Table 3 Factors associated with sustained virological response in patients with the *IL28B* non-TT genotype

Variable	Simple			Multiple		
	OR	95 % CI	*P* value	OR	95 % CI	*P* value
Host-related factor						
Age (year)	0.99	0.94–1.05	0.7963			
Sex male vs. female	1.56	0.50–4.83	0.4421			
Body weight (kg)	0.97	0.93–1.02	0.2324			
Body mass index (kg/m^2)	0.82	0.69–0.99	0.0400			
Cirrhosis absence vs. presence	1.80	0.50–6.43	0.3657			
Relapsers vs. treatment-naïve or non-responders	13.64	2.60–71.46	0.0020	9.18	1.04–81.16	0.0461
White blood cells (/μL)	1.00	1.00–1.00	0.0255			
Hemoglobin (g/dL)	1.26	0.87–1.82	0.2145			
Platelets ($\times 10^4$/μL)	1.14	1.02–1.26	0.0161			
Aspartate aminotransferase I(U/L)	0.97	0.95–1.00	0.0303			
Alanine aminotransferase I(U/L)	0.98	0.96–1.00	0.0564			
Gamma-glutamyl-transpeptidase I(U/L)	0.99	0.99–1.00	0.1852			
Albumin (g/dL)	2.30	0.42–12.72	0.3380			
Total cholesterol (mg/dL)	1.00	0.98–1.02	0.9274			
Low-density lipoprotein cholesterol (mg/dL)	1.01	0.99–1.03	0.3557			
Alpha-fetoprotein (ng/mL)	0.90	0.82–0.99	0.0304			
Virus-related factor						
HCV RNA ($\log_{10}$IU/mL)	0.67	0.28–1.59	0.3590			
Core amino acid substitution 70 wild-type vs. mutant-type	1.56	0.50–4.83	0.4421			
ISDR of NS5A non-wild-type vs. wild type	1.87	0.29–12.33	0.5130			
Treatment-response factor						
Rapid virological response + vs. −	15.27	2.96–78.81	0.0011	17.96	1.73–186.57	0.0156
Reduction in HCV RNA level at week 1 ≥4.7 $\log_{10}$/mL vs. <4.7 $\log_{10}$IU/mL	15.00	3.43–65.59	0.0003	29.35	2.88–299.22	0.0043
Treatment-related factor						
Administration intervals of telaprevir q8 vs. q12 h	0.60	0.19–1.84	0.3698			
Initial daily dose of telaprevir 2250 vs. 1500 mg	0.71	0.21–2.41	0.5781			
Duration of therapy (weeks)	1.16	0.93–1.44	0.1973			
Adherence of PEG-IFN (%)	1.04	0.99–1.08	0.1084			
Adherence of ribavirin (%)	1.01	0.98–1.04	0.4767			
Adherence of telaprevir (%)	0.99	0.96–1.03	0.6851			

HCV hepatitis C virus, *ISDR* interferon sensitivity-determining region, *Peg-IFN* PEG-interferon

($P = 0.0156$, OR = 17.96, 95 % CI = 1.73–186.57), and previous relapsers ($P = 0.0461$, OR = 9.18, 95 % CI = 1.04–81.16) (Table 3).

Combination of the *IL28B* genotype and reduction in HCV RNA levels at week 1 after the start of therapy to identify patients with a high likelihood of SVR

Figure 4 shows the schematic representation of the process used to identify patients with a high likelihood to achieve SVR by combining the two factors most strongly associated with SVR. Patients with the *IL28B* TT genotype presented a high SVR rate [102 of 106 patients (96.2 %)], regardless of the reduction in HCV RNA levels at week 1 after the start of therapy. In contrast, patients with the non-TT genotype showed a high SVR rate [15 of 18 patients (83.3 %)] if they presented a reduction of ≥4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 after the start of therapy. In contrast, the SVR rate was significantly lower [8 of 32 patients [25.0 %)] when patients did not present a reduction of ≥4.7 $\log_{10}$IU/mL at week 1 ($P = 0.0001$). In patients with the *IL28B* non-TT genotype and a reduction

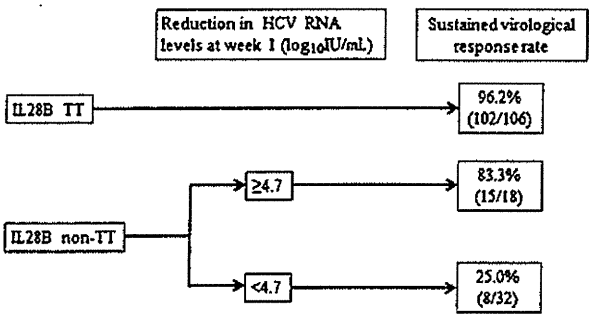

Fig. 4 Prediction of a sustained virological response (SVR) by the *IL28B* (rs8099917) genotype and reduction in HCV RNA level at week 1 after the start of therapy. In patients with the TT genotype, the SVR rate was high (96.2 %), regardless of the reduction in HCV RNA at week 1. In contrast, in patients with the non-TT genotype, the SVR rate was significantly higher in patients with a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA level at week 1 after the start of therapy than in those with a reduction of <4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 [15 of 18 patients (83.3 %) vs. 8 of 32 patients (25.0 %), $P = 0.0001$]

of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 62.5, 88.9, 83.3, 75.0, and 78.0 %, respectively. Furthermore, in patients with the non-TT genotype, when both a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 and RVR were used, the sensitivity, specificity, PPV, NPV, and accuracy for SVR were 60.9, 100, 100, 75.0, and 82.0 %, respectively.

Discussion

Multiple logistic regression analysis revealed that the *IL28B* genotype was the most significant factor predicting SVR to a 24-week regimen of TVR-based triple combination therapy. The impact of the *IL28B* genotype on SVR found for this treatment regimen was in agreement with the findings of previous studies in Japan [7, 11, 14–16, 24]. In addition, a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 after the start of therapy was identified as a strong independent on-treatment predictor for SVR in a multiple logistic regression analysis.

The reduction in HCV RNA levels at week 1 was particularly relevant in patients with the *IL28B* non-TT genotype. Whereas patients with the *IL28B* TT genotype showed a high SVR rate regardless of the on-treatment response of HCV RNA, a significant difference in SVR rate was observed based on the reduction in HCV RNA levels at week 1 in patients with the unfavorable non-TT genotype. In this patient subpopulation, the reduction in HCV RNA level at week 1 was the factor most strongly associated with SVR, and this finding is of clinical value to identify patients with a low likelihood of achieving SVR as early as possible. Furusyo et al. [11] previously reported that the serum HCV RNA levels at day 3 presented a significant difference between SVR and non-SVR patients. The ability of the very early viral response to predict SVR shown by both Furusyo et al. and our study may be explained by the strong antiviral effect of TVR. However, Furusyo et al. did not enter serum HCV RNA levels at day 3 into a multiple logistic regression analysis to identify significant independent predictors of SVR. Therefore, in that study, it was not clear whether the serum HCV RNA level at day 3 was an independent factor of SVR when including host-related, virus-related, and on-treatment factors. In the present study, the median serum HCV levels at week 1 was significantly lower for SVR patients (1.9 $\log_{10}$IU/mL) than for non-SVR patients (2.2 $\log_{10}$IU/mL) ($P = 0.0136$, data not shown). In the present study, the reduction in HCV RNA levels at week 1 after the start of therapy was an independent predictive factor for SVR. This reduction in HCV RNA level at week 1 may represent early viral kinetics closely correlated with the antiviral effect. The predictive ability of the reduction in HCV RNA level at day 3 and week 1 after the start of therapy should therefore be compared based on the *IL28B* genotype.

This study is the first report to demonstrate that a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA level at week 1 is a useful on-treatment predictive factor associated with SVR to a 24-week TVR-based triple combination therapy in clinical practice, especially in patients with the *IL28B* non-TT genotype. In 'real-world' clinical practice in some cases, it may be impossible to differentiate between previous null and partial responders because of the absence of relevant historical data from medical records. Therefore, for these treatment-experienced patients, *IL28B* genotyping may have clinical utility, as it may serve as a pretreatment marker for interferon responsiveness to guide patients and physicians. In patients with the *IL28B* non-TT genotype, both a reduction of <4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1 and positivity for HCV RNA at week 4 (non-RVR) indicated a high likelihood of treatment failure. Hence, these patients should not undergo TVR-based triple combination therapy to avoid unnecessary treatment. This study identified that measurement of the HCV RNA level not only at week 4, but also at week 1, provides important information for predicting SVR, particularly in patients with the *IL28B* non-TT genotype.

There were some limitations to this study. First, the number of patients was too low to conclusively identify factors contributing to SVR. In particular, the number of non-responders was very small. Second, TVR-resistant variants were not analyzed. Resistant variants have been reported to occur in 56 % of HCV genotype 1b patients who did not achieve SVR [35]. Therefore, resistance variants should be identified in patients with treatment

failure. Third, this study regimen was limited to T12PR24. Only a 24-week TVR-based triple combination therapy (triple therapy for 12 weeks followed by an additional 12 weeks of PEG-IFN and RBV) is allowed by the Japanese National Insurance System. In the US, Canada, and EU, triple combination therapy is administered for either 12 or 36 additional weeks after PEG-IFN and RBV, according to the response-guided regimen based on the early viral response in each category, i.e., treatment-naïve patients and previous relapsers or partial responders and null responders.

Recently, the second-generation direct-acting antiviral agent simeprevir (SMV), which is once-daily oral NS3/4A protease inhibitor, was approved in September 2013 in Japan. Hayashi et al. [36] reported a Japanese phase II study. In treatment-naïve patients, the SVR rate was 77–92 % by triple combination therapy with SMV, PEG-IFN-α-2a and RBV. During the first 3–7 days of SMV-based therapy, an initial rapid reduction in HCV RNA was evident. Mean reduction in HCV RNA at week 1 in our study in TVR-based therapy was 4.5 $\log_{10}$IU/mL (data not shown). Mean reduction in HCV RNA at week 1 was not shown with the numerical value in this SMV-based therapy, but that seems to be similar to our TVR-based therapy. However, in this study, the *IL28B* genotypes were not investigated. Therefore, in clinical practice, from now on, prospective studies should be necessary to confirm whether the reduction in HCV RNA at week 1 is predictive for SVR in SMV-based therapy based on the *IL28B* genotype as well as in TVR-based therapy.

In conclusion, this prospective, multicenter study of a 24-week TVR-based triple combination therapy for Japanese genotype 1b CHC patients showed that the *IL28B* SNP genotype is the most important baseline factor for predicting SVR, and a reduction of $\geq$4.7 $\log_{10}$IU/mL in the HCV RNA levels at week 1, i.e., viral kinetics earlier than week 4, could be a useful on-treatment predictor of SVR, especially in patients with the *IL28B* non-TT genotype. Further large-scale prospective studies including SMV-based triple combination therapy are necessary to confirm these findings and develop the individual tailoring and optimization of therapeutics.

Conflict of interest The authors declare that they have no conflict of interest.

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Clinical effectiveness of bipolar radiofrequency ablation for small liver cancers

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Abstract
Background Radiofrequency ablation (RFA) is minimally invasive and can achieve a high rate of cure of liver cancer. This study was conducted to evaluate the efficacy and safety of a bipolar RFA device (CelonPOWER System) in the treatment of Japanese liver cancer patients.
Methods The study was a multicenter, single-group, open-label trial. The indications for RFA were based on the Japanese guidelines for the management of liver cancer. The subjects had a Child-Pugh classification of A or B, and the target tumors were defined as nodular, numbering up to 3 lesions, each of which was 3 cm or less in diameter, or solitary lesions up to 4 cm in diameter. To test for the non-inferiority of the CelonPOWER System, this system was compared with the Cool-tip RF System, which has already been approved in Japan, in terms of the complete necrosis rate (CNR).
Results The CNR obtained with the CelonPOWER System was 97.8 % (88/90 patients). The CNR obtained with the Cool-tip RF System was 86.2 % (50/58 patients), confirming the non-inferiority of the CelonPOWER System ($p < 0.001$, Fisher's exact test based on binomial distribution). Throughout the treatment and follow-up periods, there were no adverse events regarding safety that were uniquely related to the CelonPOWER System and there were no cases of device failure.
Conclusions The CelonPOWER System was confirmed to be an effective and safe RFA device. It could become extensively used as a safe next-generation RFA device, reducing the physical burden on patients.

Keywords Small hepatocellular carcinoma · Radiofrequency ablation (RFA) · Bipolar RFA · Conformite Europeenne (CE) mark · Non-inferiority to monopolar RFA

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Introduction

According to a report of the Japanese Ministry of Health, Labor and Welfare in 2010, the number of deaths due to malignancies, including hepatocellular carcinoma (HCC), which is the most common type of primary liver cancer, has tended to increase annually [1]. In the 2007 report of the Japanese Ministry of Health, Labor and Welfare, the mortality of liver cancer was the 3rd highest among malignant diseases, following gastric cancer and lung cancer [2]. HCC appears in cirrhotic liver, and cirrhotic liver often results from alcohol abuse or chronic hepatitis B virus (HBV) or HCV infection. The presence of liver cirrhosis limits HCC treatment options, because surgery and systemic chemotherapy impair residual liver function and can induce fatal liver failure. In addition,

even if the primary tumor is completely resected, there is a very high recurrence rate in the residual liver [3, 4].

Radiofrequency ablation (RFA) is a minimally invasive method that can yield radical localized therapeutic results, and it has become a standard treatment for small liver cancers 3 cm or less in diameter [5].

Three different RFA systems have been introduced in Japan, all consisting of monopolar devices. One of the main problems with monopolar RFA devices is that the electrical current flows between the electrodes and the grounding pad that is used in these devices. The current flows in a wide area of the body, which may cause systemic symptoms, such as heat retention and perspiration. In addition, because the applicator is distant from the grounding pad, its low energy efficiency requires a long ablation time. Moreover, energy concentration can occur owing to an unanticipated current pathway between the applicator and grounding pad, posing a risk of burns at the grounding pad patch site and at non-treatment sites [3, 6–9].

A bipolar system, in contrast to the monopolar systems, features as its principal characteristic an electrical current flowing between two electrodes on a single probe. With a bipolar system, the current pathway is limited to only within the treatment area, thus eliminating the need for a grounding pad. A bipolar RFA system also overcomes such disadvantages of a monopolar system as the occurrence of heat retention and other side effects, low energy efficacy, and thermal injuries at electrode pad sites caused by an electrical current flowing in the body. The simultaneous use of multiple applicators with a bipolar system makes it possible to achieve a sufficiently large thermocoagulation volume with a single ablation procedure. That is, one ablation is usually sufficient for a wide area and this enables a short ablation time. In addition, ablation can be achieved even if the electrodes are not inserted directly into the tumor. The use of the bipolar system with multiple applicators with a wide ablation area maximizes the effectiveness of the bipolar system.

The purpose of this study was to evaluate the safety and efficacy of a bipolar RFA device, the CelonPOWER System, in order to obtain the clinical data necessary for an application for its regulatory approval in Japan. The study and protocol were designed in compliance with Japanese good clinical practice (GCP) based on the advice from the Pharmaceuticals and Medical Devices Agency (PMDA) of the Japanese regulatory authority. In designing this study, we were requested by the PMDA to compare this device with an existing RFA device (that had been already approved in Japan) and we selected the data from the 2002 to 2003 clinical study of the Cool-tip RF System as valid control data. The study of the Cool-tip RF System was also conducted to obtain marketing approval in Japan [10]. This study was sponsored by Olympus Medical Systems Corp.

Patients, materials, and methods

Device

Celon AG Medical Instruments (Teltow, Germany) developed a bipolar RFA device (CelonPOWER System) in order to overcome the disadvantages of monopolar RFA devices. Unlike a monopolar RFA system, the prime characteristic of this new device is its bipolar feature, i.e., two electrodes are located on the same needle (Fig. 1a, b), allowing electricity flow only between the electrodes at the treatment target site, eliminating both the need for a grounding pad and the danger of burns (Fig. 2a, b).

The bipolar characteristics of the CelonPOWER system ensure the return of power to the device, and the simultaneous use of multiple applicators yields an extensive ablated area in a single treatment, which can reduce treatment time and the burden on the patient. This eliminates the need for repeated reinsertion of single monopolar needles to perform overlapping ablation. Another advantage of the bipolar device is that electric current is immediately retrieved, preventing it from flowing to unintended sites. The CelonPOWER System was awarded the Conformite Europeenne (CE) mark in 2003, and since then its use has spread mainly in Europe [11–17].

The CelonPOWER System consists of a high-frequency power generator, a water pump, and computerized applicators for regulation of the current frequency. The basic

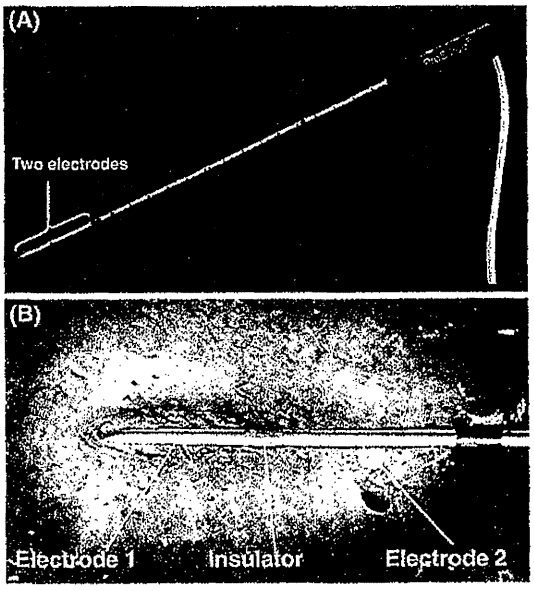


Fig. 1 In the CelonPOWER System, each applicator is needle-shaped and has two electrodes near its tip

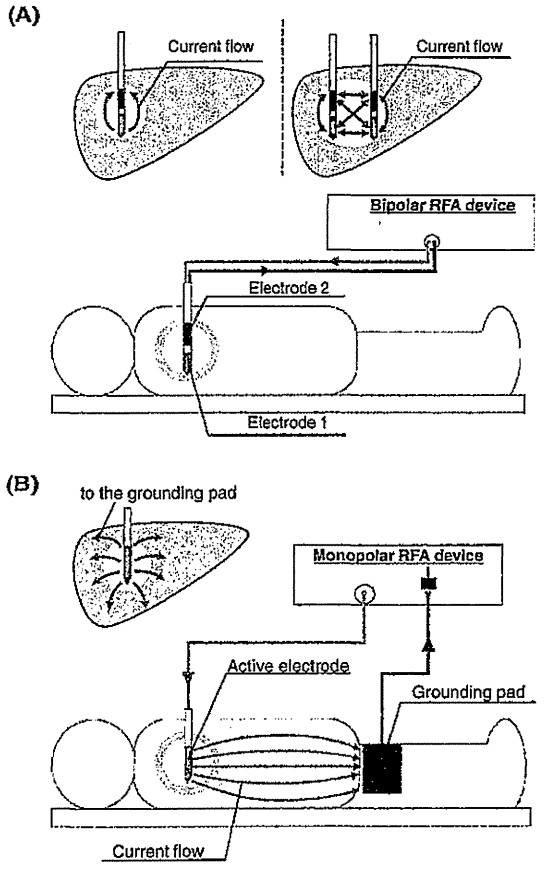


Fig. 2 Differences in the electrical flow routes of **a** the monopolar and **b** the bipolar (CelonPOWER System) radiofrequency ablation (*RFA*) systems. With the bipolar system (CelonPOWER System), the electrical current flows between the two electrodes, and for this reason the current pathway is limited to the treatment area, allowing lower power to be concentrated in a specific area and yet yielding effects equivalent to those obtained by higher energy monopolar devices, the power of which is dispersed throughout the body to the dispersion grounding pads placed under the patient

frequency of the power generator is 470 kHz, with a maximum output of 250 W. All the needles for RFA are 1.8 mm in width (15 G) but there are 3 different lengths: 20, 30, and 40 mm. The Cool-tip RF System needles are 1.5 mm in width (17 G).

Bipolar applicators

Each applicator is needle-shaped and has two electrodes near its tip. The electrical current flows between the two electrodes on the single probe, limiting the current pathway to within the treatment area. A grounding pad is unnecessary (Fig. 2a). The applicators are cooled by the internal circulation of chilled water.

Multipolar application

When simultaneously using multiple applicators (up to 3 can be employed simultaneously), it is possible to treat relatively large cancers that could not be sufficiently ablated by means of one insertion of a single applicator. The high-frequency electrical current flows sequentially between the electrodes of the applicators (6 electrode pair combinations when there are 2 applicators, 15 electrode combinations when there are 3 applicators) (Fig. 3a).

Resistance controlled automatic power (RCAP)

RCAP is a function that monitors the change of electric resistance between the electrodes, and automatically

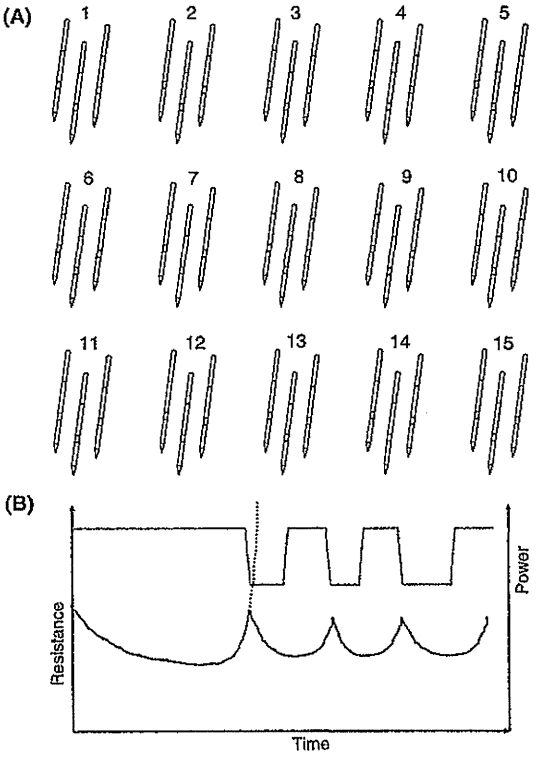


Fig. 3 When 3 applicators are employed, the high-frequency electrical current flows sequentially between 15 combinations of electrode pairs (a), and an image is generated of the automated control of the output by the resistance controlled automatic power (RCAP) function (b). RCAP is a function by which the degree of change in the electrical resistance among the electrodes (increase/decrease in slope) is monitored, and the high-frequency power output is automatically controlled

controls the high-frequency power (Fig. 3b). This function makes it possible to prevent unexpected rapid increases in electrical resistance resulting from tissue necrotization.

Patients

This clinical study was carried out based on the HCC treatment algorithm in the Scientific Data-based Clinical Practice Guidelines for Liver Cancer-2005 Version [18]. We enrolled adult male and female patients aged 20 years or older with primary or metastatic small liver cancers who had provided written informed consent. Target tumors were defined as nodular, numbering up to 3 lesions, each of which was 3 cm or less in diameter, or solitary lesions up to 4 cm in diameter. Exclusion criteria included a Child-Pugh grade of C, or platelet count below 50000/μl. Informed consent was obtained from 104 patients, of whom 96 were initially enrolled, but 5 withdrew consent before the trial started. The trial was therefore carried out in a total of 91 patients (112 treated lesions) with intention-to-treat (ITT) analysis, and 90 patients were eligible for the analysis of efficacy.

Patient details

Table 1 summarizes the data on the background characteristics of the 91 patients and 112 treated lesions treated in the study (73 patients had 1 lesion, 15 had 2, and 3 patients had 3 lesions; Table 1). The cohort consisted of 61 men and 30 women, and the mean age (±SD) was 69 ± 10 years; 84 patients had primary liver cancer, while 7 had metastatic liver cancer.

Study design

This prospective multicenter, collaborative, single-group, open-label study was conducted at 5 institutions between December 2008 and December 2009. The study protocol was approved by each center's institutional review board. The trial treatment period lasted from the acquisition of written informed consent through completion of the final treatment (maximum 3 treatments), in addition to a follow-up period from the day after the final examinations of the treatment period until the completion of examinations performed 24 weeks later. The non-inferiority of the CelonPOWER System was evaluated relative to the results obtained with a Cool-tip RF System in 2002–2003 [10].

Study methodology

Figure 4 shows the study procedures. During the treatment period, the following procedures were performed, in the order listed: registration of eligible patients, RFA treatment and examinations including computed tomography (CT) imaging, laboratory tests, and blood pressure measurement. The efficacy was evaluated from the extent of the necrotic area (tumor necrosis; TN) induced by ablation as measured on conventional and dynamic CT imaging. Additional ablation, up to a maximum of 3 sessions, was performed as necessary. The laboratory tests consisted of RBC count, WBC count, hemoglobin level, hematocrit, platelet count, prothrombin time (PT) activity, total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), and creatinine.

In the follow-up phase, at 10 ± 2 weeks (70 ± 14 days) and 24 ± 2 weeks (168 ± 14 days) following the day of the final RFA session, we performed CT imaging, laboratory tests, blood pressure measurement, measurement of alpha-fetoprotein (AFP), and measurement of protein induced by vitamin K absence or antagonist II (PIVKA-II). The CT images and tumor marker data were employed to assess the continuity of the therapeutic effect (TE) of the RFA treatment.

RFA procedure

The procedure with the CelonPOWER System device was similar to the procedure with the existing monopolar RFA devices. In all cases, the procedure was performed percutaneously under ultrasound guidance and local anesthesia.

Assessment of efficacy

TN was assessed using 5 grades, in accordance with the Criteria for Direct Effects of Liver Cancer Treatment (1994) [19]. Class V tumor necrosis (100 % TN) of liver cancer following the final RFA session was defined as "complete necrosis," and the percentage of patients achieving Class V TN was defined as the "complete necrosis rate" (CNR), the primary endpoint. The TN classification was used for short-term (during treatment) evaluation, and this was the only evaluation reported for the Cool-tip RF System in the marketing authorization holder's application for Japanese government approval. However, now the government demands not only short-term evaluation, but also long-term evaluation, for which such parameters as TE, overall response, and complete response (CR) are used.

The secondary endpoints of our study were the number of RFA sessions, the TE, and the overall assessment of the TE. The assessment of the immediate TE and the overall assessment of TE were performed in accordance with the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (2008) [20]. The TE was classified as either CR (total necrosis and normalization of all tumor

Table 1 Patient background factors and lesion characteristics

Patients ($n = 91$)		Lesions ($n = 112$)	
Background factors	*N* (%)	Characteristics	*N*
Sex		Maximum dimension (cm)	
M	61 (67.0)	<1.0	22
F	30 (33.0)	1.1–2.0	69
Age (years)		2.1–3.0	17
31–40	1 (1.1)	3.1–4.0	4
41–50	4 (4.4)	Mean ± SD	
51–60	9 (9.9)	1.6 ± 0.7	
61–70	32 (35.2)	Subsegment	
71–80	34 (37.4)	S1	0
81–90	11 (12.1)	S2	6
Cancer		S3	9
Primary	84 (92.3)	S4	8
Metastatic	7 (7.7)	S5	18
Underlying disease		S6	20
Cirrhosis	63 (69.2)	S7	18
Chronic hepatitis	22 (24.2)	S8	33
None	6 (6.6)		
Child-Pugh classification			
Grade A	83 (91.2)		
Grade B	8 (8.8)		
Number of treated lesions			
1	73 (80.2)		
2	15 (16.5)		
3	3 (3.3)		
Previous treatment of primary disease			
Yes	40 (44.0)		
No	51 (56.0)		

markers), or others. In addition, ITT analysis was performed in regard to the cumulative local recurrence rate and the overall assessment of the TE.

Assessment of safety

The following safety endpoints were assessed in all 91 patients in whom the study was conducted: overall safety assessment, adverse events, device-related adverse events, device failure, laboratory test values, and blood pressure.

Statistical analysis

Statistical analysis was performed using a one-sided significance level of 2.5 % for the primary endpoint. In principle, a two-sided significance level of 5 % was used for the other endpoints to avoid data dispersion. The CNR (the primary endpoint) was calculated as the percentage of the total number of patients who achieved Class V TN, and its exact one-sided 97.5 % confidence interval was calculated. For the secondary endpoints, the variables and their ratios were compiled, and the basic statistics for the mean and standard deviation were calculated.

Results

Patients

Written informed consent was obtained from 104 patients, including the 96 patients in the study. The study was conducted in 91 of these patients, and treatment was completed in 90 patients. Eighty-eight of the 90 patients (excluding 2 TN4 patients) were followed up. Five patients discontinued the study during the follow-up period, leaving 83 patients who completed the follow-up period. Three patients were excluded because of unacceptable enrollment dates, so the final number of patients eligible for the efficacy analysis was 80.

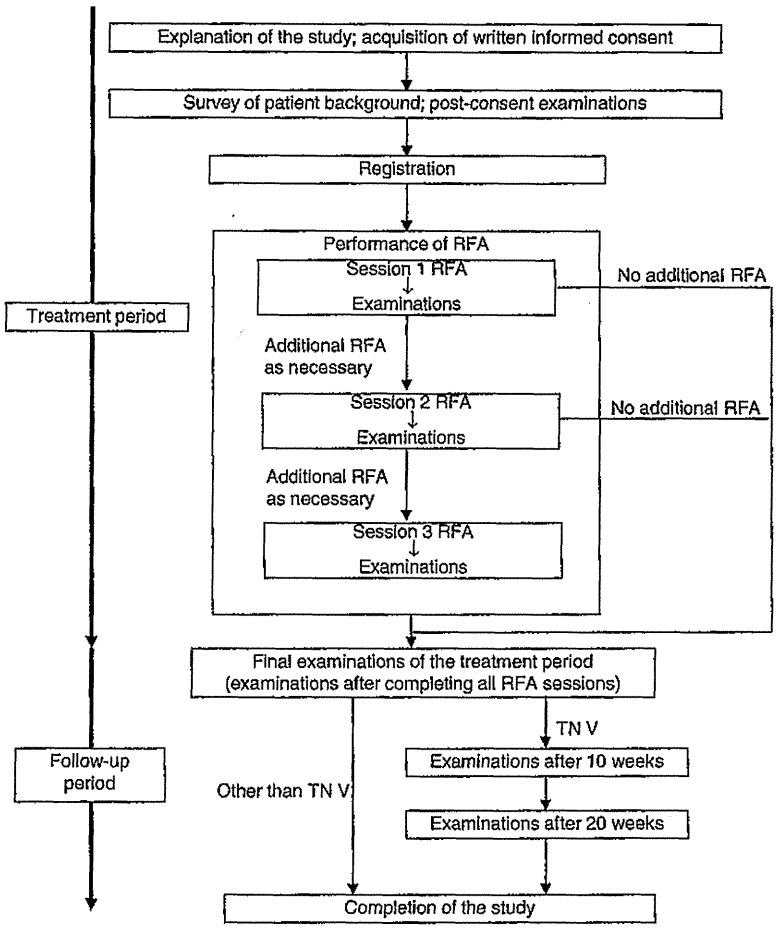


Fig. 4 Clinical study procedure. *TN* Tumor necrosis

Efficacy

Of the 90 patients who completed this clinical treatment study, 88 showed Class V TN (97.8 %). The 2 patients (2.2 %) who did not show 100 % TN both had primary liver cancers and were categorized as Class IV TN. The CNR was 100 % in patients with metastatic liver cancer (7/7 patients) and 97.6 % in patients with primary liver cancer (81/83 patients). The Japanese package insert for the Cool-tip RF System [21] states that the CNR obtained by that system was 86.2 % (50/58 patients). Assuming a 5 % non-inferiority margin, the lower limit of the confidence interval (one-sided 97.5 %) was 92.2 %, and the p value was <0.001 for the exact test based on binomial distribution.

The initial success rate (Class V TN after 1 session) was 77.8 % (70 of 90 patients), while Class V TN was seen in 16 (17.8 %) patients following a second session. The remaining 4 (4.4 %) patients underwent a third RFA session, and 2 were rated as Class V TN following that session.

We used 1 applicator in 20 patients, 2 simultaneously in 54 patients, and 3 simultaneously in 16 patients. We used 30-mm electrodes in all the patients, except in 3 of the 16 patients in whom 3 electrodes were used simultaneously; in these 3 patients we used 3 40-mm electrodes. A representative case in which 3 applicators were used is shown in Fig. 5.

Of the 88 patients who proceeded to the follow-up phase, excluding the single out-of-hospital fatality, examination at 24 weeks showed that CR was obtained in 94.3 % (82/87). The cumulative local recurrence rate at the end of 24 weeks in the follow-up period was 5.7 % (5/87 patients; ITT analysis) (Table 2).

Figure 6a, b shows a comparison of the treatment results of the Cool-tip RF System clinical trial [21] and the number of patients analyzed for the CNR and the efficacy

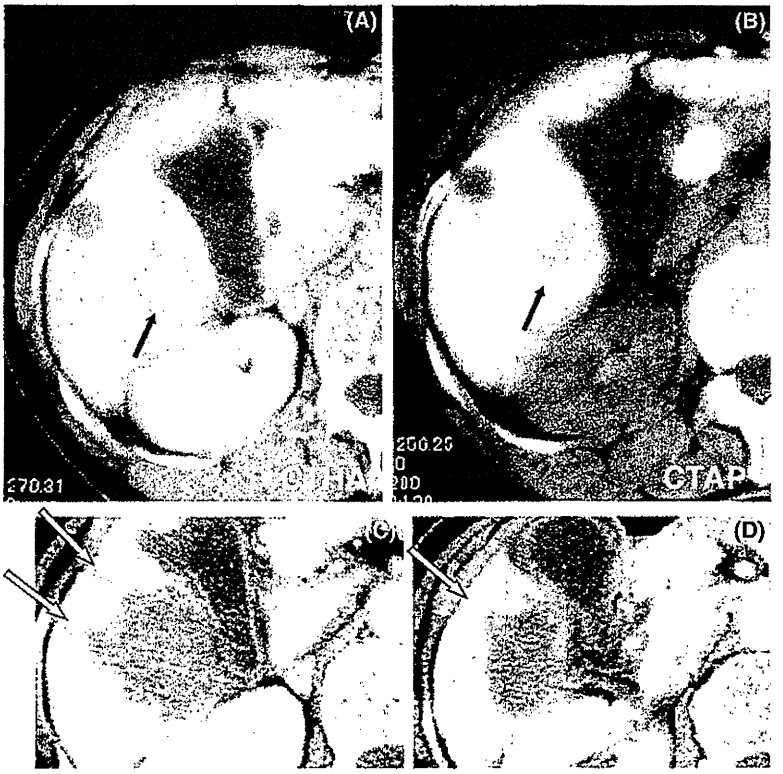


Fig. 5 Images in a female patient who had hepatocellular carcinoma (HCC) in segment VI. Before treatment, scans obtained on computed tomography during hepatic arteriography (CTHA) (a) and computed tomography during arterial portography (CTAP) (b) showed a nodular HCC (*arrow*) measuring 2.5 cm. Three applicators were placed in parallel in the HCC in liver segment VI, and then the tumor was ablated in one procedure (total ablation time 13 min 42 s, total applied energy 35.3 kJ). After the procedure, computed tomography (CT) images showed a necrotic area of 46 mm in diameter including the nodular HCC (c [*arrows* show applicator for insertion paths], d [*arrow* shows applicator for insertion paths])

Table 2 Maintenance of the therapeutic effect (TE) (overall assessment of the TE; intention-to-treat (ITT) analysis)

	This clinical study		Patients who underwent local therapy [24]	
	10 weeks	24 weeks[a]	3 months	6 months
Complete response (CR) (no. of patients)	85/88 (96.6 %)	82/87 (94.3 %)	4468/5394 (82.8 %)	4318/5378 (80.3 %)
Other (no. of patients)	3/88 (3.4 %)[b]	5/87 (5.7 %)[c]	926/5394 (17.2 %)	1060/5378 (19.7 %)

[a] One patient who died was omitted from the 24-week assessment

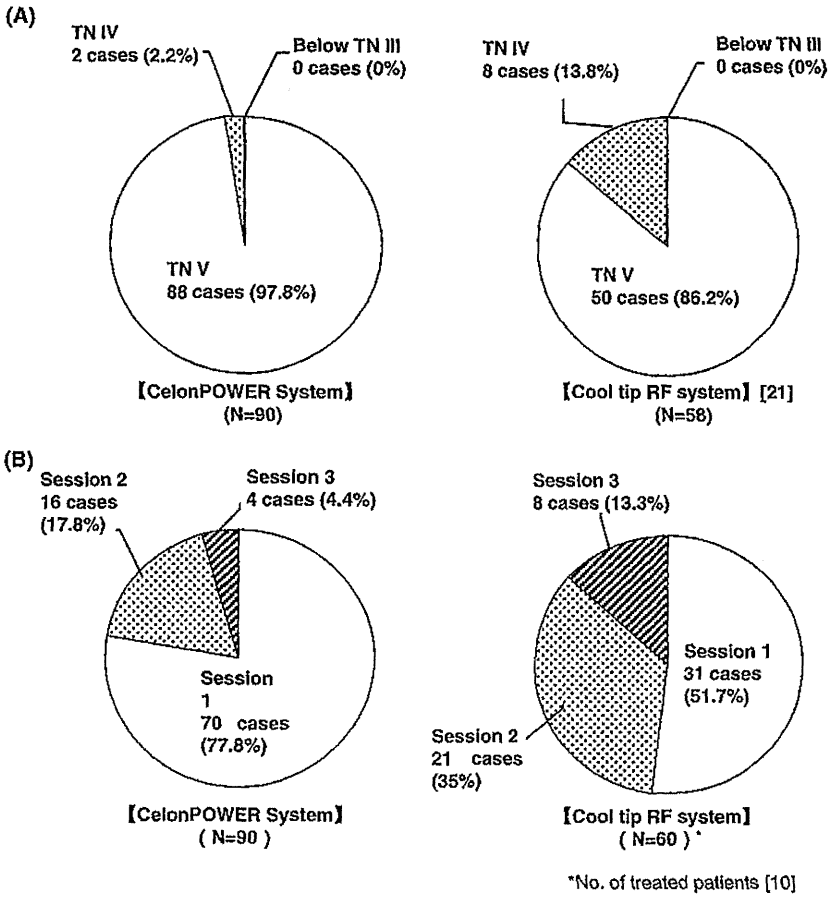
[b] Includes 3 patients who developed local recurrence within 10 weeks

[c] Includes 5 patients who developed local recurrence within 24 weeks

of each RFA session in the present clinical study. As shown in Fig. 6a, the complete necrosis (Class V TN) rate with the CelonPOWER System was 97.8 % (88/90 patients), which was higher than the rate of 86.2 % (50/58 patients) with the Cool-tip RF System. These results thus confirm the non-inferiority of the CelonPOWER System ($p < 0.001$; Fisher's exact test based on binomial distribution). As shown in Fig. 6b, the percentage of patients in whom treatment was completed in a single session was 77.8 % (70/90 patients) in the present study with the CelonPOWER System, compared with 51.7 % (31/60 patients) in the Cool-tip RF System study [10].

Safety

The overall safety assessment was performed for the entire clinical study period, i.e., inclusive of the treatment period and the follow-up period. Of the 91 patients included in the safety analysis, no procedure was rated as unsafe, although 2 procedures (2.2 %) were rated as somewhat unsafe, one with an abdominal wall burn and one with biliary peritonitis owing to bile leakage; 78 procedures (85.7 %) were rated as safe overall and 11 procedures (12.1 %) were rated as safe. There was no device failure. In the patient with biliary peritonitis, three 30-mm electrodes had been



*No. of treated patients [10]

Fig. 6 Comparison of the present results obtained with the CelonPOWER System and the clinical study results reported for the Cool-tip RF System. The percentage of Class V tumor necrosis (*TN*) (TN 100 %) cases (a) and the number of patients in whom each RFA session was completed (b)

simultaneously inserted into an S8 tumor, and treatment was finished in a single ablation.

During the course of the entire clinical study period, serious adverse events (i.e., events for which a causal relationship with the CelonPOWER System could not be ruled out) were seen in 3 patients, consisting of abdominal wall burn, pleural effusion, and biliary peritonitis. Each of those events was judged to be serious because they required prolongation of hospitalization, and each required treatment. In addition, it was judged that each of these serious adverse events was a known adverse event that had been observed with similar, already-approved RFA devices [21–23]. Also, the single fatality, which occurred at home, had occurred in a patient who had been hospitalized for treatment on the suspicion of peritonitis based on the examinations performed after 10 weeks in the follow-up period. The patient's condition had improved and the patient had been discharged, and it was later confirmed that death had occurred at home. Autopsy revealed the cause of death to have been due to the progression of cirrhosis, and it was thus thought that the death was not related to the treatment with the CelonPOWER System. Table 3 shows the most common adverse effects (those observed in 5 % of patients or more) and all of these (pleural effusion, nausea, vomiting, postprocedural pain, and fever) have been known to occur with previously approved local therapeutic devices. Moreover, all the adverse events were easily controllable.

Discussion

We set out to prospectively determine whether a bipolar RFA device (CelonPOWER System) was safe and effective in the treatment of liver cancer and whether it could be demonstrated to be non-inferior to a monopolar RFA system currently approved and employed clinically in Japan (Cool-tip RF System).

Treatment was completed in a fewer number of sessions when using the CelonPOWER System than with the Cool-

Table 3 Frequently observed adverse effects (5 % or more) (adverse reactions at an incidence of >5 % in the overall study period)

Adverse event	No. of patients	%	No. of patients treated (%)	Treatments
Aspartate aminotransferase (AST) increase	72	79.1	0 (0)	–
Alanine aminotransferase (ALT) increase	69	75.8	0 (0)	–
Lactate dehydrogenase (LDH) increase	22	24.2	0 (0)	–
Total bilirubin increase	20	22.0	0 (0)	–
Pleural effusion	12	13.2	2 (2.20)	Human serum albumin, cefmetazole sodium, tazobactam piperacillin hydrate
Vomiting	12	13.2	7 (7.69)	Metoclopramide
Nausea	10	11.0	9 (9.89)	Metoclopramide, domperidone, diazepam
Postoperative pain	9	9.9	3 (3.30)	Pentazocine, loxoprofen sodium hydrate, acetaminophen, diclofenac sodium
White blood cell count increase	8	8.8	1 (1.10)	Sulbactam sodium–cefoperazone sodium
Platelet count decrease	6	6.6	0 (0)	–
Alkaline phosphatase (ALP) increase	5	5.5	0 (0)	–
Fever	5	5.5	5 (5.49)	Loxoprofen sodium hydrate, acetaminophen, cefmetazole sodium

tip RF System, suggesting that this new system yields efficacy that is at least equivalent to that achieved with the Cool-tip RF System, while causing less of a treatment burden on the patient.

We assessed the TE level, and its maintenance in ITT cases after 10 weeks and 24 weeks (6 months) in the follow-up period of this clinical study and found that the overall TE assessment was not inferior to that of the National Follow-up Survey Report on Primary Hepatic Carcinoma (2004–2005) [24] issued by the Liver Cancer Study Group of Japan (Table 2). Considering that the method for overall TE assessment in that report was the same as that employed in the present study, it is reasonable to conclude that the TE maintenance with the CelonPOWER System is not inferior to that of other local therapy.

Nishikawa et al. reported on local recurrence when using monopolar systems clinically. They found that, in 269 patients with solitary hypervascular HCCs who had undergone RFA, the 1- and 2-year cumulative local recurrence rates were 12.8 and 23.6 %, respectively [25]. We believe that our present results for the cumulative local recurrence rate (5.7 % for 6 months) with the CelonPOWER System are comparable to those reported results.

The introduction of a new device inevitably raises the question of its safety. In our series, there were 3 adverse events–one event of abdominal wall burn and one of pleural effusion during the treatment period, and one event of biliary peritonitis during the follow-up period. These adverse events were previously known to be possible adverse events that had been observed with the Cool-tip, RITA, and Boston monopolar RFA systems that have already been approved for clinical use in Japan [21–23]. Therefore, similar caution concerning internal adverse events is necessary when using the CelonPOWER System, although the problem of external burns does not exist with this system.

The high-incidence (≥5 %) device-related adverse event rate during the course of our clinical study was similar to the rates with the Cool-tip, RITA, and Boston monopolar RFA systems [21–23].

Therefore, these events are not unique to the CelonPOWER System, and the safety of the CelonPOWER System is not inferior to that of the existing approved RFA devices.

This study has several limitations. First of all, although it was a prospective study, it was not a randomized controlled clinical study. However, all consecutive patients who satisfied the enrollment criteria were offered the opportunity to participate and the study was performed in all those who provided informed consent and decided to receive the treatment. After providing informed consent, 5 patients decided not to participate and 1 ceased treatment after 1 session, due to the difficulty posed by the proximity of the lesion to the heart and lungs.

Although we were able to compare our own results immediately after treatment with those of the Cool-tip RF

System and other systems, we were not able to compare the results 6 months after treatment because of the lack of such data for the Cool-tip RF System, because of the different GCP guidelines in force at the time of the Cool-tip RF System study. However, the 6-month follow-up data of our study were very satisfactory. Furthermore, because there were no such data available in the reports on the Cool-tip RF System, we could not compare the levels of experience of the operators in the two studies.

In conclusion, the present clinical study confirmed that the CelonPOWER System is a very safe and highly effective RFA system for liver cancer in Japanese patients. In addition, because this system is a bipolar device, it operates with high energy efficiency, and because multiple multipolar applicators can be employed simultaneously, coagulation necrosis of an extensive tumor tissue volume can be achieved in a short treatment time. Moreover, throughout the course of this clinical study, most of the patients did not experience hot flushes or perspiration. It is therefore anticipated that the CelonPOWER System will become used as a next-generation RFA system that is not only safer than existing systems, but is highly effective and places less physical burden on the patient.

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Original Article

Pegylated interferon monotherapy in patients with chronic hepatitis C with low viremia and its relationship to mutations in the NS5A region and the single nucleotide polymorphism of interleukin-28B

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Aim: Previous studies have suggested that patients with chronic hepatitis C with a low pretreatment hepatitis C virus (HCV) level have a high sustained virological response (SVR) rate, and that there would be a subpopulation of patients in which HCV can be eradicated with pegylated interferon (PEG IFN) alone without a decrease in SVR. However, the efficacy of PEG IFN monotherapy in patients with low HCV RNA levels is unclear. Several studies have reported that interferon sensitivity-determining region (ISDR) and the single-nucleotide polymorphism (SNP) of interleukin-28B (IL-28B) contribute to IFN response, but these relationships are controversial. The aim of this study was to determine whether the SNP of IL-28B (rs8099917) and amino acid substitutions in the ISDR among patients with low HCV levels affect the response to PEG IFN monotherapy.

Methods: One hundred and four patients with low-level HCV infection were studied. Low HCV level was defined as 100 KIU/mL or less.

Results: SVR was achieved in 94 patients (92.2%). HCV levels (≤50 KIU/mL) and ISDR (≥2 mutations) were associated with SVR on univariate analysis. The rates of SVR in the patients with IL-28B genotypes TT, TG and GG were 94.5%, 77.8% and 100%, respectively. The G allele tended to be associated with poor response to IFN therapy ($P = 0.0623$). On multivariate analysis, the ISDR was the factor predictive of SVR ($P = 0.004$).

Conclusion: The ISDR is significantly associated with a good response to PEG IFN monotherapy in patients with low HCV levels.

Key words: hepatitis C virus, interferon sensitivity-determining region, interferon, interleukin-28B, rapid virological response

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) that easily progresses to end-stage liver disease.[1] Because 170 000 000 persons are infected with HCV worldwide, HCV infection is a significant global health problem.

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The current recommended therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PEG IFN) and ribavirin and/or telaprevir or boceprevir.[2–6] HCV RNA levels, as well as genotypes, are an important factor associated with sustained virological response (SVR) to IFN therapy.[3,4] Patients with low HCV RNA levels have a high SVR rate, and even standard IFN monotherapy is useful for eradication of HCV in patients with low viral loads.[7–9] Several studies have succeeded in reducing the duration of treatment without risk of relapse.[10,11] Although patients with low HCV RNA have higher response rates to IFN treatment, not all patients achieve SVR. Other factors for improving the prediction of SVR in patients with low HCV RNA levels are needed. The predictive factors for SVR in patients with genotype 1b and high HCV RNA levels have been investigated, and several studies have shown that the single nucleotide polymorphism of interleukin-28B (IL-28B) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy.[12–16] However, the predictive factors for SVR among patients with low HCV RNA levels treated with PEG IFN monotherapy have been unclear.

Hepatitis C virus consists of three structural proteins (core, envelope 1 and envelope 2) and six non-structural proteins (NS2 to NS5). HCV NS5A protein was reported to have a domain associated with IFN response. This domain in the region of HCV genotype 1b is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR).[12,15–21] IFN acts to control replication of the virus by inducing the dsRNA-dependent protein kinase (PKR). The ISDR is located in the PKR-binding domain, is inhibited by PKR *in vitro*,[22] and is useful for prediction in patients with genotypes 2a, 2b and 3a.[23–28] Therefore, ISDR heterogeneity is an important factor that may affect response to IFN in patients with low HCV RNA levels. We hypothesized that ISDR heterogeneity could be predicted in patients with low HCV RNA levels in which HCV can be eradicated with PEG IFN-α alone without a decrease in SVR.

Not only genetic heterogeneity in the HCV genome but also host genetics contribute to IFN treatment outcomes. Therefore, several studies were performed to understand the host factors associated with IFN responsiveness; these showed that IL-28B polymorphisms are strongly associated with response to PEG IFN and ribavirin combination therapy in patients with genotype 1b and high viral load.[13,14,16,29] However, the associations between ISDR and IL-28B and the effects of PEG IFN-α monotherapy in patients with low HCV RNA levels are not well known.

The aim of the present study was to determine whether genomic heterogeneity of the ISDR and the SNP of IL-28B among patients with low HCV RNA levels affects the response to PEG IFN-α-2a monotherapy.

METHODS

A TOTAL OF 295 patients with chronic hepatitis C were treated by PEG IFN-α-2a monotherapy at Nagoya University Hospital and Affiliated Hospitals; 104 patients with low HCV RNA levels were selected for this study. The patients consisted of 62 men and 42 women with a mean age of 55.1 years (range, 19–78). All patients were positive for serum anti-HCV antibody by a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV RNA by a commercial polymerase chain reaction (PCR) (Roche Diagnostic Systems, Tokyo, Japan).

A low HCV level was defined as 100 KIU/mL or less, as previously reported.[4,7,9,11] No patient had hepatitis B surface antigen, co-infection with HIV, autoimmune disease or chronic alcohol abuse.

Schedule of IFN therapy

Patients received PEG IFN-α-2a (Pegasys Chugai-Roche, Tokyo, Japan) at a dose of 180 μg injected s.c. once per week for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluations of adverse events were performed once per week during treatment.

The dose of PEG IFN-α-2a was reduced to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50 000 cells/mm^3) occurred. PEG IFN-α-2a was discontinued when neutropenia of less than 250 cells/mm^3 or a platelet count of less than 25 000 cells/mm^3 was seen.

Hepatitis C virus RNA in serum samples was examined at 4 weeks, at the end of IFN therapy, and at 6 months after the end of treatment (ETR). Serum was stored at −80°C for virological examination at pretreatment.

Patients who were persistently negative for serum HCV RNA and who had a normal serum alanine aminotransferase (ALT) level at 24 weeks after withdrawal of IFN treatment were considered to have SVR. Patients who were HCV negative at the ETR but returned to HCV

positive status after withdrawal of IFN were defined as virological relapsers. Patients who did not become HCV negative with IFN therapy were defined as non-virological responders.

This study was approved by the ethics committee of each institution involved. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological tests

Hepatitis C virus was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously.[30,31] Genotypes were classified according to the nomenclature proposed by Simmonds *et al.*[32]

Nested PCR analysis and direct sequencing of the NS5A-ISDR were performed as previously reported for each genotype.[15,16,27,28] In brief, RNA was extracted from 140 μL serum using a QIAamp Viral RNA Kit (Qiagen, Valencia, CA, USA) and dissolved in 50 μL diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers with an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). NS5A-ISDR was sequenced after amplification by nested PCR as previously described.[15,16,27,28]

The primers used were as follows: NS5A-ISDR of genotype 1b, sense 5′-TGGATGGAGTGCGGTTGCACA GGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGGTGGT ATTG-3′; NS5A-ISDR of genotype 2a, sense 5′-ACGTCC ATGCTAACAGACCC-3′ and antisense 5′-GGGAATCT CTTCTTGGGGAG-3′; and NS5A-ISDR of genotype 2b, sense 5′-TCTCAGCTCCCTTGCGATCCTGA-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 30 s in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA, USA). The second PCR was done using the following sets of primers: NS5A-ISDR of genotype 1b, sense 5′-CAGGTACGC TCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGT AGGTGGCAA-3′; NS5A-ISDR of genotype 2a, sense from the first-round PCR and a new antisense primer 5′-CGAGAGAGTCCAGAACGACC-3′; and NS5A-ISDR of genotype 2b, sense 5′-AGCTCCTCAGCGAGCCA GCT-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genomic analysis

Detection of the SNP of IL-28B (rs8099917) was done by a real-time PCR system, as previously reported.[16] In brief, genomic DNA was extracted from 15 μL of whole blood using a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μL diethylpyrocarbonate-treated water. DNA (1 ng) was used for PCR with primers and probes of commercial kit (Taqman SNP Genotyping Assays; Applied Biosystems). The SNP of IL-28B (rs8099917) was amplified, and the results were analyzed by real-time PCR in a thermal cycler (7300 Real time PCR System; Applied Biosystems).

Statistical analysis

Data are expressed as mean ± standard deviation. A paired Student's *t*-test or Fisher's exact test were used to analyze differences in variables. $P < 0.05$ was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. Statview ver. 5.0 software (SAS Institute, Cary, NC, USA) was used for all analyses.

RESULTS

Background

PATIENTS' CLINICAL CHARACTERISTICS are summarized in Table 1. HCV genotypes 1b ($n = 34$), 2a ($n = 58$), 2b ($n = 9$) and unknown ($n = 3$) were detected.

Table 1 Clinical characteristics at pretreatment

Clinical characteristics	$n = 104$
Age (years)	55.1 ± 12.5
Sex: male/female	62/42
AST (IU/L)	50.0 ± 28.2
ALT (IU/L)	62.7 ± 47.3
Platelet count (10^4/uL)	18.4 ± 5.7
HCV RNA level (KIU/mL)	36 (1.6–100)
HCV genotype (1b/2a/2b/unknown)	34/58/9/3
IFN length (weeks) (24/48/<17)	49/45/10
Body mass index	22.7 ± 3.2

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon.

Table 2 Virological response in each group

(a) Virological response according to durations of IFN therapy				
	Overall ($n = 102$)	24W ($n = 48$)	48W ($n = 45$)	<17W ($n = 9$)
RVR	81.4% ($n = 83$)	87.5% ($n = 42$)	73.3% ($n = 33$)	88.9% ($n = 8$)
ETR	100% ($n = 102$)	100% ($n = 48$)	100% ($n = 45$)	100% ($n = 9$)
SVR	92.2% ($n = 94$)	93.8% ($n = 45$)	91.1% ($n = 41$)	88.9% ($n = 8$)
(b) Virological response according to HCV genotypes				
	Overall ($n = 102$)	1b ($n = 32$)	2a ($n = 58$)	2b ($n = 9$)
RVR	81.4% ($n = 83$)	81.3% ($n = 26$)	81.0% ($n = 47$)	88.9% ($n = 8$)
SVR	92.2% ($n = 94$)	87.5% ($n = 28$)	93.1% ($n = 54$)	100% ($n = 9$)

ETR, end of treatment response; HCV, hepatitis C virus; IFN, interferon; RVR, rapid virological response; SVR, sustained virological response; W, weeks.

All patients had serum HCV RNA levels of 100 KIU/mL or less, and the median HCV RNA level was 36 KIU/mL.

One hundred and four patients were initially included in this study; 49 patients were treated with PEG IFN-α-2a for 24 weeks, and 45 patients were treated for 48 weeks. Ten patients withdrew from IFN therapy within 17 weeks, and two of these 10 patients could not be followed. The reasons for discontinuing therapy were fatigue ($n = 3$), depression ($n = 1$), rash ($n = 1$), appetite loss ($n = 1$), liver failure ($n = 1$) and unknown ($n = 3$). The two patients who withdrew from follow up were excluded from the analysis, and the remaining 102 patients were followed for 6 months after the ETR.

Virological response

Virological response is shown in Table 2. Rapid virological response (RVR), which was defined as negativity for HCV after 4 weeks of treatment, for the overall group, the 48 weeks' group, the 24 weeks' group and the under 17 weeks' group was 81.4% (83/102), 73.3% (33/45), 87.5% (42/48) and 88.9% (8/9), respectively. Virological response at the ETR was 100% among all patients. Finally, 94 (92.2%) of 102 patients achieved SVR.

There was no significant difference in virological response between patients treated for 24 weeks and those treated for 48 weeks. The virological response according to HCV genotype is shown in Table 2(b). Patients with genotype 1b had a lower SVR rate than genotypes 2a and 2b, but no significant differences in genotype were noted.

Genetic heterogeneity in NS5A-ISDR and response to IFN therapy

The prevalences of the number of amino acid substitutions in ISDR according to HCV genotypes are summarized in Figure 1. The ISDR were examined by direct sequencing, and classification involved counting the number of amino acid substitutions compared to consensus strains of each genotype, as previously reported.[15,24,27,28]

Interferon sensitivity-determining region sequences were obtained in 81 patients. Five patients did not have serum at pretreatment, and 16 patients could not be amplified by PCR. Sixty-one patients (84.7%) had one mutation or more. SVR according to the ISDR is shown in Figure 2. All patients with three or more mutations in the ISDR achieved SVR, but 18 (69.2%) of 26 patients with two or less mutations in the ISDR achieved SVR. Patients with two or less mutations in the ISDR were poor responders to IFN therapy.

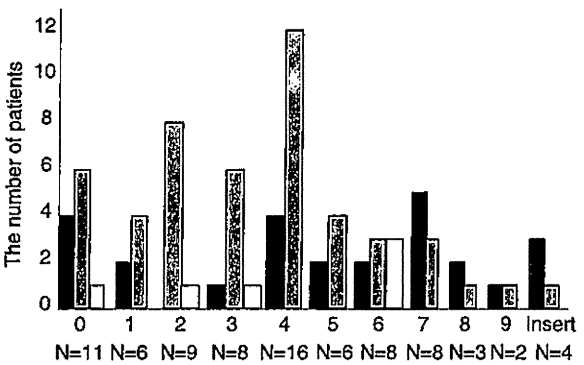

Figure 1 Number of amino acid substitutions in interferon sensitivity-determining region (ISDR) according to hepatitis C virus (HCV) genotypes. ■, HCV genotypes 1b; ▨, HCV genotypes 2a; □, HCV genotypes 2b.

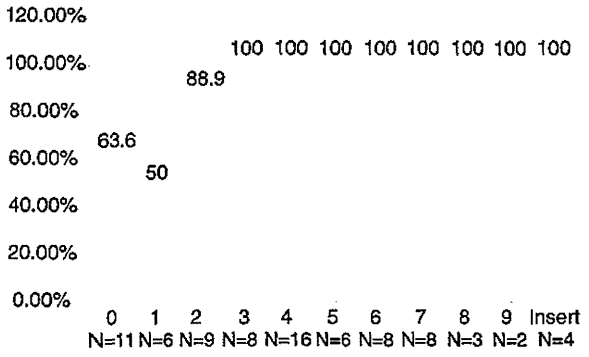

Figure 2 Sustained virological response (SVR) according to the number of amino acid substitutions in interferon sensitivity-determining region (ISDR).

Prevalence of the SNP of IL-28B (rs8099917) T (major allele) and G (minor allele) and response to IFN therapy

The frequencies of the IL-28B genotypes were: major homozygotes (TT), 73; heterozygotes (TG), 18; and minor homozygotes (GG), two. The rates of SVR in the patients with TT, TG and GG were 94.5% (69/73), 77.8% (14/18) and 100% (2/2), respectively. The SVR rate of patients with G allele of the IL-28B genotype was 80.0% (16/20), and that with T allele was 94.5% (69/73). Patients with T allele of the IL-28B genotype had a slightly higher SVR rate than did those with G allele, but there were no significant differences ($P = 0.0623$).

Analysis for factors predictive of SVR

The results of univariate analysis for factors predictive of SVR are shown in Table 3. HCV RNA levels were lower in patients with SVR than in those without SVR ($P = 0.0154$). SVR was achieved in 41.2% of patients with less than two mutations in the ISDR and 98.4% of patients with two or more mutations in the ISDR ($P = 0.0001$). HCV RNA levels and ISDR were associated with SVR on univariate analyses.

Results of multivariate analyses of factors predictive of SVR are shown in Table 4. Variables were recorded categorically as ordinal data. Background factors were age (<60 vs ≥60 years), sex (male vs female), platelet count ($<15 \times 10^4/mm^3$ vs $\geq 15 \times 10^4/mm^3$), HCV RNA level (<50 vs ≥50 KIU/mL), ALT levels (<70 vs ≥70 IU/L), aspartate aminotransferase (AST) levels (<60 vs ≥60 IU/L), HCV genotype (1 vs 2), ISDR (<2 vs ≥2 mutations), IL-28B (TT vs TG and GG) and RVR (yes vs no). As can be seen in Table 4, factors such as age, sex, platelet count, HCV RNA level, ALT levels, AST levels, HCV genotype, IL-28B and RVR did not have any effect on SVR. In contrast, the ISDR was the most influential factor.

DISCUSSION

THE HCV RNA level is one of the most important factors affecting response to IFN therapy. Patients with high HCV RNA levels respond poorly to IFN therapy, whereas patients with low HCV RNA levels have a high SVR rate to IFN therapy. Thus, most patients with low HCV RNA levels have achieved SVR, but other therapeutic options for patients who fail IFN therapy are needed. Several studies have attempted to reduce the duration of treatment, reduce the dose of IFN and/or ribavirin, or use standard IFN without risk of relapse.[8–10] The present study confirmed the high SVR rate (92.2%) in patients with low HCV RNA levels (≤100 KIU/mL)

Table 3 Univariate analysis: factors predictive of SVR

Factors	SVR ($n = 94$)	Non-SVR ($n = 8$)	*P*-value
Age (years)	54.6 ± 12.6	57.4 ± 8.8	0.5528
Sex: male/female	58/36	2/6	0.0619
ALT (IU/L)	63.2 ± 48.3	56.3 ± 32.5	0.7126
AST (IU/L)	50.7 ± 28.6	41.4 ± 21.6	0.4043
PLT ($\times 10^4/mm^3$)	18.5 ± 5.8	18.0 ± 5.0	0.8292
HCV RNA level (KIU/mL)	42.5 ± 34.8	75.0 ± 45.7	0.0154
HCV genotype: 1/2	29/63	4/3	0.4337
ISDR: <2/≥2	10/63	7/1	0.0001
IL-28B: TT/TG, GG	69/16	4/4	0.0623
RVR: yes/no	78/16	5/3	0.1661

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin-28B; ISDR, interferon sensitivity-determining region; PLT, platelets; RVR, rapid virological response; SVR, sustained virological response.

Table 4 Multivariate analysis: factors predictive of SVR

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.4556	2.837	0.183	43.891
Sex: male	0.8712	0.756	0.026	22.166
AST: <60 IU/L	0.7806	2.131	0.010	438.334
ALT: <70 IU/L	0.6063	0.239	0.001	55.563
Platelet count: $<15 \times 10^4$/uL	0.6873	0.463	0.011	19.680
HCV RNA: <50 KIU/mL	0.1046	13.170	0.585	296.318
Genotype: 2	0.1693	14.110	0.324	614.872
ISDR: <2	0.0074	0.004	0.001	0.235
IL-28B: TT	0.2684	5.978	0.252	141.852
RVR: yes	0.7495	1.756	0.055	55.696

95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin 28B; ISDR, interferon sensitivity-determining region; RVR, rapid virological response; SVR, sustained virological response.

treated by PEG IFN-α-2a monotherapy. Although the effects of shortened treatment duration of PEG IFN-α with ribavirin for patients with low HCV RNA levels are unclear, PEG IFN-α-2a monotherapy could reduce the cost and adverse events of ribavirin while maintaining a high SVR rate. This treatment would be a good therapeutic option for patients with low HCV RNA levels. However, selection by HCV RNA level alone was insufficient to predict IFN responsiveness completely, and other factors would be necessary to improve the positive predictive values for SVR in patients infected with low HCV RNA levels.

Hepatitis C virus genotype is another major factor, in addition to HCV RNA levels, that is associated with response to IFN therapy. In the present study, the SVR rates of genotypes 1 and 2 were 87.5% and 94.0%, respectively. Patients infected with genotypes 2 had a slightly higher SVR rate than did those with genotype 1, but there were no significant differences in our small study. The difference in SVR according to genotype may exist, but HCV genotype did not have enough power to be a determinant of IFN response completely among patients with low HCV RNA levels because of the bias for HCV RNA levels. However, patients infected with low HCV RNA levels respond differently to IFN therapy, suggesting that an additional factor associated with resistance to IFN exists.

The heterogeneity of the HCV NS5A region is an important factor that may affect response to IFN in patients with HCV genotype 1b and was named the ISDR.[17] Mutations in the ISDR affect the interaction with PKR and may inhibit viral replication. Therefore, ISDR of other HCV genotypes, in addition to 1b, could be used as predictors of IFN responsiveness.[23–28] In the present study, it was hypothesized that the amino acid substitutions in the ISDR would explain differences in IFN resistance in patients infected with low HCV RNA levels. Therefore, the utility of substitutions of amino acids in the ISDR for predicting IFN responsiveness was investigated. The ISDR was the most influential factor for SVR on multivariate analyses. All patients with three or more mutations in the ISDR achieved SVR, and 18 of 26 patients with less than three mutations in the ISDR achieved SVR. Thus, patients with less than three mutations in the ISDR would be resistant to PEG IFN-α-2a monotherapy and may need to receive much more powerful treatment, even if they have low HCV RNA levels. The ISDR system could be used as a diagnostic tool to predict SVR in patients infected with low HCV RNA levels. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be an important consideration to achieve optimal therapy and avoid unnecessary treatment.

Some studies of SVR to PEG IFN-α-2b and ribavirin and/or telaprevir combination therapy for chronic hepatitis C patients with genotype 1 and high viral load identified genetic variation near the IL-28B gene associated with IFN responsiveness.[13,14,16] However, the effects of genetic variation near the IL-28B gene on SVR in patients with low HCV RNA levels treated with PEG IFN monotherapy are unknown. Therefore, the utility of the SNP of IL-28B for predicting IFN responsiveness was investigated. Patients with IL-28B (rs8099917) genotypes TG and GG had a lower SVR rate than genotype TT, but no significant differences in genotype were found in this study. The SNP of IL-28B would be associated with the response to IFN, especially for poor responders, and