	4.87 ± 2.12§,¶
Day 14	10	3.96 ± 1.10	2.50 (2.42–5.75)	26.00 ± 6.77††	2.639 ± 0.556††	9.99 ± 4.37§,‡‡
Day 85	6	3.67 ± 0.87	3.24 (2.35–7.75)	25.00 ± 5.23	2.679 ± 0.355	9.06 ± 3.98§§
(b) Group B (500 mg q8h)						
Day 1	10	1.45 ± 0.83	2.54 (2.33–8.02)	6.55 ± 3.73	0.681 ± 0.412	4.03 ± 1.63§,‡‡
Day 14	10	3.06 ± 0.90	2.45 (2.33–6.00)	19.94 ± 5.97	1.914 ± 0.717	10.00 ± 6.97§,††
Day 85	7	3.16 ± 1.10	2.43 (2.33–4.00)	21.35 ± 6.88	2.105 ± 0.819	6.22 ± 3.64¶¶

Mean values ± standard deviations.
†Medians (minimum value to maximum value).
‡C_{trough} at 8 h after the first administration.
§Calculated from measured values at 8 h after the first administration.
¶$n = 7$.
††$n = 9$.
‡‡$n = 8$.
§§Calculated from measured values at 24 h after the first administration.
¶¶Calculated from measured values at 24 h after the first administration.
AUC_{0-8h}, area under the plasma concentration time curve from 0–8 h; C_{max}, maximum plasma concentration; C_{trough}, plasma trough concentrations; $t_{1/2}$, terminal half-life; t_{max}, time to reach C_{max}.

rs8099917 TG and wild-type core a.a. 70 achieved SVR. Two of four non-responders receiving the IFN monotherapy with rs8099917 TT and wild-type core a.a. 70 achieved SVR. The other two non-responders receiving the IFN monotherapy with rs8099917 TG and wild-type core a.a. 70 achieved TR. All four non-responders receiving the PEG IFN and RBV therapy with rs8099917 TG achieved TR. However, none of the pharmacokinetic parameters (C_{trough}, C_{max}, t_{max}, $AUC_{0-\infty}$ and $t_{1/2}$) of TVR were different between patients with and without SVR. Moreover, the adherence of PEG IFN and RBV did not affect SVR (Table 3).

Safety

Adverse events were observed in all patients in groups A and B. Adverse events with a frequency of more than 20% in total patients are listed in Table 4. The overall safety profile was similar in both groups. The ratios of discontinuation of all the study drugs because of adverse events were 40% (three cases of anemia, one case of malaise and vertigo) in group A and 30% (two cases of anemia, one case of severe skin disorder) in group B. Despite the modification of RBV dose, five patients (one man and four women) developed low hemoglobinemia (<8.5 g/dL) on days 22, 31, 39, 78 and 85 after the start of triple therapy. One patient (female, aged 53 years) developed IFN-related symptoms including general malaise and vertigo, and another (female, aged 56 years) developed severe skin disorder that was unable to be treated with topical steroid ointments. There was no dose-dependent trend for adverse events. During the triple therapy for 12 weeks, the amounts of hemoglobin tended to be the same or low in group A in comparison with those in group B (Fig. 2a), while serum creatinine increased more eminently in group A than in group B, with the statistical significance at weeks 4 and 8 ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 2(b). The serum creatinine recovered to the baseline level at the end of the follow-up period.

We analyzed the relationship between the above adverse events and the pharmacokinetic parameters of TVR. The AUC_{0-8h} on day 1 of patients developing low hemoglobinemia (<8.5 g/dL) was significantly higher than that of the other patients ($P = 0.040$; 9.70 ± 3.29 vs 6.15 ± 2.28). There was no correlation of creatinine elevation (>0.3 or 0.5 mg/dL from baseline) or rush with the pharmacokinetic parameters of TVR. Moreover, there was no correlation between creatinine elevation and clinical factors (age, sex, bodyweight and BMI).

DISCUSSION

THE DOSE OF TVR in the triple therapy was determined based on the TVR monotherapy study[10] as described above, in which the highest TVR C_{trough} (1054 ng/mL) and the greatest reduction of HCV RNA

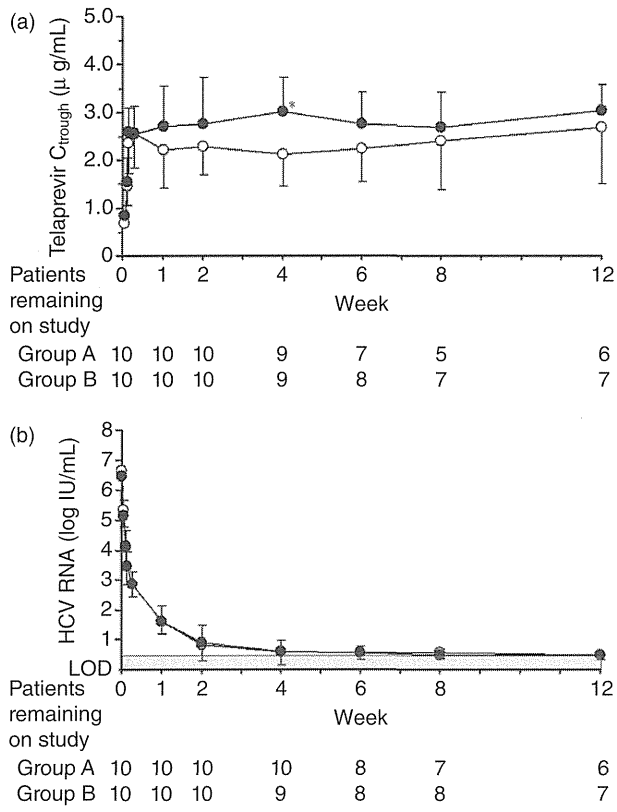

Figure 1 (a) Telaprevir C_{trough} levels and (b) change from baseline of hepatitis C virus (HCV) RNA in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. Statistical tests were performed at each point. $^*P < 0.05$ difference. The linear dynamic range of this assay was 1.2–7.8 $\log_{10}$ IU/mL, and samples with no HCV RNA detected were reported as less than 1.2 $\log_{10}$ IU/mL (no HCV RNA detectable.). The areas below the sensitivity of detection are indicated by a shaded bar (<1.2 $\log_{10}$ IU/mL, LOD: limit of detection). —●—, Group (telaprevir 750 mg q8h); —○—, group B (telaprevir 500 mg q8h).

were achieved by a 750 mg q8h regimen. Thus, no dose-finding study of TVR was conducted based on the TVR-based triple regimen. This was the first exploratory study to evaluate the antiviral response, safety and pharmacokinetics of TVR after administration at doses of 750 mg q8h and 500 mg q8h with PEG IFN and RBV. The $t_{1/2}$ of TVR on days 14 and 85 were longer than those on day 1 in both groups, probably due to the saturation of CYP3A4 activity by the repeated administration, because CYP3A4 is the major isozyme involved in the metabolism of TVR and, in addition, TVR acts as the inhibitor of this isozyme. The mean C_{max}, $AUC_{0\text{-}8h}$ and C_{trough} of TVR at steady state increased in an approximately dose-dependent manner, and those at week 2 were 3.96 μg/mL, 26.00 μg·h/mL and 2.639 μg/mL in group A, and 3.06 μg/mL, 19.94 μg·h/mL and 1.914 μg/mL in group B, respectively. The steady state pharmacokinetic parameters of TVR were similar to those obtained in the C208 study.[21] The optimum TVR dose regimen, 750 mg q8h, in Japanese CHC patients was justified based on the overseas dose-finding study and the studies on TVR-based triple therapy, because: (i) no race-related pharmacokinetic difference has been noticed in TVR between Japanese and European patients; and (ii) co-administration with PEG IFN and RBV did not notably change the exposure to TVR.

The change of mean (±SD) $\log_{10}$ HCV RNA and viral response (HCV RNA undetectable) in group A were similar to those in group B (Fig. 1b). The SVR and TR rates were 40% and 60% in group A, and 50% and 40% in group B, respectively. Although the SVR rates of all patients in this study were lower than those in the previous reports,[7,8] the rates of naïve patients (67% in group A and 75% in group B) were similar. The SVR rate of difficult to treat patients, who had not achieved SVR in the prior IFN-based therapy, was lower (20%, 2/10) in this study; the result indicating that these patients will require the TVR-based triple therapy for 24 weeks (PEG IFN, RBV and TVR were administrated for 12 weeks followed by switching to PEG IFN and RBV therapy for an additional 12 weeks).[8] Moreover, the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 were likely to achieve higher SVR than the patients with other genotypes, regardless of TVR dose (Table 3). Recent reports identify IL-28B genotype and a.a. substitution of the core region as predictors of SVR to TVR-based triple therapy.[22,23] Although these results indicate that the optimum regimen for the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 may be 500 mg q8h, the number of patients in this study was too small to reach a definitive conclusion on this point and a large-scale clinical study will be required.

The overall safety profiles of the triple regimen were similar in the two groups, and the ratios of TVR discontinuation due to anemia were 30% in group A and 20% in group B. We examined concentrations of hemoglobin and serum creatinine as the indicator of anemia and renal function, respectively (Fig. 2). The concentrations of hemoglobin were the same or higher in group B than those in group A during the dosing period, but there was no significant difference in this indicator. On the