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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1: Pie chart of variant occupation at each time point in the typical two cases. Proposed secondary resistant associated substitutions are underlined.

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Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C

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SUMMARY. The aims of this phase III study were to assess the efficacy and safety of telaprevir in combination with peginterferon alfa-2b (PEG-IFN) and ribavirin (RBV) for difficult-to-treat patients who had not achieved sustained virological response (SVR) to prior regimens in Japan. The subjects were 109 relapsers (median age of 57.0 years) and 32 nonresponders (median age of 57.5 years) with hepatitis C virus genotype 1. Patients received telaprevir (750 mg every 8 h) for 12 weeks and PEG-IFN/RBV for 24 weeks. The SVR rates for relapsers and nonresponders were 88.1% (96/109) and 34.4% (11/32), respectively. Specified dose modifications of RBV that differed from that for the standard of care were introduced to alleviate anaemia. RBV dose reductions were used for 139 of the 141 patients. The SVR rates for relapsers did not depend on RBV dose reduction for 20–100% of the planned dose (SVR rates 87.5–100%, $P < 0.05$). Skin disorders were observed in 82.3% (116/141). Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. The ratios of discontinuation of telaprevir only or of all the study drugs because of adverse events were 21.3% (30/141) and 16.3% (23/141), respectively. A frequent adverse event leading to discontinuation was anaemia. Telaprevir in combination with PEG-IFN/RBV led to a high SVR rate for relapsers and may offer a potential new therapy for nonresponders even with a shorter treatment period.

Keywords: direct-acting antiviral, peginterferon, ribavirin, sustained virological response, treatment failure.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 170 million people worldwide [1]; patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The standard of care (SOC) with peginterferon plus ribavirin (RBV) for 48 weeks is most effective for eradicating HCV genotype 1 [4], which is a dominant genotype for CHC [1]. However, the sustained virological response (SVR) rate of SOC for the treatment of naïve patients with genotype 1 is approximately <50% [5,6]. The retreatment regimen for patients who do not achieve SVR is limited to exposure to peginterferon plus RBV with modification of dose and treatment duration. Some studies have been conducted to estimate the effectiveness of peginterferon plus RBV for 48 weeks for nonresponders to prior interferon-based combination therapy, and the SVR rates in most studies did not exceed 20% [7–9]. A large randomized study of patients who had not responded to previous treatment with peginterferon alfa-2b (PEG-IFN) plus RBV gave SVR rates for peginterferon alfa-2a 180 μg/kg plus RBV for 72 weeks that were not as high as those for 48 weeks (14%, 9%) [10]. HCV patients who had failed to achieve SVR with the combination therapy displayed high risk rates of decompensated cirrhosis, HCC and liver-related mortality [11]. Therefore, it is very important to establish new regimens to increase the SVR rate and shorten the treatment period for patients who do not achieve SVR with prior treatments.

Telaprevir, classified as a direct-acting antiviral agent, is a reversible, selective, orally bioavailable inhibitor of the nonstructural NS3/4A HCV serine protease [12]. Two phase II studies (PROVE 1 and PROVE 2) on the treatment of naïve patients with genotype 1 were conducted to assess the

Abbreviations: CHC, chronic hepatitis C; ETR, end of treatment response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa-2b; RBV, ribavirin; RVR, rapid viral response; SOC, standard of care; SVR, sustained virological response.

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efficacy of telaprevir for 12 weeks in combination with peginterferon and RBV for 24 weeks [13,14]. These studies demonstrated that the SVR rates of the telaprevir regimen were significantly higher compared with SOC (PROVE 1: 61% *vs* 41%, $P = 0.02$, PROVE 2: 69% *vs* 46%, $P = 0.004$). A subsequent phase II study (PROVE 3) for treatment-failure patients with genotype 1 gave SVR rates for nonresponders, relapsers and breakthroughs in the telaprevir regimen of 39%, 69% and 57%, respectively [9].

In Japan, a phase III study was conducted for the treatment of naïve patients with genotype 1 to compare the efficacy and safety between the telaprevir regimen and SOC. It has demonstrated that the SVR rate for the telaprevir regimen was significantly higher than that for SOC (73.0% *vs* 49.2%, $P = 0.0020$) [15]. We decided to conduct a phase III study to assess the efficacy and safety of telaprevir in combination with PEG-IFN and RBV in relapsers and nonresponders who had not achieved SVR to a previously administered IFN-based regimen in Japan.

PATIENTS AND METHODS

Study patients

Relapsers and nonresponders were enrolled in Study 1 (ClinicalTrials.gov Identifier: NCT00780910) and Study 2 (ClinicalTrials.gov Identifier: NCT00781274), respectively. Relapsers were defined as patients who had been previously treated for CHC and had undetectable HCV RNA during interferon or peginterferon therapy (including combination with RBV). Nonresponders were defined as patients who were previously treated for CHC and had never had undetectable HCV RNA for more than 24 weeks with interferon or peginterferon therapy (including combination with RBV).

The patients were enrolled from 17 sites in Japan. Patients considered eligible were of 20–65 years of age, had CHC because of HCV genotype 1 (defined by NS5B sequence) [16] and ≥5.0 $\log_{10}$ IU/mL HCV RNA level at the screening test, had been previously treated for CHC with interferon or peginterferon therapy (including combination with RBV), had a body weight of 40 kg or more and below 120 kg, could be hospitalized for at least 2 weeks after the first administration, were not pregnant and agreed to contraception from the screening period to 24 weeks after the last dosing of the study drug. The patients were excluded if they had a haemoglobin level of <12 g/dL, neutrophil count of <1500/mm^3, platelet count of <100 000/mm^3, were positive for HBs antigen and HIV antibodies at the screening test, had chronic renal failure or creatinine clearance of ≤50 mL/min, depression, schizophrenia or its history, history of suicide attempt, decompensated cirrhosis, previous or current HCC or other malignancies, autoimmune hepatitis, alcoholic liver disease or haemochromatosis.

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and conducted in accordance with good clinical practice and the Declaration of Helsinki.

Study design

All patients received PEG-IFN (PegIntron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week subcutaneously, RBV (Rebetol®; MSD) at a dose of 600 mg per day (for body weight ≤60 kg), 800 mg per day (for body weight >60 to ≤80 kg) or 1000 mg per day (for body weight >80 kg) and telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 h after food. The patients were treated with telaprevir, PEG-IFN and RBV for 12 weeks, followed by PEG-IFN and RBV (PEG-IFN/RBV) for 12 weeks. All patients had a 24-week follow-up period after the last dosing of study drugs to assess SVR.

Dose modification of study drugs

Specified dose modification of RBV that differed from the dose for SOC was introduced to alleviate anaemia. The initial dose of RBV was reduced by 200 mg per day in case of a haemoglobin level <13 g/dL at baseline. The RBV dose was reduced by 200 mg per day in patients receiving 600 or 800 mg per day (by 400 mg per day in those receiving 1000 mg) when the haemoglobin level was <12 g/dL and was reduced by an additional 200 mg per day when the haemoglobin level was <10 g/dL. The RBV dose was also reduced by 200 mg per day if the haemoglobin level dropped ≥1 g/dL within 1 week, and this level was <13 g/dL. Telaprevir was withdrawn when the haemoglobin level was <8.5 g/dL. PEG-IFN/RBV were withdrawn or interrupted when the haemoglobin level was <8.5 g/dL. The dose modifications of PEG-IFN were followed by SOC. Dose modification and interruption of telaprevir were not allowed. Telaprevir was withdrawn if serious adverse events appeared. The use of erythropoietin was not allowed for elevating the haemoglobin level.

Stopping rules

Patients could be discontinued from the study at any time if the investigator or sponsor determined that it was not in the interest of the patient to continue the study or the patient wished to withdraw from the study. The study drugs were discontinued if the patients had a haemoglobin level of <8.5 g/dL, white blood cell count of <1000/mm^3, neutrophil count of <500/mm^3 or platelet count of <50 000/mm^3.

In case of the following criteria for serum HCV RNA viral kinetics measured during the treatment period, discontinuation of the study drugs was decided at the investigator's discretion. (i) When the following criteria applied twice consecutively: (a) the amount of change from the lowest value for HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL and (b)

HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL after it had been confirmed to be <1.2 $\log_{10}$ IU/mL. (ii) When the serum HCV RNA level at 13 weeks after administration of study drugs did not decrease by >2.0 $\log_{10}$ IU/mL from the baseline level.

Efficacy assessments

Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co. Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 $\log_{10}$ IU/mL. Samples with undetectable HCV RNA were reported as '<1.2 $\log_{10}$ IU/mL (no detectable HCV RNA)'. Measurements were obtained at week 4 before day 1 of the screening period: at days 1 (predose), 2 and 3; weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 of the treatment period; and weeks 2, 4, 8, 12, 16, 20 and 24 of the follow-up period.

The primary endpoint was a SVR defined as an undetectable HCV RNA level 24 weeks after the end of treatment. Relapse, breakthrough, and nonresponse were defined based on AASLD Guidelines as follows [4]: 'relapse' was a state of undetectable serum HCV RNA at the end of treatment and reappearance of serum HCV RNA during the follow-up period; 'breakthrough' was a state of undetectable serum HCV RNA and reappearance of serum HCV RNA during the treatment period; and 'nonresponse' was a state of continuously detectable serum HCV RNA during the treatment period.

Safety assessments

All adverse events were recorded up to the last visit and coded using MedDRA/J version 13.0. (MedDRA Japanese Maintenance Organization, Tokyo, Japan) Measurements for chemical laboratory data were obtained at week 4 before day 1 of the screening period: at day 1 (predose); weeks 1, 2, 4, 8, 10, 12, 14, 16, 18, 20 and 24 of the treatment period; and weeks 2, 4, 8, 12 and 24 of the follow-up period. Electrocardiogram (ECG) and fundus examinations were performed once during the screening period. Adverse events, haematological and chemical laboratory data, and vital signs were assessed and summarized. The severity of rash was categorized into three grades.

Statistical analysis

Sustained virological response rates were evaluated for the full analysis set. Categorical variables were compared by Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA), and a P value < 0.05 was considered significant.

Table 1 Baseline characteristics of study patients

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n* (%)		
Men	66 (60.6)	17 (53.1)
Women	43 (39.4)	15 (46.9)
Age, years – median (range)	57.0 (20, 65)	57.5 (40, 65)
Weight, kg – median (range)	62.50 (41.0, 92.5)	61.30 (44.9, 92.5)
BMI, kg/m^2 – median (range)*	23.10 (18.0, 32.4)	22.60 (17.1, 31.2)
ALT (IU/L) – median (range)†	36.0 (16, 302)	48.0 (17, 190)
Haemoglobin (g/dL) – median (range)	14.70 (12.0, 17.8)	14.50 (12.3, 16.6)
White blood cell count (/mm^3)	4680.0 (2490, 15940)	4830.0 (3040, 8000)
Platelet count ($\times 10^4$/mm^3) – median (range)	17.80 (9.9, 33.8)	17.85 (9.1, 26.2)
HCV RNA ($\log_{10}$ IU/mL) – median (range)‡	6.75 (5.2, 7.6)	6.78 (6.0, 7.7)
HCV genotype 1 subtype – *n* (%)		
1a	0 (0.0)	1 (3.1)
1b	109 (100.0)	31 (96.9)
Prior therapy for chronic hepatitis C – *n* (%)		
Interferon	13 (11.9)	1 (3.1)
Interferon plus ribavirin	14 (12.8)	2 (6.3)
Peginterferon	3 (2.8)	0 (0.0)
Peginterferon plus ribavirin	79 (72.5)	29 (90.6)

HCV, hepatitis C virus.
*The body mass index (BMI) is the weight in kilograms divided by the square of the height in metres; †Alanine aminotransferase; ‡The HCV RNA level was measured using the COBAS TaqMan HCV test (Roche).

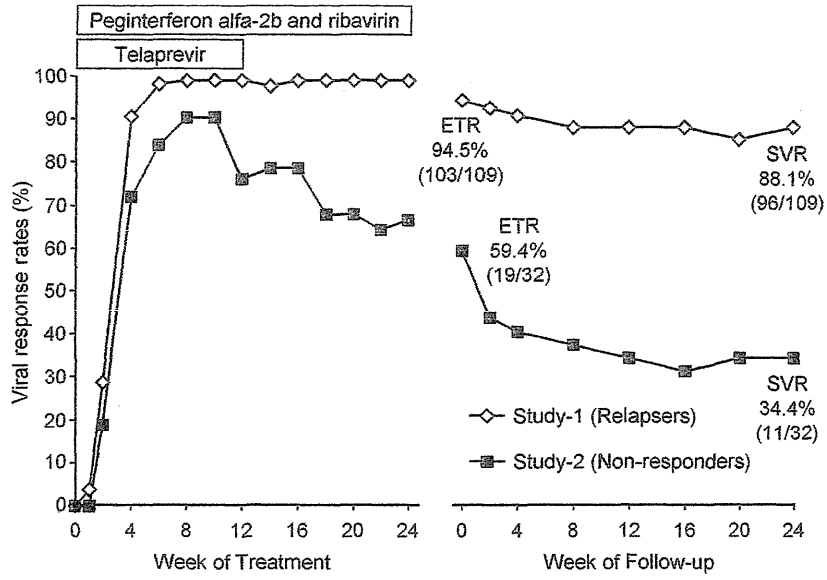

Fig. 1 Undetectable hepatitis C virus RNA rates at each measurement point. SVR, sustained virological response; ETR, end-of-treatment response.

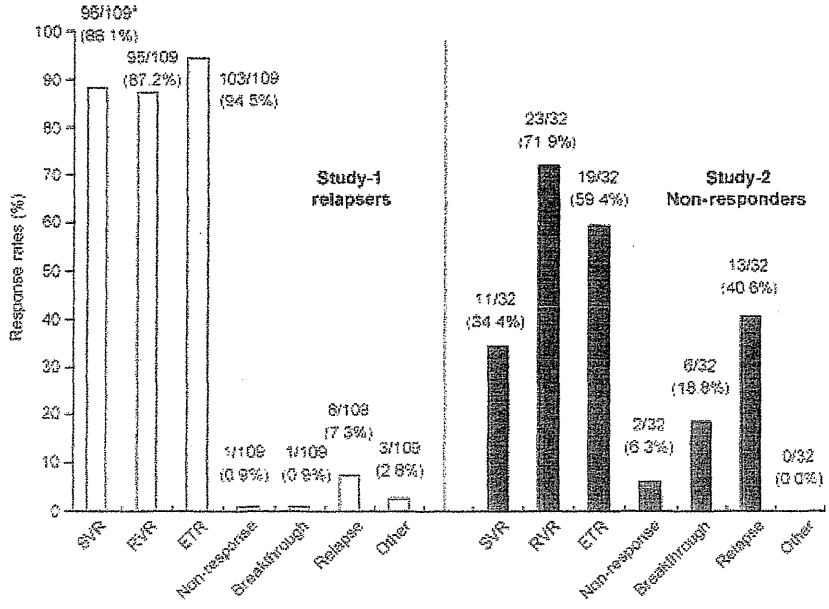

Fig. 2 Response rates of patients with virological response. *Number of patients who achieved SVR in each subgroup/*N* (%). SVR, sustained virological response; RVR, rapid viral response; ETR, end-of-treatment response.

RESULTS

Study patients

From November 2008 to August 2009, a total of 168 patients [Study 1 (*N* = 135) and Study 2 (*N* = 33)] were screened, and 141 patients [Study 1 (*N* = 109) and Study 2 (*N* = 32)] received at least one dose of a study drug. The baseline characteristics of the study patients are shown in Table 1. Patients previously treated with PEG-IFN (with or without RBV) and IFN (with or without RBV) in Study 1 and Study 2 accounted for 75.2% (82 of 109) and 24.7% (27 of 109) and 90.6% (29 of 32) and 9.4% (3 of 32), respectively. The median of age, weight, haemoglobin level, platelet count and HCV RNA level for Study 1 and Study 2 were 57.0 and 57.5 years, 62.5 and 61.3 kg, 14.7 and 14.5 g/dL, 17.8

and $17.85 \times 10^4/mm^3$, and 6.75 and 6.78 log_{10} IU/mL, respectively. Patients over 50 years of age accounted for 81.7% (89 of 109) and 81.3% (26 of 32), respectively.

Efficacy in study 1 (relapsers)

Figure 1 shows the change in the undetectable HCV RNA rates at each measurement point. The rapid viral response (RVR) rate and the end of treatment response (ETR) rate were 87.2% (95/109) and 94.5% (103/109), respectively. The SVR rate, nonresponse, breakthrough and relapse were 88.1% (96/109), 0.9% (1/109), 0.9% (1/109) and 7.3% (8/109), respectively (Fig. 2).

Factors influencing the SVR rate are compared in Table 2. The SVR rate in the patients who achieved undetectable HCV RNA at ≤week 4 was significantly higher than that in the patients who achieved undetectable HCV RNA at >week 4 (91.8% *vs* 66.7%, $P = 0.0487$). Also, the SVR rate for men was significantly higher than that for women (93.9% *vs* 79.1%, $P = 0.0316$). The SVR rate with discontinuation of all the study drugs was significantly lower than that with discontinuation of only telaprevir or no discontinuation of the study drugs (all the study drugs: 60.0%, only telaprevir: 95.0% and no discontinuation: 94.2%, $P = 0.0007$). In contrast, there was no difference in the SVR rate in relation to HCV RNA level and prior therapy for CHC. SVR rates by the ratio of the actual total RBV dose to the anticipated total RBV dose were evaluated (Fig. 3). The SVR rates did not depend on RBV dose reduction for 20–100% of the planned dose (87.5–100%, $P < 0.05$).

Efficacy in study 2 (nonresponders)

The RVR and ETR rates were 71.9% (23/32) and 59.4% (19/32), respectively (Fig. 1). The SVR rate, nonresponse, breakthrough and relapse were 34.4% (11/32), 6.3% (2/32), 18.8% (6/32) and 40.6% (13/32), respectively (Fig. 2). There was no difference in the SVR rate in relation to

Table 2 SVR rates stratified by demographic, undetectable HCV RNA and discontinuation of study drug treatment

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n/N* (%)		
Male	62/66 (93.9)	8/17 (47.1)
Female	34/43 (79.1)	3/15 (20.0)
P-value	0.0316	0.1475
Age – *n/N* (%)		
≤49	18/20 (90.0)	2/6 (33.3)
≥50	78/89 (87.6)	9/26 (34.6)
P-value	1.0000	1.0000
HCV RNA (log_{10} IU/mL) – *n/N* (%)		
≥7.0	26/30 (86.7)	5/10 (50.0)
<7.0	70/79 (88.6)	6/22 (27.3)
P-value	0.7498	0.2515
Prior therapy for chronic hepatitis C – *n/N* (%)		
Interferon	12/13 (92.3)	1/1 (100.0)
Interferon plus ribavirin	13/14 (92.9)	2/2 (100.0)
Peginterferon	3/3 (100.0)	– (–)
Peginterferon plus ribavirin	68/79 (86.1)	8/29 (27.6)
P-value	0.9271	0.0333
Undetectable – *n/N* (%)		
≤Week 4	90/98 (91.8)	9/23 (39.1)
>Week 4 ≤end of treatment	6/9 (66.7)	2/7 (28.6)
P-value	0.0487	1.0000
Discontinuation of study drug treatment – *n/N* (%)		
No discontinuation	65/69 (94.2)	9/20 (45.0)
Telaprevir only	19/20 (95.0)	2/7 (28.6)
All study drugs	12/20 (60.0)	0/5 (0.0)
P-value	0.0007	0.1711

SVR, sustained virological response; HCV, hepatitis C virus.
SVR was defined as an undetectable HCV RNA level 24 weeks after the end of treatment.

baseline characteristics, HCV RNA level and prior treatment for CHC. The SVR rates for the patients who received 40–80% RBV dose reduction were over 30% (Fig. 3).

Safety

Adverse events were observed in all the patients in Study 1 and Study 2. Adverse events observed in at least 15% of the patients in each clinical study are listed in Table 3. Adverse events were similar between Study 1 and Study 2. Most of the adverse events were mild and moderate. Serious adverse events in Study 1 and Study 2 were reported in 11.9% (13/109) and 9.4% (3/32) of the patients, respectively. The ratios of discontinuation of all the study drugs because of adverse events in Study 1 and Study 2 were 17.4% (19/109) and 12.5% (4/32), respectively. A frequent adverse event leading to discontinuation was anaemia. Discontinuation rates of all the study drugs because of anaemia in Study 1 and Study 2 were 10.1% (11/109) and 9.4% (3/32), respectively. One death was reported in Study 1. One patient in Study 1 died of pulmonary embolism. Causality of PEG-IFN and RBV was classified as 'probably related' and that of telaprevir was classified as 'possibly related'.

Adverse events related to skin disorders were observed in 82.3% (116/141) of the patients. Skin disorders reported in over 10% of the patients were rash in 39.0% (55/141), drug eruption in 24.1% (34/141), injection site reaction in 12.8% (18/141) and injection site erythema in 12.8% (18/141) of the patients. Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. Grade 3 (severe) skin disorders in Study 1 and Study 2 were reported in 6.4% (7/109) and 6.3% (2/32) of the patients, respectively. Discontinuation of all the study drugs because of skin disorders in Study 1 amounted to 3.7% (4/109). No discontinuation because of skin disorders occurred in Study 2.

Figure 4 shows the changes in haemoglobin levels, platelet counts and neutrophil counts during the treatment and follow-up periods. Changes in the haematological parameters were similar between Study 1 and Study 2. The platelet count and neutrophil count decreased sharply within 4 weeks and then gradually decreased. Despite the modification of RBV, the median haemoglobin levels in Study 1 and Study 2 decreased to 10.6 and 10.4 g/dL at week 12, respectively. No patient discontinued all the study drugs because of neutrophil decrease. The haematological parameters recovered to the baseline level at the end of the follow-up period.

DISCUSSION

This phase III study was planned and conducted to assess the efficacy and safety of telaprevir in combination with PEG-IFN/RBV for relapsers and nonresponders. Most of the patients who participated in this study had received a prior PEG-IFN/RBV regimen. Despite a shorter treatment period, the SVR rates for relapsers and nonresponders were 88.1% and 34.4%, respectively. The result indicates that the HCV RNA response to previous treatment history should be one of the diagnostic factors for predicting SVR.

The SVR rate for men was significantly higher than that for women in the relapser group (93.9% *vs* 79.1%, $P = 0.0316$). There was no significant difference in other characteristics of the patients in that group. Once the relapsers had achieved undetectable HCV RNA, this condi-

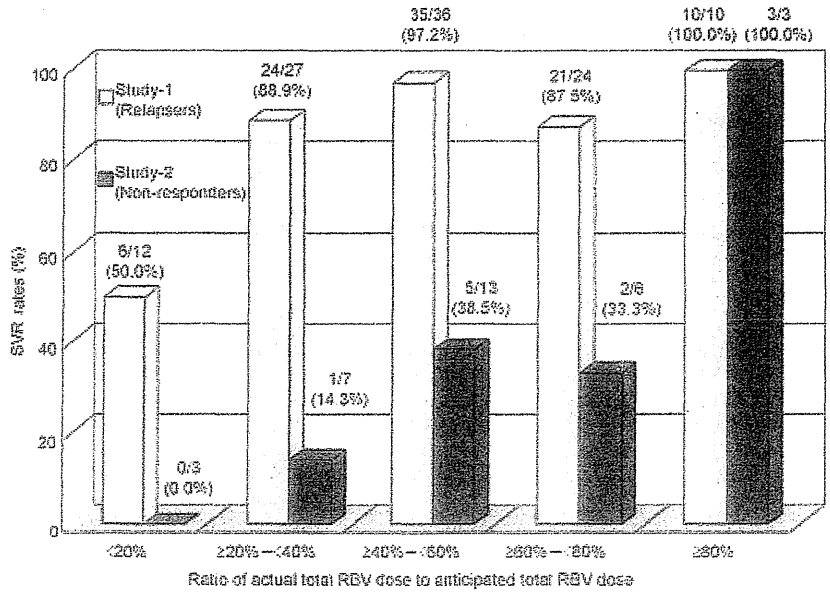

Fig. 3 Sustained virological response rates according to adherence to the ribavirin dose.

Table 3 Most common adverse events

MedDRA/J (Version.13.0) preferred term – *n* (%)	Study 1 (relapsers) *N* = 109	Study 2 (nonresponders) *N* = 32	Total *N* = 141
Anaemia	96 (88.1)	32 (100.0)	128 (90.8)
Pyrexia	90 (82.6)	30 (93.8)	120 (85.1)
White blood cell count decreased	83 (76.1)	22 (68.8)	105 (74.5)
Blood uric acid increased	72 (66.1)	25 (78.1)	97 (68.8)
Platelet count decreased	73 (67.0)	22 (68.8)	95 (67.4)
Malaise	60 (55.0)	23 (71.9)	83 (58.9)
Decreased appetite	56 (51.4)	15 (46.9)	71 (50.4)
Hyaluronic acid increased	56 (51.4)	15 (46.9)	71 (50.4)
Rash	39 (35.8)	16 (50.0)	55 (39.0)
Headache	42 (38.5)	10 (31.3)	52 (36.9)
Blood creatinine increased	36 (33.0)	12 (37.5)	48 (34.0)
Insomnia	34 (31.2)	11 (34.4)	45 (31.9)
Blood bilirubin increased	34 (31.2)	10 (31.3)	44 (31.2)
Alopecia	35 (32.1)	7 (21.9)	42 (29.8)
Diarrhoea	31 (28.4)	7 (21.9)	38 (27.0)
Dysgeusia	29 (26.6)	6 (18.8)	35 (24.8)
Vomiting	26 (23.9)	8 (25.0)	34 (24.1)
Drug eruption	24 (22.0)	10 (31.3)	34 (24.1)
Nausea	24 (22.0)	4 (12.5)	28 (19.9)
Abdominal discomfort	22 (20.2)	6 (18.8)	28 (19.9)
Blood triglycerides increased	19 (17.4)	8 (25.0)	27 (19.1)
Pruritus	20 (18.3)	2 (6.3)	22 (15.6)
Arthralgia	18 (16.5)	4 (12.5)	22 (15.6)
Nasopharyngitis	19 (17.4)	2 (6.3)	21 (14.9)
Stomatitis	13 (11.9)	6 (18.8)	19 (13.5)
Back pain	12 (11.0)	5 (15.6)	17 (12.1)
Blood phosphorus decreased	10 (9.2)	6 (18.8)	16 (11.3)

The adverse events listed are those that were reported in at least 15% of patients in each clinical study.

tion was sustained until the end of the treatment period. The patients who achieved RVR had a higher SVR rate than the patients who had no RVR in the relapser group (91.8% *vs* 66.7%, $P = 0.0487$).

In contrast, there was no significant difference related to characteristics in the nonresponder group. The SVR rates between men and women and undetectable *HCV* RNA were, however, slightly different. As Study 2 for the nonresponders was of a small scale, it will be necessary to evaluate a larger number of patients. The breakthrough ratio in the nonresponders during the PEG-IFN/RBV treatment period and relapse ratio were 18.8% and 40.6%, respectively. Two patients were nonresponders with high telaprevir-resistant variants; one was subtype 1a and the only patient with this characteristic in the study.

Triple therapy for 12 weeks, followed by PEG-IFN/RBV for 12 weeks for the relapsers led to a high SVR rate. In contrast to the relapsers, all breakthroughs were observed in 18.8% of nonresponder patients after the end of telaprevir treatment, and relapse were observed in 40.6% of nonresponder patients after the end of treatment period. Continuation of telaprevir over 12 weeks and PEG-IFN/RBV over 24 weeks might be needed to achieve a higher SVR rate for nonresponders.

Dose modification of RBV that differed from that for SOC was introduced to prevent anaemia in the patients [17]. Dose reductions of RBV were observed in 98.6% of the patients, and those who had 200 mg RBV per day as a minimum dose and those who discontinued it accounted for 41.8% and 29.8%, respectively. The haemoglobin level recovered to the baseline level at the end of the follow-up period. As a result of dose modification, the change in the haemoglobin level in this study was similar to that in PROVE 3 [9]. Checking the haemoglobin level once a week during the treatment period is important. The SVR rates did not depend on RBV dose reduction among the relapsers who had over 20% of the anticipated total RBV dose (87.5–100%). Thus, it is important to monitor haemoglobin levels and continue RBV dosing appropriately to achieve SVR, even with a low RBV dose.

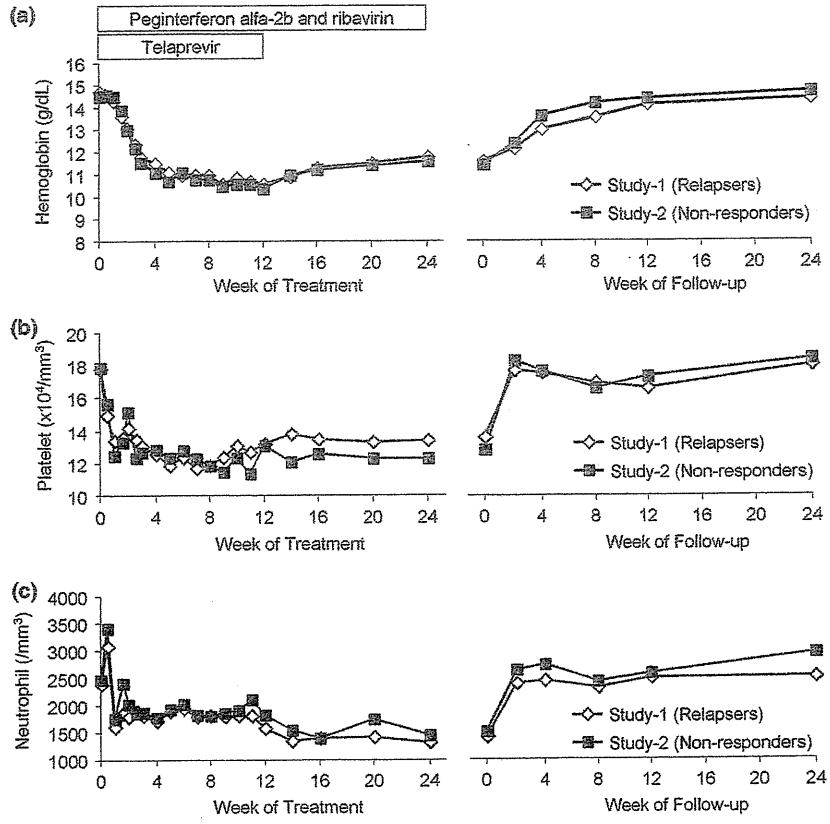

Fig. 4 Changes in hematology parameters. Median haemoglobin levels (a), median platelet counts (b) and median neutrophil counts (c) were plotted during treatment and follow-up periods.

Adverse events related to skin disorder were reported by 82.3% of the subjects. Of the nine cases of severe skin disorders, seven occurred within 8 weeks. Telaprevir was likely to be related to the occurrence of the severe skin disorders. The mechanism of skin disorders is unknown. All the patients who discontinued treatment received immediate care from dermatologists and recovered eventually. Skin disorders should be carefully monitored by physicians in collaboration with dermatologists.

The relationship between the SVR rates and the difference in SNPs in gene IL28B or near IL28B has become clear [18,19]. With genetic variation in rs8099917, SVR rates of 83.8% and 27.6% were achieved for patients with genotype TT and non-TT who were treated with telaprevir in combination with PEG-IFN/RBV, respectively [20]. Also, genetic variations in gene ITPA related to haemoglobin decrease and reduction of RBV has been discussed for patients treated with PEG-IFN/RBV [21,22]. We did not evaluate IL28B and ITPA in this study. As anaemia was the most frequent adverse event leading to the discontinuation of the study drugs in the present study, it should become a valuable pharmacogenetic diagnostic tool to optimize the triple therapy.

In conclusion, this phase III study conducted in Japan demonstrated that telaprevir in combination with PEG-IFN/RBV had a high SVR rate for relapsers and shows promise as a potential therapy for nonresponders even with a short treatment period. Prolongation of telaprevir and PEG-IFN/RBV treatment should be a better option for achieving high SVR for nonresponders. As the data demonstrated convincingly that the benefits greatly outweigh the risks, telaprevir-based regimen is at the lead for the next generation of HCV therapies.

DISCLOSURES

None to declare.

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APPENDIX

The members of the phase III study were as follows: Sapporo Kosei General Hospital, Toranomon Hospital, Juntendo University Hospital, Musashino Red Cross Hospital, Toranomon Branch Hospital, University of Yamanashi Hospital, Shinshu University Hospital, Gifu Municipal Hospital, Ogaki Municipal Hospital, Nagoya University Hospital, Osaka University Hospital, Ikeda Municipal Hospital, Saiseikai Suita Hospital, Hiroshima University Hospital, Shin-Kokura Hospital, Kurume University Hospital and Kagoshima University Medical and Dental Hospital.

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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 2012;000:000-000)

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/mL, and the undetectable samples were defined as negative. A sustained virological response (SVR) was

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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.

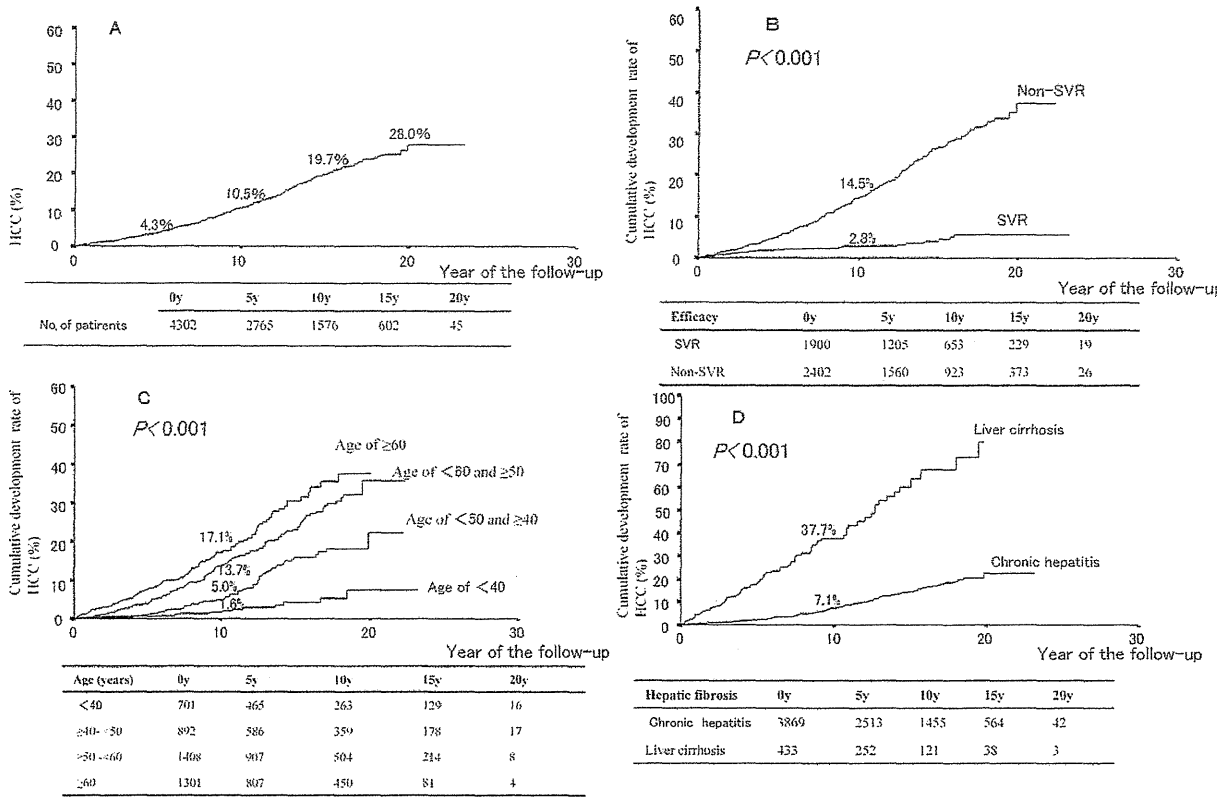
‡Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, n = 335, total dose of IFN = 508 ± 184 megaunit, total dose of ribavirin = 160 ± 68 g; natural IFN-β + ribavirin, n = 101, total dose of IFN = 502 ± 176 megaunit, total dose of ribavirin = 156 ± 67 g; pegylated IFN-α2b+ribavirin, n =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

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



No. of patients	0y	5y	10y	15y	20y
	4302	2765	1576	602	45

Efficacy	0y	5y	10y	15y	20y
SVR	1900	1205	653	229	19
Non-SVR	2402	1560	923	373	26

Age (years)	0y	5y	10y	15y	20y
<40	701	465	263	129	16
≥40- <50	892	586	359	178	17
≥50- <60	1408	907	504	214	8
≥60	1301	807	450	81	4

Hepatic fibrosis	0y	5y	10y	15y	20y
Chronic hepatitis	3869	2513	1455	564	42
Liver cirrhosis	433	252	121	38	3

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

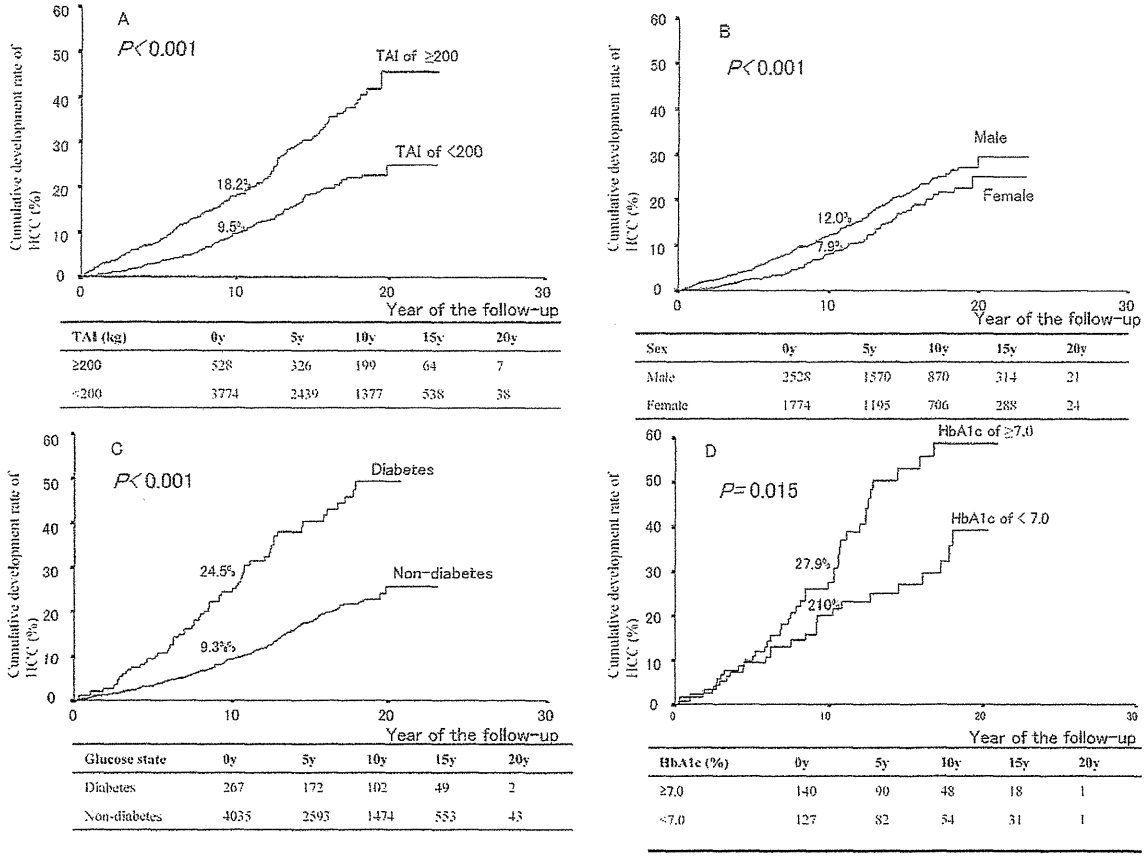


Fig. 2. Cumulative development rate of HCC based on the difference of (A) TAI, (B) sex, (C) diabetic state, and (D) mean HbA1c level during follow-up in T2DM patients.

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.

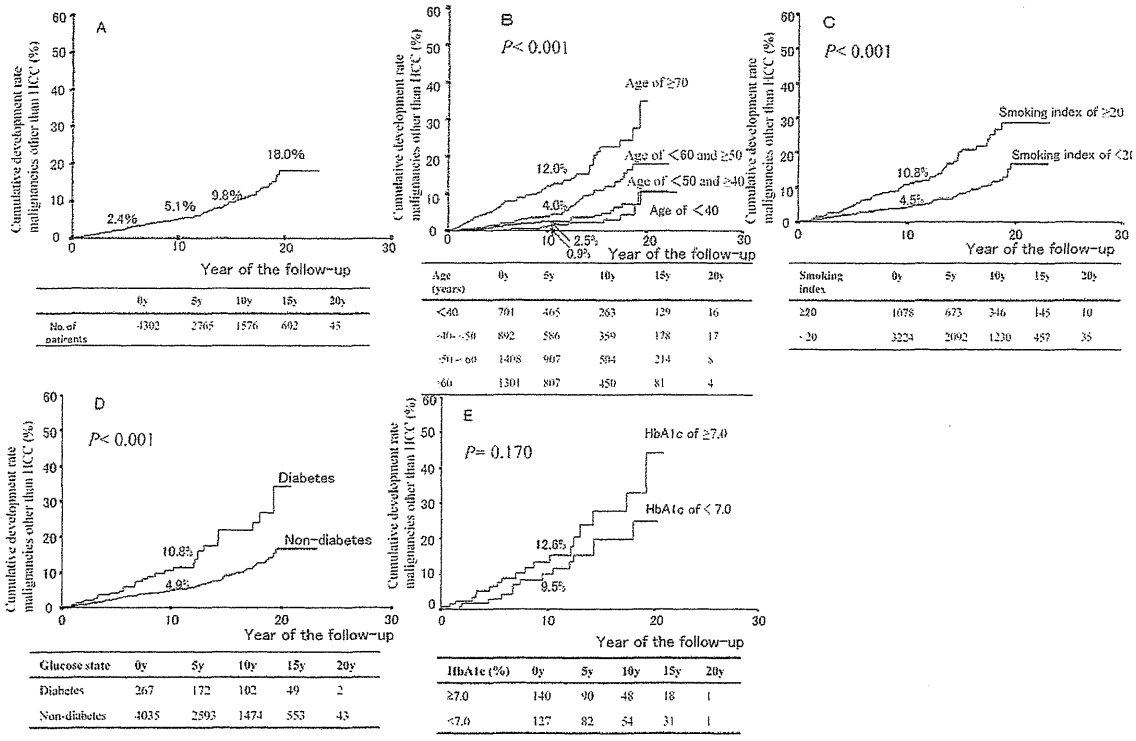


Fig. 3. Cumulative development rate of malignancies other than HCC (A) in total HCV patients treated with IFN therapy and based on the difference of (B) age, (C) smoking index, (D) diabetic state, and (E) mean HbA1c level during follow-up in T2DM patients.

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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