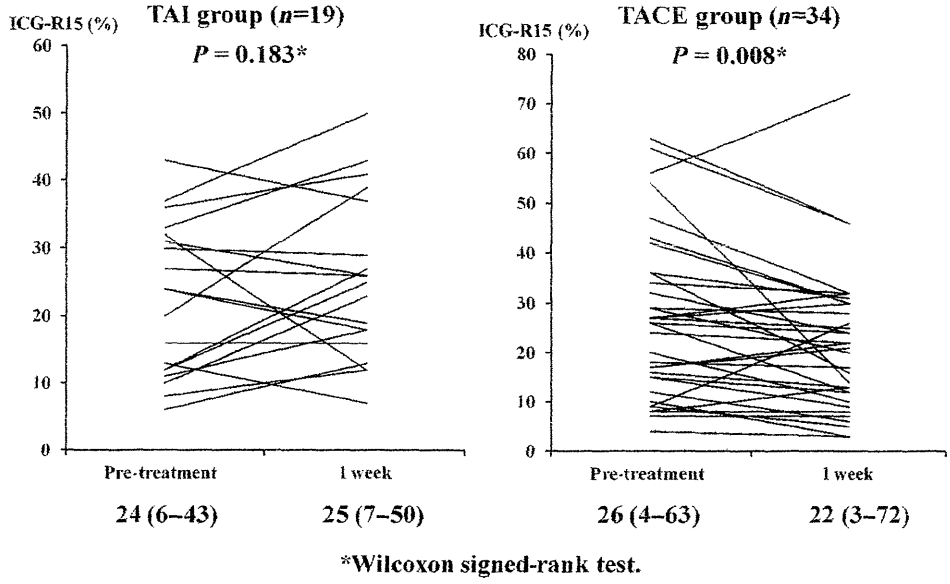

Figure 1. Comparison of indocyanine green retention rate at 15 min (ICG-R15) before and 1 week after miriplatin administration. Although there were no significant changes in ICG-R15 before and after miriplatin administration in the TAI group, ICG-R15 values were significantly lower 1 week after miriplatin administration in the TACE group.

values determined before and 1 week after miriplatin administration [AFP ($P = 0.004$) in the TAI group; AFP ($P < 0.0001$) and DCP ($P = 0.001$) in the TACE group (α level was $P = 0.016$)]. ICG-R15 was assessed before and 1 week after miriplatin administration in 53 patients from the two groups. Although there were no significant differences seen in the TAI group, ICG-R15 values were significantly decreased 1 week after miriplatin administration in the TACE group (Fig. 1).

Multivariate Analysis for Factors Associated with Objective Response

We evaluated variables for association with objective response (complete or partial) after treatment using miriplatin. Univariate analysis identified the following 10 factors that were associated with objective response: a *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) $\leq$10% ($P = 0.0005$), first-time transcatheter arterial chemotherapy ($P = 0.005$), the use of gelatin particles (TACE, $P = 0.007$), solitary tumor ($P = 0.004$), injection artery (peripheral to segmental hepatic artery, $P = 0.049$), AFP $\leq$ 30 μg/l ($P = 0.053$), DCP $\leq$ 40 AU/l ($P = 0.03$), total bilirubin $\leq$1.0 mg/dl ($P = 0.011$), lactate dehydrogenase $\leq$210 IU/l ($P = 0.057$) and hemoglobin >11.0 g/dl ($P = 0.051$; Table 4).

These parameters were subjected to multivariate logistic regression analysis. Objective response was significantly associated with AFP-L3 $\leq$10% [$P = 0.004$; risk ratio = 3.09; 95% confidence interval (CI) = 1.42–6.70], first-time transcatheter arterial chemotherapy ($P = 0.007$; risk ratio = 4.41; 95% CI = 1.49–13.07) and patients undergoing TACE ($P = 0.021$; risk ratio = 2.97; 95% CI = 1.17–7.49; Table 4).

Adverse Effects

The adverse effects occurring after miriplatin administration are summarized in Table 5.

Fever, anorexia and elevation of serum transaminase levels were observed in most patients after miriplatin administration. Grade 4 neutrocytopenia was seen in one patient (1%) in the TACE group; Grade 4 aspartate aminotransferase elevations were seen in one patient (3%) in the TAI group and four patients (3%) in the TACE group; and Grade 4 alanine aminotransferase elevation was seen in one patient (1%) in the TACE group. Increases in serum alanine aminotransferase levels and anorexia tended to occur more frequently in the TACE group. Hepatic abscess was observed in one patient (3%) in the TAI group and one patient (1%) in the TACE group ($P = 0.403$). Resolution of all abscesses was achieved using continuous administration of antibiotic drugs without drainage.

All patients with adverse effects recovered within 2 weeks. No vascular complications involving the hepatic artery were observed among the 68 patients who again underwent angiography 3–6 months after miriplatin administration. No other serious complications or treatment-related deaths were observed. There were no other significant differences in adverse effects between the two groups.

DISCUSSION

TACE is widely performed for patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized control trials and meta-analysis (8–10,12,13). Various anticancer drugs, such as doxorubicin hydrochloride, epirubicin hydrochloride,

Table 4. Univariate and multivariate analyses for predictors of objective response (logistic regression analysis)

	Category	Univariate		Multivariate	
		Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Lens culinaris agglutinin-reactive fraction of AFP	1: ≤10; 0: >10	3.53 (1.73–7.20)	0.0005	3.09 (1.42–6.70)	0.004
First-time transcatheter arterial chemotherapy	1: yes; 0: no	3.32 (1.42–7.74)	0.005	4.41 (1.49–13.07)	0.007
Use of gelatin particles	1: yes; 0: no	2.79 (1.31–5.93)	0.007	2.97 (1.17–7.49)	0.021
Tumor multiplicity	1: solitary; 0: multiple	4.12 (1.56–10.85)	0.004		
Tumor size	1: ≤19 mm; 0: >19 mm	–	0.725		
Injection artery	1: peripheral to segmental hepatic artery; 0: others	2.45 (1.00–6.03)	0.049		
AFP (μg/l)	1: ≤30; 0: >30	1.88 (0.99–3.56)	0.053		
DCP (AU/l)	1: ≤40; 0: >40	2.04 (1.07–3.88)	0.030		
Total bilirubin (mg/dl)	1: ≤1.0; 0: >1.0	2.25 (1.19–4.25)	0.011		
Lactate dehydrogenase (IU/l)	1: ≤210; 0: >210	1.84 (0.98–3.45)	0.057		
Hemoglobin (g/dl)	1: ≤11.0; 0: >11.0	0.514 (0.26–1.00)	0.051		

CI, confidence interval.

Table 5. Adverse effects after miriplatin administration

Grade	Number of TAI patients ($n = 40$)				Number of TACE patients ($n = 122$)				*P* value[a]
	1	2	3	4	1	2	3	4	
White blood cell decreased	2 (5%)	12 (30%)	1 (3%)	0	1 (1%)	27 (22%)	7 (6%)	0	0.204
Neutrophil count decreased	1 (3%)	8 (20%)	0	0	2 (2%)	21 (17%)	5 (4%)	1 (1%)	0.694
Anemia	10 (25%)	8 (20%)	3 (8%)	0	40 (33%)	21 (17%)	3 (2%)	0	0.425
Platelet count decreased	18 (45%)	12 (30%)	2 (5%)	0	72 (59%)	21 (17%)	11 (9%)	0	0.203
Aspartate aminotransferase increased	16 (40%)	8 (20%)	9 (22%)	1 (3%)	55 (45%)	23 (19%)	30 (25%)	4 (3%)	0.983
Alanine aminotransferase increased	15 (37%)	8 (20%)	2 (5%)	0	54 (44%)	12 (10%)	19 (16%)	1 (1%)	0.080
Fever	17 (42%)	2 (5%)	0	0	67 (55%)	14 (11%)	0	0	0.082
Anorexia	10 (25%)	0	0	0	56 (46%)	1 (1%)	0	0	0.050
Nausea	6 (15%)	0	0	0	23 (19%)	0	0	0	0.581
Abdominal pain	3 (8%)	3 (8%)	0	0	22 (18%)	4 (3%)	0	0	0.168
Hepatic infection	0	0	1 (3%)	0	0	0	1 (1%)	0	0.403

[a]*P* values were analyzed by the χ^2 test.

mitomycin C, cisplatin and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified (13–15).

Although TACE can be repeated in most patients, therapeutic efficacy cannot be maintained by repeating TACE using the same anticancer drug if the tumor is thought to be resistant to it. Various types of resistance to therapy can occur during repeated TACE. Platinum derivatives are frequently administered to patients with advanced HCC that has become unresponsive to anthracycline and antibiotic drugs (24,25).

Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin (16–19). Miriplatin–lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where active derivatives of miriplatin are gradually released. According to pharmacokinetic studies, the plasma concentration of total platinum in patients treated with miriplatin–lipiodol

suspension is much lower than the concentration in patients who are administered intra-arterial cisplatin; the C_{max} is ~300-fold lower and the T_{max} roughly 500-fold longer than the corresponding values for intra-arterial cisplatin. Miriplatin–lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC), which is the active platinum compound that binds to nuclear DNA and mediates miriplatin–lipiodol cytotoxicity. Also, in a cisplatin-resistant rat hepatoma cell line model, DPC did not show cross-resistance with cisplatin (26).

Clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy and safety of adding embolizing agents has not been evaluated. Some clinical studies have demonstrated the efficacy and safety of TACE with miriplatin (27–29). To the best of our knowledge, there have not been any clinical studies comparing TACE with TAI. There is an ongoing Phase III trial comparing miriplatin and epirubicin used as TACE agents (JapicCTI-080632[ja]). Although the endpoint of this trial is overall survival, results on the survival benefits of each therapy will be reported within the next several years.

In the present study using miriplatin–lipiodol suspension, the addition of an embolizing agent led to a more favorable result compared with TAI alone, as assessed 1–3 months after TACE and TAI treatments. Additionally, no serious adverse events and no vascular complications were observed with the addition of embolizing agents. In comparisons of ICG-R15 values before and 1 week after administration of miriplatin–lipiodol suspension, the ICG-R15 was only significantly decreased in the TACE group. Improvement of hepatic arterial flow caused by tumor artery embolization may be a reason that the ICG-R15 values decreased in the TACE group.

By multivariate analysis, AFP-L3 values, no previous transcatheter arterial chemotherapy, and the use of gelatin particles (TACE) were highly correlated with objective response after miriplatin–lipiodol suspension administration. Among these factors, AFP-L3 and no previous transcatheter arterial chemotherapy might be considered as surrogate markers for tumor sensitivity to chemotherapy and grade of malignancy.

Previous studies have reported that complete tumor necrosis after TACE provided favorable long-term survival in HCC patients (7,30). In this study, tumor response occurred after TACE using miriplatin–lipiodol suspension. Our results together with the results of previous studies suggest that transcatheter arterial chemotherapy using miriplatin–lipiodol suspension and embolizing agents may provide a more favorable prognosis than arterial infusion alone for patients with HCC.

Recently, a drug-eluting bead has been developed to enhance drug delivery to tumors and reduce systemic exposure. Conventional TACE and TACE with drug-eluting beads are increasingly being performed in Western countries. A prospective, controlled, randomized study comparing TACE using doxorubicin-loaded microspheres with TACE using conventional doxorubicin showed that there were no significant differences in the rates of CR, objective response, and control of disease (31). Patients with the Child–Pugh class B disease, ECOG score of 1, bilobar disease or recurrence after curative treatment benefited more from TACE using doxorubicin-loaded microspheres than from conventional TACE. Both conventional TACE and TACE using drug-eluting beads are potent palliative options for the treatment of HCC. Additional clinical studies are needed to assess patient selection and verify the survival benefits of conventional TACE using miriplatin and TACE using miriplatin-eluting beads.

Since this was a retrospective study, the patients were not randomized with respect to TACE or TAI treatments. A prospective study is needed to assess the safety and efficacy of TACE using miriplatin–lipiodol suspension. In addition, there should be more study to determine the most effective, least toxic anticancer agent among the various available antitumor agents used for TACE.

In conclusion, the combination of embolizing agents with miriplatin–lipiodol suspension can be used safely for patients with unresectable HCC. Assessments performed shortly after treatments showed that the rate of objective response was significantly higher in the TACE patient group than in the TAI group after transcatheter arterial chemotherapy using miriplatin–lipiodol suspension.

Authors' contribution

N.I.: study concept and design, database management and statistical analysis, and writing the paper; K.I.: study concept and design and study supervision; Y.K.: data collection; H.S.: data collection; T.H.: data collection; N.A.: data collection; M.K.: data collection; S.S.: data collection; F.S.: data collection; Y.S.: data collection; Y.A.: study supervision; and H.K.: study supervision.

Conflict of interest statement

The following authors have received honoraria (lecture fees) from Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan: Hiromitsu Kumada, Kenji Ikeda, Yasuji Arase, Yoshiyuki Suzuki, Fumitaka Suzuki and Norio Akuta.

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Hepatology Research 2012; 42: 264–272 doi: 10.1111/j.1872-034X.2011.00915.x

Original Article

Difference in malignancies of chronic liver disease due to non-alcoholic fatty liver disease or hepatitis C in Japanese elderly patients

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Aim: Malignancies that include hepatocellular carcinoma often occurred in patients with chronic liver disease. The aim of this retrospective match control study was to assess the cumulative development incidence and predictive factors for total malignancies in elderly Japanese patients with non-alcoholic hepatic diseases (NAFLD) or hepatitis C virus (HCV).

Methods: A total of 1600 NAFLD patients with age of ≥60 years were enrolled, and 1600 HCV patients with age of ≥60 years were selected as control by matching 1:1 with NAFLD group for age, sex, and follow-up period. The primary goal is the first development of malignancies. Evaluation was performed by the use of the Wilcoxon rank sum test, the Kaplan–Meier method, and Cox proportional hazard model. The mean observation period is 8.2 years in both NAFLD and HCV group, respectively.

Results: The number of patients with the development of malignancies was 167 in the NAFLD group and 395 in the HCV group. The 10th development rate of malignancies was 13.9% in the NAFLD group and 28.2% in the HCV group (risk ratio 2.27; $P < 0.001$). The incident rates of hepatocellular carcinoma in all the malignancies were 6.0% (10/167) in the NAFLD group and 67.6% (267/395) in the HCV group ($P < 0.001$). The malignancies in the NAFLD group were observed in the following order: gastric cancer 34 cases (20.4%) > colon cancer 31 cases (18.6%) > prostate cancer 21 cases (12.6%).

Conclusions: The incident rates of hepatocellular carcinoma in all the malignancies were approximately 6% in the NAFLD group and two-thirds in the HCV group.

Key words: carcinogenesis, hepatitis C virus, non-alcoholic fatty liver disease

INTRODUCTION

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) is one of the more common causes of chronic liver disease worldwide.[1–6] NAFLD is considered to be the liver component of metabolic syndrome.[7,8] It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and diabetes mellitus type 2

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Specific author contributions: Yasuji Arase: design, data collection, data analysis, manuscript development and overseeing; Mariko Kobayashi: design, data collection, data analysis, manuscript development; Fumitaka Suzuki, Yoshiyuki Suzuki, Norio Akuta, Norihiro Imai, Hitomi Sezaki, Masahiro Kobayashi, Naoki Matsumoto, Satoshi Saito, Tetsuya Hosaka, Kenji Ikeda: data collection; Yusuke Kawamura, Yuki Ohmoto, Kazuhisa Amakawa, Shiun Dong Hsieh, Kyoko Ogawa, Maho Tanabe, Hirhoshi Tsuji: data collection; Hiromitsu Kumada: design, data collection, data analysis, manuscript development and overseeing; Tetsuro Kobayashi: manuscript development and overseeing.

Conflict of interest: None.
Financial support: None.
Potential competing interests: None.
Received 14 July 2011; revision 13 September 2011; accepted 16 September 2011.

(T2DM).[9–13] In addition, NAFLD sometimes progressed to non-alcoholic steatohepatitis (NASH). In patients with cirrhotic NASH, liver-related events such as hepatocellular carcinoma (HCC) and liver failure are one of the main causes of morbidity and mortality.[14] However, studies on prolonged prognosis of NAFLD are few in Japan. Thus, the true prevalence and natural history of NAFLD in Japanese patients are still unclear.

On the other hand, hepatitis C virus (HCV) often causes liver cirrhosis and HCC.[15–18] The majority of HCC is ascribed to hepatitis viruses, of which 70–80% corresponding to approximately 35 000 per year is attributed to the persistent infection with HCV in Japan. However, studies on malignancies other than HCC are few in the HCV patients.

With this background in mind, the present study was initiated to investigate the cumulative incidence and risk factors of malignancies that includes HCC after prolonged follow-up in elderly Japanese patients with NAFLD or HCV. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

METHODS

Patients

THE NUMBER OF patients who were diagnosed with fatty liver by the ultrasonography (US) between January 1994 and December 2007 in the Health Management Center and/or Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 10 810. Of these, 1600 Japanese patients satisfied the following enrolled criteria; (i) age of ≥60 years; (ii) daily alcohol intake of <20 g/day; (iii) negativity for hepatitis B surface antigens (HBsAg), hepatitis C virus antibodies, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (iv) the absence of malignancies by gastrofiberscope, abdominal US, chest X-ray, and/or chest computed tomography (CT); (v) annual examination for health screening; and (vi) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Patients with either of the following criteria were excluded from the study: (i) they had illnesses that could seriously reduce their life expectancy; and (ii) they had history of carcinogenesis. In the same period, 7189 HCV patients without fatty liver determined by US were followed in the same hospital. Seven inclusion criteria and two exclusion criteria described in NAFLD group were applied to 2575 of these 7189 HCV patients without fatty liver. Thus, a total of 1600 NAFLD patients with age of ≥60 years were enrolled, and 1600 HCV patients with age of ≥60 years were selected as controls by matching 1:1 with the NAFLD group for age, sex, and follow-up period.

Patients were classified into three groups according to fasting plasma glucose (FPG): (i) those with FPG level of <109 mg/dL (normal glucose group); (ii) those with FPG level of 109–125 mg/dL (pre-diabetes group); and (iii) those with FPG level of ≥126 mg/dL (diabetes group).[19] Patients were regarded as hypertensive by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic on at least three visits. We considered persons smokers if they had smoked a cigarette at the initiation of follow-up.

The primary goal is the development of malignancies. The diagnosis of malignancies was made due to tumor marker, imaging (US, CT or magnetic resonance imaging [MRI]), and/or histological examination.[20–27] All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Medical evaluation

Diagnosis of fatty liver was based on the presence of an ultrasonographic pattern consistent with bright liver with stronger echoes in the hepatic parenchyma than in the renal parenchyma.[28] US test was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo Japan. Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured in light clothing and without shoes to the nearest 0.1 Kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg)/height (in m^2). All the patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits including questions on alcohol intake and smoking history.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche, Tokyo,

Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The used serum samples were stored at −80°C at the first consultation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA.

Follow-up

We used 60 years of age as the starting point for observations in 1417 patients (NAFLD, 694 patients; HCV, 723 patients) who came to our hospital before the age of 60. In 1783 patients (NAFLD, 906 patients; HCV, 877 patients) who came after the age of 60, the day of first visit was used as the start of observations. All patients were followed up at least twice a year by monitoring hematological and biochemical data. Imaging examinations were done approximately once a year for each patient, using abdominal-US and Chest X-ray. Moreover, the patients were checked for tumor marker (carcinoembryonic antigen [CEA], α-fetoprotein [AFP], and prostate-specific antigen [PSA]), gastrofiberscope (or gastrography), and occult blood test of feces at least one year. Two hundred and eighty-two patients were lost to follow-up. Because the appearance of malignancy was not identified in these 282 patients, they were considered as censored data in statistical analysis.[29] Patients treated with antiviral agents were regarded as withdrawals at the time of having the negativity of HCV RNA level by the Amplicor method.

Statistical analysis

Clinical differences between the NAFLD group and HCV group were evaluated by Wilcoxon rank sum test or Fisher's exact test. The cumulative development rates of malignancies were calculated by using the Kaplan–Meier technique, and differences in the curves were tested using the log-rank test.[30] Independent risk factors associated with malignancies were studied using the stepwise Cox regression analysis.[31] The following 15 variables were analyzed for potential covariates for incidence of primary goals in NAFLD group and HCV group: age, gender, body mass index, hypertension, current smoking, albumin, triglyceride, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), fasting plasma glucose, platelet, and AFP at the initiation time of follow-up. A *P*-value of less than 0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL, USA).

RESULTS

Characteristics of the patients enrolled

TABLE 1 SHOWS the baseline characteristics of the 1600 patients in NAFLD group and the 1600 patients in the HCV group at the initiation of follow-up. There are significant differences in several baseline characteristics such as body mass index, AST, ALT, triglyceride, total cholesterol, fasting plasma glucose, platelet, AFP between the HCV group and NAFLD group as shown in Table 1.

Development of malignancy

A total of 562 subjects (167 in NAFLD group and 395 in HCV group) developed malignancy during follow-up. The cumulative development rate of carcinogenesis at the 10th year was determined to be 13.9% in the NAFLD group and 28.2% in the HCV group by the use of the Kaplan–Meier method (Fig. 1). The development rate of each malignancy in both groups is shown in Table 2. The malignancies in the NAFLD group were observed in the following order: gastric cancer 34 cases (20.4%) > colon cancer 31 cases (18.6%) > prostate cancer 21 cases (12.6%). On the other hand, HCC in the HCV group accounted for two-thirds of malignancy. The development rates per 1000 person years in HCC and malignant lymphoma in the HCV group was statistically higher than those in the NAFLD group. However, there were no significant differences in gastric cancer, colon cancer, prostate cancer, and lung cancer between both groups. The incidence rates of HCC in all of the malignancies were 6.0% (10/167) in the NAFLD group and 67.6% (267/395) in the HCV group ($P < 0.001$). Seven of 10 NAFLD patients with development of HCC were evaluated as having histological liver condition at the time of development of HCC. One patient had simple steatosis, and another six patients had non-alcoholic steatohepatitis (NASH). The grade of liver fibrosis in six NASH patients with development of HCC was as follows: grade 1, one patient; grade 2, two patients; grade 3, two patients; grade 4, one patient.

The development rates of each malignancy between the NAFLD group and the HCV group based on the difference of gender are shown in Table 3. The development rates of HCC expressed by 1000 person years in the HCV group were two orders of magnitude higher than those in the NAFLD group in both males and females. There were no significant differences in other malignancies except for HCC between the

Table 1 Patient characteristics at the starting time of follow up†

	NAFLD group	HCV group	*P*-value
n	1600	1600	
Age (years)	62.5 ± 9.5	62.6 ± 8.7	0.936
Gender (male/female)	1200/400	1200/400	1.000
Body mass index	25.1 ± 2.6	21.8 ± 4.0	<0.001
Blood pressure			
(systolic, mmHg)	132 ± 17	133 ± 18	0.972
(diastolic, mmHg)	76 ± 11	77 ± 12	0.937
Hypertension (+/−)	279/1321	306/1294	0.252
Smoking (+/−)	421/1179	396/1141	0.807
AST (IU/L)	29 ± 15	77 ± 64	<0.001
ALT (IU/L)	37 ± 25	104 ± 97	<0.001
GGT (IU/L)	73 ± 79	83 ± 97	0.196
Albumin (g/dL)	4.2 ± 0.3	4.1 ± 0.4	0.883
Triglyceride (mg/dL)	161 ± 105	99 ± 51	<0.001
Total cholesterol (mg/dL)	211 ± 33	176 ± 38	<0.001
FPG (mg/dL)	104.1 ± 10.5	95.8 ± 9.3	<0.001
FPG (DM/pre-DM /normal)	208/330/1062	184/276/1140	<0.001
Platelet ($\times 10^4/mm^3$)	22.1 ± 6.5	15.8 ± 5.8	<0.001
AFP (ng/mL)	3.4 ± 2.4	10.8 ± 10.0	<0.001
Follow-up period (year)	8.2 ± 3.8	8.2 ± 3.9	0.928

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease.

NAFLD group and the HCV group in both males and females.

Predictive factors for the development of malignancies

The factors associated with the development of malignancies in the NAFLD group and HCV group are shown in Tables 4 and 5. In the NAFLD group, multivariate Cox proportional hazards analysis shows that malignancies occurred when patients had an age of ≥70 years (hazard ratio [HR] : 2.10; 95%CI = 1.38–3.17; $P < 0.001$), current smoking (HR : 1.64; 95%CI = 1.18–2.27; $P = 0.003$), and elevated glucose level (HR : 1.32; 95%CI = 1.08–1.61; $P = 0.007$).

On the other hand, in HCV group, multivariate Cox proportional hazards analysis shows that malignancies development rate was high with statistical significance when patients had elevated AFP (HR : 2.52; 95%CI = 1.94–3.44; $P < 0.001$), elevated glucose level (HR : 1.35; 95%CI = 1.18–1.59; $P < 0.001$), elevated AST level (HR : 1.75; 95%CI = 1.13–2.70; $P = 0.010$), hypoalbuminemia (HR : 1.51; 95%CI = 1.15–1.97; $P = 0.002$), male (HR : 1.49; 95%CI = 1.16–1.94; $P = 0.002$), and thrombocytopenia (HR : 1.49; 95%CI = 1.14–1.96; $P = 0.002$).

DISCUSSION

THE DEVELOPMENT INCIDENCE of malignancies in elderly patients with NAFLD or HCV has been described in the present study. The reason for selecting elderly patients is that development of malignancies in patients with age of ≥60 years occur frequently compared with young patients. Thus, it is likely that the difference between NAFLD and HCV patients tends to become clear.

The present study shows several findings with regard to the development of malignancies in elderly Japanese patients with NAFLD or HCV. First, HCC in the NAFLD group accounted for approximately 6% of the cause of malignancies. The four malignancies of the stomach, colon, prostate, and lung accounted for about 60% in the NAFLD group. Matsuda *et al.* have reported the cancer incidence in Japan.[32] According to their report, the outbreak of malignancies in a Japanese male popu-

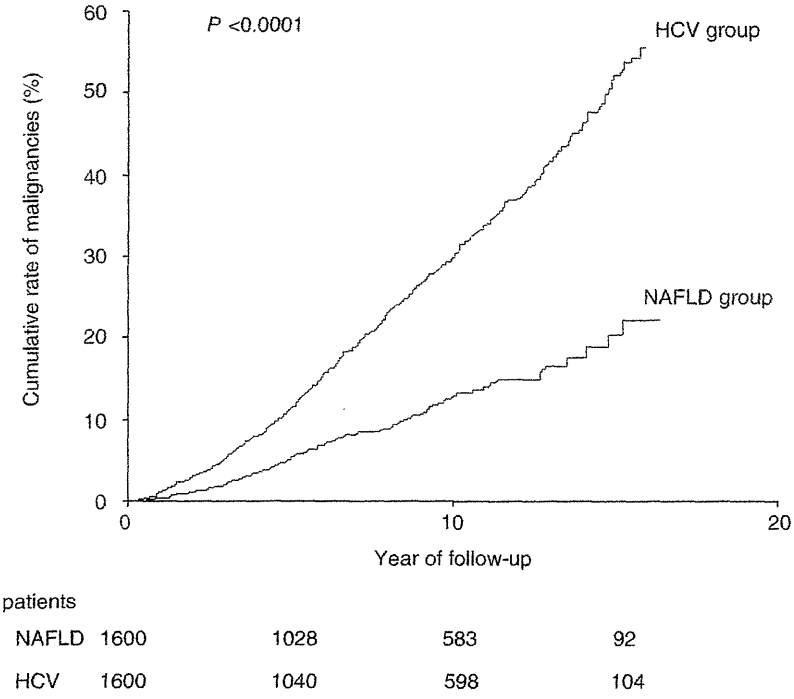

Figure 1 Cumulative development rate of malignancies in non-alcoholic hepatic diseases (NAFLD) or hepatitis C virus (HCV) patients.

lation was observed in the following order in 2005: gastric cancer 20.4% > colon cancer 16.0% > lung cancer 15.4% > prostatic cancer 10.9% > HCC 7.4%. On the other hand, the outbreak of malignancies in a Japanese female population was observed in the following order in 2005: mammary cancer 18.0% > colon cancer 16.2% > gastric cancer 13.6% > lung cancer 9.3% > uterine cancer 6.8%. The incidence of prostate cancer in NAFLD was greater than that in a total Japanese population. Renehan *et al.* showed that body mass index is connected with prostate carcinogenesis relative to other tumours.[33] NAFLD patients might tend to have

Table 2 Development rate of each malignancy in the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group†

Malignancies	NAFLD group		HCV group		*P*‡
	n (%)†	1000 person years	*n* (%)†	1000 person years	
Total	167 (100%)	12.96	395 (100%)	30.88	<0.001
Hepatocellular carcinoma	10 (6.0%)	0.78	267 (67.9%)	20.86	<0.001
Gastric cancer	34 (20.4%)	2.66	28 (7.1%)	2.19	0.522
Colon cancer	31 (18.6%)	2.42	26 (6.6%)	2.03	0.593
Prostate cancer	21 (12.6%)	1.64	14 (3.5%)	1.10	0.308
Lung cancer	17 (10.2%)	1.33	13 (3.3%)	1.02	0.583
Malignant lymphoma	1 (0.6%)	0.08	9 (2.3%)	0.70	0.021
Other cause	46 (27.5%)	3.59	31 (7.8%)	2.43	0.106
Unknown origin	6 (3.6%)	0.46	7 (1.8%)	0.55	1.000

†Data are number of patients (%) and development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between both groups by log rank test.

Table 3 Development rate of Each Malignancy between the non-alcoholic fatty liver disease (NAFLD) group and the hepatitis C virus (HCV) group based on the difference of gender†

Malignancies	Male		*P*‡	Female		*P*‡
	NAFLD ($n = 1200$)	HCV ($n = 1200$)		NAFLD ($n = 400$)	HCV ($n = 400$)	
Total	13.96	34.17	<0.001	10.31	20.93	<0.001
Hepatocellular carcinoma	0.83	23.75	<0.001	0.63	10.83	<0.001
Gastric cancer	2.91	2.40	0.571	1.88	1.39	1.000
Colon cancer	2.42	2.19	0.655	1.88	1.39	1.000
Lung cancer	1.33	1.05	0.676	1.25	0.93	1.000
Malignant lymphoma	0.08	0.63	0.124	0.00	0.93	0.577
Prostate cancer	1.64	1.10	0.306			
Breast cancer				1.81	1.41	1.000
Other cause	3.59	4.38	0.604	2.43	1.71	0.577
Unknown origin	0.46	0.52	1.000	0.30	0.62	1.000

†Data are development rates of each malignancy per 1000 person years. ‡Comparison of new development in each malignancy between NAFLD group and HCV group based on the difference of gender by log rank test

carcinogenesis of prostate based on obesity. Our results show that physicians in charge of NAFLD patients should pay attention to the malignancies of stomach, colon, prostate, and lung in addition to development of HCC. Moreover, aging, hyperglycemia, and smoking were dominating factors to enhance the development of malignancies in NAFLD group.

Second, HCC in the HCV group accounted for about two-thirds of the outbreak of malignancies. In the present study, the development rates of HCC and malignant lymphoma in the HCV group were statistically higher than those in the NAFLD group. The high incidences of HCC and malignant lymphoma have been reported by many reseachers.[15–19,34] Male, hyperglycemia, elevated AST, hypoalbuminemia, thrombocytopenia, and elevated AFP were dominating factors to enhance the development of malignancies in the HCV group. Hypoalbuminemia, thrombocytopenia,

Table 4 Predictive factors for malignancies in the non-alcoholic fatty liver disease (NAFLD) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, ≥70/<70)	2.34 (1.60–3.44)	<0.001	2.09 (1.42–3.07)	<0.001
Gender (M/F)	1.11 (0.76–1.60)	0.631		
BMI (≥25/<25)	0.74 (0.52–1.04)	0.079		
Hypertension (−/+)	1.27 (0.88–1.84)	0.197		
Smoking (+/−)	1.62 (1.18–2.24)	0.003	1.64 (1.18–2.27)	0.003
AST (IU/L, ≥34/<34)	1.03 (0.62–1.70)	0.973		
ALT (IU/L, ≥36/<36)	1.27 (0.76–2.08)	0.357		
GGT (IU/L, ≥109/<109)	1.26 (0.79–2.01)	0.350		
Albumin (g/dL, <3.9/≥3.9)	1.41 (0.90–2.04)	0.145		
Triglyceride (mg/dL, ≥150/<150)	1.20 (0.85–1.69)	0.282		
Total cholesterol (mg/dL, ≥220/<220)	1.39 (0.87–2.23)	0.170		
Glucose (DM/ pre-DM/non-DM)	1.39 (1.14–1.69)	0.001	1.32 (1.08–1.61)	0.007
Platelet ($\times 10^4/mm^3$, <15/≥15)	1.41 (1.02–1.96)	0.036		
AFP (ng/mL, ≥10/<10)	1.11 (0.35–3.48)	0.338		

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase.

Table 5 Predictive factors for malignancies in the hepatitis C virus (HCV) group†

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, ≥70/<70)	1.41 (1.11–1.78)	0.003		
Gender (M/F)	1.78 (1.4692.10)	<0.001	1.49 (1.16–1.94)	0.002
BMI (≥25/<25)	1.85 (0.71–4.85)	0.201		
Hypertension (+/−)	1.20 (1.01–1.44)	0.045		
Smoking (+/−)	1.71 (1.43–2.10)	<0.001		
AST (IU/L, ≥36/<36)	2.26 (1.73–3.01)	<0.001	1.75 (1.13–2.70)	0.010
ALT (IU/L, ≥30/<30)	1.69 (1.33–2.16)	<0.001		
GGT (IU/L, ≥109/<109)	1.99 (1.53–2.58)	0.014		
Albumin (g/dL, <3.9/≥3.9)	2.07 (1.65–2.56)	<0.001	1.51 (1.15–1.97)	0.002
Triglyceride (mg/dL, ≥150/<150)	1.15 (0.56–2.41)	0.789		
Total cholesterol (mg/dL, ≥220/<220)	0.51 (0.19–1.35)	0.159		
Glucose (DM/pre-DM/non-DM)	1.37 (1.23–1.55)	<0.001	1.35 (1.18–1.59)	<0.001
Platelet ($\times 10^4/mm^3$, <15/≥15)	2.28 (1.81–2.92)	<0.001	1.49 (1.14–1.96)	0.002
AFP (ng/mL, ≥10/<10)	3.10 (2.46–4.11)	<0.001	2.50 (1.94–3.44)	<0.001

†Data are number of patients or mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, GGT, gamma-glutamyltransferase.

and elevated AFP indicate the advanced liver fibrosis: it is probable that these factors enhance the HCC development as reported before.[35] Our result shows that HCV positive males with hyperglycemia, hypoalbuminemia, elevated AST, thrombocytopenia, and elevated AFP should be carefully checked for HCC.

Third, there were no significant differences in the development of each malignancy between males and females in the NAFLD group. On the other hand, rare development of HCC in males was statistically higher than that of females. However, there are no significant differences in the development of each malignancy except for HCC between males and females in the HCV group. This result suggests that development differences based on gender except for HCC in HCV group might be not important.

Cirrhotic NASH enhances the liver-related events such as HCC and liver failure. However, most patients with NAFLD do not have NASH. According to Japanese annual health check reports, 9–30% of Japanese adults demonstrate evidence of NAFLD by US. Since it is known that about 10% of individuals with NAFLD have NASH, the prevalence of NASH is estimated to be 1–3% of the adult Japanese population.[14] In patients with cirrhotic NASH, HCC and liver failure are the main causes of morbidity and mortality (5-year cumulative HCC development rate 11.3%, 5-year survival rate 75.2%, respectively). However, in the present study, most NAFLD was thought to be non-NASH. Our results suggest that patients with NAFLD before progression to NASH should be followed up to closely check the malignancies other than HCC in addition to HCC. On the other hand, patients with HCV should be followed up to take care to check liver-related disease containing HCC

The present study was limited that most of the NAFLD patients were not undergoing histological or morphological assessment by peritoneoscopy or liver biopsy before the starting time of follow up owing to their advanced age on the day of the first consulting or normal transaminase. Another limitation was that there are several differences in clinical background such as liver fibrosis between the NAFLD and HCV groups. This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up with a large number of patients included.

Our results indicate the following: (i) Physicians in charge of NAFLD patients should pay attention to the carcinogenesis development of stomach, colon, prostate, and lung containing HCC; and (ii) physicians in charge of HCV patients should closely check for HCC.

ACKNOWLEDGMENTS

THE PRESENT WORK was supported in part by grants-in-aid from Okinaka memorial institute for medical research and Japanese Ministry of Health, Labour and Welfare.

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Research Article

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Dual oral therapy with daclatasvir and asunaprevir for patients with HCV genotype 1b infection and limited treatment options

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See Editorial, pages 643–645

Background & Aims: Improved therapeutic options for chronic hepatitis C virus (HCV) infection are needed for patients who are poor candidates for treatment with current regimens due to anticipated intolerability or low likelihood of response.

Methods: In this open-label, phase 2a study of Japanese patients with chronic HCV genotype 1b infection, 21 null responders (<2 $\log_{10}$ HCV RNA reduction after 12 weeks of peginterferon/ribavirin) and 22 patients intolerant to or medically ineligible for peginterferon/ribavirin therapy received dual oral treatment for 24 weeks with the NS5A replication complex inhibitor daclatasvir (DCV) and the NS3 protease inhibitor asunaprevir (ASV). The primary efficacy end point was sustained virologic response at 12 weeks post-treatment (SVR_{12}).

Results: Thirty-six of 43 enrolled patients completed 24 weeks of therapy. Serum HCV RNA levels declined rapidly, becoming undetectable in all patients on therapy by week 8. Overall, 76.7% of patients achieved SVR_{12} and SVR_{24}, including 90.5% of null responders and 63.6% of ineligible/intolerant patients. There were no virologic failures among null responders. Three ineligible/intolerant patients experienced viral breakthrough and four relapsed post-treatment. Diarrhea, nasopharyngitis, headache, and ALT/AST increases, generally mild, were the most common adverse events; three discontinuations before week 24 were due to adverse events that included hyperbilirubinemia and transaminase elevations (two patients).

Conclusions: Dual therapy with daclatasvir and asunaprevir, without peginterferon/ribavirin, was well tolerated and achieved high SVR rates in two groups of difficult-to-treat patients with hepatitis C virus genotype 1b infection.

Introduction

Therapies for chronic hepatitis C virus (HCV) infection have improved markedly over the past decade. The recent approval of the first direct-acting antivirals (DAAs) was an important milestone in the evolution of HCV therapy, establishing that DAAs can enhance regimen efficacy and provide durable viral clearance. These new agents in combination with peginterferon and ribavirin (PegIFN-α/RBV) achieve overall sustained virologic response (SVR) rates of approximately 70% in treatment-naïve patients with HCV genotype 1 infection [1,2].

Despite these advances, current treatment options remain inadequate for some patients. Patients with prior null response to PegIFN-α/RBV (<2 $\log_{10}$ decline in HCV RNA after 12 weeks) have a particularly acute need for further therapeutic improvements. Null responders generally respond poorly to retreatment with PegIFN-α/RBV; fewer than 10% achieve SVR [3]. Retreatment of null responders with PegIFN-α/RBV combined with telaprevir or boceprevir increases SVR rates to approximately 30–38%, suggesting that addition of a DAA to PegIFN-α/RBV increases efficacy, but that more potent regimens are still urgently needed [4,5]. There are also many patients who cannot be treated with current therapies; this group includes patients with prior intolerance to PegIFN-α/RBV and patients who are ineligible for PegIFN-α/RBV -containing therapy for medical reasons.

There is precedence for use of combination antiviral regimens to treat human immunodeficiency virus (HIV) infections;

Keywords: Daclatasvir; Asunaprevir; Hepatitis C; Antiviral.
Received 15 May 2012; received in revised form 5 September 2012; accepted 30 September 2012; available online 23 November 2012
* DOI of original article: http://dx.doi.org/10.1016/j.jhep.2013.01.007.
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Abbreviations: HCV, hepatitis C virus; DAA, direct-acting antiviral; PegIFN-α/RBV, peginterferon alfa and ribavirin; SVR, sustained virologic response; HIV, human immunodeficiency virus; NS5A, non-structural protein 5A; NS3, non-structural protein 3; ALT, alanine aminotransferase; ULN, upper limit of the normal reference range; INR, international normalized ratio; CYP3A4, cytochrome P450 3A4.

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evidence is mounting that DAA regimens can also provide durable clearance of HCV infections. Thus, there is a strong rationale for exploration of dual DAA regimens, without PegIFN-α/RBV. In combination, DAAs with different molecular targets can increase regimen potency and raise the barrier to resistance, potentially eliminating the need for PegIFN-α/RBV and providing a viable therapy for patients who are anticipated to be poorly responsive or intolerant to current PegIFN-α/RBV-containing regimens. The improved tolerability and convenience that can be anticipated with dual DAA regimens suggests that they may also benefit treatment-naïve patients and other groups. Previous studies of DAA-only regimens, or DAAs combined with RBV, have demonstrated marked antiviral effects in treatment-naïve and experienced patients, including null responders, supporting the further evaluation of dual DAA therapy reported here [6–10].

Daclatasvir (DCV; BMS-790052) is a first-in-class, highly selective NS5A replication complex inhibitor with picomolar potency and broad genotypic coverage; asunaprevir (ASV; BMS-650032) is a potent NS3 protease inhibitor active against genotypes 1 and 4. Daclatasvir and asunaprevir have different modes of action and resistance-associated variants, and in combination show increased antiviral potency *in vitro* and a high genetic barrier to resistance [11,12]. Daclatasvir and asunaprevir had no clinically meaningful pharmacokinetic interaction in healthy volunteers [13]. Initial efficacy evaluations of daclatasvir and asunaprevir (DUAL therapy) showed potent antiviral effects and SVR rates ⩾90% in Japanese and US/European null responders with HCV genotype 1b infection [7,8].

We present final results of an open-label trial evaluating DUAL oral therapy with daclatasvir and asunaprevir in Japanese patients with chronic HCV genotype 1b infection. Initial results from a sentinel cohort of 10 patients with prior null response to PegIFN-α/RBV have been reported [7]. The present report combines these data with results for 11 additional null responders, together with results for 22 patients with prior intolerance to PegIFN-α/RBV or who were medically ineligible for PegIFN-α/RBV-containing therapy.

Materials and methods

Study design

This open label, phase 2a study (AI447-017; clinicaltrials.gov identifier NCT01051414) was conducted in two populations of patients with HCV genotype 1 infection, including null responders (<2 $\log_{10}$ decline of serum HCV RNA levels after 12 weeks of prior PegIFN-α/RBV), and PegIFN-α/RBV ineligible/intolerant patients. The latter group discontinued prior therapy with PegIFN-α/RBV due to intolerance after <12 weeks, or patients were treatment-naïve but poor candidates for PegIFN-α/RBV for medical reasons such as advanced age or complications of depression, anemia, myelosuppression, diabetes, or cardiovascular or renal dysfunction.

Patients were enrolled in two cohorts of null responders and two cohorts of PegIFN-α/RBV ineligible/intolerant patients. One cohort of each population included intensive sampling for pharmacokinetic analyses; both cohorts of each population were combined for efficacy and safety assessments. The sentinel cohort of null responders, reported previously, provided 4-week safety data for review by the study Safety Committee, prior to initiation of the other cohorts [7]. The primary efficacy end point was the proportion of patients with undetectable HCV RNA at 12 weeks post-treatment (SVR_{12}). Key secondary end points included the proportions of patients with HCV RNA undetectable at week 4, week 12, the end of treatment, and post-treatment week 24 (SVR_{24}).

Written informed consent was obtained from all patients. The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local regulatory requirements.

Patients

Eligible patients were men and women aged 20–75 years with HCV genotype 1 infection ⩾6 months and HCV RNA ⩾10^5 IU/ml. Women of childbearing potential were using adequate contraception. Patients were excluded if they had evidence of liver cirrhosis within 24 months of screening by laparoscopy, imaging studies, or liver biopsy; a history of hepatocellular carcinoma, other chronic liver disease, variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis; co-infection with hepatitis B virus or HIV; other clinically significant medical conditions; exposure to any investigational drug or placebo within 4 weeks, or any previous exposure to NS5A or NS3 protease inhibitors.

Exclusionary laboratory findings included alanine aminotransferase (ALT) >5× upper limit of normal (ULN), total bilirubin ⩾2 mg/dl, direct bilirubin >1.5× ULN, international normalized ratio (INR) ⩾1.7, albumin ⩽3.5 g/dl, hemoglobin <9.0 g/dl, white blood cells <1500/mm^3, absolute neutrophils <750/mm^3, platelets <50,000/mm^3, and creatinine >1.8× ULN. Prohibited concomitant medications included CYP3A4 inducers or moderate/strong CYP3A4 inhibitors, non-study medications with anti-HCV activity, prescription or herbal products not prescribed for treatment of a specific condition, proton pump inhibitors, and erythropoiesis-stimulating agents. Prescribed H2 receptor antagonists were administered ⩾2 h after and ⩾10 h prior to daclatasvir; other acid modifying agents were administered ⩾2 h prior and ⩾2 h after daclatasvir.

Study drug dosing

Patients received 24 weeks of treatment with daclatasvir 60 mg once daily (two 30 mg tablets), combined with asunaprevir 200 mg twice daily, with 24 weeks of post-treatment follow-up. In the sentinel cohort of null responders, asunaprevir was initially administered as three 200 mg tablets twice daily (600 mg BID), subsequently reduced to 200 mg BID during treatment following reports from another study of greater and more frequent aminotransferase elevations with the higher dose [14].

Patients with HCV RNA <15 IU/ml on or after week 4 continued treatment to week 24; patients discontinued treatment if HCV RNA decreased <2 $\log_{10}$ IU/ml from baseline on or after week 2. Patients with viral breakthrough on or after week 2, or quantifiable HCV RNA (⩾15 IU/ml) on or after week 4, either discontinued treatment or weight-based PegIFN-α/RBV was added (null responders only), for up to 48 additional weeks, at the discretion of the investigator based on anticipated tolerability. Viral breakthrough was defined as confirmed ⩾1 $\log_{10}$ IU/ml increase from nadir of HCV RNA, or HCV RNA ⩾15 IU/ml after confirmed undetectable. Post-treatment relapse was defined as confirmed HCV RNA ⩾15 IU/ml during follow-up in patients with undetectable HCV RNA at the end of treatment.

Safety and efficacy assessments

HCV RNA, physical examinations, adverse events, laboratory parameters, and concomitant medications were assessed at screening, study days 1 (baseline), weeks 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24, and post-treatment weeks 4, 8, 12, and 24. Twelve-lead electrocardiograms were recorded at all visits except weeks 3 and 6.

Serum HCV RNA levels were determined at a central laboratory using the Roche COBAS® TaqMan® HCV Auto assay, (Roche Diagnostics KK, Tokyo, Japan), lower limit of quantitation 15 IU/ml. HCV genotype and subtype and *IL28B* genotype (rs12979860) were determined by PCR amplification and sequencing. Baseline liver fibrosis was assessed by serum blood markers (APRI; AST and Platelet Ratio Index) [15]. HCV resistance-associated polymorphisms were analyzed in stored baseline samples from all patients and post-failure samples from patients with viral breakthrough or post-treatment relapse. Polymorphisms were analyzed by PCR amplification and population sequencing of the HCV NS3 protease and NS5A domains.

Statistical analysis

Categorical variables were summarized using counts and percents; continuous variables were summarized with univariate statistics.

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Table 1. Baseline demographic and disease characteristics.

Parameter		Null responders n = 21	Ineligible/intolerant n = 22
Age, median yr (range)		61 (31-70)	68 (47-75)
Male, n (%)		8 (38.1)	6 (27.3)
HCV genotype 1b, n (%)		21 (100)	22 (100)
IL28B genotype, n (%)			
(rs12979860)	CT	18 (85.7)	6 (27.3)
	CC	3 (14.3)	16 (72.7)
HCV RNA, mean $\log_{10}$ IU/ml (SD)		6.8 (0.47)	6.6 (0.64)
ALT, mean U/L (SD)		57.9 (24.86)	45.7 (25.79)
APRI score			
	Score >2, n (%)	3 (14.3)	1 (4.5)
	Median (range)	0.96 (0.24-3.41)	0.57 (0.40-2.79)
PegIFN-α/RBV ineligible, n (%)		n.a.	18 (81.8)
PegIFN-α/RBV intolerant, n (%)		n.a.	4 (18.2)

n.a., Not available.

Results

Patient characteristics and disposition

Forty-nine patients were screened of which six failed to meet entry criteria; 21 null responders and 22 ineligible/intolerant patients were enrolled and treated (Table 1). The enrolled population was generally older (median 62 years), consistent with HCV epidemiology in Japan, and primarily female (67%); all patients were Japanese. No patient had prior exposure to HCV DAAs. Although any HCV genotype 1 subtype was permitted, all enrolled patients had genotype 1b infection, reflecting the high proportion of this subtype in Japan [16]. Null responders were primarily *IL28B* genotype CT (rs12979860) as expected [17]; ineligible/intolerant patients were primarily genotype CC, consistent with the distribution of *IL28B* genotypes in Japan [18]. Eighteen ineligible/intolerant patients were treatment-naïve and considered ineligible for PegIFN-α/RBV due to anticipated difficulty in completing therapy due to advanced age (≥70 years) (seven patients), cytopenia (two), depression (two), hypertension (one), or other reasons (six), consistent with common clinical practice in Japan. Four patients had prior PegIFN-α/RBV intolerance due to cytopenia (two patients), depression (one), or other reasons (one). Baseline HCV RNA and ALT levels were similar across patient groups. Although patients with cirrhosis by imaging criteria were excluded, four enrolled patients had APRI scores >2 at baseline, indicating probable cirrhosis [15].

Thirty-six of 43 enrolled patients completed 24 weeks of therapy (Fig. 1). Two null responders discontinued study medication due to hyperbilirubinemia (week 2) and aminotransferase elevation (week 12), respectively. One null responder achieved very low HCV RNA (50 IU/ml) at week 4; however, stringent protocol-defined rules required discontinuation from DAA-only therapy and addition of PegIFN-α/RBV to the dual DAA regimen at week 6. Study drugs were discontinued in four ineligible/intolerant patients due to aminotransferase elevation (week 16), viral breakthrough (week 16), or patient request (weeks 8 and 16); all four patients remained on study for assessment of SVR.

Virologic response

High rates of virologic response were seen at all time points in both study populations (Table 2). Overall, 77% of patients achieved SVR_{12} and SVR_{24}. HCV RNA was undetectable in more ineligible/intolerant patients than null responders at week 4, suggesting a more rapid initial antiviral effect, but HCV RNA was undetectable in similar proportions of both populations at week 12 and the end of treatment. Rates of SVR_{24} were higher in null responders (91%) than in ineligible/intolerant patients (64%) due to virologic failures in the latter group (3 breakthroughs and 4 relapses). Assessment of virologic response by *IL28B* genotype (rs12979860) showed slightly greater responses at weeks 2, 3, and 4 in patients with genotype CC; however, similar proportions of patients with genotypes CC and CT achieved SVR_{24} (Fig. 2). All four patients with possible cirrhosis based on APRI score achieved SVR_{24}.

HCV RNA declined rapidly after initiation of therapy in all patients (Fig. 3). Mean reductions of HCV RNA from baseline at week 4 were 5.6 and 5.4 $\log_{10}$ IU/ml in null responders and ineligible/intolerant patients, respectively; HCV RNA was undetectable by week 8 in all patients on therapy. In the ineligible/intolerant group, initial virologic response in the four intolerant patients was similar to that of the cohort overall; three of these patients subsequently achieved SVR_{24} and one relapsed. The null responder who discontinued at week 2 with hyperbilirubinemia had low-level HCV RNA at discontinuation and undetectable HCV RNA at all post-treatment assessments. The null responder who added PegIFN-α/RBV at week 6 received 46 weeks of quadruple therapy and HCV RNA remained undetectable 24 weeks post-treatment. Among the four ineligible/intolerant patients who discontinued study drugs before week 24, HCV RNA was undetectable at discontinuation (weeks 8 or 16) in three patients and remained undetectable in the two patients who completed post-treatment follow-up.

Viral breakthrough and relapse

No null responders experienced virologic breakthrough or relapse (Table 2). Three ineligible/intolerant patients experienced viral breakthrough at weeks 10 or 16 after ≥4 weeks with undetectable

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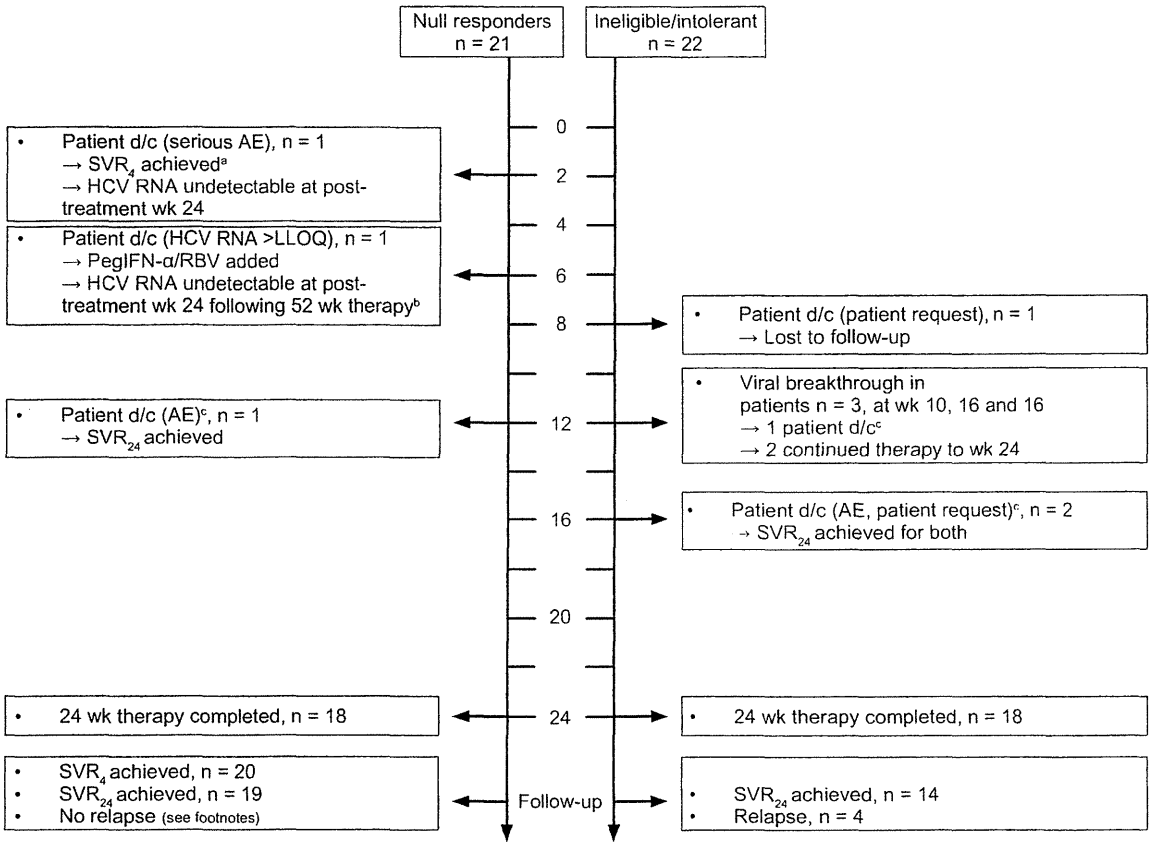

Fig. 1. Patient disposition. Patient flow through treatment and follow-up is shown. d/c, Discontinuation of study medication; SVR_4, SVR_{12} and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment. [a]On-study follow-up continued to post-treatment week 4; HCV RNA remained undetectable at post-treatment week 24 after study discontinuation, reported as failure for SVR_{24} per statistical protocol requirements; [b]HCV RNA was undetectable at post-treatment week 24 after study discontinuation due to addition of PegIFN-α/RBV, reported as failure for SVR per statistical protocol requirements; [c]on-study follow-up to assess SVR continued after discontinuation of study drugs.

Table 2. Virologic outcomes.

n (%)	Null responders, n = 21	Ineligible/intolerant, n = 22
HCV undetectable		
Wk 4 (RVR)	11 (52.3)	19 (86.4)
Wk 12 (cEVR)	19 (90.5)	20 (90.9)
End of treatment	19 (90.5)	19 (86.4)
SVR_4	20 (95.2)[1]	15 (68.2)[2]
SVR_{12}	19 (90.5)[1]	14 (63.6)[2]
SVR_{24}	19 (90.5)[1]	14 (63.6)[2]
Viral breakthrough	0	3 (13.6)
Post-treatment relapse	0	4 (18.2)

Intention to treat (missing = failure) analysis. End of treatment is week 24 or last on-treatment visit for patients who discontinued early.
RVR, rapid virologic response; cEVR, complete early virologic response; SVR_4, SVR_{12}, and SVR_{24}, sustained virologic response 4, 12 or 24 weeks post-treatment.
[1]Two patients discontinued from the study before completion of follow-up. One patient received added PegIFN-α/RBV per protocol criteria and is counted as failure for SVR_4, SVR_{12}, and SVR_{24} for DAA only therapy; one patient had missing HCV RNA data for follow-up weeks 12 and 24 and is counted as failure for SVR_{12} and SVR_{24} per statistical protocol.
[2]One patient was lost to follow-up for assessment of SVR_{12} and SVR_{24}.

serum HCV RNA, and four patients relapsed at post-treatment week 4 (three patients) or 12 (one patient) after ⩾18 weeks with undetectable HCV RNA. All three patients with viral breakthrough were *IL28B* genotype CT (rs12979860), compared with 6/22 ineligible/intolerant patients overall. Three patients who relapsed were *IL28B* genotype CC; one was genotype CT.

Resistance-associated polymorphisms in NS5A and/or NS3 protease were found pretreatment in 33/43 patients overall, most of whom achieved SVR. Daclatasvir and asunaprevir resistance-associated variants were detected post-failure in all seven patients with virologic failure (Table 3). The NS5A-Y93H variant pre-existed in 10/43 study patients, of which five (50%) experienced virologic failure and five (50%) achieved SVR. NS5A-L31 and NS3-D168 substitutions emerged in all failures, but were not detected pretreatment except for NS5A-L31M in one patient.

In general, patients with virologic failure had concurrent asunaprevir and daclatasvir trough concentrations below median values, but within the expected range (Fig. 4). Notably, most patients with trough concentrations below median values achieved SVR. There were no strong associations between virologic failure and pretreatment parameters that included gender, age, baseline HCV RNA level, *IL28B* genotype, reason for PegIFN-

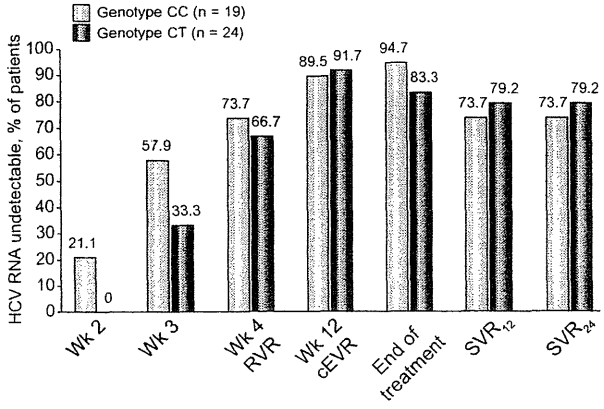

Fig. 2. Outcomes by *IL28B* genotype. Virologic outcomes at milestone time points are shown for the overall population by *IL28B* (rs12979860) genotype. End of treatment is week 24 or the last on-treatment visit for patients who discontinued early. RVR, rapid virologic response; cEVR, complete early virologic response; SVR_{12} and SVR_{24}, sustained virologic response 12 or 24 weeks post-treatment.

α/RBV ineligibility, and fibrosis stage. Adherence to treatment, assessed by pill counts at study visits, was high in six of the seven patients with virologic failure.

Safety

The most frequently reported adverse events were generally mild headache, nasopharyngitis, aminotransferase elevations, and diarrhea (Table 4). The most frequent grade 3 or 4 laboratory abnormalities were serum aminotransferase elevations. There were six serious adverse events in five patients, including grade 2/3 pyrexia (three patients), grade 2 exacerbation of hypochondriasis, and grade 2 gastroenteritis (unrelated to study drugs) with grade 4 hyperbilirubinemia (described in detail previously)

Table 3. Resistance-associated polymorphisms in patients with virologic failure.

	Patient		NS5A				NS3	
			L31	Q54	P58	Y93	Q80	D168
Viral breakthrough	1	Baseline	L/M			Y/H		
		Post-VBT	M		A	H		A
	2	Baseline		Y		Y/H	L	
		Post-VBT	M	Y		H		V
	3	Baseline		Y		H		
		Post-VBT	M	Y		H		V
Post-treatment relapse	4	Baseline			P/S	Y/H		
		Post-relapse	M			H		A
	5	Baseline			L			
		Post-relapse	M		L	H		V/D
	6	Baseline						
		Post-relapse	V			H		V
	7	Baseline				H		
		Post-relapse	V/M			H		V

[7]. All three pyrexia events resolved after 4–10 days with continued study treatment; the hypochondriasis persisted for approximately six months and resolved after completion of study treatment. In the patient who discontinued with hyperbilirubinemia, bilirubin normalized four weeks post-treatment [7]. Serum aminotransferases normalized by four weeks post-treatment in the two patients who discontinued for elevations.

Discussion

High rates of SVR_{24} were achieved after 24 weeks of dual oral DAA therapy in null responders and PegIFN-α/RBV ineligible or

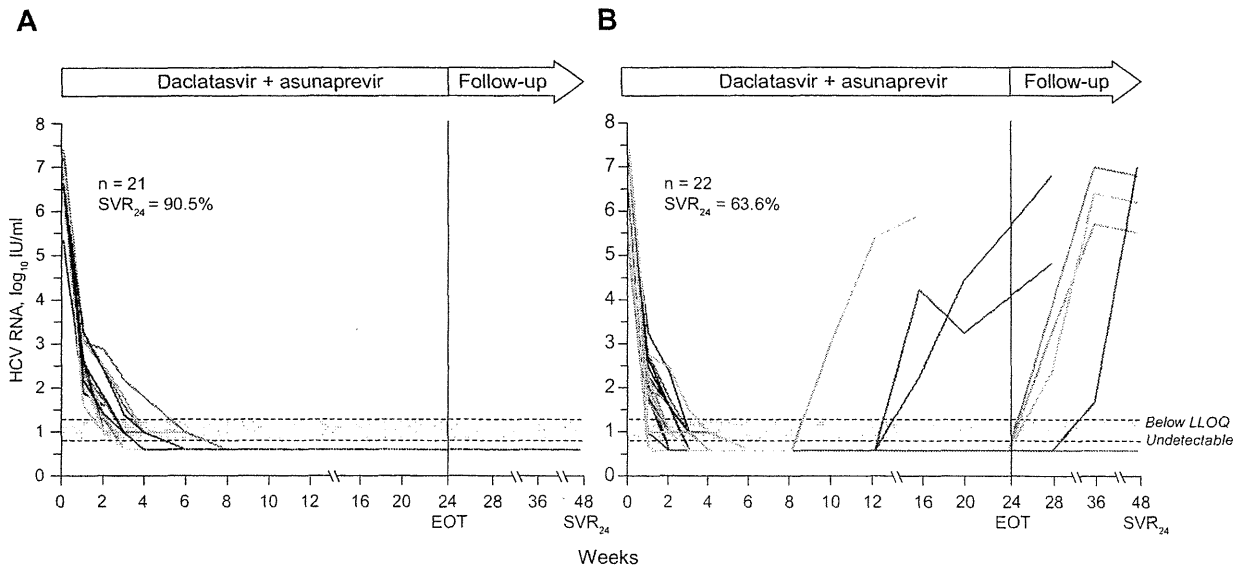

Fig. 3. HCV RNA levels, individual patients. Serum HCV RNA levels over time are shown for each patient. (A) Null responders; (B) ineligible/intolerant patients. EOT, end of treatment; SVR_{24}, sustained virologic response 24 weeks post-treatment; LLOQ, lower limit of quantitation = 15 IU/ml.

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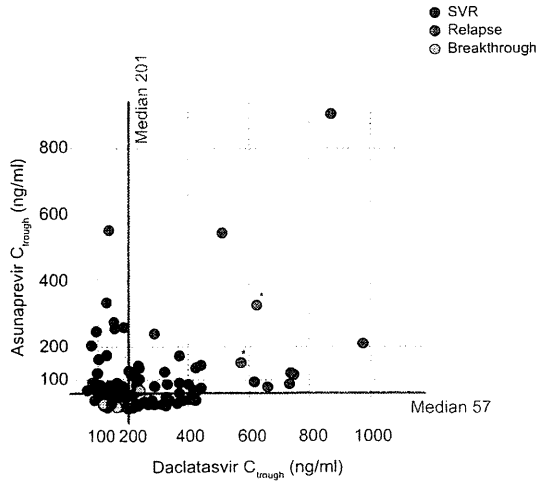

Fig. 4. Daclatasvir and asunaprevir trough plasma concentrations. Available trough plasma concentrations of asunaprevir and daclatasvir for individual patients are plotted and color-coded according to each patient's virologic outcome. Multiple determinations are shown for some patients. *Indicates values from a single patient with documented non-compliance.

Table 4. Most frequent adverse events and laboratory abnormalities.

Event, n (%)		Null responders (n = 21)	Ineligible/ intolerant (n = 22)
Adverse events occurring in ≥3 patients in either group	Headache	8 (38)	6 (27)
	Nasopharyngitis	6 (29)	8 (36)
	ALT increase	6 (29)	6 (27)
	Diarrhea	9 (43)	2 (9)
	AST increase	6 (29)	4 (18)
	Pyrexia	3 (14)	5 (23)
	Eosinophilia	1 (5)	4 (18)
	Abdominal discomfort	3 (14)	2 (9)
	Malaise	2 (10)	3 (14)
	Constipation	2 (10)	3 (14)
	Back pain	3 (14)	1 (5)
	Decreased appetite	0	3 (14)
Grade 3 or 4 lab abnormalities	ALT	2 (10)	2 (9)
	AST	1 (5)	2 (9)
	Lymphocytes	2 (10)	1 (5)
	Phosphorus	1 (5)	1 (5)
	Bilirubin, total	1 (5)	0
	Leukocytes	1 (5)	0

intolerant patients, representing two populations that are particularly difficult to treat due to limited therapeutic options. SVR rates were comparable at post-treatment weeks 4, 12, and 24; only one relapse occurred more than 4 weeks post-treatment. The 90.5% SVR rate in null responders is substantially higher than the generally poor response to PegIFN-α/RBV retreatment and the 37% SVR rate reported for genotype 1b null responders treated with PegIFN-α/RBV and telaprevir [4,19]. Therefore, therapy of this population with daclatasvir and asunaprevir appeared to overcome the poor interferon responsiveness, which may be less relevant to the efficacy of this DAA-only regimen. The SVR rate of 63.6% in ineligible/intolerant patients, although lower than results in null responders, is the first demonstration of a potentially effective treatment for these patients who currently have no therapeutic options. High SVR rates in both populations were achieved despite multiple adverse predictors of response to PegIFN-α/RBV therapy, including older age, high viral load, and a high proportion of *IL28B* genotype CT in the null responders.

Detectable HCV RNA was cleared rapidly; viral suppression was greater at all time points compared to reported results with PegIFN-α/RBV combined with telaprevir or TMC435 in genotype 1 null responders [4,20]. The slightly greater early viral suppression in ineligible/intolerant patients may reflect the higher frequency of *IL28B* CC genotype in this group. In the overall population, early virologic response was greater in patients with CC genotype, although this difference disappeared by week 12. Potentially, CC genotype may increase early viral suppression by increasing responsiveness to endogenous interferons that are released as a result of the rapid antiviral activity of the dual DAA therapy, allowing reversal of HCV-induced immunosuppression [21].

These results in patients with HCV genotype 1b differ from those reported for genotype 1a. In a similar study of US/European null responders, 2/9 patients with genotype 1a achieved SVR with daclatasvir + asunaprevir dual therapy, compared with 10/10 patients with genotype 1a who received quadruple therapy combining daclatasvir and asunaprevir with PegIFN-α/RBV [8]. This difference suggests that viral genotype can influence responses to DAA regimens, and outcomes can be optimized by individualized therapy that considers viral genotype.

The two populations included in this study represent substantial numbers of patients worldwide. Approximately 10% of HCV genotype 1-infected patients receiving PegIFN-α/RBV have a null response [22]. The cumulative prevalence of PegIFN-α/RBV null responders and the frequent failure of retreatment with current regimens, together suggest that a large population of null responders is awaiting improved therapies. The population of PegIFN-α/RBV ineligible or intolerant patients has not been extensively studied but may be substantial. In the IDEAL study, 23.2% of the 4469 patients screened were considered ineligible for PegIFN-α/RBV therapy; of these, 30.3% had hematologic or psychiatric conditions that may not preclude DAA-only regimens [23]. In registration trials, 9.7–14% of patients receiving PegIFN-α/RBV discontinued study treatment due to intolerance [24,25]. Moreover, these clinical trial data are likely to underestimate the true size of the ineligible and intolerant populations in community practice.

Virologic failures occurred relatively late in therapy after extended periods with undetectable HCV RNA. All seven patients with virologic failure had emergent NS5A and NS3 mutations that together confer high-level resistance to both daclatasvir and asunaprevir *in vitro* [11,12]. Pretreatment, NS5A-Y93H was detected in five of the seven patients with virologic failure and in five additional patients who achieved SVR, suggesting that pre-existing Y93H is loosely associated with virologic failure but is not an absolute predictor. Pharmacokinetics may also have contributed; nearly all patients with virologic failure had trough plasma concentrations of daclatasvir and asunaprevir below their respective median values. However, SVR was achieved by most patients with trough drug levels below the median, and by

several patients who discontinued study treatment after 2–16 weeks. Thus, the relationship of drug exposure to virologic outcome remains uncertain; further study is needed to define on-treatment predictors of outcome and the optimal duration of therapy.

Current data do not fully explain the observed differences in rates of virologic failure and SVR, between the two study populations. *IL28B* genotype was the primary difference between the two populations pretreatment. All three breakthroughs occurred in ineligible/intolerant patients with the unfavorable *IL28B* CT genotype; however, null responders had no breakthroughs, despite a much higher frequency of this genotype. Differing proportions of patients with concurrent pre-existing resistance-associated polymorphisms and low plasma drug concentrations may have contributed to differing rates of virologic failure between the two populations. Analysis of baseline parameters failed to identify other factors that may have influenced outcomes. However, these analyses were limited by the relatively small study population and may have been confounded by unreported non-adherence or baseline parameters not quantified absolutely, such as the stage of liver fibrosis. This issue requires further study in larger populations to confirm the apparent difference in outcomes and to identify factors predictive of virologic failure.

The adverse event profile of the study regimen was generally more favorable than that typically observed with PegIFN-α/RBV-containing regimens [26]. There were no significant hematologic or psychiatric abnormalities; the most common adverse events were non-specific in nature and generally mild to moderate in intensity. Mild diarrhea was experienced by 26% of study patients, consistent with previous studies of asunaprevir and other drugs of this class [4,6,14]. The four observed grade 3/4 ALT elevations resolved with continued therapy or after discontinuation and were not associated with significant clinical events. A role for study drugs in the reported serious adverse events cannot be ruled out except for gastroenteritis; however, four of the six events resolved spontaneously with continued treatment. The case of hyperbilirubinemia with gastroenteritis was complicated by multiple confounding factors, and the contribution of study drugs is uncertain [7].

In conclusion, dual oral therapy with daclatasvir and asunaprevir elicited rapid clearance of detectable HCV RNA and achieved high rates of SVR in two difficult-to-treat patient populations. These results confirm initial findings that HCV genotype 1b infections can be cured with daclatasvir combined with asunaprevir, without PegIFN-α/RBV [7,8]. Thus, this regimen has potential to offer effective treatment to null responders who have previously shown little or no response to PegIFN-α/RBV, and to PegIFN-α/RBV ineligible/intolerant patients who have no current treatment options. Further research will assess the benefits of this and other DAA combinations in larger and more diverse patient populations, but the promise of all oral and well-tolerated HCV therapy is on the horizon.

Financial support

This study was funded by Bristol-Myers Squibb.

Conflicts of interest

K Chayama has received research grants and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma, Mitsubishi Tanabe Pharma, Daiichi Sankyo, Toray Industries, Otsuka Pharmaceutical Company, and GlaxoSmithKline KK. Hiroki Ishikawa, Hideaki Watanabe, Wenhua Hu, Timothy Eley, Fiona McPhee, and Eric Hughes are employees of Bristol-Myers Squibb. All other authors have no conflicts to report.

Acknowledgments

The authors thank the patients and their families, and research staff, investigators and safety committees at all participating sites. Marc Bifano, MS, and Bing He, MS, contributed to analysis and interpretation of pharmacokinetic data. Editorial assistance for preparation of this manuscript was provided by Richard Boehme, PhD, of Articulate Science and was funded by Bristol-Myers Squibb.

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