December 31, 2008. The cumulative survival probability was calculated using the Kaplan-Meier method. The cause of death was investigated meticulously using medical records. To develop a scoring system as a prognostic predictor for patients with extrahepatic metastasis, a split-sample method was applied. Our 342 patient cohort was divided randomly into 2 groups: a training set (n = 171) and a testing set (n = 171). The clinical data obtained at the diagnosis of extrahepatic metastasis were assessed as predictors of survival using a Cox proportional hazards model in the training set. The following variables were included in this analysis: age, sex, Eastern Cooperative Oncology Group (ECOG) performance status,[21] hepatitis B surface antigen (HBsAg), HCV antibody, Child-Pugh classification, the size and number of intrahepatic lesion(s), the presence of macroscopic vascular invasion, the presence of symptoms of extrahepatic metastasis, HCC-specific tumor marker levels (AFP, AFP-L3, and DCP), and response to treatment. Each variable was assessed first in a univariate analysis, and the variables that reached a P value $< .05$ were evaluated in a multivariate analysis with stepwise variable selection using Akaike information criterion (AIC). Then, the ratio of regression coefficients of the final model was determined and was rounded to whole digits for convenience. This scoring system was validated in the test group using the chi-square trend test and the Harrell concordance index (c-index).[22] Data were expressed as the mean ± standard deviation unless specified otherwise. All P values $< .05$ were considered statistically significant. All analytical procedures were performed with S-plus (version 7.0; Insightful Corp., Seattle, Wash).

Table 1. Patient Characteristics at the Diagnosis of Extrahepatic Metastasis (n = 342)

Variable	No. of Patients (%)
Age: Mean ± SD, y	66.9 ± 9.0
Men	270 (78.9)
Performance status	
0-1	314 (91.8)
≥2	28 (8.2)
Viral infection	
HBsAg, positive	62 (18.1)
Anti HCVAb, positive	268 (78.4)
Both positive	15 (4.4)
Both negative	27 (7.9)
Child-Pugh class	
A	167 (48.8)
B	153 (44.7)
C	22 (6.4)
Status of intrahepatic lesions	
None	61 (17.8)
≤3 cm and 1-3 lesions	110 (32.2)
>3 cm or ≥4 lesions	171 (50)
Macroscopic vascular invasion, present	65 (19)
Site of extrahepatic metastasis[a]	
Lung	135 (39.5)
Lymph node	117 (34.2)
Bone	87 (25.4)
Adrenal gland	30 (8.8)
Brain	4 (1.2)
Spleen	2 (0.6)
Breast	1 (0.3)
Symptoms of extrahepatic metastasis, present	80 (23.4)
AFP>400 ng/mL	158 (46.2)
AFP-L3>15%[b]	169 (64.8)
DCP>100 mAU/mL	196 (57.3)

SD indicates standard deviation; HCVAb, hepatitis C virus antibody; AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxy prothrombin.
[a] Including overlap.
[b] Missing in 81 patients.

RESULTS

Patient Background Data

Table 1 indicates that the average age at diagnosis for patients with primary extrahepatic metastasis from HCC was 66.9 ± 9.0 years, and ratio of men to women was 4:1. The distribution of the metastases among patients was the lung in 135 patients (39.5%), lymph node in 117 patients (34.2%), bone in 87 patients (25.4%), adrenal in 30 patients (8.8%), brain in 4 patients (1.2%), spleen in 2 patients (0.6%), and breast in 1 patient (0.3%), for a total of 376 extrahepatic occurrences in 342 patients. Metastases that were detected within 2 weeks after diagnosis of the first metastasis were considered synchronous. Viable, coexisting intrahepatic HCC lesions were identified in 281 patients (82.2%) when the extrahepatic metastasis was diagnosed. Intrahepatic vascular tumor invasion was evident in 65 patients (19%) patients: Portal vein invasion was evident in 57 patients, hepatic vein and inferior vena cava invasion was evident in 13 patients, and invasion into the bile duct was evident in 4 patients. The ECOG performance status was 0 in 229 patients, 1 in 85 patients, 2 in 19 patients, 3 in 5 patients, and 4 in 4 patients. Eighty patients (23.4%) had symptoms caused by extrahepatic metastasis, including dyspnea caused by multiple lung metastases; bone fracture, nerve paralysis, and pain caused by bone metastasis; abdominal pain and obstructive jaundice caused by abdominal lymph node metastasis; and disturbance of consciousness caused by bleeding from brain metastasis.

Table 2. Treatments Received for Extrahepatic Metastasis in the Study Cohort[a]

		No. of Patients (%)					
Organ	Total No.	Resection	Ablation	TACE	Radiation	Chemotherapy	No Treatment
Lung	135	19 (14.1)	—	1 (0.7)	4 (3)	42 (31.1)	69 (51.1)
Lymph nodes	117	8 (6.8)	5 (4.3)	2 (1.7)	26 (22.2)	27 (23.1)	49 (41.9)
Bone	87	—	3 (3.4)	—	68 (78.2)	2 (2.3)	14 (16.1)
Adrenal gland	30	5 (16.7)	7 (23.3)	11 (36.7)	1 (3.3)	—	6 (20)
Brain	4	—	—	—	2 (50)	—	2 (50)
Spleen	2	1 (50)	—	—	—	—	1 (50)
Breast	1	—	—	—	1 (100)	—	—

TACE indicates transarterial chemoembolization
[a] Including overlap.

Treatment of Patients With Extrahepatic Metastasis

Retrospectively reviewed, the treatments for extrahepatic metastatic lesions in our study cohort were considered only in those patients who had Child-Pugh Class B or better liver function and an ECOG performance status ≥ 2 and when intrahepatic lesions, if any, generally were controlled or controllable. Patients also received treatment when they were suffering from symptoms caused by extrahepatic metastasis. Table 2 indicates that these treatments included resection, chemotherapy, irradiation, TACE, and percutaneous ablation.

Surgical resection was undergone by 19 patients who had a lung metastasis (including 13 patients who underwent video-assisted thoracoscopic surgery), 8 patients who had lymph node metastasis, 5 patients who had adrenal metastasis, and 1 patient who has a spleen metastasis. Percutaneous ablation, using either ethanol or radiofrequency, was undergone by 7 patients with adrenal metastasis, 5 patients with lymph node metastasis, and 3 patients with bone metastasis, and TACE was undergone by 11 patients, 2 patients, and 1 patient with of adrenal, lymph node, and lung metastasis, respectively. Irradiation was received by other patients with metastasis as follows: 68 patients with bone metastasis, 26 patients with lymph node metastasis, 4 patients with lung metastasis, 2 patients with brain metastasis, 1 patient with an adrenal metastasis, and 1 patient with a breast metastasis. Systemic chemotherapy was received by an additional 42 patients with lung metastasis, 27 patients with lymph node metastasis, and 2 patients with bone metastasis in our cohort. The most often used chemotherapeutic regimen was cis-diamminedichloroplatinum (CDDP) monotherapy (29 patients) followed by 5-fluorouracil (5-FU) plus interferon (IFN) (24 patients), TS-1 alone (7 patients), CDDP plus 5-FU (6 patients), etoposide alone (6 patients), and TSU-68 (5 patients).

Percutaneous ablation of the intrahepatic lesions, which was indicated only when any extrahepatic lesions had been completely resected or ablated or controlled by irradiation, was performed in 60 patients. TACE treatment of intrahepatic lesions was indicated for patients who had Child-Pugh Class A or B liver function and when the vast majority of the total tumor volume was located in the liver. By using a combination of systemic chemotherapy and/or locoregional therapy to treat intrahepatic lesions, 22 of the patients in our study group achieved a CR as evaluated by the overall response according to RECIST.

Prognosis After the Diagnosis of Extrahepatic Metastasis

In the current study, during the observation period, 301 patients died. The cause of death was related to HCC in 273 patients (90.7%) patients and to liver dysfunction in 15 patients (5%), and death was unrelated to the liver in another 13 patients (4.3%). Extrahepatic metastasis of HCC was related directly to death in 23 patients (7.6%) patients, including 17 deaths from respiratory failure because of a lung metastasis, 5 incidents of cerebral hemorrhage from a brain metastasis, and death in 1 patient who had a bone metastasis and suffered liver failure that caused by hemorrhaging from a bone fracture that was the result of this lesion.

Gastroesophageal varices rupture sometimes became a critical event at the terminal phase of advanced HCC. In the current study, gastroesophageal varices rupture occurred in 25 patients at the end of life. Portal hypertension in these patients was caused either by portal vein tumor thrombus or cirrhosis, which may often coexist and are difficult to discriminate accurately.

The cumulative survival rates at 1 year, 2 years, 3 years, and 5 years after the diagnosis of extrahepatic metastasis in our cohort were 39.3%, 15.3%, 7.4%, and 4%,

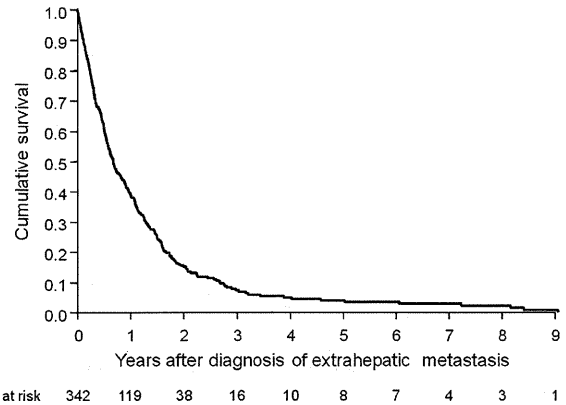

Figure 1. Cumulative survival is illustrated for patients with hepatocellular carcinoma who had a diagnosis of extrahepatic metastasis.

respectively (Fig. 1), and the median survival was 8.1 months (range, from 1 day to 108.7 months). The cumulative survival rates at 1 year, 2 year, and 3 years were 48.9%, 21.2%, and 10.6%, respectively, when the patients had received some treatment for extrahepatic metastasis; and the rates were 19%, 2.3%, and 0%, respectively, when no treatment had been indicated.

Predictors of Prognosis

Prognostic predictors after the diagnosis of extrahepatic metastasis were analyzed in the training set of 171 patients using a Cox proportional hazards model. These predictors were based on clinical factors that were recorded at diagnosis. In univariate analysis, the following factors were associated significantly with a poor prognosis: performance status, Child-Pugh classification, number and size of intrahepatic lesions, the presence of macroscopic vascular invasion, a symptomatic extrahepatic metastasis, AFP level, and CR to therapy (Table 3). Clinical factors that were statistically significant in univariate analysis were analyzed further in multivariate analysis with a stepwise selection of variables to minimize the AIC. To simplify the scoring system using multivariate analysis, intrahepatic tumor extension was categorized as none, a viable lesion without vascular invasion, or a viable lesion with vascular invasion. Only intrahepatic tumor extension at the diagnosis of extrahepatic metastasis and performance status were selected by a stepwise selection as factors in the final model (Table 4). Scores were assigned to each factor according to the estimated regression coefficient in the final model, and the prognosis score was defined as the sum of each score (Table 5). Our scoring system was validated using the testing set of 171 patients. A Kaplan-Meier plot was used to illustrate distinct survival curves according to the prognosis score (chi-square linear trend test: P thinsp;< .001) (Fig. 2). The c-index for the scoring system in the testing set was 0.73, thus reflecting good prognostic discrimination (Table 6).

Table 3. Predictors of Survival After a Diagnosis of Extrahepatic Metastasis: Univariate Analysis (n = 171)

Variable	β	HR (95% CI)	P
Age	0.02	1.02 (1.00-1.03)	.12
Men	0.07	1.08 (0.72-1.61)	.72
Performance status			
0		1.00	
1	0.36	1.44 (1.00-2.07)	.05
2	1.08	2.96 (1.29-6.79)	.01
3	2.61	13.5 (3.90-47.04)	<.0001
4	1.07	2.93 (0.40-21.26)	.29
HBsAg positive	−0.17	0.84 (0.53-1.33)	.46
Anti-HCVAb-positive	−0.27	0.76 (0.51-1.15)	.19
Child-Pugh class			
A		1.00	
B	0.37	1.44 (1.03-2.02)	.03
C	0.64	1.90 (0.97-3.69)	.06
Size of intrahepatic lesion, cm			
Absent		1.00	
≤3.0	0.71	2.04 (1.18-3.51)	.01
>3.0	1.41	4.12 (2.31-7.32)	<.0001
No. of intrahepatic lesion			
Absent		1.00	
1-3	0.67	1.96 (1.16-3.30)	.01
>3	0.93	2.52 (1.55-4.11)	.0002
Macroscopic vascular invasion, present	0.78	2.18 (1.46-3.25)	.0001
Symptom of extrahepatic metastasis, present	0.37	1.45 (1.01-2.09)	.047
AFP>400 ng/mL	0.54	1.71 (1.23-2.39)	.002
AFP-L3>15.0%	0.30	1.34 (0.92-1.96)	.12
DCP>100 mAU/mL	0.08	1.09 (0.78-1.51)	.62
Response to treatment, CR[a]	−0.77	0.46 (0.21-1.00)	.049

HR indicates hazard ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; DCP, des-gamma-carboxy prothrombin; CR, complete response

[a] Response was evaluated using overall responses according to Response Evaluation Criteria in Solid Tumors (RECIST); treatments included locoregional therapy and systemic chemotherapy for both intrahepatic lesions and extrahepatic lesions.

DISCUSSION

The prognosis for patients with extrahepatic metastasis of HCC was poor in the current study, consistent with previous reports that the 1-year survival rate is approximately 40% for patients with this disease.[23-27] However, from our current analyses, we observed that extrahepatic

Table 4. Predictors of Survival After a Diagnosis of Extrahepatic Metastasis: Multivariate Analysis (n = 171)

Variable	β	HR (95% CI)	*P*
Intrahepatic viable lesion			
None		1.00	
Without macroscopic vascular invasion	0.67	1.96 (1.21-3.18)	.006
With macroscopic vascular invasion	1.31	3.70 (2.08-6.57)	<.0001
Performance status			
0		1.00	
1	0.30	1.36 (0.94-1.96)	.11
2	1.11	3.05 (1.32-7.06)	.009
3-4	1.78	5.94 (2.09-16.9)	.0008

HR indicates hazard ratio; CI, confidence interval.

Table 5. Scoring System to Predict Survival in Patients With HCC and Extrahepatic Metastasis

Variable	Score
Intrahepatic viable lesion	
None	0
Present without macroscopic vascular invasion	1
Present with macroscopic vascular invasion	2
Performance status	
0-1	0
2	2
3-4	3

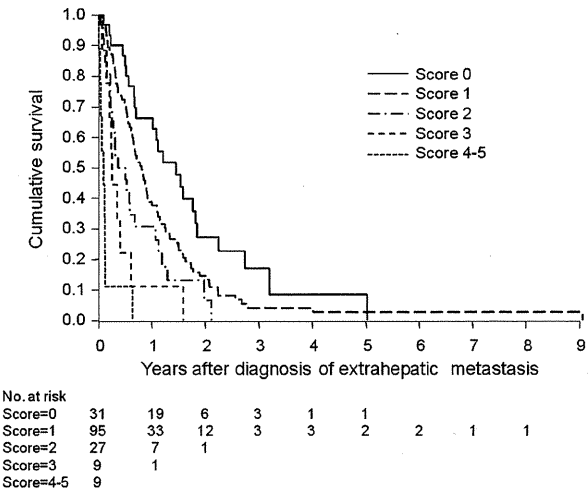

Figure 2. Stratified cumulative survival is illustrated for patients with hepatocellular carcinoma who had a diagnosis of extrahepatic metastasis based on prognostic scores. The prognosis for patients in the testing set could be stratified clearly by the scoring system based on an analysis of patients in the training set.

Table 6. Median Survival According to Prognostic Scores (n = 171)

Score	No. of Patients	Median Survival, mo
0	31	17.5
1	95	9.7
2	27	6.1
3	9	3.0
4-5	9	1.2

metastasis was not the direct cause of death in the majority of affected patients: the exceptions included respiratory failure from a bilateral lung metastasis and cerebral hemorrhage as a result of a brain metastasis, which accords with a previous report.[28] Hence, the presence of extrahepatic metastasis is an indicator of the aggressiveness of the primary HCC as a whole rather than an independent prognostic determinant.

In contrast to extrahepatic metastases, the progression of intrahepatic lesions was identified as the cause of death in 81% of patients in our current cohort, indicating the importance of controlling intrahepatic tumors in patients with HCC whenever possible. Repeated percutaneous ablations or TACE generally are considered for patients with HCC who develop an intrahepatic recurrence.[29,30] Intrahepatic arterial chemotherapy also reportedly is effective against advanced HCC with portal venous tumor invasion.[31] Thus, these locoregional treatments should be considered for intrahepatic lesions in selected patients who have extrahepatic metastasis, although the liver function reservoir should be evaluated cautiously in these patients.

In the current study cohort, patients received treatment for extrahepatic metastasis when their intrahepatic tumor was under control and liver function was maintained. Extrahepatic metastases also were treated when metastasis-related symptoms were strong or when further progression of the metastatic lesions was considered life-threatening. The prognosis was better among the current patients with HCC who received some treatment for their extrahepatic metastasis compared with those who were untreated. However, the contribution of these treatments to the overall prognosis remains unknown, because the patients who received them generally were in better condition. Nevertheless, our current findings indicate that treatments for extrahepatic metastases can be considered in patients who have hepatic lesions under control, because long-term survival was achieved only in those who had received such therapies.

Our current analyses indicated that resection of metastatic lesions produced a satisfactory local response, consistent with previous reports.[32-36] Locoregional therapy for extrahepatic metastasis also was discussed in earlier studies, including irradiation for bone,[37] lymph node,[38] brain,[39] and adrenal[40] metastases; TACE for adrenal metastasis[41]; and percutaneous ablation for adrenal[42] and bone metastases.[43] We also used these methods to treat some patients in our cohort. According to the conventional treatment strategy for solid tumors, the presence of metastatic disease is a contraindication for locoregional therapy, because it is believed that these tumor cells already have spread systemically. However, from the viewpoint of reducing tumor burden, locoregional therapy may be an adequate strategy when the target lesions account for the major portion of the total tumor volume. When resection and other locoregional therapies were contraindicated for extrahepatic metastasis, we sometimes used systemic chemotherapy. However, the overall response rate to conventional chemotherapy in the current study was only 25.4%. The establishment of an effective chemotherapeutic regimen still is needed for these patients, and molecular targeted agents, such as sorafenib,[16,17] are expected to improve their prognosis.

The scoring system we propose in the current study incorporates the presence of intrahepatic lesions, the extent of vascular invasion, and performance status. The progression of an intrahepatic lesion was the major cause of death among our patients, as described above. In patients who had extrahepatic metastases, evaluation of the size and number of intrahepatic lesions often is difficult because of disease progression. From the standpoint of these patients, the proposed scoring system is both simple and convenient. Vascular invasion is 1 of the most important prognostic factors for HCC.[12] Our current results demonstrated that macroscopic vascular invasion is significant even in patients who have extrahepatic metastasis. Performance status, which is an important biologic factor in clinical oncology, also is included in our scoring system.[13] Liver function no doubt is a prognostic determinant for patients with HCC; however, the Child-Pugh classification did not retain significance in our multivariate analysis. This may be because the Child-Pugh class is strongly correlated with performance status, which also includes other significant aspects of cancer biology.

Our current results indicate that the median survival of patients with HCC who have extrahepatic metastases varies widely from within 1 month to 1.5 years and can be discerned using the prognosis factors that were evaluated in this study. Patients who have a prognostic score ≥ 2, which indicates an estimated median survival ≥ 6 months, can be considered for intensive treatment, including surgical procedures. In addition, our scoring system may be used for the enrollment of patients into clinical trials of newly developed agents for which patients with extrahepatic metastasis or vascular invasion may be candidates, although further detailed research will be required to establish such use. We compared the prognosis of patients who were treated in the 1990s and the 2000s and observed no statistical difference between the 2 decades (data not shown). During the study period, newly developed agents, such as sorafenib and drug-eluting beads, were not available in Japan.

There were some limitations in this retrospective cohort study. First, a variety of treatments was provided for various intrahepatic and extrahepatic lesions. Substantial heterogeneity existed in patient background. Second, the proportion of patients who had vascular invasion in our cohort was relatively small despite the presence of extrahepatic metastasis, and this may indicate that the total tumor burden also was relatively small. This may have been because most extrahepatic metastasis in our cohort emerged while treatment for intrahepatic lesions was being repeated. Moreover, the proportion of patients with vascular invasion was not very high, even among the patients who had extrahepatic metastasis at initial presentation. Supposedly, this is because our hospital is a tertiary care center, and patients with an apparent indication for percutaneous ablation were referred to us selectively. Third, the number of patients who had prognostic scores of 3, 4, 5 was not large enough for confirmation, although the linearity of median survival (Table 6) suggests the relevance of the scoring system.

In conclusion, the major cause of death in patients with HCC who have extrahepatic metastases is progression of the intrahepatic HCC lesion. We contend that treatment of intrahepatic lesions should not be contraindicated merely because of the presence of an extrahepatic metastasis. Moreover, radical treatments for extrahepatic metastases may be considered when hepatic lesions are under reasonable control or if the metastasis is accompanied by severe symptoms.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

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Will There Be an HCV Meeting in 2020? Summary of the 17th International Meeting on Hepatitis C Virus and Related Viruses

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Hepatitis C virus (HCV), which was discovered in 1989, is a major etiologic agent in human liver disease. Approximately 130 million people, or 2% of the population, worldwide are infected. The 17th International Meeting on Hepatitis C Virus and Related Viruses was held September 10–14, 2010, in Yokohama, Japan. The meeting was attended by almost 700 scientists from all over the world who are interested in the fundamental aspects of the molecular virology, immunology, pathogenesis, prevention, and treatment of HCV infection. Two special opening lectures given by Masaaki Komatsu and Takashi Gojobori focused attention on the related research fields of autophagy and genome biology, respectively. In the subsequent sessions, the latest research, original studies, and controversies were presented in 9 keynote lectures, 82 oral presentations, and 329 poster presentations.

Viral Entry

The opening scientific session of this meeting focused on the viral host cell entry processes. Thomas Baumert presented the keynote lecture, which included an overview of the HCV cell entry process and recent advances at his laboratory. These included the finding that HCV variants that reinfect the liver after transplantation demonstrate more efficient cell entry and are less susceptible to neutralization by host antibodies. He also described the isolation of monoclonal antibodies against claudin-1 that do not inhibit either extracellular or direct cell-to-cell HCV transfer.

Alexander Ploss described the establishment of a mouse model for studying HCV cell entry. They utilized an HCV cell culture virus (HCVcc) expressing recombinase and transgenic mice bearing a recombinase-activatable fluorescent protein. Bioluminescent imaging indicated that only mice transduced with CD81 and occludin supported HCVcc entry. The presence of an intact immune system in these animals makes it particularly important for the testing of HCV vaccine candidates. Danyelle N. Martin described a role for transferrin receptor 1 (TfR1) in mediating HCV cell entry. The inhibition of HCV entry with TfR1 antibodies and silencing, suggest this factor should be added to the growing list of cellular proteins required for HCV cell entry. Joachim Lupberger presented results from a study showing an essential role for the epidermal growth factor receptor (EGFR) in HCV cell entry. He found that EGFR is required for both mediating the interactions between two other entry factors, CD81 and CLDN1, and catalyzing the fusion activity of viral glycoproteins.

Translation/Replication

Volker Lohmann began the session by describing what is known of the functions of viral nonstructural proteins and their associated host cellular factors in viral translation and replication. He included an overview of viral isolates and model systems currently used, and presented data addressing the mechanisms for efficient replication of the JFH-1 isolate.

Several reports have focused on the molecular basis of the architecture and composition of membrane-associated sites for HCV replication, which often induce membrane alterations, such as the so-called membranous web. Brenno Wolk demonstrated that NS4B is sufficient to direct all nonstructural proteins into the viral replication complex compartment, and that intragenotype-specific interactions are required for NS4B-dependent recruitment of NS5A. Ines Romero-Brey showed that the membranous web predominantly contains double-membrane vesicles with various diameters. These vesicle structures were connected to the endoplasmic reticulum (ER) through funnel-like structures.

Several DDX DEAD-box RNA helicases were identified as host factors associated with HCV replication. Yasuo Ariumi presented the cross-talk of HCV with DDX proteins and the role of distinct DDX proteins in viral replication. Tetsuro Shimakami and Selena M. Sagan reported the importance of miR-122 to not only enhance IRES-mediated translation, but stabilize positive-strand HCV RNA by binding to its 5′ extremity. Enzymatic activity of host phosphatidyl-inositol-4 kinase III alpha was shown to be critically involved in HCV replication and the activity is regulated by HCV NS5A (Simon Reiss). Nam-Joon Cho reconstituted a functionally active full-

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length HCV polymerase on a biomimetic membrane platform. Deborah Harrus found that guanosine triphosphate specifically stimulates the initial step of de novo initiation by stimulating transition of newly formed linker primer.

Assembly and Release

In the keynote lecture, Guangxiang G. Luo presented an overview of particle assembly and release, and the impact of apolipoprotein (Apo) E in the entry and assembly of HCV. He demonstrated the inhibition of HCVcc entry by treatment with anti-ApoE antibody and the direct interaction of ApoE with NS5A.

Ann L. Wozniak showed an important role for p7 in the production of infectious particles. Their data suggest that p7 stimulates virus production through the alkalization of intracellular vesicles. Ophelia Granio showed that both p7 and NS2 are required for the recruitment of core from lipid droplets (LDs) to ER. Costin-Ioan I. Popescu showed that NS2 accumulated in dotted structures in the ER in juxtaposition with Core and LDs. They concluded that cross-talk among Core, E1, E2, p7, and NS2 was essential for virion assembly. Vlastimil Jirasko demonstrated point mutations in the transmembrane regions of NS2 impaired the particle production and suggested that NS2 serves as a platform of viral and cellular proteins that coordinates HCV assembly. Qisheng Li identified the proviral function of IKKα by genome wide siRNA screening. IKKα regulates lipid metabolism and biogenesis of LDs and may enhance production of virus particles. The very low-density lipoproteins are secreted via a Golgi-dependent pathway. Bryan R. Bishe demonstrated the important role of phosphatidylinositol-4-phosphate and its interacting protein GOLPH3 in HCV secretion in the trans-Golgi network. Roland Remenyi showed 3-dimensional visualization of the HCV life cycle in cultured cells by electron tomography. They detected virus-like particles at various cytoplasmic locations. Viral particles in the proximity of LDs and within sponge-like inclusion were observed. These results provide ultrastructural visualization of putative assembly sites close to LDs.

Host Factors

In the invited lecture, Sara Cherry presented an overview of high-throughput screening toward the identification of host factors required for viral infection.

The contribution of autophagy to the HCV life cycle was also presented in this section, most notably, host factors linked with lipids. Tsubasa Munakata showed that the fatty acid synthase is required for efficient HCV replication. They also suggested the importance of palmitate for HCV replication. Samantha L. Blackham presented both the thioredoxin-interacting protein and the peroxisome proliferator activated receptor-α have significant effects on HCV replication. The host factors functioning on infectious HCV particle production were also reported. Takayuki Hishiki demonstrated the isoform dependent binding affinities of ApoE for low-density lipoprotein receptors and they affect infectivity of HCV. Laurent Chatel-Chaix found that Y-box binding protein interacted with HCV NS3 protein and viral RNA and was relocalized from nucleocytoplasmics site to the core-containing surface of LDs. Mohsan Saeed reported that the ER-associated degradation pathway was activated by HCV infection in a viral envelope protein-dependent manner. Po-Yuan Ke showed that HCV infection induces the unfolded protein response and activates the autophagic pathway. They proposed that autophagy contributes to the suppression of HCV in an autolysosome formation-dependent manner. Hiroto Kambara did not find any effects on HCV replication by inhibition of autophagosome formation in replicon cells. They proposed a role for autophagy induced by HCV infection to avoid the generation of vacuolation harmful to cell survival. Qisheng Li reported the network map of cellular pathways and machineries that are associated with HCV life cycle.

Very low-density lipoprotein is now considered to be one of a component of HCV particles. LDs are composed of fatty acid, triglyceride, and cholesterol, surrounded by several types of lipoproteins. In addition, Daniel J. Felmlee reported that chylomicron-associated viruses may be generated by virion association while in the vascular compartment. Francois Jean showed that the serine protease inhibitor protein Spn4A was modified to be directed to Site-1 protease specifically and was introduced into adenovirus vector to inhibit cholesterol and fatty acid syntheses for down-regulation of HCV propagation. The modified serpin could suppress Site-1 protease activity, reduce the LD, and block HCVcc infection. Nicolas Menzel tried to identify novel cellular factors involved in HCV assembly and release and found ERK inhibitor and cytosolic phospholipase A2 (cPLA2) inhibitor reduce viral production. cPLA2 inhibitor also reduced the amount of LD-associated core and supernatant ApoB/E. cPLA2 may be crucial for assembly of infectious HCV particles, possibly through participating in the formation of lipoproteins. Kohji Moriishi reported that the proteasome activator PA28γ participates in HCV propagation. PA28γ may participate in the propagation of HCV by regulating the degradation of Core in both ubiquitin-dependent and -independent manners. NS5A is regulated by phosphorylation of several host protein kinases. Takahiro Masaki identified 79 serine threonine protein kinases that were tightly associated with NS5A. Two of these may regulate the production of viral particles and/or viral replication.

Innate Immunity

The early phase of host defense against viral infection has largely been delineated based on recent advances in innate immunity. In the invited lecture, Manoj N. Krishnan introduced his comprehensive study on the Toll-like receptor 3–TRIF (TICAM-1) pathway. Using RNAi and polyI:C, he screened the genes specifically up-regulated via the TRIF (TICAM-1) pathway. He expected that some viral infections are selectively blocked by the IPS-1 pathway, while others are blocked by the TRIF pathway.

Michael Gale, Jr., identified IFITM1 inhibits HCV infection. IFITM1 assembles with CD81 and tranlocates to the tight junction. This translocation of CD81 hampers the receptor function of CD81. They also discovered a novel pathway for ISGF3 activation. A non-receptor type tyrosine kinase-1 triggers activation of ISGF3 independent from the classical IFNAR pathway. IP-10 is a chemokine and is a negative predictor for pegylated interferon (IFN)/ribavirin therapy. Matthew L. Albert indicated that there is a 2-amino-acid–deleted form of IP-10 that serves as an antagonist for intact IP-10, and this form abrogates an early virologic response. As this IP-10 truncation is mediated by dipeptidylpeptidase IV, they believed that dipeptidylpeptidase IV is a novel therapeutic target for HCV patients during IFN therapy. Joo Chun Yoon suggested that activation of natural killer cells is inhibited by HCV-infected hepatocytes. They claimed that the early phases of HCV infection may be established through the failure of virus-inducible natural killer cell activation. Shin-ichiro Nakagawa reported that polyI:C induces both type I IFN and IFN-λ in human hepatocytes. The antiviral effect appears to parallel the induction of IFN-λ. This, together with the report by Emmanuel Thomas, suggests that the IFN-λ system is activated in HCV infected hepatocytes.

Adaptive Immunity

In a keynote lecture, Robert Thimme summarized the mechanisms of HCV-induced T-cell dysfunction. Multifaceted factors contribute to the hyporesponsiveness of T cells, including viral mutations, primary T-cell failure, lack of support from dendritic cells, expression of inhibitory molecules on T cells, and abundance of regulatory T cells (Tregs). Whether the ability of HCV-specific CTLs is comparable with that of CTLs having other specificities remains controversial. Bianca Seigel showed that HCV-specific CTLs are functionally impaired when compared with other CTLs, irrespective of their expression of inhibitory receptors or differentiation stages. CD161 is a C-type lectin that is expressed in HCV-specific $CD8^+$ T cells with tissue homing phenotype. Vicki M. Fleming found that $CD4^+CD161^+$ T cells produce large amounts of inflammatory cytokines and accumulate in the liver, where they are thought to exert pro-inflammatory roles. Naruyasu Kakita reported that certain adaptive Tregs, known as interleukin (IL)-10–producing type 1 Tregs, are increased in HCV-positive hepatocellular carcinoma patients, and their significance in hepatocellular carcinoma was greater than that of natural Tregs. Even in patients who have attained a sustained virologic response, trace amounts of HCV RNA are sporadically detectable in plasma. Barbara Rehermann reported the inoculation studies of such plasma. Residual HCV RNA in patients was able to infect chimpanzees and induced broad, HCV-specific T-cell responses. HCV RNA levels continued to be high when T-cell responses declined, suggesting that such HCV remains transmissible as hepatotropic pathogens.

Pathogenesis

In the invited lecture, Michael Diamond presented new mechanisms for West Nile virus immune evasion via 2'O methylation of viral RNA to subvert host innate immunity.

Genome-wide analysis of quantitative data (transcriptomics, proteomics, and metabolomics) facilitates systems biology analysis of HCV infection. Deborah L. Diamond analyzed the pathways involved in the progression of chronic hepatitis, namely, fibrosis and carcinogenesis, and found that molecules relating to cell metabolism including fatty acid oxidation enzymes and antioxidant systems may be master regulators of liver disease progression in HCV infection. HCV core protein has been shown to play a key role in the development of steatosis in HCV infected liver, especially in patients with genotype 3a HCV infection. Sophie Clement-Leboube showed that PTEN expression was down-regulated in the HCV infected liver. Analysis of lipo-viral-particle from hepatitis C patients by Olivier Diaz revealed that empty lipo-viral-particle lacking HCV RNA outnumbers those with RNA. The presence of virus-modified lipoproteins in HCV-infected patients may play a role in the pathogenesis of hepatitis C. Massimiliano Pagani used serum miRNA signatures to monitor liver disease in HCV infection and found miRNome candidates that are specific for HCV disease progression. Shuhei Taguwa showed that Con1 replicon induces incomplete autophagy through the dysfunction of autolysosomal acidification, which results in the secretion of immature cathepsin B in cells. Because the secretion of the protein is enhanced in many types of tumors, this observation may be associated with the pathogenesis of liver tumorigenesis in HCV infection.

The existence of extrahepatic manifestations is another issue of interest. Essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, and Sjögren syndrome are conditions that have been shown to correlate

with HCV infection. Nicola A. Fletcher reported that brain microvascular endothelial cells express all the recognized entry factors for HCV, and brain microvascular endothelial cells actually support infection by HCVpp and HCVcc. This suggests potential disorders of the central nervous system in HCV infection.

Treatment

In the keynote lecture, Masashi Mizokami presented "Genome-wide association study and its application for HCV treatment." He emphasized that the functional relevance of IL-28B single nucleotide polymorphisms should be elucidated to further advance the progress of research on the mechanisms of chronic HCV infection and treatment.

Yasuhiro Asahina presented that genetic variation in IL-28B is associated with gene expression involving innate immunity. Minor alleles of IL-28B, as well as a higher RIG-I/IPS-1 ratio are associated with null viral response. Martin Laggins correlated IL-28B genetic variation with pretreatment levels of IP-10 and HCV RNA throughout therapy. The favorable genetic variation of IL-28B single nucleotide polymorphisms (major allele) was significantly associated with lower baseline IP-10. Masao Honda revealed that hepatic IFN-stimulated genes (ISGs) are associated with genetic variation in IL-28B and the outcome of IFN therapy for chronic hepatitis C using microarray gene expression profiling of the biopsied liver samples. Multivariate logistic regression analysis showed that ISGs, fibrosis stage, and ISDR mutations were strongly associated with viral response. Hepatic ISGs were associated with the IL-28B polymorphism and expression was significantly higher in patients with the minor genotype than in those with the major genotype. Takashi Motomura also analyzed ISG expression using liver transplantation samples. Expression of ISGs in recipients' liver carrying the minor allele of IL-28B was significantly up-regulated when compared with the major allele. Surprisingly, IFN sensitivity for recurrent hepatitis C after liver transplantation is influenced by IL-28B genetic variation not only in recipients, but also in donors.

Drug Development

This session opened with a keynote lecture by Raffaele De Francesco describing the current state of drug development for patients with chronic hepatitis C. Because of the rapid development of NS3/4A, NS5A, and NS5B inhibitors, he finally presented the hopeful message "Will there be an HCV meeting in 2020?".

Lotte Coelmont characterized an NS5A D320E variant showing low-level resistance to DEB025, a cyclophilin (Cyp)-binding molecule. This study suggests that DEB025 presents a high barrier to resistance, and that D320E confers low-level resistance to DEB025 by reducing the need for CypA-dependent isomerization of NS5A. Paul Targett-Adams reported that NS5A inhibitors stimulated redistribution of NS5A from the ER to ring-like structures in the cytoplasm, and disrupted colocalization with NS5B. This study suggests that NS5A inhibitors perturb formation of new replication complexes rather than acting on preformed complexes. Luis M. Schang developed a family of small synthetic rigid amphiphiles with large hydrophilic heads and small, planar and rigid hydrophobic tails, called RAFIs (rigid amphipathic fusion inhibitors), which inhibit the infectivity of enveloped virions including HCV. Emmanuel Thomas screened host genes involving the anti-HCV activity of ribavirin. Among 64 host genes, several candidate genes were identified as host factors involving ribavirin's anti-HCV activity. Interestingly, silencing of the *ITPA* gene increased the anti-HCV activity of ribavirin. Pablo Gastaminza identified a novel family of 1,2-diamines as an anti-HCV reagent from a chemical library. The analysis of ~300 derivatives identified several compounds with enhanced potency and low cytotoxicity.

Vaccines/Epidemiology

HCV therapeutic vaccines are aimed to induce effective T-cell responses. Marianne Mikkelsen reported that vaccination of mice with recombinant adenovirus expressing HCV NS3 fused to the MHC class II chaperon protein invariant chain significantly enhanced NS3 specific $CD8^+$ T-cell responses, and protected mice against NS3-expressing vaccinia virus challenge. This vaccination induced polyfunctional $CD8^+$ memory T cells. Lars Frelin aimed to restore immunologic function through vaccination in a transgenic mouse model with impaired HCV-specific T-cell responses owing to a persistent presence of hepatic HCV NS3/4A antigens. They found that heterologous sequences improved activation and expansion of NS3/4A-specific T cells in a wild-type host, as well as in a tolerant NS3/4A-transgenic mouse model. The authors also suggested an important role for Tregs in the impaired HCV-specific T-cell responses.

Livia M.G. Rossi examined antibody cross-immunoreactivity against different HVR1 variants to identify antigens with a possible application of HCV vaccine development. The authors identified a small set of HVR1 variants that cross-immunoreacted with a large number of HVR1 peptides, thus suggesting their potential use in the development of HCV vaccine candidates.

Conclusion

HCV2010 in Yokohama was successful and contributed to the progress of research in the field. HCV infection remains one of the most serious worldwide health problems. The goals of this symposium were to

increase the scientific understanding of this virus and gain insights applicable to future efforts to control its infection. From this point of view, we gained further fundamental understanding about HCV at the meeting. The discovery of IL-28B as a new host factor involved in HCV treatment and pathogenesis had a major impact on HCV research. New treatment advances have been made in recent years and will continue in the near future. We would like to conclude that this meeting was successful in providing opportunities for exchanging up-to-date information and international collaboration. The next meeting will take place in Seattle, Washington, from September 8–12, 2011 (http://www.hcv2011.org/).

Reprint requests

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Conflicts of interest

The authors disclose no conflicts.

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Original Article

Cancer preventive effect of pegylated interferon α-2b plus ribavirin in a real-life clinical setting in Japan: PERFECT interim analysis

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Aim: This study was conducted to clarify the incidence of hepatocellular carcinoma (HCC) and the factors contributing to its occurrence by following chronic hepatitis C patients who received pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination therapy.

Methods: Patients who received PEG-IFN α-2b and RBV combination therapy with no history of HCC or HCC within 3 months after the start of treatment were observed for the onset of HCC at 67 centers.

Results: Sustained virological response (SVR) was observed in 999 (53.5%) of 1865 patients eligible for analysis. During the observation period (median duration: 4 years and 3 months), HCC developed in 59 patients (3.1%). A significant difference was observed in the 5-year cumulative incidence of HCC between SVR and non-SVR patients (1.1% vs. 7.1%). Factors contributing to HCC selected in multivariate analysis were therapeutic efficacy, sex, age, alanine aminotransferase (ALT) level at 24 weeks after the end of treatment, and platelet count. Non-SVR patients with ALT improvement after the end of treatment had a significantly lower 5-year cumulative incidence of HCC than those without (3.4% vs. 11.0%). HCC developed in 10 patients who achieved SVR, and multivariate analysis indicated that ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC.

Conclusion: Several known risk factors for HCC contributed to HCC in patients who received PEG-IFN α-2b and RBV combination therapy, and ALT abnormality after the end of treatment contributes to the onset of HCC in both non-SVR and SVR patients.

Key words: alanine aminotransferase, chronic hepatitis C virus, hepatocellullar carcinoma, pegylated inteferon, ribavirin

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; BR, biochemical response; CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; IFN, interferon; LVR, late virological response; NR, no response; NVR, non-virological response; PEG-IFN, pegylated interferon; ; RBV, ribavirin; SVR, sustained virological response; TR, transient response.

INTRODUCTION

THE INCREASE IN the incidence of hepatocellular carcinoma (HCC) in Japan peaked in 2004 and is now in a declining trend.[1] The HCC mortality rate, however, is still particularly high among developed countries,[2] and even now nearly 35 000 people die

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annually from HCC. In Japan, about 70% of patients diagnosed with HCC are positive for hepatitis C virus antibody.[3] The hepatitis C virus infection rate[2] and incidence of HCC both increase with the age of the patient,[4] and curing chronic hepatitis C (CHC) to reduce HCC and deaths due to HCC is a pressing issue.

With the discovery of interferon (IFN), CHC became a curable disease, and with the addition of ribavirin (RBV), therapeutic outcomes have improved dramatically. Currently, about 50%[5–8] of patients with HCV genotype 1b and high virus load and more than 80%[9] of genotype 2 patients achieve sustained virologic response (SVR), and the SVR rate is reported to improve further with long-term treatment[10,11] and with combination therapy plus a statin.[12]

The efficacy achieved with these IFN therapies is also reported to lead to the inhibition of the onset of HCC and deaths due to HCC[13–19], but only a few reports are available of long-term observation of patients receiving PEG-IFN α plus RBV combination therapy.

We therefore examined the HCC preventive effect of combination therapy in 1865 patients who received PEG-IFN α-2b and RBV.

METHODS

Patients and treatment

PERFECT (THE PEG-IFN and Ribavirin, Find Evidence of Chronic Hepatitis C Therapy in Tokyo) Study Group, consisting of 67 centers in Tokyo and Yamanashi Prefecture, conducted a retrospective study to investigate the efficacy and safety of PEG-IFN α-2b plus RBV in CHC patients in a real-life clinical setting. The participating centers, targeted patients, and the treatment method have already been reported[10] and are summarized below.

Patients seen from December 2004 who completed PEG-IFN α-2b plus RBV combination therapy by September 2007 were registered regardless of genotype, history of IFN treatment, or alanine aminotransferase (ALT) levels. Excluded from this study were pregnant or possibly pregnant and lactating women, and patients with severe heart disease, chronic kidney failure or creatinine clearance of ≤50 mL/min, current or history of severe psychiatric disorder, and autoimmune hepatitis. Doses of PEG-IFN α-2b and RBV and dose adjustment followed the Japanese package insert. The duration of treatment was 48 weeks, the standard of care for patients with genotype 1 and high virus load. In patients with late viral response (LVR) who did not achieve viral negativity by week 12, treatment could be extended up to 72 weeks. Patients other than those with genotype 1 and high virus load were treated for 24 weeks.

Included in this analysis were the patients registered in the PERFECT Study who had no history of HCC and for whom SVR/non-SVR status could be confirmed. The patients who developed HCC within 3 months of the start of treatment were excluded from analysis to rule out the possibility of inclusion of patients with HCC already present at the start of treatment.

The start of the follow-up period was defined as the first day of PEG-IFN α-2b and RBV treatment. The patients were monitored for the onset of HCC by routine follow-up methods practiced by each center. The diagnosis of HCC was based on the presence of typical hypervascular characteristics on angiography in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy specimens was performed in patients whose angiograms did not demonstrate a typical image of HCC.

This multicenter study was approved by the institutional review board of each participating center. The study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from each patient.

Statistical analysis

All statistical analyses were performed using SAS, version 9.13 (SAS Institute, Cary, NC, USA). Intergroup comparison of background variables was performed by Fisher's exact test and Mann–Whitney *U*-test.

The cumulative incidence of HCC was calculated by the Kaplan–Meier method, and intergroup comparison was conducted using the log-rank test. The determination of the factors contributing to HCC was conducted by Cox proportional hazards regression model using a stepwise procedure, incorporating the factors exhibiting $P < 0.2$ by the log-rank test and excluding factors with more than 30% of values missing. The determination of factors associated with biochemical response (BR) was conducted by a stepwise procedure using the results of logistic univariate analysis ($P < 0.2$) in logistic multivariate analysis.

All tests were two-sided, with a significance level set at $P < 0.05$.

RESULTS

Study population

A TOTAL OF 1865 subjects, consisting of 999 SVR patients (SVR rate 53.5%) and 866 non-SVR patients, were eligible for analysis. Of the non-SVR patients, 441 had transient response (TR) defined as viral negativity achieved during treatment (relapse: 408, virus breakthrough: 33), 400 patients had non-virological response (NVR) defined as viral negativity not being achieved, and the change in viral load during treatment was not known for 25 patients.

The duration of observation ranged from 3 months to 5 years and 8 months, with a median of 4 years and 3 months.

During the observation period, HCC developed in 59 patients (3.1%). Between patients who developed HCC and those who did not, significant differences in background factors were detected in age ($P < 0.0001$), hepatic fibrosis ($P = 0.0002$), virological efficacy ($P < 0.0001$), ALT levels ($P = 0.0089$), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L) ($P < 0.0001$), platelet count ($P = 0.0001$), serum albumin ($P = 0.0062$), and alpha fetoprotein (AFP) ($P < 0.0001$) (Table 1).

Virological efficacy and incidence of HCC

The 5-year cumulative incidence of HCC by the Kaplan–Meier method was 1.1% in SVR patients and 7.1% in non-SVR patients, a difference that was significant ($P < 0.001$) (Fig. 1). No significant difference was observed in the incidence of HCC between TR and NVR patients among non-SVR patients, but the difference between TR and SVR patients was significant ($P < 0.0001$) (Fig. 2). This trend was also observed regardless of gender, with no significant difference in the incidence of HCC observed between TR and NVR in either male or female patients and a significant difference observed between TR and SVR in both male patients ($P = 0.0007$) and female ($P = 0.0065$) patients.

Factors contributing to HCC

The factors contributing to HCC selected in the multivariate analysis were therapeutic efficacy (SVR vs. NVR), sex, age (<60 vs. ≥60 years), ALT level at 24 weeks after the end of treatment (≤40 vs. >40 IU/L), and platelet count (<10 vs. ≥10 × 10 000/mm^3) (Table 2).

Biochemical response and incidence of HCC in non-SVR patients

Since ALT levels at 24 weeks after the end of treatment was selected as one factor contributing to HCC, the changes in ALT levels and onset of HCC were examined in 514 non-SVR patients with a pretreatment ALT level of more than 40 IU/L whose ALT level at 24 weeks after the end of treatment was obtained. Of these 514

Table 1 Patient background by onset of hepatocellular carcinoma (HCC) (1865 patients)

Factor	With onset of HCC ($n = 59$)	Without onset of HCC ($n = 1806$)	*P*-value
Gender (male/female)	40/19	1014/792	0.0832
Age	62 (44–74)	56 (17–77)	<0.0001
Diabetes (yes/no/unknown)	6/33/20	100/1040/666	0.1539
Hypertension (yes/no/unknown)	4/6/49	116/569/1121	0.0763
Alcohol abuse (yes/no/unknown)	11/16/32	195/493/1118	0.1930
Fibrosis (0/1/2/3/4/unknown)	0/12/13/15/4/15	57/573/355/205/56/560	0.0002
Genotype (1/2/3/unknown)	52/5/0/2	1421/365/2/18	0.0876
Effect of IFN (SVR/non-SVR)	10/49	989/817	<0.0001
Body mass index (kg/m^2)	22.6 (14.2–34.0)	22.9 (14.9–41.2)	0.8546
ALT (IU/L)	79 (24–343)	60 (8–984)	0.0089
ALT at 24 weeks after end of treatment (IU/L) (≤40/>40/unknown)	16/30/13	1105/352/349	<0.0001
Platelet count (×10 000/mm^3)	13.3 (4.3–22.2)	16.3 (3.6–213.3)	0.0001
Serum albumin (g/dL)	3.9 (2.9–4.7)	4.1 (2.8–5.9)	0.0062
AFP (ng/mL)	13 (2.2–327.9)	5 (0–875)	<0.0001

Median (minimum – maximum).
AFP, alpha fetoprotein; ALT, alanine aminotransferase; IFN, interferon; SVR, sustained virological response.

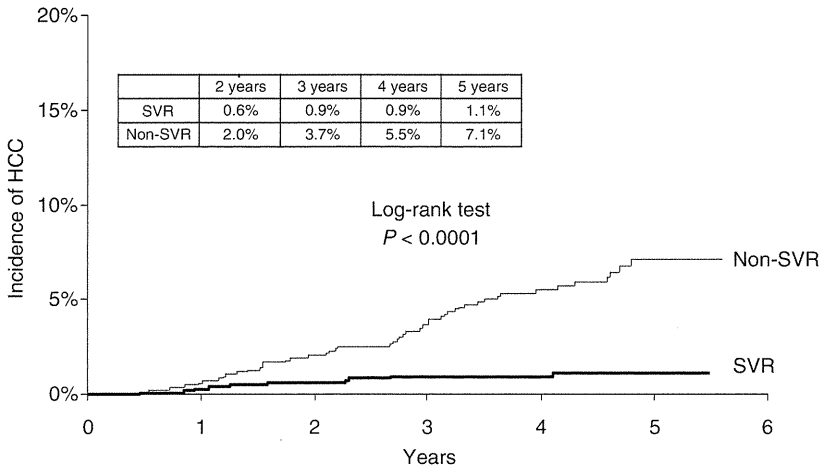


Figure 1 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (1865 patients) (sustained virological response [SVR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between SVR and non-SVR was examined using the log-rank test.

patients, ALT level at 24 weeks after the end of treatment was reduced to less or equal to 40 IU/L (biochemical response: BR) in 234 patients, and the remaining 280 patients had values of more than 40 IU/L (non-BR). There were significant differences between BR and non-BR patients in the background factors of pretreatment ALT level, age, hepatic fibrosis, platelet count, AFP, and treatment duration. Selected as the factors contributing to BR in non-SVR patients in the multivariate analysis were TR, long treatment duration, and high platelet count before the start of treatment (Table 3).

The 5-year cumulative incidence of HCC was 3.4% in BR patients and 11.0% in non-BR patients, and the difference in incidence was significant ($P = 0.0012$) (Fig. 3). The 5-year cumulative incidence of HCC in male patients was 3.6% in BR patients and 13.9% in non-BR patients, and the difference was significant ($P = 0.0012$). In female patients, however, it was 3.5% in BR patients and 7.6% in non-BR patients, and although the incidence of HCC was lower in BR patients, the difference was not significant ($P = 0.0706$).

Incidence of HCC in patients with normal pretreatment ALT levels

When the incidence of HCC was compared between SVR (288) and non-SVR (214) patients among 502 patients with pretreatment ALT levels less or equal to 40 IU/L, the 5-year cumulative incidence of HCC was 0% in SVR patients and 4.8% in non-SVR patients, indicating a significant difference ($P = 0.0005$) between the groups

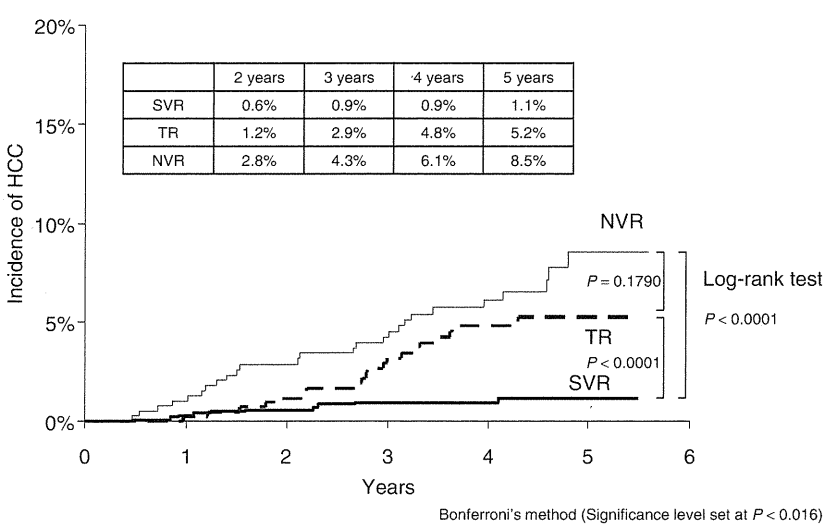


Figure 2 Onset of hepatocellular carcinoma (HCC) by therapeutic efficacy (sustained virological response [SVR] vs. transient response [TR] vs. non-virological response [NVR]). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. The difference between each group was examined using the log-rank test (Bonferroni's Method, significance level set at $P < 0.016$).

Table 2 Factors contributing to hepatocellular carcinoma (all patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
Therapeutic efficacy	SVR	1		
	TR	2.055	0.709–5.955	0.1845
	NVR	2.985	1.036–8.601	0.0428
Sex	Male	1		
	Female	0.486	0.243–0.969	0.0405
Age	<60	1		
	≥60	2.005	1.035–3.883	0.0391
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	3.940	1.754–8.850	0.0009
Platelet count (×10 000/mm^3)	<10	1		
	≥10	0363	0.169–0.779	0.0093
Serum albumin (g/dL)	<4	1		
	≥4	0.594	0.310–1.140	0.1175

Factors examined: Of the 15 factors exhibiting $P < 0.2$ by log-rank test (therapeutic efficacy [1: SVR, 2: TR, 3: NVR], genotype [1: 1, 2: 2 or 3], sex [1: male, 2: female], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1 <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], hypertension [1: absent, 2: present], diabetes [1: absent, 2: present], heavy drinking [1: absent, 2: present], and treatment duration [1: ≤48 W, 2: >48 W]), nine factors were examined. Excluded were factors for which approximately 30% of values were missing (AFP, grade, stage, diabetes, hypertension, and heavy drinking).
AFP, alpha fetoprotein; ALB, albumin; ALT, alanine aminotransferase; NVR, non-virological response; PLT, platelet count; SVR, sustained virological response; TR, transient response.

(Fig. 4). This tendency is also observed with the 280 patients having pretreatment ALT levels of less or equal to 30 IU/L.

Onset of HCC in SVR patients

Hepatocellular carcinoma developed in 10 patients who achieved SVR. Multivariate analysis indicated that in SVR patients, the ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC ($P = 0.0007$) (Table 4). In SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment, the 5-year cumulative incidence of HCC was 5.6% while the incidence in patients with an ALT level of less or equal to 40 IU/L was 0.7%, indicating a significant difference ($P = 0.0004$) between the groups (Fig. 5).

DISCUSSION

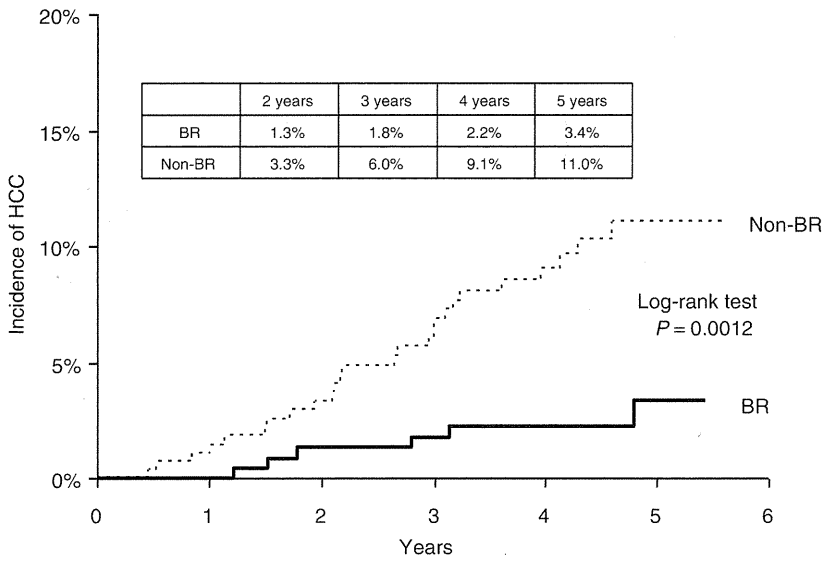
THIS STUDY INDICATED that the risk factors for HCC after PEG-IFN α-2b plus RBV combination therapy are NVR, male sex, older age, low platelet count, and an ALT level of more than 40 IU/L at 24 weeks after the end of treatment.

Kurokawa *et al.*[16] tracked 403 patients receiving PEG-IFN α-2b plus RBV combination therapy for a median

Table 3 Factors contributing to biochemical response in non-sustained virological response patients Logistic regression analysis (multivariate)

		Odds ratio	95% confidence interval	*P*-value
Virological response	NVR	1	1.480–3.203	0.0001
	TR	2.177		
Treatment duration	per week	1	1.000–1.022	0.0424
		1.011		
Platelet count	per 10 000/mm^3	1	1.018–1.099	0.0043
		1.058		

Factors examined were those exhibiting $P < 0.2$ by log-rank test: Genotype, virological response (TR/NVR), treatment duration, pre platelet count, diabetes, stage, and alanine aminotransferase (ALT).

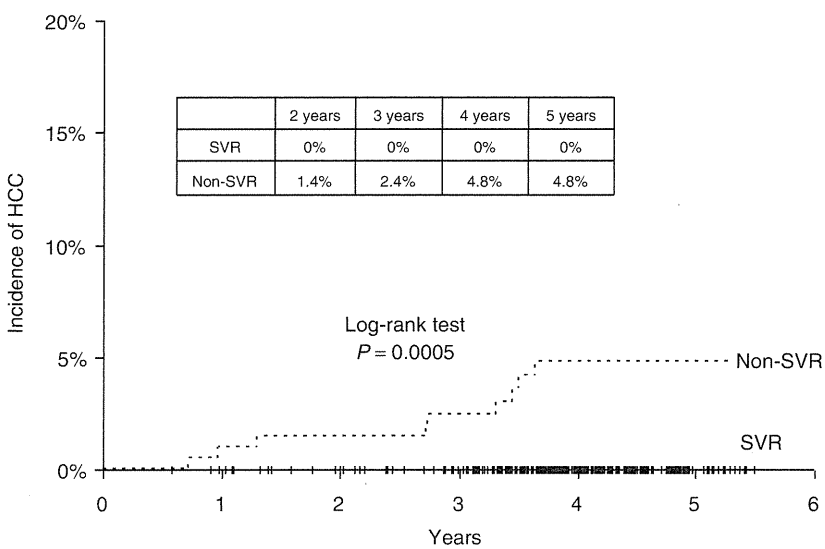


	2 years	3 years	4 years	5 years
BR	1.3%	1.8%	2.2%	3.4%
Non-BR	3.3%	6.0%	9.1%	11.0%

Figure 3 Alanine aminotransferase (ALT) normalization and hepatocellular carcinoma (HCC) in non-virological response [NVR] patients. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between biochemical response (BR) and non-BR.

duration of 36.5 months and reported that in multivariate analysis, virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis were selected as the factors contributing to HCC. Arase *et al.*[15] tracked 500 patients 60 years of age and older receiving IFN alone or in combination with RBV for an average duration of 7.4 years and also reported that the factors contributing to HCC are virological efficacy (SVR vs. non-SVR), age, and hepatic fibrosis. In our study, hepatic fibrosis was not tested with multivariate analysis because more than 30% of values were missing, but it was selected as a significant factor in the univariate analysis. Platelet count was selected in multivariate analysis, and the results in our study are therefore considered to be generally consistent with these reports.

The results of the present study indicated no significant difference between TR and NVR in non-SVR in stratified cumulative incidence of HCC, and although there was a significant difference between SVR and both TR and NVR, TR was not significant against SVR in multivariate analysis, and NVR was the only significant factor. Kurokawa *et al.*[16] reported the same results by



	2 years	3 years	4 years	5 years
SVR	0%	0%	0%	0%
Non-SVR	1.4%	2.4%	4.8%	4.8%

Figure 4 Therapeutic efficacy and hepatocellular carcinoma (HCC) in patients with pretreatment alanine aminotransferase (ALT) of ≤40. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between sustained virological response (SVR) and non-virological response (NVR).

Table 4 Factors contributing to hepatocellular carcinoma (sustained virological response [SVR] patients) Cox regression analysis (multivariate)

		Hazard ratio	95% confidence interval	*P*-value
ALT at 24 weeks after end of treatment (IU/L)	≤40	1		
	>40	16.054	3.235–79.681	$P = 0.0007$
Serum albumin (g/dL)	<4	1		
	≥4	0.196	0.036–1.073	$P = 0.0603$

Factors examined: Of the 10 factors exhibiting $P < 0.2$ by log-rank test (Genotype [1: 1, 2: 2 or 3], age [1: <60, 2: ≥60], pre ALT [1: ≤40, 2: >40], +24 w ALT [1: ≤40, 2: >40], pre PLT [1: <10, 2: ≥10], pre ALB [1: <4, 2: ≥4], pre AFP [1: <20, 2: ≥20], grade [1: A0–1, 2: A2–3], stage [1: F0–1, 2: F2–4], and diabetes [1: absent, 2: present]), 5 factors were examined. Excluded were pre ALT, with which HCC did not occur in the ≤40 group, and AFP, grade, stage, and diabetes, the factors for which approximately 30% of values were missing.
ALB, albumin; ALT, alanine aminotransferase; PLT, platelet count;

comparing cumulative incidences of HCC among SVR, TR and NVR (the results of multivariate analysis are not known). On the other hand, Morgan *et al.*,[19] in their follow-up study of the HALT-C Trial, reported that there was no difference between TR and NVR in the incidence of HCC or death related to hepatic disease/liver transplantation, but when all hepatic-related outcomes were examined, a significantly superior inhibition was observed with TR compared to NVR. Our results also demonstrate that although the difference is not significant, the cumulative incidence of HCC is lower in TR patients than in NVR patients, especially in male patients (5-year cumulative incidence of HCC: 6.0% vs. 10.7%). It is therefore necessary to continue to observe this for an extended number of years.

Our results study indicated that in non-SVR patients, whether or not ALT level is normalized after treatment is a greater contributing factor for the onset of HCC than virological response. Normalization of ALT has already been reported to contribute to the inhibition of the onset of HCC even under HCV-positive conditions,[13,20] and this was found to apply also to non-SVR patients receiving PEG-IFN α plus RBV combination therapy.

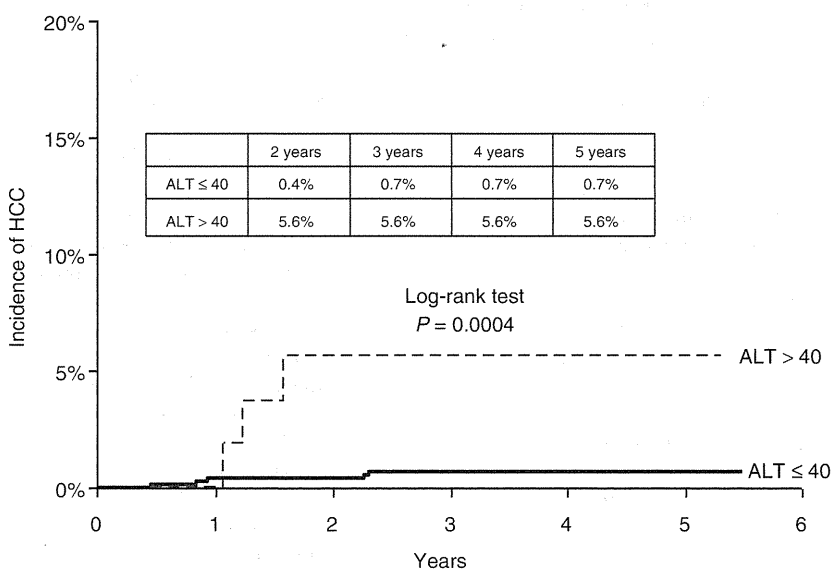


Figure 5 Alanine aminotransferase (ALT) levels at 24 weeks after end of treatment and hepatocellular carcinoma (HCC) in patients with sustained virological response (SVR). The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Log-rank test was used to study the difference between SVR patients with an ALT level of more than 40 IU/L at 24 weeks after the end of treatment and those with an ALT level of less or equal to 40 IU/L.

Our investigation also indicated that abnormal ALT levels also contribute to the onset of HCC in SVR patients. In multivariate analysis, the only contributing factor to the development of HCC in SVR patients was ALT levels at 24 weeks after the end of treatment. However, the onset of HCC is also observed in patients who achieve ALT normalization after treatment, and it is therefore difficult to conclude that ALT is the only risk factor for the onset of HCC in SVR patients. The potential involvement of hepatic fibrosis as well as hepatic steatosis, which persists after viral clearance[21] and small amounts of virus remaining in the liver[22] have also been suggested as risk factors for the onset of HCC in SVR patients. Further detailed investigation is therefore necessary. Nevertheless, regardless of whether or not SVR is achieved, it is clear that abnormal ALT is a factor affecting the onset of HCC. Careful monitoring of changes in ALT and instituting measures to normalize ALT are therefore important regardless of whether or not SVR is achieved.

With the administration of PEG-IFN α plus RBV combination therapy tailored for individual patients and the addition of direct-acting antivirals to current combination therapy, the therapeutic outcomes for CHC will continue to further improve, and the number of patients who develop hepatic cirrhosis and HCC from hepatitis C can be expected to decrease in the future. HCC can occur even in patients achieving SVR, and even if SVR is not achieved, as long as the possibility to inhibit the onset of HCC remains, there will be a need for various treatment innovations to achieve the prevention of HCC, the ultimate goal of treatment of CHC.

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