Follow-up Results of *HCV* GT2 Patients After Sofosbuvir/Ribavirin Therapy: Careful Attention to Occurrence of HCC

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Follow-up Results of *HCV* GT2 Patients After Sofosbuvir/Ribavirin Therapy: Careful Attention to Occurrence of HCC

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Abstract. *Background: We examined treatment the efficacy and data on long-term outcomes in real-world Japanese patients infected with hepatitis C virus (HCV) genotype 2 treated with 12-week sofosbuvir/ribavirin combination therapy. Patients and Methods: In a total of 86 patients who were treated with sofosbuvir/ribavirin, sustained virological response (SVR) rates and long-term-outcomes were retrospectively analyzed. Results: The adherence to this combination therapy was 98.8%. The rates of SVR at week 24 (SVR24) achieved with this treatment according to the 'intention-to-treat' and 'per-protocol' analyses were 89.5% and 96.2%, respectively. Two patients who experienced relapse did not have any previously reported resistance-associated substitutions in the HCV non-structural protein 5B (NS5B) polymerase region. We did not observe any patients who experienced late relapse but did observe that 50% and 1.3% of patients with and without a previous history of hepatocellular carcinoma (HCC), respectively, developed HCC after achieving SVR24 (with a mean follow-up period of 2.7±0.8 years). Conclusion: Patients with SVR should be carefully followed-up to screen for the occurrence of HCC, although it is infrequent.*

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Key Words: HCV, SVR, HCC, late relapse, RAS.

Chronic hepatitis C virus (HCV) infection is a major cause of hepatocellular carcinoma (HCC) and end-stage liver disease in Japan and Southern Europe (1, 2). Recent interferon-free therapy with direct-acting antivirals (DAAs) against HCV resulted in higher sustained virological response (SVR) rates with shorter treatment durations and few adverse events (3).

In Japan, the estimated proportion of the general population with *HCV* infection is 1.0-2.0%, and *HCV* genotype 2 (GT2) accounts for 30% of chronic HCV infections (4). The 12-week combination therapy of the HCV non-structural protein 5B (NS5B) inhibitors sofosbuvir and ribavirin was supported by the Japanese health insurance system as the very first DAA therapy for *HCV* GT2-infected patients (3, 5-7). However, the efficacy and follow-up data of this treatment in a group of real-world Japanese patients infected with *HCV* GT2 are limited and complex (5-7). Although there are different results between phase III clinical trials and real-world data, the 12-week combination therapy of sofosbuvir/ribavirin led to 90-95% SVR rates in Japanese DAA-naïve patients infected with *HCV* GT2 (3, 5-7).

Recently, pan-genotypic interferon-free therapies became approved in Japan. The 12-week combination of sofosbuvir/*HCV* NS5A inhibitor ledipasvir without ribavirin has been available for both HCV GT1 and GT2 infection (8, 9). This combination led to 100% and 96% SVR rates in patients infected with *HCV* GT1 and GT2, respectively (8, 9).

The 8-week combination of the *HCV* NS3 inhibitor glecaprevir/*HCV* NS5A inhibitor pibrentasvir without ribavirin has also been available for patients without cirrhosis infected with both *HCV* GT1 and GT2 (10). This combination led to 99.2% and 98.2% SVR rates in patients infected with *HCV* GT1 and GT2, respectively (10). Thus,

interestingly, the SVR rates of patients with *HCV* GT2 infection may be inferior to those of patients with *HCV* GT1 infection who receive DAA therapy.

It is possible that the results after achieving SVR with interferon-free therapy may be different from those after achieving SVR with interferon-containing treatment because the population is aging in Japan (6) and rapid immunological changes are also observed (11). Unexpectedly high rates of early tumor recurrence and occurrence have been reported in patients with *HCV*-related HCC undergoing interferon-free therapy (12, 13), although they are a controversial (14). Late relapse of *HCV* RNA has also been reported (15, 16).

These findings prompted us to analyze resistance-associated substitutions (RASs) in *HCV* GT2 patients with treatment failure and the data on long-term outcomes in *HCV* GT2-infected patients treated with the 12-week combination therapy of sofosbuvir/ribavirin. In particular, we followed up patients who achieved SVR at week 24 (SVR24). We focused on the virological response and the occurrence of HCC in real-world Japanese *HCV*-GT2-positive patients who were treated with sofosbuvir/ribavirin.

Patients and Methods

Patients. Patients were retrospectively enrolled at Nihon University School of Medicine Itabashi Hospital, Tokyo, Japan. Patients were eligible if they met the following criteria: i) Chronically infected with *HCV* GT2; ii) age more than 20 years; iii) negative for the HBs antigen; iv) negative for human immunodeficiency virus; v) no severe anemia; vi) no severe renal diseases; vii) no severe heart diseases; viii) no severe mental diseases; ix) no current intravenous drug abuse ; x) no pregnancy ; xi) no previous exposure to DAAs ; xii) no Child–Pugh B or C cirrhosis (class A acceptable) ; xiii) no current HCC; and xiv) the initiation of combination therapy with sofosbuvir/ribavirin between 2013 and 2017.

Study design. Data for Japanese *HCV* GT2-infected patients who were previously treated with a 12-week combination of sofosbuvir/ribavirin were analyzed. We included 86 consecutive patients in the present study (Figure 1). Participation in the present study has been posted at our institutions. Written informed consent was obtained from the patients for the analysis of HCV RASS. This study was approved by the Nihon University School of Medicine Itabashi Hospital Institutional Review Board (RK-161213-7 and RK-181009-4) and conformed to the ethical guidelines of the Declaration of Helsinki. In the present study, 400 mg of sofosbuvir per day plus a weight-based amount of ribavirin (400-1,000 mg) per day were perorally administered for 12 weeks (3). Clinical and laboratory assessments were performed at least every 4 weeks before and during treatment, and every 24 weeks after the end of treatment (EOT). Adverse events (17) were noted in patient interview, physical examinations and laboratory tests.

Serum biochemical tests and hematological tests. Serum liver function tests, determination of the estimated glomerular filtration rate (eGFR) and hematological tests were performed according to standard methods at least every 4 weeks before and during treatment and every 24 weeks after EOT.

Diagnosis of HCC and liver cirrhosis. Patients were subsequently followed-up through an HCC surveillance program based on tumor markers with or without ultrasonography evaluations every 4-6 months. An intrahepatic nodule was considered to be HCC on contrast-enhanced computed tomography (CT) or gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid magnetic resonance imaging when both a hypervascular nodule in the arterial phase and a hypovascular nodule in the late phase were demonstrated. Cirrhosis was diagnosed by ultrasonography (sign of cirrhosis) with or without transient elastography [Fibroscan (Echosens, Paris, France) liver stiffness more than 12 kPa] (5).

Determination of HCV RNA and HCV GTs. Serum *HCV* RNA levels were determined using the COBAS 6800/8800 system (Roche Diagnostics K.K., Minato-ku, Tokyo, Japan) with detection limits of ~40 IU/ml. *HCV* GTs were determined by previously described methods (18).

Assessment of treatment efficacy. SVR12, SVR24 and SVR48 were defined as undetectable serum *HCV* RNA at 12, 24 and 48 weeks after EOT, respectively (19). Relapse was defined as undetectable *HCV* RNA at EOT followed by the reappearance of *HCV* RNA (5).

Determination of RASs. Serum was obtained from the two patients who experienced relapse (patients 1 and 2) and stored at −80˚C until analysis. Two sets of amplification primers were generated for *HCV* NS4B, NS5B and the 3'-nontranslated region of *HCV* GT2 based on the sequences previously reported (20-23).

Nucleic acids were extracted from 140 μl of serum using the QIAamp Viral RNA Mini kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. cDNA was synthesized using random primers and Super-Script III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Firstly, polymerase chain reaction (PCR) was performed using KOD-Plus-Neo (Toyobo, Osaka, Japan) with 5'-CTGGAGCCTGAGCAGGTAGAGC-3' and 5'-GGGAGTAGGAA AAGGCCTACCC-3' under the following conditions: 2 min denaturation at 94˚C, 35 cycles at 98˚C for 10 s and 68˚C for 80 s, and 5 min extension at 68˚C on the GeneAmp PCR system 9700 (Applied Biosystems, Foster, CA, USA). A nested PCR was performed using KOD-Plus-Neo (Toyobo) with 5'-CTCCGT CGTGTGCTGCTCCATG-3' and 5'-AGTTTGAG CTTGGTC TTCACCG-3' under the same PCR conditions described above. PCR products were cloned into Zero Blunt TOPO (Thermo Fisher Scientific, Waltham, MA, USA) and directly sequenced. Nucleotide sequences in the present study were compared with those of *HCV* GT1a H77 (GenBank accession no. AF009606) (20), *HCV* GT1b Con1 (GenBank accession no. AJ238799) (21), *HCV* GT2a JFH1 (GenBank accession no. AB047639) (22) and *HCV* GT2b HC-J8 (GenBank accession no. D10988) (23). Nucleotide sequences were analyzed with GENETYX 10 (GENETYX Corp., Tokyo, Japan). All nucleotide sequences from the present study have been deposited in the DNA Data Bank of Japan (https://www.ddbj.nig.ac.jp/index-e.html)under accession number LC480953 – LC480956.

Statistical analysis. Data are expressed as the means±standard deviations (SDs). Statistical analyses of patient characteristic were performed by univariate analysis with the Student's *t*-test or the chi-squared test. Values of $p<0.05$ was considered statistically significant. Statistical analysis was performed with DA Stats software version PAF01644 (NIFTY Corp., Tokyo, Japan) and Excel Statistics program for Windows 2010 (SSRI, Tokyo, Japan).

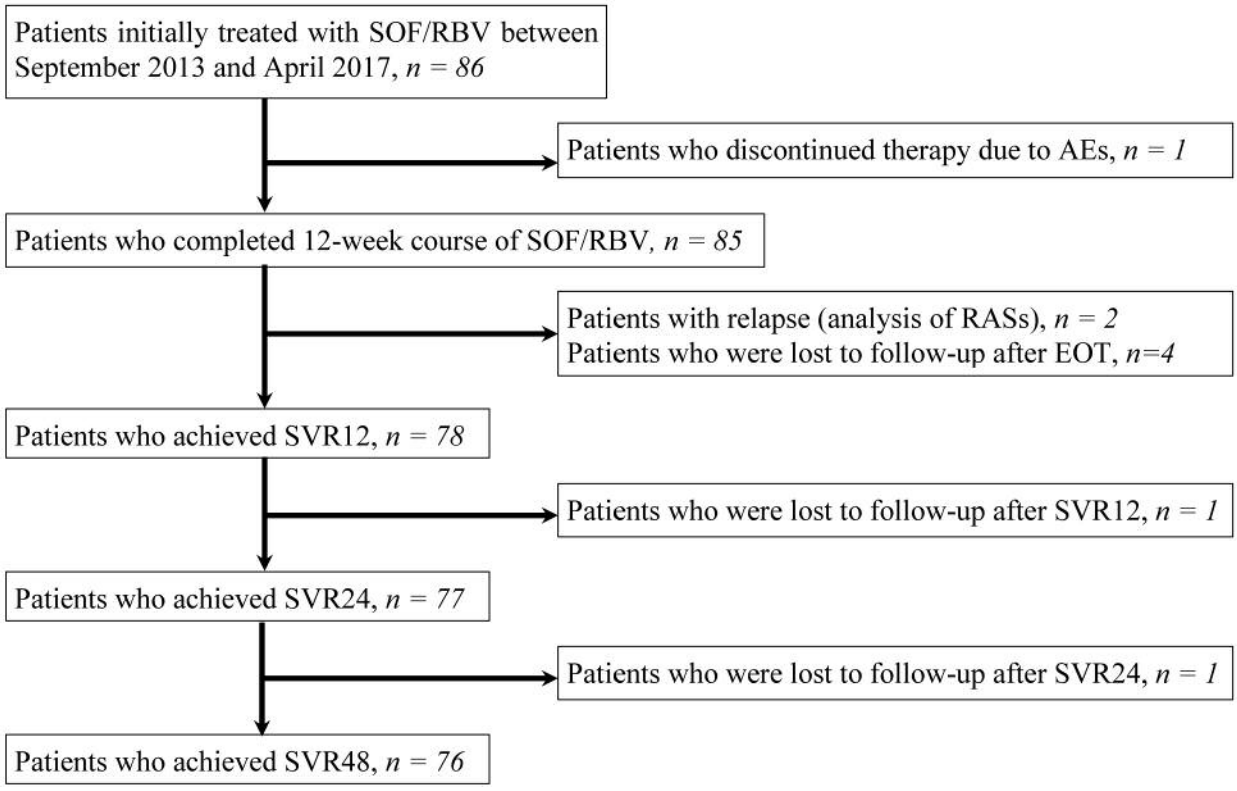

Figure 1. *Flowchart showing the patients who were enrolled in this study. SOF: Sofosbuvir; RBV: ribavirin; AEs: adverse events; EOT: end of treatment; SVR: sustained virological response: RASs: Resistance-associated substitutions.*

Results

Patient characteristics. The characteristics of the 86 patients in the present study are shown in Table I. The mean age of patients in the present study was older than that of the patients in the Japanese phase III study (57 years) (3). In total, 10.4% of patients were interferon-experienced. The mean liver stiffness was 8.9±7.0 kPa, and 22% of the patients had cirrhosis. Concerning the prevalence of *HCV* GTs, *HCV* GT2a was the predominant subtype in the present study.

Notably, two patients with a previous history of HCC were also included: A 69-year-old man infected with *HCV* GT2b who had undergone radical surgery for HCC 5 months before the initiation of DAA therapy, and a 64-year-old man infected with *HCV* GT2b who had undergone radical surgery for HCC 4.5 years and 1.5 years before the initiation of DAA therapy.

Adherence to the combination therapy of sofosbuvir/ribavirin. In total, 86 Japanese patients underwent treatment with combination therapy with sofosbuvir/ribavirin (Figure 1). Only one patient discontinued taking this combination due to an elevated serum creatinine level (1.45 mg/dl) (Table II). In this patient, *HCV* RNA was undetectable at week 4, but *HCV* RNA reappeared after the cessation of therapy. This patient has been followed-up for 2.5 years, during which time he has not receive antiviral treatment, but HCC has not yet developed. It is noteworthy that 85 patients (98.8%) completed the 12-week course of sofosbuvir/ribavirin, meaning that adherence to this combination therapy was superior to that of interferon-including treatment (24). No other serious adverse events were observed.

Efficacy of the combination therapy of sofosbuvir/ribavirin. The SVR12 rates according to the 'intention-to-treat' and 'per-protocol' analyses were 90.6% (78/86) and 96.2% (78/81), respectively. The SVR24 rates according to the 'intention-to- treat' and 'per-protocol' analyses were 89.5% (77/86) and 96.2% (77/80), respectively. The SVR48 rates according to the 'intention-to-treat' and 'per-protocol' analyses were 88.3% (76/86) and 96.2% (76/79), respectively (Figure 1). Of the nine interferon-experienced patients, eight, seven and seven patients achieved SVR12, SVR24 and SVR48, respectively, and one experienced relapse. Thus, the efficacy of sofosbuvir/ribavirin combination therapy appears to be excellent at least 48 weeks after EOT.

Analysis of RASs. Two patients experienced relapse after the EOT with the 12-week sofosbuvir/ribavirin combination therapy (Table II), and these two both had infections with *HCV* GT2b. We sequenced the *HCV* NS5B polymerase regions in post-treatment sera collected from these two patients by Sanger methods and compared them with previously reported RASs (25). We did not identify any sofosbuvir-related RASs at positions L159, S282, C316, L320 and V321 (Table III). Among previously reported RASs, M414Q, a RAS related to *HCV* NS5B inhibitor dasabuvir and *HCV* GT1, was identified in both patients with relapse, and A421V, aa RAS related to *HCV* NS5B inhibitor beclabuvir and *HCV* GT1a, was identified in both patients with relapse; however, these RASs were also found in *HCV* GT2a JFH1 and GT2b HCJ8, which were from DAA-treatment naïve patients. Fortunately, the two patients with relapse in the present study achieved SVR after retreatment with a 12-week course of glecaprevir/pibrentasvir combination therapy.

Virological response after EOT in patients with SVR24. In the present study, 77 patients achieved SVR24 (Figure 1). It has been reported that late relapse can occur in *HCV* RNA-positive patients with SVR after interferon-including and interferon-free therapies against HCV infection (16). Although we followed-up these patients with SVR24 for a mean of 2.7±0.8 years, we did not observe any with *HCV* RNA relapse or HCV reinfection among the *HCV* GT2-infected patients who achieved SVR24 after the 12-week combination therapy with sofosbuvir/ribavirin (Table IV).

Occurrence of HCC after EOT in patients with SVR24. We did not observe any patients in whom HCC occurred during therapy with the combination of sofosbuvir/ribavirin or during the 24 weeks after therapy. After SVR24 was achieved, two patients developed HCC (2.5%, 2/77) (Table V). Among patients with and without a previous history of HCC, 50% (1/2) and 1.3% (1/75), respectively, developed HCC after SVR24.

We also compared the characteristics of patients who did and did not develop HCC (Table VI). Univariate analysis demonstrated that patients with the HCC occurrence after SVR tended to be male and had a lower platelet count, a history of HCC and longer follow-up period. Multivariate logistic regression analysis of factors among those investigated failed to demonstrate that any associated with the development of HCC because the number of patients was too small.

Among the three patients who experienced reappearance of *HCV* RNA, only one patient developed HCC. In this patient, HCC developed while *HCV* RNA was undetectable after the 12-week retreatment with glecaprevir/pibrentasvir (Table II, patient 1).

Table I. *Clinical characteristics at the baseline of the 'intention-to-treat' patients*.

Characteristic	All patients (n=86)
Male/female, n	40/46
Mean age±SD, years	60.5±11.1
Mean BMI±SD, kg/m^2	23.2±3.8
Mean *HCV* RNA±SD, IU/ml	2,430,000±3,324,000
Genotype: 2a/2b/unknown, n	53/32/1
Interferon-naïve/-experienced, n	77/9
Chronic hepatitis/cirrhosis, n	67/19
Mean AST±SD, IU/l	52.7±41.8
Mean ALT±SD, IU/l	55.9±53.8
Mean hemoglobin±SD, g/dl	13.6±1.7
Mean platelet count±SD, 10^3/μl	182±67
Mean eGFR±SD, ml/min/1.73 m^2	76.8±15.4
History of HCC: Yes/no, n	84/2
History of diabetes mellitus: Yes/no, n	72/14

BMI: Body mass index; *HCV*: hepatitis C virus; AST: aspartate aminotransferase; ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; HCC: hepatocellular carcinoma; SD: standard deviation.

Discussion

Although the current interferon-free combination therapies for *HCV* GT2-infected patients with chronic hepatitis and cirrhosis result in higher SVR rates, SVR rates in Japanese patients infected with *HCV* GT2 need to be more improved further (8-10). This retrospective study also demonstrated that the SVR rates and adherence to the 12-week combination of sofosbuvir plus weight-based ribavirin were better than those for the interferon-containing regimens (24), supporting the results of previous studies (3, 5-7).

Sofosbuvir/ribavirin treatment was associated with few serious adverse events; discontinuation of therapy due to serum creatinine elevation was observed in one patient (1.1%). Before the initiation of this combination therapy, his eGFR was 75.7 ml/min/1.73 m^2. Saxena *et al*. reported that patients with eGFRs ≤45 ml/min/1.73 m^2 more frequently had anemia, worse renal function and more serious adverse events than their counterparts when taking sofosbuvir-containing regimens (26). During combination therapy with sofosbuvir/ ribavirin, close monitoring of renal function is also required.

The clinical impact of DAA RASs in the *HCV* NS5B region on sofosbuvir/ribavirin treatment failure is still unclear (5-7, 25). The two patients who experienced relapse had cirrhosis, which is also one of the risk factors for *HCV* RNA reappearance (6), and did not have any RASs in the *HCV* NS5B polymerase region, which had been previously reported (25). As in *HCV* NS5B, 1,256 base pairs need to be covered to include all clinically relevant DAA RASs; it may

Table II. *Baseline data on patients who experienced hepatitis C virus (HCV) relapse after combination therapy with sofosbuvir and ribavirin.*

Patient	Age (years)/ gender	*HCV* GT	*HCV* RNA (IU/ml)	Previous treatment	BMI (kg/m^2)	Liver stiffness (kPa)/cirrhosis	AST (IU/l)	ALT (IU/l)	Hemoglobin (g/dl)	Platelet count ($10^3/\mu l$)	eGFR (ml/min/ 1.73 m^2)
1*	55/Male	2b	630,000	None	25.4	25.3/Yes	231	218	15.7	62	75.7
2**	58/Male	2b	3,162,000	IFN	NA	32/Yes	86	94	16	200	81.7
3#	54/Male	2b	12,589,000	None	19.9	9.2/No	93	91	14.7	136	75.7

BMI: Body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; HCC: hepatocellular carcinoma; IFN: interferon; NA: not available. None of these patients had a history of HCC or *diabetes mellitus*. *HCV* RNA: *Relapse 8 weeks after the end of treatment; **relapse at 4 weeks after the end of treatment; #undetectable at week 4, but therapy was discontinued on day 45 due to the elevation of serum creatinine level (1.45 mg/dl), with subsequent relapse.

Table III. *Resistance associated substitutions in patients with hepatitis C virus (HCV) GT2b infection who experienced relapse after combination therapy with sofosbuvir/ribavirin in the present study.*

	Substitution												
AA position	L159	S282	M289	C316	L320	V321	S368	N411	M414	A421	E446	Y448	P495
GT1a (H77)	–	–	C	–	–	–	–	–	–	–	–	–	A
GT1b (Con1)	–	–	C	–	–	–	–	–	–	–	Q	–	–
GT2a (JFH-1)	–	–	–	–	–	–	–	–	Q	V	–	–	N
GT2b (HC-J8)	–	–	–	–	–	–	–	–	Q	V	–	–	–
GT2b Patient 1	–	–	–	–	–	–	–	–	Q	V	–	–	–
GT2b Patient 2	–	–	–	–	–	–	–	–	Q	V	–	–	–

AA: Amino acid; –, no substitution compared to AA in the top line. H77 (AF009606) (20); Con1 (AJ238799) (21); JFH1 (AB047639) (22); HC-J8 (D10988) (23).

be more difficult to determine the RASs in *HCV* NS5B regions than in *HCV* NS3 or NS5A (20). Further studies will be needed regarding sofosbuvir-related RASs.

Hayashi *et al*. reported that late reappearance of the original *HCV* strain was confirmed by direct sequencing in 0.96% (4/413) of patients with SVR after treatment with the *HCV* NS3/4A inhibitor asunaprevir and the *HCV* NS5A inhibitor daclatasvir, and patients redeveloped serum *HCV* RNA at 6, 12, 12 and 26 months after achieving SVR24 (16). In the present study, no patient who achieved SVR24 after treatment with sofosbuvir/ribavirin redeveloped *HCV* RNA. Pisaturo *et al*. reported that 5.4% of patients with DAA-related SVR12 experienced late relapse (after the achievement of a SVR12; median=24 weeks, range=24-72 weeks) (27). Although late relapse is infrequent, posttreatment follow-up is very important in the DAA era, as it was in the interferon era (28, 29). When liver function is worse after the achievement of SVR, *HCV* RNA should be examined to rule out late relapse.

Although it is likely that exposure to DAAs was associated with a decrease in all-cause mortality and HCC (30), we observed that 3.4% (3/86) of the patients who received sofosbuvir/ribavirin, including two who achieved SVR24, developed HCC, suggesting that careful follow-up of patients after DAA therapy is needed. After SVR was achieved with sofosbuvir/ribavirin treatment, HCC occurred in one out of two patients or 1.3% (1/75) of patients with and without a history of HCC, respectively. Together, these findings indicate that careful attention should be paid to patients with a history of HCC after they achieve SVR.

Table IV. *Virological response after the end of treatment (EOT) in patients with sustained virological response at week 24 (n=77).*

Time after EOT (years)	Total patients, n	Patients with undetectable *HCV* RNA, n (%)
1	76	76 (100)
1.5	71	71 (100)
2	70	70 (100)
2.5	63	63 (100)
3	45	45 (100)
3.5	16	16 (100)
4	6	6 (100)
4.5	1	1 (100)
5	1	1 (100)

HCV: Hepatitis C virus.

Table V. *Patients in whom hepatocellular carcinoma (HCC) occurred after sustained virological response was achieved.*

Patient	Age (years)/ gender	*HCV* GT	*HCV* RNA (IU/ml)	Previous treatment	BMI (kg/m^2)	Liver stiffness (kPa)/cirrhosis	AST (IU/l)	ALT (IU/l)	Hemoglobin (g/dl)	Platelet count ($10^3/\mu l$)	eGFR (ml/min/ 1.73 m^2)	History of HCC
4*	64/Male	2b	5,011,000	Naïve	24.7	NA/Yes	50	66	15.4	153	76.8	Yes
5**	65/Male	2a	316,000	Naïve	20.2	NA/Yes	49	36	11.3	207	79	No

BMI: Body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; HCC: hepatocellular carcinoma; IFN: interferon; NA: not available. None of these patients had a history of *diabetes mellitus*. *HCC recurred 2.5 years after initiation of sofosbuvir/ribavirin. **HCC occurred 1.5 years, and 2.5 years after the initiation of sofosbuvir/ribavirin.

Table VI. *Clinical characteristics of the patients who underwent a follow-up examination at least 1 year after the end of treatment.*

	HCC occurrence			
Characteristic	Total patients (n=77)	Patients without (n=75)	Patients with (n=2)	*p*-Value*
Male/female, n	35/42	33/42	2/0	0.116
Mean age±SD, years	60.9±11.1	60.8±11.2	64.5±0.7	0.646
Mean BMI±SD, kg/m^2	23.3±3.9	23.3±3.9	22.5±3.1	0.774
Mean *HCV* RNA±SD, IU/ml	2,175,000±2,995,000	2,162,000±3,009,000	2,664,000±3,320,000	0.816
Genotype: 2a/2b/unknown, n	47/29/1	46/28/1	1/1/0	0.745
Interferon naïve/experienced, n	70/7	69/6	1/1	0.650
Chronic hepatitis/cirrhosis, n	62/15	61/14	1/1	0.269
Mean AST±SD, IU/l	50.4±38.0	50.4±38.5	59.5±0.7	0.973
Mean ALT±SD, IU/l	54.9±52.7	55.0±33.4	51.0±21.2	0.916
Mean hemoglobin±SD, g/dl	13.5±1.6	13.5±1.5	13.3±2.8	0.855
Mean platelet count±SD, $10^3/\mu l$	183±68	183±68	180±38	0.0618
Mean eGFR±SD, ml/min/1.73m^2	76.8±16.0	76.7±16.2	77.9±1.5	0.917
History of HCC: Yes/no, n	75/2	74/1	1/1	<0.00001
History of *diabetes mellitus*: Yes/no, n	60/17	59/16	1/1	0.334
Follow-up period (years)	2.7±0.8	2.7±0.7	3.5±0.7	0.182

BMI: Body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate.*By Student's *t*-test or chi-square test.

The 12-week combination therapy with sofosbuvir/ribavirin led to higher SVR rates, with better adherence and fewer adverse effects. Late relapse was infrequent. The limitation of the present study is that the number of study patients may have been too small. In conclusion, continued follow-up will be needed to determine whether unexpected events occur in *HCV* GT2-infected patients who achieve SVR after treatment with sofosbuvir/ribavirin. However, patients who achieve SVR should be carefully followed-up to allow screening for the development of HCC.

Conflicts of Interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors' Contributions

Conception and design: Tomohiro Kaneko, Tatsuo Kanda, Mitsuhiko Moriyama; Collection and assembly of data: Tomohiro Kaneko, Tatsuo Kanda, Kazushige Nirei; Data analysis and interpretation: Tomohiro Kaneko, Tatsuo Kanda, Mitsuhiko Moriyama; All Authors; Article writing: Tomohiro Kaneko, Tatsuo Kanda, Mitsuhiko Moriyama; Final approval of article: All Authors.

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(This text is a combination of advice and suggestions contributed by Editors, Authors, Readers and the Managing Editor of AR).