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Short Communication

Open-label phase 2 study of faldaprevir, deleobuvir and ribavirin in Japanese treatment-naive patients with chronic hepatitis C virus genotype 1 infection

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Aim: The safety and efficacy of the NS3/4A protease inhibitor faldaprevir in combination with the non-nucleoside NS5B polymerase inhibitor deleobuvir and ribavirin in Japanese treatment-naive patients with chronic genotype 1 hepatitis C virus (HCV) infection was evaluated.

Methods: In this multicenter, open-label phase 2 study, patients were assigned to 8 weeks of treatment with 80 mg (group 1) or 120 mg (group 2) faldaprevir once daily (q.d.) in combination with deleobuvir 600 mg twice daily and weight-based ribavirin. This was followed by a 24-week treatment with faldaprevir 120 mg q.d. in combination with peginterferon-α-2a and ribavirin. The primary objective was safety; virological response at weeks 4 and 8 was a secondary endpoint.

Results: Twelve and 13 patients were treated in group 1 and 2, respectively; all were infected with HCV genotype 1b. All patients experienced a drug-related adverse event (AE). The frequency of individual events was generally numerically greater in group 2 than 1. The most common AEs were nausea (66.7%, group 1; 76.9%, group 2) and vomiting (33.3%, group 1; 61.5%, group 2). Virological response at weeks 4 and 8 was achieved by 11 (91.7%) patients in group 1; in group 2, 12 (92.3%) patients achieved virological response at week 4 and all at week 8. All patients who achieved the week 8 endpoint achieved sustained virological response at week 12.

Conclusion: Faldaprevir 80 or 120 mg q.d. in combination with deleobuvir and ribavirin was tolerable and had similar efficacy in Japanese patients with HCV genotype 1 infection. ClinicalTrials.gov: NCT01528735.

Key words: clinical trial, faldaprevir, hepatitis C, interferon-free, Japanese, phase 2

INTRODUCTION

CURRENT RECOMMENDED TREATMENT for chronic hepatitis C virus (HCV) genotype 1 infection in Japanese patients is triple therapy with the protease inhibitor simeprevir, and pegylated interferon plus ribavirin (PegIFN/RBV).[1] Sustained virological response (SVR) rates of approximately 90% were achieved using this combination in Japanese treatment-naive patients with genotype 1 infection.[2,3] However, because PegIFN-based therapies are associated with serious side effects leading to premature discontinuations and have a number of contraindications,[4] there has been increasing focus on identifying interferon-free treatment strategies.[5]

Faldaprevir, a potent NS3/4A protease inhibitor with pharmacokinetic profile that supports once-daily (q.d.) dosing,[6,7] has been evaluated in a predominantly Caucasian population in combination with the non-nucleoside NS5B polymerase inhibitor deleobuvir and RBV in treatment-naive patients with HCV genotype 1 infection.[8–11] In the SOUND-C2 and SOUND-C3 studies, faldaprevir dosed at 120 mg q.d. with deleobuvir 600 mg twice daily (b.i.d.) and RBV for 28 and 16 weeks, respectively, demonstrated high antiviral activity and good tolerability in Caucasian patients with HCV genotype 1b infection.[8,9]

The objectives of this phase 2 study were to investigate the safety and efficacy of 8 weeks of treatment with the interferon-free combination of faldaprevir (80 mg and 120 mg q.d.), deleobuvir (600 mg b.i.d.) and RBV in Japanese treatment-naive patients with chronic HCV genotype 1 infection to inform dosing in phase 3 studies for Japanese HCV-infected patients. A lower dose of

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faldaprevir, 80 mg q.d., was assessed because the exposure had previously been demonstrated to be higher in Japanese than Caucasian patients, which could affect tolerability.[12]

Since the completion of the study, the development of faldaprevir and deleobuvir has been terminated in view of the fast progress in development of interferon-free regimens. However, this small study provides valuable information about protease inhibitor therapy in Japanese patients that may be relevant to other direct-acting antivirals.

METHODS

Study design and treatment

THIS WAS A multicenter, ascending dose, open-label phase 2 study (ClinicalTrials.gov: NCT01528735) carried out in five sites in Japan between February 2012 and August 2013. Twenty-four patients were planned to be treated in two treatment groups with 12 patients in each group (Supplementary Fig. 1). Patients in group 1 received 8 weeks of faldaprevir 80 mg q.d. and deleobuvir 600 mg b.i.d. in combination with standard weight-based dose of RBV. Patients in group 2 received 8 weeks of faldaprevir 120 mg q.d. and deleobuvir 600 mg b.i.d. in combination with standard weight-based dose of RBV. Both groups received treatment with faldaprevir 120 mg q.d. in combination with PegIFN-α-2a and RBV during weeks 9–32. A loading dose of 160 mg (for group 1) or 240 mg (for group 2) was given on the first day of faldaprevir administration. Progression from group 1 to group 2 was based on the assessment of safety-related data until week 4 and available pharmacokinetic data by the data monitoring committee (DMC).

Temporary interruptions and dose reduction of faldaprevir or deleobuvir administration were not allowed. Patients were to discontinue their respective treatment before week 32 in case of lack of virological response, including virological breakthrough defined as an increase of HCV RNA of 1 $\log_{10}$ or more from nadir or HCV RNA of 25 IU/mL or more following an initial decrease to less than 25 IU/mL in two consecutive measurements within 2 weeks if the first measurement was less than 1000 IU/mL; lack of virological response at visit 14 (week 20), defined as an absence of drop by 2 $\log_{10}$ or more from baseline plasma HCV RNA. Patients could be withdrawn from the trial if they experienced adverse events (AEs) that warranted drug discontinuation; for example, a concurrent increase in bilirubin (>5 × upper limit of normal [ULN] with a ratio of conjugated/total >0.5) and alanine aminotransferase (ALT; >5 × ULN and with an absolute increase in ALT >100 U/L compared with baseline). Patients with virological breakthrough during faldaprevir/deleobuvir/RBV treatment before visit 10 (day 57) were to be immediately switched to PegIFN/RBV treatment for 48 weeks. All patients were to be followed up for 24 weeks after the end of study treatment.

Study documentation was approved by the appropriate institutional review board. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation guidelines. All patients provided written informed consent before study participation.

Patient population

Eligible patients were treatment naive, aged 20–70 years, with chronic HCV genotype 1 infection determined by positive anti-HCV antibodies and HCV RNA of 100 000 IU/mL or more at screening in conjunction with either a liver biopsy consistent with chronic HCV infection, or positive anti-HCV antibodies or detectable HCV RNA at least 6 months before screening. Patients were required to have undergone a liver biopsy within 3 years or FibroScan within 6 months prior to study treatment to determine the stage of fibrosis. Patients with compensated liver cirrhosis were excluded from the study. Other key exclusion criteria were: mixed genotype HCV; evidence of non-HCV-related liver disease; hepatitis B infection; HIV infection; decompensated liver disease; clinically significant comorbidities; ongoing photosensitivity or recurrent rash; hemoglobin of less than 12 g/dL (women) or less than 13 g/dL (men); total bilirubin of more than 2 mg/dL with ratio of direct/indirect bilirubin of more than 1; and hypersensitivity to the study treatments.

Endpoints

The primary objective of the study was to evaluate safety. A primary endpoint was defined *post-hoc* as number (%) of patients with drug-related AEs. Other safety endpoints included AEs, discontinuations due to AEs, laboratory tests, vital signs and 12-lead electrocardiogram. AEs were classified using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events.

No primary efficacy endpoint was defined. Secondary efficacy endpoints were virological response (plasma HCV RNA level <25 IU/mL [undetected or detected]) at week 4 or at week 8. A number of other efficacy endpoints were analyzed including, but not limited to, sustained virological response at 12 and 24 weeks post-treatment (SVR12 and SVR24, respectively). Plasma HCV RNA level was measured using the quantitative Roche COBAS® TaqMan HCV/HPS assay (Roche Applied Science, Indianapolis, IN, USA) with a lower limit of quantification of 25 IU/mL and lower limit of detection of 10 IU/mL.

Plasma HCV RNA levels of less than 25 IU/mL were to be analyzed and reported as being undetectable or detectable.

Statistical analysis

All analyses were descriptive in nature and no hypothesis was tested. Safety analyses were carried out on all patients who received at least one dose of study medication (treated set). Efficacy analyses included all patients in the treated set who had at least one on-treatment value of HCV RNA viral load.

RESULTS

Patients

IN GROUP 1, 15 patients were enrolled and 12 were treated; in group 2, 17 patients were enrolled and 13 were treated. Of those, 11 (91.7%) patients in group 1 and 12 (92.3%) in group 2 completed the 8-week interferon-free treatment with faldaprevir, deleobuvir and RBV. Baseline patient demographics and disease characteristics were generally similar between the two groups (Table 1). All patients were infected with HCV genotype 1b. The proportion of patients with viral load of 800 000 IU/mL or more or *IL28B* (rs12979860) CC genotype was 91.7% or 75.0% in group 1 and 61.5% or 53.8% in group 2, respectively.

Safety

During the interferon-free faldaprevir, deleobuvir and RBV treatment period, all patients experienced at least one drug-related AE (Table 2). The frequency of each AE was generally numerically higher in group 2 than group 1. The profile of frequently reported AEs was generally similar to that of drug-related AEs with nausea, vomiting and diarrhea the most common. Most AEs were of mild or moderate intensity with only one patient in group 2 experiencing a severe AE (weight decreased). Protocol-defined AEs of special interest (special search category) were reported for one (8.3%) patient in group 1 (photosensitivity grade 2) and one (7.7%) patient in group 2 (rash grade 2). No serious AEs were reported. One patient in each group discontinued all treatment due to AEs; one in group 1 on day 8 (asthma) and the other in group 2 on day 56 (abnormal hepatic function). Another patient in group 2 discontinued faldaprevir after taking the last dose in the interferon-free treatment phase (day 57) because of decreased appetite and vomiting.

Table 1 Baseline demographics and disease characteristics

	Group 1 $n = 12$	Group 2 $n = 13$
Male, *n* (%)	4 (33.3)	5 (38.5)
Mean age, years (SD)	57.7 (7.38)	55.5 (8.42)
Mean BMI, kg/m^2 (SD)	23.27 (5.14)	23.42 (4.92)
HCV genotype 1b, *n* (%)	12 (100)	13 (100)
IL28B genotype (rs12979860), *n* (%)		
CC	9 (75.0)	7 (53.8)
CT	3 (25.0)	6 (46.2)
TT	0	0
Mean HCV RNA, $\log_{10}$ IU/mL (SD)	6.5 (0.41)	6.1 (0.68)
Viral load ≥800 000 IU/mL, *n* (%)	11 (91.7)	8 (61.5)
Fibrosis stage†, *n* (%)		
≤F2	11 (91.7)	13 (100)
F3	1 (8.3)	0

†FibroScan results were used to determine stage of fibrosis for participants without a liver biopsy (F0–F2, <9.5 kPa; F3–F4, ≥9.5 kPa). Patients with cirrhosis were not eligible for inclusion in the study.
BMI, body mass index; HCV, hepatitis C virus; SD, standard deviation.

Table 2 Summary of adverse events (AEs) during the 8-week interferon-free treatment period

Patients with AEs, *n* (%)	Group 1 $n = 12$	Group 2 $n = 13$
Any AE	12 (100)	13 (100)
Any drug-related AE	12 (100)	13 (100)
AEs leading to discontinuation of study medication(s)	1 (8.3)	2 (15.4)
All medications	1 (8.3)	1 (7.7)
Faldaprevir only	0	1 (7.7)
AEs leading to RBV dose reduction†	3 (25.0)	2 (15.4)
Severe AEs	0	1 (7.7)
Serious AEs	0	0
AEs of interest of at least moderate intensity (≥grade 2)‡		
Photosensitivity reaction	1 (8.3)	0
Rash	0	1 (7.7)
Most frequent AEs (>20% in any group)§		
Nausea	8 (66.7)	10 (76.9)
Vomiting	4 (33.3)	8 (61.5)
Diarrhea	4 (33.3)	4 (30.8)
Decreased appetite	3 (25.0)	5 (38.5)
Anemia	3 (25.0)	0
Pruritus	2 (16.7)	3 (23.1)
Rash	0	7 (53.8)
Hyperbilirubinemia	0	4 (30.8)

†Events were associated with decrease in hemoglobin (three patients with anemia, one patient with iron deficiency anemia and one patient with hemoglobin decreased).
‡As special search category, by Medical Dictionary for Regulatory Activities (MedDRA) preferred term.
§By MedDRA preferred term. No deaths were reported.

Changes in laboratory parameters for the interferon-free phase are shown in Table 3. An increase in hemoglobin DAIDS grades was observed in both groups, with one grade 3 event in group 2. AEs related to hemoglobin were manageable by dose reduction of RBV. Grade 3–4 increases in total bilirubin were more frequent in group 2 (50%) than in group 1 (16.7%); these were mainly driven by increases in unconjugated bilirubin.

Efficacy

There was a rapid decrease in mean $\log_{10}$ viral load with initiation of treatment with no difference in viral load decline between the two groups (Supplementary Fig. 2). Eleven out of 12 (91.7%) patients in group 1 achieved HCV RNA of less than 25 IU/mL detected or undetected at week 4 and at week 8 (Table 4). In group 2, this endpoint was achieved at week 4 by 12 out of 13 (92.3%) patients and by all at week 8. The patient who did not achieve the week 8 endpoint in group 1 had discontinued all treatment on day 8 due to worsening of pre-existing asthma. All patients in both groups who had HCV RNA of less than 25 IU/mL detected or undetected at week 8 went on to achieve SVR12 and SVR24, including two patients who did not receive the 24-week interferon-based treatment as planned. No patients experienced virological breakthrough in either group during the interferon-free treatment phase. No relapse occurred in the trial.

Table 3 Changes in laboratory parameters for the interferon-free treatment phase

n (%)†	Group 1 *n* = 12	Group 2 *n* = 12‡
Hemoglobin		
All grades	4 (33.3)	5 (41.7)
Grade ≥3	0	1 (8.3)
Platelets		
All grades	0	0
Grade ≥3	0	0
AST		
All grades	1 (8.3)	0
Grade ≥3	0	0
ALT		
All grades	1 (8.3)	0
Grade ≥3	0	0
Total bilirubin		
All grades	9 (75.0)	12 (100)
Grade ≥3	2 (16.7)	6 (50.0)

†Worst Division of AIDS grade on treatment.
‡Only patients without missing values included. ALT, alanine aminotransferase; AST, aspartate transaminase.

Table 4 Efficacy results

Response, *n* (%)	Group 1 *n* = 12	Group 2 *n* = 13
Secondary efficacy endpoints		
HCV RNA <25 IU/mL, undetected or detected		
At week 4	11 (91.7)	12 (92.3)
At week 8	11 (91.7)	13 (100)
Other efficacy endpoints		
HCV RNA <25 IU/mL, undetected		
At week 4	10 (83.3)	10 (76.9)
At week 8	10 (83.3)	13 (100)
HCV RNA <25 IU/mL at week 4 (detected or undetected) and undetected at week 8	10 (83.3)	12 (92.3)
ETR†	11 (91.7)	13 (100)
SVR12	11 (91.7)	13 (100)
SVR24	11 (91.7)	13 (100)
Virological failure		
Breakthrough	0	0
Relapse‡	0/10	0/12

†ETR, the proportion of patients achieving HCV RNA <25 IU/mL undetected at the end of all treatment.
‡Relapse, HCV RNA ≥25 IU/mL in patients who completed the planned active treatment duration and had undetected HCV RNA at the end of treatment. ETR, end of treatment response; HCV, hepatitis C virus; SVR, sustained virological response.

CONCLUSIONS

IN THIS PHASE 2 trial, the interferon-free combination of faldaprevir 80 or 120 mg q.d., deleobuvir 600 mg b.i.d. and RBV for 8 weeks was tolerable and efficacious in Japanese treatment-naive patients with HCV genotype 1b infection.

All patients experienced at least one drug-related AE during treatment; these were mostly mild or moderate in intensity and manageable. In general, the AE profile was consistent with other studies on faldaprevir, deleobuvir and RBV conducted with Caucasian patients outside Japan,[8–11] with gastrointestinal events the most common AEs. Some AEs, including rash, vomiting and hyperbilirubinemia, were more frequently reported with faldaprevir 120 mg q.d. than faldaprevir 80 mg q.d.. All rash and photosensitivity events were manageable and did not require discontinuation of trial medication. Consistent with the mechanism of action of faldaprevir,[13] increased levels of total bilirubin were observed during treatment which were mainly driven by increases in unconjugated bilirubin and with no signs of liver injury.

No apparent difference in antiviral activity was observed between the regimens with faldaprevir 80 mg q.d. and

120 mg q.d. The secondary efficacy endpoint (plasma HCV RNA <25 IU/mL, undetected or detected) was achieved by more than 90% of patients at both weeks 4 and 8, and no patient experienced virological breakthrough or relapse. All patients who completed the interferon-free period in both groups reached SVR, including two patients who were treated for only 8 weeks with the oral combination without extended PegIFN/RBV treatment. These results suggest that faldaprevir dosed at 80 mg q.d. is efficacious in Japanese patients.

In conclusion, 8-week treatment of both doses of faldaprevir (80 or 120 mg q.d.) in combination with deleobuvir and RBV was tolerable and had similar efficacy in Japanese patients; although the regimen with faldaprevir 80 mg q.d. was favorable in terms of safety profile.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found in the online version of this article at the publisher's web site:

Figure S1 Study design.
Figure S2 Mean plasma HCV RNA level (log10) over time by treatment group for the interferon-free treatment phase.

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RESEARCH ARTICLE

Serum Wisteria Floribunda Agglutinin-Positive Mac-2 Binding Protein Values Predict the Development of Hepatocellular Carcinoma among Patients with Chronic Hepatitis C after Sustained Virological Response

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Abstract

Measurement of *Wisteria floribunda*agglutinin-positive human Mac-2 binding protein (WFA^+-M2BP) in serum was recently shown to be a noninvasive method to assess liver fibrosis. The aim of this study was to evaluate the utility of serum WFA^+-M2BP values to predict the development of hepatocellular carcinoma (HCC) in patients who achieved a sustained virological response (SVR) by interferon treatment. For this purpose, we retrospectively analyzed 238 patients with SVR who were treated with interferon in our department. Serum WFA^+-M2BP values were measured at pre-treatment (pre-Tx), post-treatment (24 weeks after completion of interferon; post-Tx), the time of HCC diagnosis, and the last clinical visit. Of 238 patients with SVR, HCC developed in 16 (6.8%) patients. The average follow-up period was 9.1 years. The cumulative incidence of HCC was 3.4% at 5 years and 7.5% at 10 years. The median pre-Tx and post-Tx WFA^+-M2BP values were 1.69 (range: 0.28 to 12.04 cutoff index (COI)) and 0.80 (range: 0.17 to 5.29 COI), respectively. The WFA^+-M2BP values decreased significantly after SVR ($P < 0.001$). The median post-Tx WFA^+-M2BP value in patients who developed HCC was significantly higher than that in patients who did not ($P < 0.01$). Multivariate analysis disclosed that age (> 60 years), sex (male), pre-Tx platelet count (< $15.0 \times 10^3/\mu L$), and post-Tx WFA^+-M2BP (> 2.0 COI) were associated with the development of HCC after SVR.

Conclusion

Post-Tx WFA+-M2BP (> 2.0 COI) is associated with the risk for development of HCC among patients with SVR. The WFA+-M2BP values could be a new predictor for HCC after SVR.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world [1]. Chronic hepatitis C virus (HCV) infection is a major cause of HCC. Millions of people are persistently infected with HCV globally [2–4] and these individuals are at high risk of developing HCC [5–7]. Several studies have demonstrated that interferon (IFN) treatment in chronic hepatitis C patients reduces the risk for progression of liver disease, HCC, liver-related death, and all-cause mortality [8–13], especially in patients who exhibit a sustained virological response (SVR). However, some risk for HCC—albeit a small one—remains even after achieving viral eradication [10,14–19]. Several factors have been reported to affect HCC development among patients with SVR.

Recently, an assay for the measurement of *Wisteria floribunda* agglutinin-positive human Mac-2 binding protein (WFA+-M2BP) was reported as a novel, noninvasive, and rapid bedside method to assess liver fibrosis [20]. M2BP has been shown to have multibranching and sialylated N-glycans. WFA is considered to recognize the GalNAc residue of N-glycans and O-glycans or the clustered LacNAc (Gal-GlcNAc) structure. Currently, we are analyzing the glycan structures of WFA+-M2BP in detail using MS-based technology [21]. Glycans can reflect the differentiation stage of cells but not necessarily the level of cellular damage, and therefore they can be very effective markers for chronic disease. Several reports performed with proteome analysis have identified Mac-2 binding protein as a potential marker of liver fibrosis progression [22–25]. Kuno et al. were the first to report that a rapid and simple glycan-based immunoassay for WFA+-M2BP can quantify fibrosis [20,26]. On the other hand, we reported that AFP and WFA+-M2BP values are noninvasive predictive markers for the development of HCC in patients with HCV [27,28]. In this report we evaluated the utility of WFA+-M2BP values to predict the development of HCC in patients who had achieved SVR after IFN treatment.

Patients and Methods

Patients

From December 1989 to December 2010, a total of 601 consecutive HCV patients who received IFN treatment and achieved SVR at the National Hospital Organization Nagasaki Medical Center were enrolled in this retrospective study. The diagnosis of chronic HCV infection was based on continuous positivity for both anti-HCV by a second or third-generation enzyme-linked immunoadsorbent assay (ELISA) and positivity for serum HCV RNA by polymerase chain reaction (PCR). Before treatment, HCC was definitively ruled out either by ultrasonography (US), dynamic computed tomography (CT), or magnetic resonance imaging (MRI) on enrollment. Exclusion criteria for this study were: (1) positivity for hepatitis B surface antigen; (2) positivity for human immunodeficiency virus; (3) autoimmune hepatitis or primary biliary cirrhosis; (4) a shorter follow-up period (< 12 months) after the completion of IFN treatment; (5) a history of HCC at the time of IFN treatment; (6) development of HCC within 12 months after the completion of IFN treatment; (7) administration of low dose long-term IFN treatment; and (8) absence of properly stored serum samples or insufficient archival material. After

the exclusions, 238 patients who achieved SVR were analyzed retrospectively for the risk factors of HCC.

For all patients in our cohort, a blood sample was taken on the days of the administration of IFN treatment (pre-treatment; pre-Tx), 24 weeks after completion of IFN treatment (post-treatment; post-Tx), and on the days of HCC diagnosis and last clinical visit. All separated serum samples were stored at -20°C until use. Medical histories, along with the results of routine tests for blood cell counts, liver biochemistry and HCV viral load/genotype at the time of IFN treatment and thereafter, were retrieved from medical records. Complete blood cell counts and biochemical tests were performed using automated procedures in the clinical pathologic laboratories of our hospital.

Histological evaluation

Liver biopsies were undertaken using fine-needle aspiration (16G or 18G sonopsy) guided by US. Liver tissue specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. The histological assessment was made by two independent pathologists according to the classification of Desmet et al. [29].

Interferon treatment

Among the 238 patients, 123 received IFN monotherapy for 24 weeks, 28 patients received pegylated (PEG-)IFN monotherapy for 48 weeks, and 87 patients received IFN plus ribavirin or PEG-IFN plus ribavirin combination therapy for 48–72 weeks.

HCV RNA and HCV genotypes

The presence of HCV RNA was determined by reverse transcriptase (RT-) PCR using a commercial kit (Amplicor HCV; Roche Diagnostic Systems, Basel, Switzerland). Genotypes of HCV were determined by RT-PCR with genotype-specific primers (HCV RNA core genotype; Roche Diagnostics, Tokyo, Japan) [30,31]. In patients treated before the availability of PCR, the presence of HCV RNA was investigated by using sera stored at -20°C.

Definitions of response to interferon treatment

SVR was defined as the absence of detectable HCV RNA at 24 weeks after the end of IFN treatment. There was no relapse of viremia after 24 weeks among the patients who achieved SVR.

Measurement of Wisteria floribunda agglutinin-positive human Mac-2 binding protein (WFA$^+$-M2BP)

WFA$^+$-M2BP quantification was performed based on a lectin-antibody sandwich immunoassay using a fully automatic HISCL-2000i immunoanalyzer (Sysmex Co., Hyogo, Japan) [20]. The measured values of WFA$^+$-M2BP conjugated to WFA were indexed with the values obtained using the following equation:

$$\text{Cutoff index(COI)} = ([\text{WFA}^+\text{-M2BP}]_{\text{sample}} - [\text{WFA}^+\text{-M2BP}]_{\text{NC}}) / ([\text{WFA}^+\text{-M2BP}]_{\text{PC}}) - [\text{WFA}^+ - -\text{M2BP}]_{\text{NC}}$$

Here, $[\text{WFA}^+\text{-M2BP}]_{\text{sample}}$ represents the WFA$^+$-M2BP count of the serum sample (PC, positive control; NC, negative control). The positive control was supplied as a calibration solution preliminarily standardized to yield a COI value of 1.0 [26].

Follow-up and diagnosis of hepatocellular carcinoma

All patients were followed up at an interval of 1–12 months by measurement of blood count and liver biochemistry, along with quantitative detection of HCV RNA, AFP, AFP-L3, and DCP. Diagnostic imaging either by US, CT, or MRI was performed at least once per year. A diagnosis of HCC was made based on positive results of typical vascular patterns, as revealed by either contrast-enhanced CT, contrast-enhanced MRI or angiography. Otherwise, the pathological diagnosis was made by fine-needle biopsy of space-occupying lesions detected in the liver.

Ethical considerations

Informed consent to utilize medical records and specimens was obtained from each patient. We obtained the written consent of participants at the time of serum collection. These processes and the study protocol were approved by the Ethical Committee of National Hospital Organization Nagasaki Medical Center (confirmation number: 25102), and conformed with the 1975 Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research (Ministry of Health, Labor, and Welfare of Japan, Ethical Guidelines for Clinical Research, 2008). Our research is available on the National Hospital Organization Nagasaki Medical Center website (http://www.nagasaki-mc.jp/).

Statistical analysis

Continuous variables (AST, ALT, albumin, total bilirubin, γ-GTP, fasting blood sugar, HbA1c, triglyceride, total cholesterol, BMI, platelet counts, AFP, WFA^+-M2BP) were dichotomized with respect to the median value or clinically meaningful values in the multivariate analysis. Statistical analysis was performed using a Wilcoxon signed rank test and Mann-Whitney U-test. To estimate the cumulative risk of developing HCC, the Kaplan-Meier method and the log-rank test were used. Cox proportional hazards regression analysis was performed to evaluate risk factors for HCC. The diagnostic performances of WFA^+-M2BP and AFP for censored development of HCC were assessed by examining the area under the time-dependent receiver operating characteristic (ROC) curves (AUROC) [32]. Inclusion of variables was assessed using a stepwise selection method. A P value of 0.05 was considered statistically significant. Data analysis was performed with SPSS ver. 22.0 (SPSS, Chicago, IL).

Results

Patient characteristics

The baseline characteristics of the 238 patients are summarized in Table 1. The median age was 55.0 years; 147 (61.8%) patients were male; and 104 (43.7%), 68 (28.6%), 42 (17.6%) and 24 (10.1%) patients were diagnosed histologically with fibrosis stage F1, F2, F3 and F4, respectively. The median value of the pre-Tx platelet count was 16.0 (range: 6.4 to 33.2 $\times 10^3/\mu L$) and that of the post-Tx platelet count was 16.8 (range: 6.5 to 36.3 $\times 10^3/\mu L$). The median value of pre-Tx AFP was 5 (range: 1 to 200 ng/mL) and that of post-Tx AFP was 3 (range: 1 to 46 ng/mL). The average follow-up period was 9.1 years.

Cumulative incidence of HCC

During the follow-up period, HCC developed in 16 (6.8%) of the 238 patients. The cumulative incidences of HCC at 5 and 10 years were 3.4% and 7.5%, respectively.

Table 1. Characteristics of Patients Enrolled in the Present Study.

Factors	Value
Patients, n	238
Age, year	55.0 (18–75)
Male, n (%)	147 (61.8)
BMI (kg/m^2)	23.20 (16.7–34.9)
Alcohol intake (> 20g/day), n (%)	64 (26.9)
Fibrosis stage, n (%) F 1/2/3/4	104 (43.7)/68 (28.6)/42 (17.6)/24 (10.1)
Steatosis (≥ 10%), n (%)	25(10.5)
Pre-Tx platelet counts (×10^3/μL)	16.0 (6.4–33.2)
Post-Tx platelet counts (×10^3/μL)	16.8 (6.5–36.3)
Albumin (g/dL)	4.30 (2.9–5.5)
Pre-Tx AST (IU/mL)	60.0 (12–365)
Post-Tx AST (IU/mL)	20.0 (10–54)
Pre-Tx ALT (IU/mL)	100.0 (12–519)
Post-Tx ALT (IU/mL)	17.0 (7–64)
γ-GTP (IU/L)	37.0 (7–1790)
T. Bilirubin (mg/dL)	0.70 (0.3–1.9)
HbA1c (%)	5.70 (4.4–8.1)
Pre-Tx AFP (ng/mL)	5.0 (1–200)
Post-Tx AFP (ng/mL)	3.0 (1–46)
Pre-Tx WFA$^+$-M2BP (COI)	1.70 (0.28–12.04)
Post-Tx WFA$^+$-M2BP (COI)	0.80 (0.17–5.29)
HCV serogroup, n (%)	
1	111 (46.6)
2	101 (42.4)
Unknown	26 (11.0)
IFN regimen, n (%)	
IFN monotherapy	123 (51.6)
PEG-IFN monotherapy	28 (11.8)
IFN/PEG-IFN+RBV	87 (36.6)
Observation period, years	9.1 (5.6) *

Data are given as the medians with ranges.

*Results are expressed as the means ± standard deviation. Unless otherwise indicated, data were collected at pre-treatment (before administration of IFN therapy; pre-Tx). Several biochemical measurements were made at both pre-Tx and post-treatment (24 weeks after completion of IFN therapy; post-Tx).

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin; BMI, body mass index; AFP, α-fetoprotein; HCV, hepatitis C virus; PEG-IFN, pegylated interferon; RBV, ribavirin.

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Risk factors for HCC

Univariate analysis demonstrated factors that increase the risk for HCC development after SVR. Cox regression analysis was performed on 20 variables: age, sex, BMI, alcohol intake, fibrosis stage, degree of steatosis, pre-Tx platelet counts, post-Tx platelet counts, albumin, pre-Tx AST, post-Tx AST, pre-Tx ALT, post-Tx ALT, γ-GTP, T.bilirubin, HbA1c, pre-Tx AFP, post-Tx AFP, pre-Tx WFA$^+$-M2BP, post-Tx WFA$^+$-M2BP. Cutoff values for AFP and WFA$^+$-M2BP were determined by time-dependent ROC analysis as 5 ng/ml and 2.0 COI, respectively.