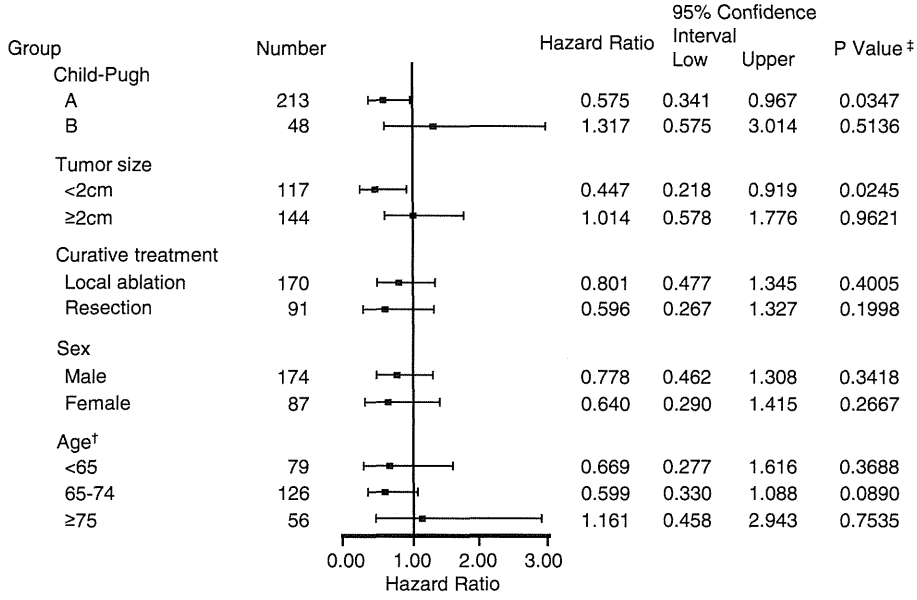

Fig. 3 Sub-group analysis: peretinoin 600 mg/day vs. placebo. †Age at the time of registration to the randomized trial. ‡Log-rank test

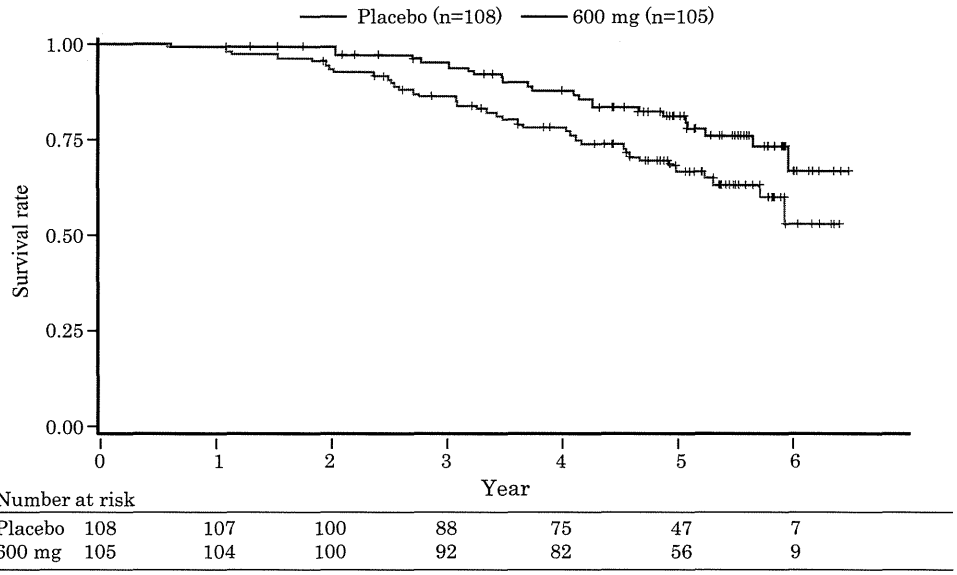

Fig. 4 Kaplan–Meier plot of overall survival in Child-Pugh A: peretinoin 600 mg/day vs. placebo. Hazard ratio 0.575; 95 % CI 0.341–0.967; log-rank test, $P = 0.0347$

patients with tumor size of under 2 cm, and peretinoin is particularly effective at suppressing multicentric recurrence in such cases.

From the results of this study and the randomized trial, we speculate that peretinoin improves patient survival time by suppressing HCC recurrence. Among those classified as Child-Pugh A, the hazard ratio of recurrence-free survival for peretinoin and placebo in the randomized trial was 0.60 (95 % CI 0.41–0.89) [18], and the hazard ratio of overall survival for peretinoin 600 mg/day and placebo was 0.575 (95 % CI 0.341–0.967). The similar hazard ratios could be explained by the following reasons. First, the investigational drug was administered for 2 years maximum in the randomized trial, and the median follow-up duration of this cohort study was nearly 5 years. Peretinoin is particularly well-known for its capacity to suppress multicentric recurrence, so given that a certain duration of time is required for a pre-cancerous lesion to develop into cancer, we surmise that peretinoin may have continued to work effectively in suppressing recurrence even after completion of peretinoin treatment. A follow-up survey [21] of the randomized trial conducted by Muto et al. [16] found that after administering this drug for 1 year, continued effects were noted for at least 150 weeks (roughly 3 years) following treatment completion. In addition, peretinoin has been found to suppress platelet-derived growth factor C

[22, 23], and thus it may be continuously suppressing recurrence by inhibiting the progression of hepatic fibrosis.

The limitation of this study was that the randomized trial was designed to evaluate recurrence-free survival in peretinoin-treated patients, and thus the number of patients may have been insufficient for evaluating the primary endpoint of this cohort study, i.e., overall survival. However, being able to collect and evaluate data from all patients who were administered the investigational drug in the randomized trial improved the statistical accuracy of our analysis.

Despite the limitation, the important point of this study is that by focusing on survival versus death, which is an index with no room for subjectivity, we were able to determine that among those classified as Child-Pugh A, the 600 mg/day group had a significantly longer overall survival compared to the placebo group. Notably, independent of this cohort study, a phase III trial is currently underway to examine the effects of peretinoin on controlling HCC recurrence among those classified as Child-Pugh A who have completed curative treatment for HCV-HCC. In conclusion, administration of 600 mg/day peretinoin to patients who have completed curative treatment for HCV-HCC is anticipated to improve survival of patients with relatively stable liver function, such as those classified as Child-Pugh A. Our finding provides novel insights into the understanding of 5-year survival after treatment with investigational drug in patients with HCV-HCC.

Acknowledgments The authors would like to thank all patients and their families who participated in this study. We also thank all who were involved in the research and would like to acknowledge Non-Profit Organization Japan Clinical Research Support Unit for their assistance in data management and statistical analyses, and Statcom Co., Ltd. for their assistance in preparing and editing this manuscript. This study was presented in part at the seventh annual conference of the International Liver Cancer Association, Washington, D.C., September 13-15, 2013. This study was funded by the Comprehensive Support Project (CSP) of the Public Health Research Foundation. The corporate and individual sponsors of this study are listed on the CSPOR website (http://www.csp.or.jp/cspor/kyousan_e.html). The pharmaceutical manufacturer/distributor who provided financial contribution as a corporate sponsor took no part in this study other than providing information relevant to proper use of the study drug(s). The following institutions provided data: Ehime University Hospital, Fukuoka University Hospital, Gifu Municipal Hospital, Gifu University Hospital, Hiroshima Prefectural Hospital, Hiroshima University Hospital, Hokkaido University Hospital, Hyogo College of Medicine, Iwate Medical University Hospital, Jichi Medical University Hospital, Kagoshima University Medical And Dental Hospital, Kansai Medical University Takii Hospital, Kinki University Hospital, Kitano Hospital, Kitasato University East Hospital, Kokura Memorial Hospital, Kumamoto University Hospital, Musashino Red Cross Hospital, Nagasaki University Hospital, National Cancer Center Hospital, National Cancer Center Hospital East, National Hospital Organization Nagasaki Medical Center, Oita University Hospital, Osaka City General Hospital, Osaka City University Hospital, Osaka General Medical Center, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka Red Cross Hospital, Osaka Rosai Hospital, Saiseikai Fukuoka General Hospital, Sapporo Kosei General Hospital, Shimonoseki Kosei Hospital, Showa University Hospital, Teine-Keijinkai Hospital, The University of Tokyo Hospital, Tokyo Medical And Dental University Hospital Faculty of Medicine, Tokyo Medical University Hospital, Toranomon Hospital, Toyohashi Municipal Hospital, Yamaguchi University Hospital and Yokohama City University Medical Center.

Conflict of interest Okita has received lecture fees from Kowa. Izumi has received lecture fees from MSD, Chugai Pharmaceutical, Daiichi Sankyo, and Bayer Yakuhin. Masafumi Ikeda has received research funding from Kowa. Kokudo has received research funding from Dainippon Sumitomo Pharma, Bayer Yakuhin, Merk Serono, Bristol-Myers Squibb, Chugai Pharmaceutical, Taiho Pharmaceutical, and Yakult Pharmaceutical. Ueshima has received lecture fees from Bayer Yakuhin, MSD, Ajinomoto Pharmaceuticals, Eisai, Dainippon Sumitomo Pharma, Eidia, Takeda Pharmaceutical, Janssen Pharmaceutical, Daiichi Sankyo, and Boehringer Ingelheim Japan. Kudo has received lecture fees from Bayer Yakuhin and Eisai. Okusaka has received research funding from Kowa. Ohashi received executive salaries from Statcom, lecture fees from Chugai Pharmaceutical and Shionogi, manuscript fee from DNP Media Create, and research funding from Kowa Pharmaceutical, Astellas Pharma, Takeda Pharmaceutical, and Kyowa Hakko Kirin. Kumada holds a patent on SRL, and has received lecture fees from MSD, Bristol-Myers Squibb, Mitsubishi Tanabe Pharma, Dainippon Sumitomo Pharma, Toray Industries, and Ajinomoto Pharmaceuticals. The other authors declare that they have no conflict of interest.

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J Gastroenterol (2015) 50:191–202
DOI 10.1007/s00535-014-0956-9

The Japanese Society of Gastroenterology

Peretinoin after curative therapy of hepatitis C-related hepatocellular carcinoma: a randomized double-blind placebo-controlled study

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Received: 8 November 2013 / Accepted: 19 March 2014 / Published online: 13 April 2014

Abstract

Background Effective prophylactic therapies have not been established for hepatocellular carcinoma recurrence. Peretinoin represents one novel option for patients with hepatitis C virus-related hepatocellular carcinoma (HCV-HCC), and it was tested in a multicenter, randomized, double-blind, placebo-controlled study.

Methods Patients with curative therapy were assigned to one of the following regimens: peretinoin 600, 300 mg/day, or placebo for up to 96 weeks. The primary outcome was recurrence-free survival (RFS).

Results Of the 401 patients initially enrolled, 377 patients were analyzed for efficacy. The RFS rates in the 600-mg group, the 300-mg group, and the placebo group were 71.9, 63.6, and 66.0 % at 1 year, and 43.7, 24.9, and 29.3 % at 3 years, respectively. The primary comparison of peretinoin (300 and 600-mg) with placebo was not significant ($P = 0.434$). The dose–response relationship based on the hypothesis that "efficacy begins to increase at 600 mg/day" was significant ($P = 0.023$, multiplicity-adjusted $P = 0.048$). The hazard ratios for RFS in the 600-mg group vs. the placebo group were 0.73 [95 % confidence interval (CI) 0.51–1.03] for the entire study period and 0.27 (95 % CI 0.07–0.96) after 2 years of the randomization. Common adverse events included ascites, increased blood pressure, headache, presence of urine albumin, and increased transaminases.

Conclusions Although the superiority of peretinoin to placebo could not be validated, 600 mg/day was shown to be the optimal dose, and treatment may possibly reduce the recurrence of HCV-HCC, particularly after 2 years. The

This study was presented in part at the 46th annual meeting of the American Society of Clinical Oncology, Chicago, IL, June 4–8, 2010, the annual conference of the International Liver Cancer Association, Montreal, September 10–12, 2010, and the 2011 American Society of Clinical Oncology Gastrointestinal Cancers Symposium, San Francisco, CA, January 20–22, 2011.

Electronic supplementary material The online version of this article (doi:10.1007/s00535-014-0956-9) contains supplementary material, which is available to authorized users.

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efficacy and safety of peretinoin 600 mg/day should continue to be evaluated in further studies.

Keywords Liver neoplasms · Recurrence · Multicentric de novo carcinogenesis · Retinoids · Dose–response relationship

Abbreviation

CI	Confidence interval
CT	Computed tomography
HCC	Hepatocellular carcinoma
HR	Hazard ratio
RFS	Recurrence-free survival

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world, affecting 740,000 people annually [1]. The incidence of HCC has been rising due to an increase in hepatitis-C virus infections [2–4]. Curative resection or ablation is indicated for early HCC [5–7]. However, the 3-year recurrence rate after curative treatment in the general population is 50 % [8, 9]. Furthermore, the recurrence rate is >70 % in hepatitis C virus-positive patients [10]. Recurrence within 2 years of curative treatment is mainly associated with intrahepatic metastasis, whereas multicentric de novo carcinogenesis (second primary HCC) is the common cause of recurrence after 2 years [11, 12]. Most importantly, no effective approach has been established to prevent HCC recurrence [13–15].

The concept of chemoprevention using retinoids has been proposed as a means to delay or prevent recurrence after treatment of HCC [16]. Peretinoin [(2*E*,4*E*,6*E*,10*E*)-3,7,11,15-Tetramethylhexadeca-2,4,6,10,14-pentaenoic acid] is a synthetic retinoid with a retinoic acid receptor and retinoid X receptor agonist activity [17]. Peretinoin is known to suppress tumor growth in the human liver by inducing apoptosis and differentiation of liver cancer cells [18, 19]; it also acts by increasing p21 protein levels and reducing cyclin D1 levels to inhibit proliferation of these cells [20]. Recently, a small-scale, randomized study has demonstrated that peretinoin (600 mg/day) reduced HCC recurrence and increased the survival of patients treated with curative therapy [21, 22].

Multiple other studies have evaluated the safety of peretinoin dosages. A phase I study evaluated the proportionality of blood concentration using three peretinoin doses (300, 600, and 900 mg/day) [23]. Based on nonclinical studies and phase I studies, a dose of 300 mg/day was assumed to result in a drug concentration in the liver sufficient to produce medicinal action (apoptosis and induction). The 900 mg/day dose resulted in unacceptable hypertension.

Based on these findings and the hypothesis that peretinoin efficacy saturates at 300 mg/day, this clinical study evaluated the efficacy and dose–response relationship of peretinoin in a randomized, double-blind, placebo-controlled study.

In Japan, approximately 70 % of HCC patients are HCV-positive, and there is a higher risk of HCC recurrence due to HCV than due to other causes of recurrence. To verify an inhibitory effect on recurrence in a population that is uniform in having a high risk of recurrence, this

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study was conducted in HCV-positive patients after having a complete response to treatment.

Methods

Patients

Patients were recruited from outpatient groups at 41 institutions in Japan. Patients with primary HCV-HCC or first recurrence successfully treated with resection or radiofrequency ablation were included in the study. HCC was diagnosed based on the finding of a typical vascular pattern (hypervascularity in the arterial phase and wash-out in the portal equilibrium phase) on dynamic CT, according to the Consensus-Based Clinical Practice Manual proposed by the Japan Society of Hepatology [5]. Complete response was defined similarly to that of the modified Response Evaluation Criteria in Solid Tumors (modified RECIST) definition and required a diagnosis of a complete cure. Three independent radiologists reviewed all CT images to confirm complete cure. Eligibility criteria included: positive for serum hepatitis C virus RNA; Child-Pugh liver function class A or B (Table S1); platelet count $\geq$50000/μL; and age $\geq$20 years. Exclusion criteria included: positive for hepatitis B surface antigen; portal invasion with HCC; concurrent use of transcatheter arterial embolization/chemoembolization for curative treatment; use of other investigational drugs, antitumor drugs, interferon, or vitamin K2; uncontrollable blood pressure under drug therapy (systolic blood pressure $\geq$160 mmHg or diastolic blood pressure $\geq$100 mmHg); serious complications; allergy to retinoids or contrast agents for CT; past total gastric resection; and being pregnant or breastfeeding. This study was approved by the institutional review board at each center and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent. The study protocol is registered at JAPIC Clinical Trials: JapicCTI-060250 (http://www.clinicaltrials.jp/user/cteSearch.jsp).

Study design

This study was a multicenter, parallel-group, double-blind, randomized, placebo-controlled study (Fig. S1). Patients who satisfied the eligibility criteria were assigned to receive peretinoin 600 mg/day, peretinoin 300 mg/day, or placebo at a 1:1:1 ratio. The randomization was centralized, and assignment to study groups was conducted by computer using the minimization method with adjustment for primary tumor/first recurrence and curative treatment (resection/ablation). Equal intra-institutional distribution was ensured. Patients orally ingested the assigned study drug twice daily for up to 96 weeks. Follow-up of individual patients was to be discontinued and study completion would occur when the number of events (HCC recurrence or death) reached 180–200. Patients visited the institutions once every 4 weeks during the study treatment period and once every 12 weeks thereafter. Treatment compliance was evaluated by pill counts. Use of antitumor drugs, interferon, vitamin K2, vitamin A, or an antiviral drug ribavirin was prohibited during the study duration. All those involved in this study, including patients, were blinded to the treatment regimen and a placebo identical in external appearance to the study drug was used.

Endpoints

The primary endpoint was recurrence-free survival (RFS), defined as the time from randomization to HCC recurrence or death from any cause, whichever occurred first. Abdominal dynamic CT was performed every 12 weeks. Tumor markers (α-fetoprotein, Lens culinaris agglutinin-reactive α-fetoprotein isoform, and protein induced by vitamin K absence or antagonist-II) were measured every 12 weeks. If tumor markers rapidly increased, abdominal dynamic CT was additionally performed. Recurrence of HCC was confirmed based on findings of hypervascularity (nodules enhanced in the arterial phase and washout in the late phase) by dynamic CT images. Recurrence of HCC was judged by three independent radiologists, who reviewed all dynamic CT images. The secondary endpoint was disease-free survival, which was defined as the time from randomization to HCC recurrence, death from any cause, or onset of secondary tumor, whichever occurred first. For safety assessments, incidences of adverse events were evaluated based on periodic examinations and tests. Laboratory tests were performed collectively at the central laboratory.

Statistical analysis

The primary comparison of this study was to examine the superiority of peretinoin (300 and 600-mg) to placebo by RFS. RFS was estimated and presented as survival curves for the treatment groups using the Kaplan–Meier method. Subsequently, the dose–response relationship of peretinoin was evaluated. A stratified log-rank test was performed according to the type of curative treatment (resection/ablation) in the pre-specified three sets of contrasts (Fig. S2). The contrast reflecting the hypothesis that the efficacy of peretinoin saturates at 300 mg/day was tested as the "primary comparison". To evaluate the dose–response relationship of peretinoin, tests were also performed for the two other sets of contrasts ("efficacy increases linearly"

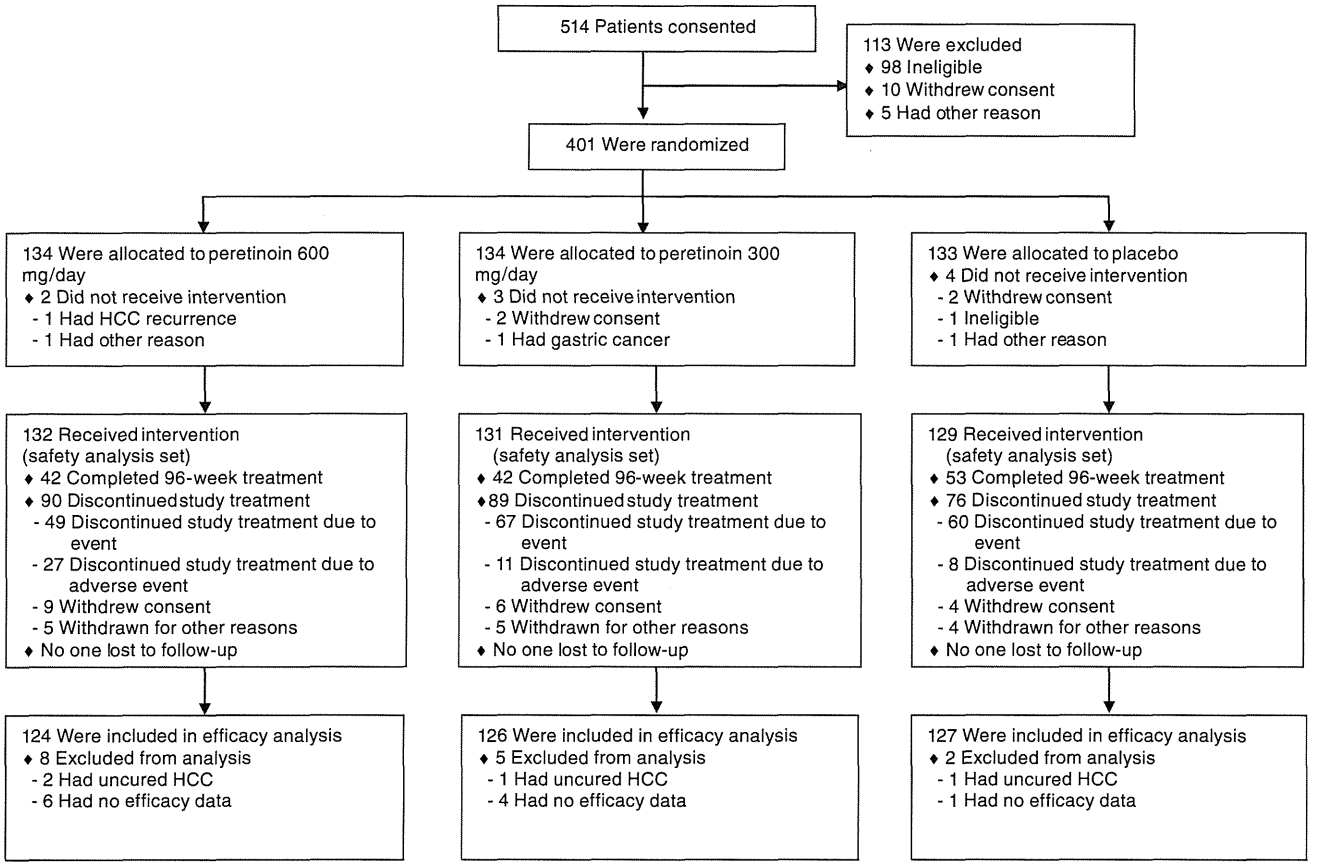


Fig. 1 Flow diagram of study patients

and "efficacy begins to increase at 600 mg/day"). The multiplicity of the three sets of contrasts was adjusted using a permutation test [24].

Sample size was determined based on the primary aim. The number of events required when the clinically useful hazard ratio (HR) supporting a reduction in recurrence was around 0.60 with a 0.025, one-sided significance level, a 0.90 power, and a 2:1 patient ratio [peretinoin (300 and 600-mg) vs. placebo] was calculated using Freedman's formula [25]. Based on 180 expected events and the 3-year mean duration of follow-up, 120 patients in each treatment group (360 patients in total) were required.

Hazard ratios and 95 % confidence intervals (CIs) for RFS in the peretinoin 600-mg or the 300-mg group vs. placebo group were calculated for the entire study period and at predefined intervals (within 1 year of randomization, at 1–2 years, and after 2 years) with Cox regression analyses using curative treatment as a covariate.

In exploratory, post-hoc, subgroup analysis, the Cox proportional-hazard model was used to evaluate the interaction between baseline characteristics and the effect of peretinoin (peretinoin 600-mg vs. placebo). Factors chosen as baseline characteristics were sex, age, HCC (primary or first recurrence), treatment (local ablation or surgical resection), number of tumor masses, tumor size, and Child-Pugh class.

Efficacy analysis was performed after excluding ineligible patients, patients whose efficacy data were missing, and patients who had never taken the study drug based on the intention-to-treat principle. A two-sided significance level of 0.05 was used. The analysis of disease-free survival was performed in the same manner as the RFS analysis. All patients who had taken the study drug at least once were included in the safety analysis set. Adverse events were classified according to Medical Dictionary for Regulatory Activities (MedDRA) Version 12.0. The dose–response relationship for safety was evaluated using the Cochran–Armitage test.

The independent data and safety monitoring committee performed an interim analysis twice in accordance with the study protocol as follows: first, safety was analyzed when approximately 60 patients had been in the study for at least 1 year; second, safety and efficacy were analyzed when the number of events reached approximately 100. The study was continued after these two interim analyses because none of the discontinuation criteria defined in the study protocol were applicable.

Table 1 Baseline characteristics of study patients

Variable	Peretinoin 600 mg/day $n = 124$ Number (%)	300 mg/day $n = 126$	Placebo $n = 127$
Gender			
Male	81 (65.3)	73 (57.9)	87 (68.5)
Female	43 (34.7)	53 (42.1)	40 (31.5)
Age (year)			
<65	37 (29.8)	38 (30.2)	40 (31.5)
65–75	63 (50.8)	61 (48.4)	57 (44.9)
≥75	24 (19.4)	27 (21.4)	30 (23.6)
Mean (SD)	68.1 (7.1)	68.2 (7.7)	68.6 (7.8)
BMI (kg/m^2)			
<25	95 (76.6)	98 (78.4)	106 (83.5)
≥25	29 (23.4)	27 (21.6)	21 (16.5)
Platelet ($\times 10^4/\mu L$)			
<10	44 (35.5)	49 (38.9)	58 (45.7)
≥10	80 (64.5)	77 (61.1)	69 (54.3)
Means (SD)	12.0 (5.1)	11.5 (4.0)	11.4 (4.3)
Child-Pugh class[a]			
A	100 (80.6)	104 (82.5)	106 (83.5)
B	24 (19.4)	22 (17.5)	21 (16.5)
HCC			
Primary	111 (89.5)	111 (88.1)	115 (90.6)
First recurrence	13 (10.5)	15 (11.9)	12 (9.4)
Treatment			
Local ablation	78 (62.9)	81 (64.3)	83 (65.4)
Surgical resection	46 (37.1)	45 (35.7)	44 (34.6)
Number of tumor masses			
1	104 (83.9)	106 (84.1)	105 (82.7)
2–3	19 (15.3)	20 (15.9)	21 (16.5)
≥4	1 (0.8)	0 (0.0)	1 (0.8)
Tumor size (cm)			
<2	56 (45.2)	55 (43.7)	57 (44.9)
≥2	68 (54.8)	71 (56.3)	70 (55.1)
AFP (ng/mL)			
≤10	57 (46.0)	47 (37.3)	45 (35.4)
>10	67 (54.0)	79 (62.7)	82 (64.6)
Means (SD)	44.8 (152.8)	37.3 (76.3)	39.4 (82.4)
AFP-L_3 (%)			
≤10	113 (91.1)	116 (92.1)	117 (92.1)
>10	11 (8.9)	10 (7.9)	10 (7.9)
Means (SD)	3.9 (10.1)	3.2 (4.8)	4.3 (8.3)
PIVKA-II (mAU/mL)			
≤40	115 (92.7)	118 (93.7)	118 (92.9)
>40	9 (7.3)	8 (6.3)	9 (7.1)
Means (SD)	42.9 (227.0)	26.8 (44.1)	86.5 (711.3)

BMI body mass index, *HCC* hepatocellular carcinoma, *AFP* alpha-fetoprotein, *AFP-L3* alpha-fetoprotein L3, *PIVKA-II* protein induced by vitamin K absence or antagonist-II, *SD* standard deviation

[a] Severity of hepatic dysfunction evaluated on the scale of Child-Pugh class A to C. The analysis excluded patients with class C hepatic dysfunction

Results

Patients

Patients were recruited from March 14, 2005, through July 30, 2007. The study was terminated on August 27, 2009, because the target number of events (180–200) was achieved. The median follow-up period was 911 days (95 % CI 845–937 days). A total of 401 patients were randomized (Fig. 1). Patient characteristics were comparable among the three treatment groups (Table 1). Patients aged ≥65 years accounted for about 70 % of the study population, and those who were Child-Pugh class A accounted for about 80 % of patients in each treatment group. The mean duration of the study treatment was 416 days (95 % CI 392–441 days) (600-mg group, 398 days; 300-mg group, 410 days; placebo group, 442 days). Of those who were included in the efficacy analysis, 368 patients (97.6 %) complied with the study treatment for at least 70 % of the time (96.0, 98.4, and 98.4 %, respectively).

Efficacy

A total of 377 patients were analyzed (600-mg group: 124, 300-mg group: 126, placebo group: 127). Figure 2 shows 300, 600-mg Kaplan–Meier curves of the 600-mg group, the 300-mg group, and the placebo group for RFS. In the primary comparison, the effect of peretinoin (300 and 600-mg) on RFS compared to that of the placebo was not significant ($P = 0.434$). As shown in the Kaplan–Meier curves, RFS in the 600-mg group during the entire course of the study trended slightly higher than in the other two groups. The proportions of patients with RFS in the 600-mg group, the 300-mg group, and the placebo group, respectively, were 71.9, 63.6, and 66.0 % in year 1, 48.3, 43.4, and 42.3 % in year 2, and 43.7, 24.9, and 29.3 % in Year 3 (Table S2). Hazard ratios for the RFS results of peretinoin 600 mg/day vs. placebo and peretinoin 300 mg/day vs. placebo are shown in Table 2. The risks were comparable in the 300-mg and placebo groups (HR during the entire study period, 1.06; 95 % CI 0.78–1.45; HR after 2 years, 1.19; 95 % CI 0.55–2.60). In contrast, the risk of recurrence after 2 years of randomization in the 600-mg group decreased by 70 % compared to the placebo group (HR during the entire study period, 0.73; 95 % CI 0.51–1.03; HR after 2 years, 0.27; 95 % CI 0.07–0.96). During the study, RFS events were defined as HCC recurrence or death of any cause; there were 53 RFS events observed in patients in the 600-mg group, 80 events in the 300-mg group, and 77 events in the placebo group (Table S2). The analysis of disease-free survival showed comparable results (Fig. S3).

Dose–response relationship

Table 3 shows the results of log-rank tests on the three prespecified comparisons, which were based on the three hypotheses for the dose–response relationship of peretinoin. No significant dose–response relationship was seen in the comparison that tested whether "peretinoin efficacy saturates at 300 mg/day" ($P = 0.434$, the same test applied to the "primary comparison" described previously). When testing whether "peretinoin efficacy increases linearly", no significance was obtained ($P = 0.079$). In contrast, the dose–response relationship was significant in the comparison that tested whether "peretinoin efficacy begins to increase at 600 mg/day" ($P = 0.023$, multiplicity-adjusted $P = 0.048$).

Post-hoc subgroup analysis

The effect of the 600 mg dosage of peretinoin observed in the entire study population was consistent with each subgroup except in patients with tumor size ≥2 cm and Child-Pugh B (Fig. 3a). There were significant treatment effect interactions with tumor size and Child-Pugh class ($P = 0.039$ and $P = 0.035$, respectively), and the interaction with Child-Pugh class was the largest both in magnitude of the effect size and in statistical significance. Kaplan–Meier curves of the 600-mg group vs. placebo group for RFS in patients with Child-Pugh A are shown in Fig. 3b.

Safety

A total of 392 patients were analyzed (600-mg group: 132, 300-mg group: 131, placebo group: 129). The overall incidence of adverse events was 95.5 % (126/132) in the 600-mg group, 93.9 % (123/131) in the 300-mg group, and 90.7 % (117/129) in the placebo group. Common adverse events that occurred in ≥10 % of patients in either the 600 or 300-mg of the treatment groups included ascites, diarrhoea, oesophageal varices, nasopharyngitis, back pain, headache, oedema peripheral, albumin urine present, increased blood pressure and increased transaminases (Table 4). Incidences of these events mainly increased with peretinoin dose. Most of these events were mild or moderate in severity and controllable. The proportion of adverse events which resulted in discontinuation of the study treatment increased with dose: 15.9 % (21/132) in the 600-mg group, 6.9 % (9/131) in the 300-mg group, and 4.7 % (6/129) in the placebo group ($P = 0.002$). These events included onychoclasis, headache, anemia, renal impairment, edema, peripheral edema, and increased transaminases.

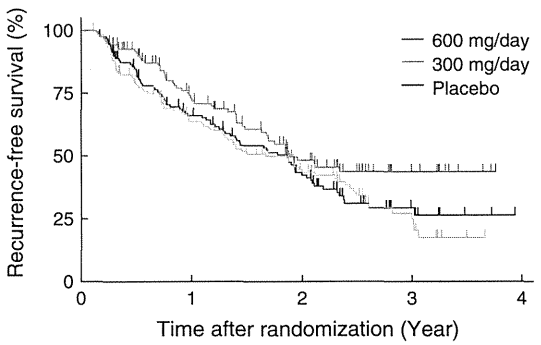
The overall incidence of serious adverse events increased with peretinoin dose: 31.1 % (41/132) in the 600-mg group, 29.8 % (39/131) in the 300-mg group, and 20.2 % (26/129) in the placebo group ($P = 0.048$). Seven patients treated with peretinoin died from serious adverse events. They were all aged ≥65 years (including three aged ≥75 years). The baseline Child-Pugh class was A in three patients and B in four. The causes of death varied: three patients in the 600-mg group died of cardiorespiratory arrest, sepsis, and hepatic failure, and four in the 300-mg group died of pneumonia, hepatic failure, sudden death, and diffuse large B-cell lymphoma.

Discussion

This study could not confirm the efficacy of peretinoin (300 and 600 mg/day) for reducing the recurrence of HCV-HCC. Based on the previous Phase I study [26], peretinoin was assumed to be effective from the 300 mg/day dose level; thus, this study was designed to compare peretinoin (300 and 600-mg) dosage groups to a placebo group as the primary objective. Unfortunately, the results did not achieve statistical significance. The number of recurrences and the HR observed in the peretinoin 300-mg group were equivalent to that of the placebo group, which indicated the dose level of peretinoin 300 mg/day was ineffective in this patient population. Sample size of the study was determined to test the primary comparison of peretinoin (300 and 600-mg) group vs. placebo group with number of patients in 2:1 ratio. Since there was no statistically significant difference, the comparisons between peretinoin 300-mg or 600-mg to the placebo group were underpowered.

The gene expression pattern in the liver before and after peretinoin treatment was examined in a clinical pharmacological study performed in humans simultaneously with this study. Genes expected to result in inhibition of recurrence, including retinoid-induced genes, interferon- and tumor suppressor-related genes, and hepatocyte differentiation genes, were among the genes that changed markedly before and after treatment with 600 mg/day. However, there was little change or no change in expression of these genes with treatment at 300 mg/day [27]. Consequently, while it was assumed that non-clinical studies and pharmacokinetic results showed that treatment of 300 mg/day elicited sufficient medicinal action, the above results of treatment with 300 mg/day in humans shows insufficient change in gene expression, and as such, probably does not confirm the previous result.

The optimal dose of peretinoin was found to be 600 mg/day, and it was possibly effective, particularly after 2 years



Number at risk					
600 mg/day	124	70	36	10	0
300 mg/day	126	71	39	11	0
Placebo	127	75	40	10	0

Fig. 2 Kaplan–Meier curves for recurrence-free survival

Table 3 Dose–response relationship of peretinoin for recurrence-free survival

Set of contrast [Placebo, 300 mg/day, 600 mg/day]	Standardized log-rank score[a]	*P*-value	Adjusted *P*-value
[−2, 1, 1] Efficacy saturates at 300 mg/day	−0.782	0.434	–
[−1, 0, 1] Efficacy increases linearly	−1.756	0.079	–
[−1, −1, 2] Efficacy begins to increase at 600 mg/day	−2.269	0.023	0.048[b]

[a] Stratified log-rank test based on surgical procedure

[b] Multiplicity between contrasts adjusted by permutation test

Table 2 Hazard ratios for recurrence-free survival

	Hazard ratio (95 % CI)			
	<1 year	1–2 years	≥2 years	Overall study period
Peretinoin 600 mg/day vs. placebo	0.72 (0.45–1.17)	0.93 (0.52–1.66)	0.27 (0.07–0.96)	0.73 (0.51–1.03)
Peretinoin 300 mg/day vs. placebo	1.11 (0.73–1.70)	0.89 (0.50–1.60)	1.19 (0.55–2.60)	1.06 (0.78–1.45)

Hazard ratios for the predetermined periods starting on the day of patient registration and for the entire study period, estimated for peretinoin 600 and 300 mg/day against placebo in a Cox regression analysis with surgical procedure as a covariate. Patients with no events and uncensored patients at the start of predetermined period were included; those with an event and censored patients at the end of predetermined period were censored

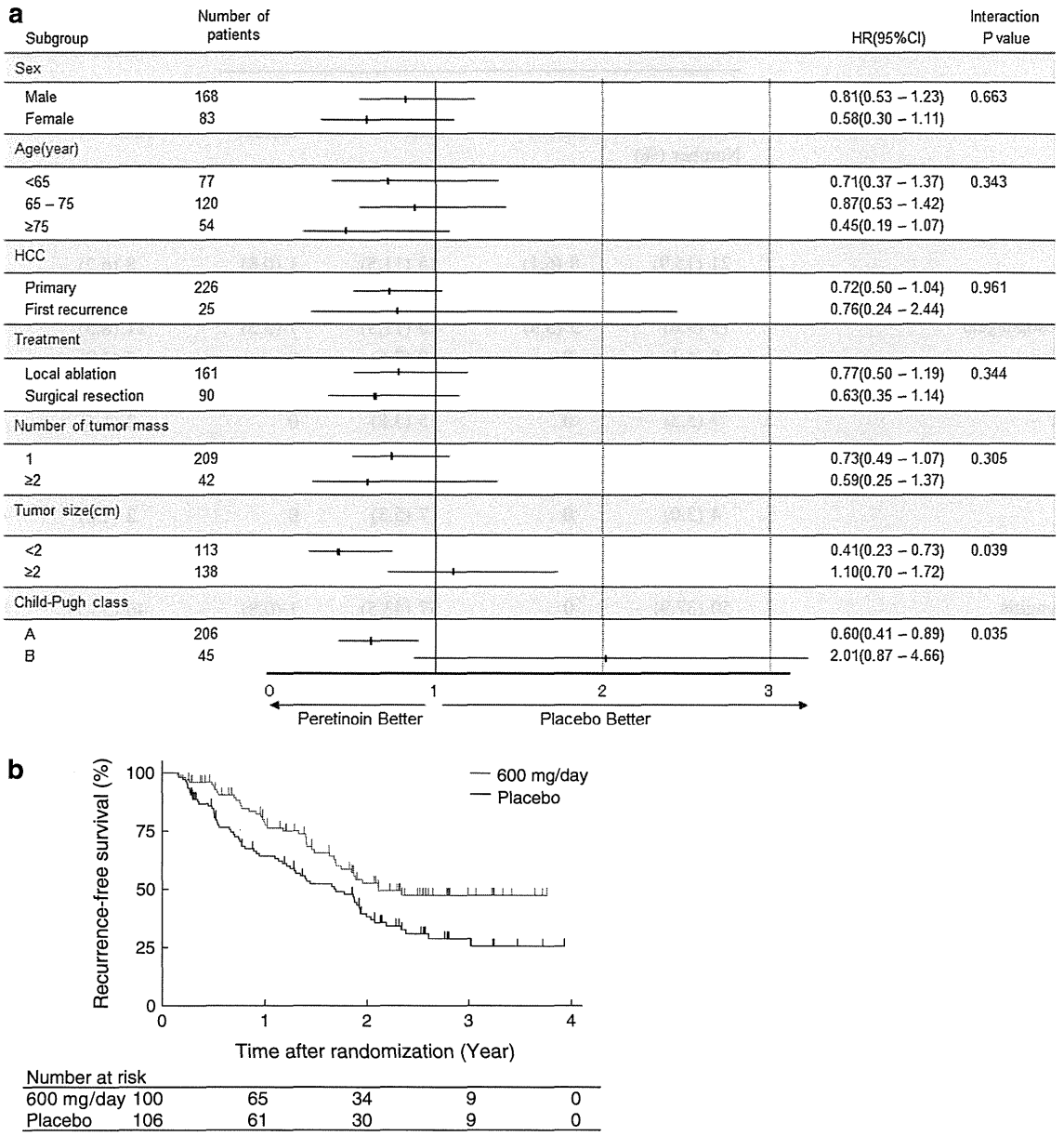


Fig. 3 **a** Exploratory, post-hoc, subgroup analysis of hazard ratio for recurrence-free survival. **b** Exploratory, post-hoc, subgroup analysis for recurrence-free survival

from curative treatment. A previous study reported that the overall HR for peretinoin 600 mg/day vs. placebo was 0.31 (95 % CI 0.12–0.78) [21]. This result is comparable to the HR after 2 years (0.27; 95 % CI 0.07–0.96) in our study. While the previous study evaluated recurrence of only de novo carcinogenesis (second primary HCC), this study evaluated both intrahepatic metastasis and de novo carcinogenesis. However, multicentric de novo carcinogenesis is the common cause of recurrence after 2 years [11, 12].

Adverse reactions associated with other retinoids [26] (e.g., mucocutaneous symptoms such as cheilitis and peeling skin, abnormal lipid metabolism, musculoskeletal disorders, and ocular symptoms) occurred infrequently in patients treated with peretinoin. Ascites and increased blood pressure were specific to peretinoin. Blood pressure should be periodically monitored before and during treatment with peretinoin and controlled with antihypertensives as appropriate. Seven patients died from various adverse events. Associations between peretinoin and these adverse events were undeterminable, because all seven patients who died from adverse events were aged ≥65 years, and four among them had Child-Pugh B hepatic impairment. Thus, age and advanced hepatic impairment could have been the causative factor rather than the treatment. The

Table 4 Adverse events in safety analysis set

Adverse events	Peretinoin				Placebo $n = 129$	
	600 mg/day $n = 132$		300 mg/day $n = 131$			
	Total Number (%)	Serious	Total	Serious	Total	Serious
Overall incidence	126 (95.5)	41 (31.1)	123 (93.9)	39 (29.8)	117 (90.7)	26 (20.2)
Gastrointestinal disorders						
Ascites	21 (15.9)	8 (6.1)	15 (11.5)	1 (0.8)	8 (6.2)	1 (0.8)
Diarrhoea	16 (12.1)	1 (0.8)	10 (7.6)	0	7 (5.4)	0
Varices oesophageal	13 (9.8)	5 (3.8)	15 (11.5)	7 (5.3)	11 (8.5)	4 (3.1)
Constipation	8 (6.1)	0	10 (7.6)	0	5 (3.9)	0
Abdominal discomfort	8 (6.1)	0	4 (3.1)	0	2 (1.6)	0
Stomatitis	7 (5.3)	0	5 (3.8)	0	2 (1.6)	0
Nausea	7 (5.3)	0	2 (1.5)	0	6 (4.7)	0
Cheilitis	7 (5.3)	0	1 (0.8)	0	0 (0.0)	0
Gastritis	4 (3.0)	0	7 (5.3)	0	2 (1.6)	0
Gastric polyps	1 (0.8)	0	12 (9.2)	0	10 (7.8)	0
Infections and infestations						
Nasopharyngitis	50 (37.9)	0	57 (43.5)	1 (0.8)	46 (35.7)	0
Cystitis	9 (6.8)	0	6 (4.6)	0	4 (3.1)	0
Urinary tract infection	8 (6.1)	0	6 (4.6)	3 (2.3)	0 (0.0)	0
Eye disorders						
Cataract	5 (3.8)	2 (1.5)	7 (5.3)	3 (2.3)	4 (3.1)	2 (1.6)
Musculoskeletal and connective tissue disorders						
Back pain	17 (12.9)	0	11 (8.4)	0	10 (7.8)	0
Arthralgia	7 (5.3)	0	5 (3.8)	0	8 (6.2)	0
Muscle spasms	3 (2.3)	0	7 (5.3)	0	7 (5.4)	0
Blood and lymphatic system disorders						
Anemia	7 (5.3)	0	1 (0.8)	0	2 (1.6)	0
Vascular disorders						
Hypertension	12 (9.1)	0	10 (7.6)	0	4 (3.1)	0
Respiratory, thoracic and mediastinal disorders						
Cough	4 (3.0)	0	7 (5.3)	0	9 (7.0)	0
Upper respiratory tract inflammation	2 (1.5)	0	5 (3.8)	0	7 (5.4)	0
Injury, poisoning and procedural complications						
Contusion	8 (6.1)	0	7 (5.3)	0	7 (5.4)	0
Nervous system disorders						
Headache	17 (12.9)	0	15 (11.5)	0	11 (8.5)	0
Dizziness	9 (6.8)	0	5 (3.8)	0	4 (3.1)	0
General disorders and administration site conditions						
Edema peripheral	16 (12.1)	0	11 (8.4)	0	11 (8.5)	0
Pyrexia	12 (9.1)	2 (1.5)	13 (9.9)	1 (0.8)	8 (6.2)	0
Edema	10 (7.6)	0	3 (2.3)	0	4 (3.1)	0
Metabolism and nutrition disorders						
Diabetes mellitus	3 (2.3)	1 (0.8)	7 (5.3)	0	9 (7.0)	0
Skin and subcutaneous tissue disorders						
Pruritus	11 (8.3)	0	12 (9.2)	0	9 (7.0)	0
Rash	7 (5.3)	0	9 (6.9)	0	9 (7.0)	0
Nail disorder	4 (3.0)	0	7 (5.3)	0	2 (1.6)	0

Table 4 continued

Adverse events	Peretinoin				Placebo $n = 129$	
	600 mg/day $n = 132$		300 mg/day $n = 131$			
	Total Number (%)	Serious	Total	Serious	Total	Serious
Investigations						
Albumin urine present	29 (22.0)	0	14 (10.7)	0	8 (6.2)	0
Blood pressure increased	26 (19.7)	0	20 (15.3)	0	19 (14.7)	1 (0.8)
Transaminases increased	23 (17.4)	0	10 (7.6)	0	15 (11.6)	0
Protein urine present	8 (6.1)	0	2 (1.5)	0	0 (0.0)	0
Blood urine present	5 (3.8)	0	7 (5.3)	0	3 (2.3)	0
Gamma-glutamyl transferase increased	3 (2.3)	0	5 (3.8)	0	12 (9.3)	0

Adverse events occurred in ≥5 % of patients in any of the treatment groups in the safety analysis set, as shown in Medical Dictionary for Regulatory Activities (MedDRA) Version 12.0. Adverse events were classified as being serious or non-serious in accordance with definition adopted by the International Conference on Harmonization

fact that 70 % of study patients were aged ≥65 years attests to the overall tolerability of peretinoin in elderly patients. The Child-Pugh class could also affect peretinoin efficacy. The guidelines for clinical studies of HCC recommend inclusion of patients with Child-Pugh A in clinical trials because death from cirrhosis in patients with Child-Pugh B or C could mask the efficacy of treatment [28]. The proportion of patients with Child-Pugh A was about 80 % in this study. Subgroup analysis revealed that the effect of peretinoin 600-mg in Child-Pugh A patients was significant (HR, 0.60; 95 % CI 0.41–0.89), even though these analyses were post-hoc and exploratory. Recently, a confirmatory peretinoin study was initiated and will be focused on Child-Pugh A patients.

Systemic chemotherapy, immunotherapy, and interferon have not been established as a standard treatment for the prevention of HCV-HCC [13–15]. Through its antiviral action, interferon delays the progression of cirrhosis and hepatic impairment and prolongs survival (pooled risk ratio, 0.65; 95 % CI 0.52–0.80). However, it does not significantly reduce recurrence (pooled risk ratio, 0.86; 95 % CI 0.76–0.97) [13]. Peretinoin was previously shown to decrease the risk of recurrence of de novo carcinogenesis by about 70 % [20], and this result was reproduced in this study. Considerably prolonged survival was observed (risk ratio, 0.3; 95 % CI 0.1–0.8) [21].

A previous study revealed that 25 % of adverse reactions to interferon were severe, resulting in treatment discontinuation or dose reduction [13]. While safety issues prevent the use of interferon in elderly patients [29, 30], this study indicated that peretinoin was well tolerated by such elderly patients.

One limitation of this study is that the median follow-up period of 2.5 years may be considered short. Since recurrences due to de novo carcinogenesis peak after 4 years postoperatively [11], the reduction of de novo carcinogenesis due to peretinoin may have been more accurately reflected with a longer follow-up period. However, the sample size at 2 years in this study (about 40 patients in each group) was comparable to the sample size of a previous study [21] and adequate to statistically evaluate the efficacy of peretinoin. Future studies should, however, include longer follow-up analysis if feasible.

In this study, the superiority of peretinoin (300 and 600-mg) to placebo could not be evaluated. Peretinoin 600 mg/day was found to be the optimal dose, and it could possibly reduce the recurrence of HCV-HCC. Although the HR for the RFS in the peretinoin 600 mg/day vs. placebo was not statistically significant for the entire study period, the significant reduction by >70 % in recurrence after 2 years is clinically meaningful and consistent with previous study results [21]. Further confirmatory studies on Child-Pugh class A patients are worth conducting and would continue to ensure the efficacy of the peretinoin 600 mg/day regimen and thoroughly explore its safety.

Acknowledgments The authors would like to thank Yoshiro Niitsu, Yutaka Aoyagi, Kunihiko Hayasi, Shigehiro Katayama, and Akira Itabashi, who all served on the independent data and safety monitoring committee; special thanks also goes to Tomoaki Ichikawa and Kazuhiko Ueda who served as independent CT image reviewers. The authors would like to acknowledge Statcom Co., Ltd, for their assistance in the preparation and editing of this manuscript. This study was financially supported by Kowa Company, Ltd. Additionally, the authors thank the following principal investigators who enrolled patients in the peretinoin study: (The principal investigators listed below are members of the Peretinoin Study Group) Toshiya Kamiyama, Yoshiyasu Karino, Kunihiko Tsuji, Kazuhiro Kasai, Kenichi Ido, Norio Isoda, Norihiro Kokudo, Fuminori Moriyasu, Shigeki Arii, Takuji Okusaka, Junji Furuse, Kohei Nakachi, Katsumi Sasaki, Kenji Ikeda, Namiki Izumi, Shigehiro Kokubu, Takahide Nakazawa,

Kazushi Numata, Hisataka Moriwaki, Yoichi Nishigaki, Fumihiro Urano, Susumu Shiomi, Masatoshi Kudo, Kiyohide Kioka, Yukio Osaki, Michio Kato, Eiji Mita, Hiroshi Kasugai, Kazuho Imanaka, Harumasa Yoshihara, Toshihito Seki, Takefumi Nakamura, Toyokazu Fukunaga, Shuhei Nishiguchi, Kazuaki Chayama, Mikiya Kitamoto, Isao Sakaida, Satoyoshi Yamashita, Norio Horiike, Yoichi Hiasa, Makoto Tokumatsu, Nobuhiko Higashi, Shotaro Sakisaka, Yozo Iida, Hiromi Ishibashi, Takashi Kanematsu, Masataka Seike, Yutaka Sasaki, and Hirohito Tsubouchi.

Conflict of interest Izumi has received lecture fees from MSD, Chugai Pharmaceutical, Daiichi Sankyo, and Bayer. Matsui has received consulting fees from Kowa, a research grant from Bayer, a grant from PSP, and other financial support from Eisai and Bayer. Kaneko has received research grants from Bayer and Nippon Shinyaku, and grants from Chugai Pharmaceutical, MSD, Kowa, Mitsubishi Tanabe Pharma, Bristol-Myers Squibb, Eli Lilly, Boehringer Ingelheim Japan, Kyowa Hakko Kirin, Dainippon Sumitomo Pharma, Takeda Pharmaceutical, Eisai, Daiichi Sankyo, AstraZeneca, Novartis Pharma, Astellas Pharma, Teijin Pharma, Zeria Pharmaceutical, Sanofi, Toray Industries, Shionogi, Novo Nordisk Pharma, and Pfizer Japan. Moriwaki has received grants from Ajinomoto Pharmaceuticals, Chugai Pharmaceutical, MSD, Mitsubishi Tanabe Pharma, Otsuka Pharmaceutical, and Bristol-Myers Squibb, and lecture fees from Otsuka Pharmaceutical and Ajinomoto Pharmaceuticals. Kokudo has received a research grant from Bayer, and grants from Chugai Pharmaceutical and Yakult. Nishiguchi has received grants from Chugai Pharmaceutical, MSD, Ajinomoto Pharmaceuticals, and Otsuka Pharmaceutical. Okusaka has received research grants from Chugai Pharmaceutical, Kowa, Otsuka Pharmaceutical, Yakult, Eli Lilly, Taiho Pharmaceutical, Boehringer Ingelheim Japan, Kyowa Hakko Kirin, Ono Pharmaceutical, Takeda Bio Development Center, and the Shizuoka Industrial Foundation. Shiomi has received a research grant from Nihon Medi-Physics. Kudo has received lecture fees from Bayer, and grants from MSD and Takeda Pharmaceutical. Ido has received consulting fees from Kowa. Hayashi has a received grant from Chugai Pharmaceutical. Ohashi has received lecture fees from Kowa Pharmaceutical, and grants from Kowa Pharmaceutical and Astellas Pharma. Kumada holds a patent on SRL, and has received lecture fees from MSD, Dainippon Sumitomo Pharma, Ajinomoto Pharmaceuticals, Mitsubishi Tanabe Pharma, and Bristol-Myers Squibb. The other authors declare that they have no conflicts of interest.

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Antiviral Therapy 2014; 19:479–490 (doi: 10.3851/IMP2729)

Original article

Virological escape in HCV genotype-1-infected patients receiving daclatasvir plus ribavirin and peginterferon alfa-2a or alfa-2b

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Background: Daclatasvir (DCV; BMS-790052) is a picomolar inhibitor of HCV non-structural protein 5A (NS5A) and has demonstrated efficacy in patients chronically infected with HCV.

Methods: In the double-blind, randomized studies AI444021 and AI444022, 71 Japanese patients chronically infected with HCV genotype 1 (predominantly genotype 1b) received DCV (10 mg or 60 mg) plus peginterferon alfa-2b or alfa-2a and ribavirin. Virological failure occurred in 14% (5/36) of treatment-naive patients and 54% (19/35) of prior alfa/ribavirin non-responders. Resistance testing was performed on baseline samples and samples with HCV RNA≥1,000 IU/ml at week 1 through post-treatment week 24.

Results: Baseline NS5A resistance-associated polymorphisms had less impact on virological response rates than IL28B genotype. All patients with virological failure had NS5A DCV-resistant variants at the time of failure. The predominant NS5A variants were L31V/M/I plus Y93H; this combination was detected in 100% (5/5) of treatment-naive patients and 74% (14/19) of non-responders with failure. Emergent resistance variants in prior non-responders (four viral breakthroughs, one relapse) were more varied with novel combinations such as L31F-ΔP32 and L28M-R30Q-A92K detected. Significant loss in DCV antiviral activity was generally only seen with ≥ two resistance-associated NS5A substitutions. All DCV-resistant variants were still detected at end of study.

Conclusions: Virological failure in HCV genotype 1b treatment-naive Japanese patients receiving DCV plus alfa-2a/ribavirin or alfa-2b/ribavirin was associated with enrichment of NS5A resistance variants L31V/M-Y93H. In prior non-responders, emergent variants associated with failure also included NS5A-A92K or NS5A-ΔP32. As with L31-Y93 variants, these variants persisted.

Introduction

Treatment of chronic hepatitis C has improved substantially with the introduction of direct-acting antivirals targeting HCV NS3 protease, particularly for patients infected with HCV genotype 1 (GT1), the predominant genotype in the USA (GT1a), north Asia (GT1b) and Europe [1,2]. Addition of the NS3 protease inhibitors boceprevir or telaprevir to peginterferon alfa and ribavirin (alfa/RBV) yields sustained virological response (SVR) rates of 70–75% in treatment-naive patients [3,4]. However, response rates remain low in prior alfa/RBV

non-responders [5–7]. Telaprevir and boceprevir also have complicated dosing schedules and drug interaction profiles, and are associated with frequent adverse events [8,9]. Thus, new therapies are needed with broader efficacy across HCV genotypes and improved tolerability.

Daclatasvir (DCV; BMS-790052) is a once-daily, pan-genotypic HCV non-structural protein 5A (NS5A) replication complex inhibitor with picomolar potency *in vitro* [10,11]. In clinical studies, DCV combined with alfa/RBV has shown an adverse event profile comparable with that of alfa/RBV alone, and has achieved rapid, dose-dependent declines in HCV RNA [12,13]. In a study in treatment-naive patients with HCV GT1 infection, 83% of patients receiving DCV+ alfa/RBV for 48 weeks experienced an SVR 24 weeks post-treatment (SVR_{24}) [13].

DCV resistance has been mapped to the N-terminal region of HCV NS5A using subgenomic GT1 replicons [14,15]. In GT1a, signature resistance substitutions were identified at NS5A amino acid positions M28, Q30, L31 and Y93, while NS5A-L31V and Y93H were the major signature substitutions in GT1b. Single substitutions at these sites confer only modest reductions in DCV susceptibility in GT1b replicons compared with substantially greater reductions in GT1a; the requirement for multiple substitutions in GT1b to confer significant loss in DCV susceptibility reflects a higher barrier to resistance. Clinical data support this concept: in a study that assessed the alfa/RBV-free combination of DCV and the NS3 protease inhibitor asunaprevir in prior null responders, emergence of drug-resistant variants associated with viral breakthrough occurred in six of nine patients with GT1a infection and in neither of two patients with GT1b [16].

Two Phase II studies of DCV combined with alfa/RBV have recently been completed in Japanese treatment-naive patients and prior alfa/RBV non-responders with primarily GT1b infection [17,18]. DCV combined with RBV and alfa-2a or alfa-2b achieved SVR rates of 90–100% in treatment-naive patients; among previous alfa/RBV non-responders, SVR rates of 78% and 33% were observed in the alfa-2a and alfa-2b studies, respectively. We conducted genotypic and phenotypic analyses of samples from study patients to assess the impact of baseline polymorphisms, viral load, and IL28B genotype on virological outcome and to identify emerging drug-resistant variants associated with virological failure.

Methods

Study designs

Studies AI444021 (Clinicaltrials.gov identifier NCT01016912) and AI444022 (Clinicaltrials.gov identifier NCT01017575) were double-blind, randomized, Phase IIa studies assessing the antiviral activity and safety of DCV combined with alfa/RBV in Japanese adults (20–70 years old) with chronic HCV GT1 infection (HCV RNA$\geq 10^5$ IU/ml), including both treatment-naive patients and prior alfa/RBV non-responders (null or partial). Treatment-naive patients had never received interferon-based or direct-acting antiviral HCV therapy; non-responders had failed to achieve ≥ 2 $\log_{10}$ HCV RNA reduction at week 12 (null responder) or HCV RNA never became undetectable after ≥12 weeks (partial responder) of alfa/RBV. Eligible patients received DCV 10 mg or 60 mg or placebo (treatment-naive only) once daily in combination with RBV and weekly alfa-2b (AI444021) or alfa-2a (AI444022). Patients in the placebo group received alfa/RBV for 48 weeks. DCV recipients who achieved a protocol-defined response (PDR; HCV RNA below the lower limit of quantification [LLOQ, 15 IU/ml] at week 4 and undetectable at week 12) were treated for 24 weeks; those without a PDR received 48 weeks of treatment [17,18].

Study assessments

Plasma samples were collected at baseline and at weeks 1, 2, 4, 6, 8 and 12, and then every 4 weeks on-treatment, week 4 post-treatment for patients with virological breakthrough, and weeks 4, 12 and 24 post-treatment for treatment responders. HCV RNA levels were determined using the Roche COBAS® TaqMan® HCV Auto assay (Roche Diagnostics KK, Tokyo, Japan; LLOQ, 15 IU/ml). Resistance analysis was performed on all samples at baseline and following virological failure when HCV RNA levels exceeded 1,000 IU/ml. Virological failure categories comprised the following: failure to achieve early virological response (EVR; <2 $\log_{10}$ HCV RNA decrease from baseline at week 12); virological breakthrough (confirmed detectable on treatment after previously undetectable or >1 $\log_{10}$ HCV RNA increase from nadir); HCV RNA detectable at end of therapy (including early discontinuation) or HCV RNA detectable post-treatment when HCV RNA was undetectable at end of therapy (relapse).

Genotypic analyses

Viral RNA was isolated from plasma (QIAmp MinElute Vacuum Kit; Qiagen, Gaithersburg, MD, USA) and the patient-derived HCV NS5A region was amplified by reverse transcriptase PCR and population-sequenced [15]. For clonal analysis, PCR amplicons were cloned into the TOPO vector and transformed into TOP10 *Escherichia coli* using a commercial kit (TOPO® TA cloning® kit; Invitrogen, Carlsbad, CA, USA) with ≥20 individual colonies expanded and sequenced for each analysis. Consensus sequences from clinical samples were compared with

reference sequences for GT1a (H77) or GT1b (Con1) as appropriate.

Phenotypic analyses

Phenotypic analyses of resistance-associated NS5A substitutions were performed using established *in vitro* HCV replicon systems [14,19]. The parental Con 1 replicon construct used in these studies carried a Renilla luciferase reporter gene and the S2204I cell culture-adaptive mutation. Amino acid substitutions were introduced into the NS5A coding region by recombinant PCR; PCR products were infused onto digested Con1 replicon DNA, which was cut with Eco47III (in NS4B) and EcoRI (in NS5A) per the manufacturer's instructions (In-Fusion Cloning kit; Clontech Laboratories, Mountain View, CA, USA) and confirmed by sequence analysis. For phenotyping of patient-derived NS5A sequences, NS5A was deleted from the Con1 replicon and SacII and ClaI restriction sites flanking the NS5A coding region were added. An S2204I adaptive mutation was added to NS5A amplified from patient plasma samples, then infused onto the modified Con1 replicon. Replicon clones were linearized with ScaI and transcribed *in vitro* with a T7 RiboMAX kit (Promega Co., Madison, WI, USA). For transient transfection assays, 10 to 20 μg of RNA were transfected into Huh-7 cells using DMRIE-C reagent (Invitrogen) per the manufacturer's instructions. After 16 h, cells were transferred to 96-well assay plates (Corning Inc., Corning, NY, USA) and dilutions of inhibitors in DMSO were added (1 μl/well). After an additional 72 h, plates were assayed with a Renilla luciferase assay system (Promega Co.). Where signal strength was poor in the transient assay, stable cell lines were selected to determine DCV susceptibility changes.

Results

Patients and virological responses

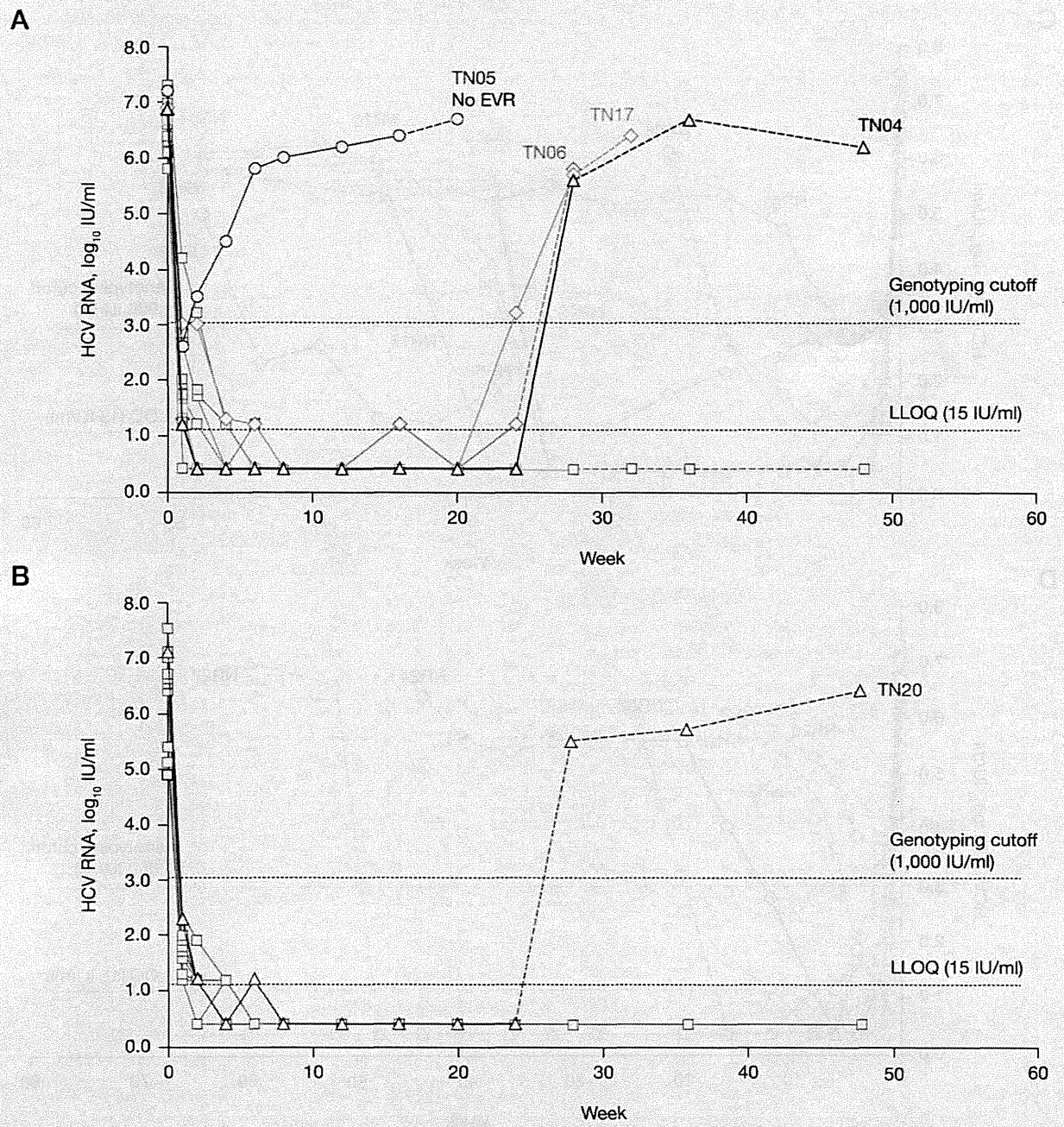
A total of 71 study patients (36 treatment-naive, 35 non-responders [22 null/13 partial responders]) received DCV plus alfa-2b/RBV (study AI444021) or alfa-2a/RBV (study AI444022) and were included in the analysis. All patients were infected with HCV GT1b except two treatment-naive patients with GT1a in the 10 mg arm of AI444022. Patients generally experienced rapid reduction in viral load after 1 week (Figure 1). HCV RNA was undetectable in most treatment-naive patients at the end of treatment and 24 weeks post-treatment (Table 1). In total, 5 of 36 treatment-naive patients experienced virological failure, 5 in the DCV 10 mg arm (3 receiving alfa-2b/RBV, 1 receiving alfa-2a/RBV) and 1 in the DCV 60 mg arm (alfa-2b/RBV). Among non-responders, HCV RNA was undetectable at the end of treatment in most patients but many relapsed post-treatment, particularly with DCV 10 mg (Table 1). In total, 19 of 35 non-responders (14/22 null, 5/13 partial responders; 13 with alfa-2b/RBV, 6 with alfa-2a/RBV) experienced virological failure. All 6 failures (4 receiving DCV 10 mg, 2 receiving DCV 60 mg) in the alfa-2a study were prior null responders, whereas the 13 failures (7 receiving DCV 10 mg, 6 receiving DCV 60 mg) in the alfa-2b study included both null responders (4 receiving DCV 10 mg, 4 receiving DCV 60 mg) and partial responders (3 receiving DCV 10 mg, 2 receiving DCV 60 mg).

Treatment-naive patients

Baseline analysis

Virological responses among treatment-naive patients did not appear to be influenced by baseline HCV RNA levels or baseline NS5A polymorphisms associated with DCV resistance (Additional file 1 and Additional file 2). Baseline HCV RNA levels were 6.8–7.2 (median 6.9) $\log_{10}$ IU/ml among patients with virological failure versus 4.9–7.5 (median 6.6) $\log_{10}$ IU/ml among patients with SVR_{24}. Although baseline NS5A polymorphisms at amino acid positions associated with DCV resistance were observed in 24/36 treatment-naive patients, only 5 (1 GT1a and 4 GT1b) patients had the NS5A-Y93H variant (Additional file 1 and Additional file 2). This variant was previously associated with virological failure in patients with GT1b [19]. In GT1a, NS5A-Y93H is associated with a >5,000-fold decrease in DCV susceptibility, whereas in GT1b, it is associated with a <50-fold decrease in DCV susceptibility. However, Y93H at baseline lowers the GT1b resistance barrier to DCV [14,15]. Of the five treatment-naive patients with virological failure, four had baseline DCV resistance-associated NS5A polymorphisms, of which only one had Y93H. This patient (TN20, GT1b, IL28B genotype CT) received DCV 60 mg with alfa-2b/RBV and relapsed post-treatment. Four other patients from the alfa-2a study (one GT1a, three GT1b) had Y93H at baseline; these patients had the more favourable IL28B CC genotype and achieved SVR_{24}. Q54 variants (H or N), which alone do not change DCV susceptibility, were detected in three patients (one with no EVR, two with virological breakthrough). As with TN20, these three patients had non-CC IL28B genotypes, as did one patient who relapsed and had no baseline NS5A polymorphisms. The 16 remaining patients with baseline DCV resistance-associated polymorphisms achieved SVR_{24}; all but 1 (TN34, Q30Q/R) were IL28B genotype CC. Overall, virological failure appeared to be associated with non-CC IL28B genotype. There were 11/36 treatment-naive patients with non-CC IL28 genotype, 7 receiving alfa-2b and 4 receiving alfa-2a (Additional file 1); these included all 5 patients with virological failure (1 receiving alfa-2a, 4 receiving alfa-2b).

Figure 1. HCV RNA levels on and off treatment, individual patients



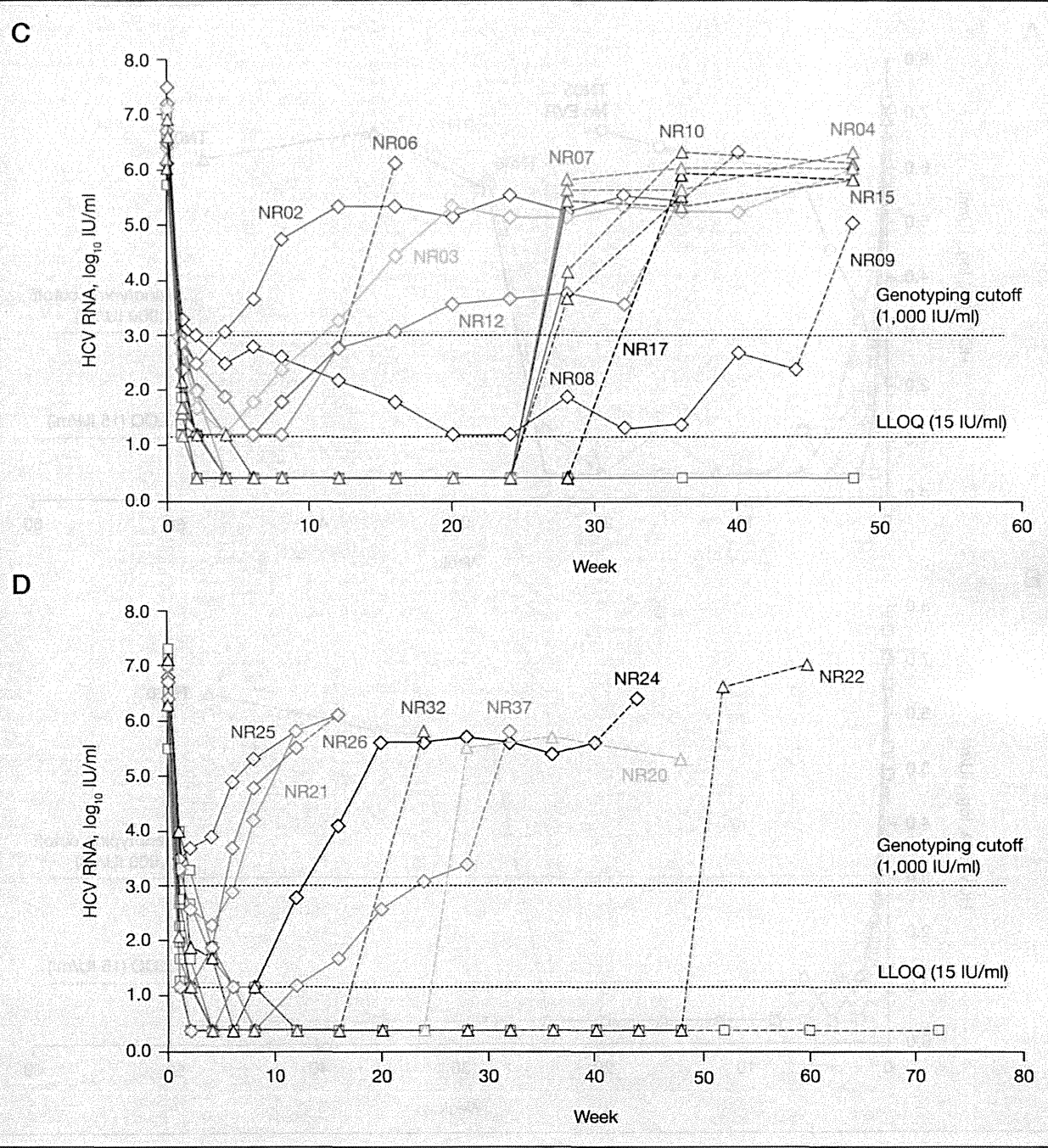
HCV RNA levels through week 24 of follow-up in treatment-naive patients receiving **(A)** daclastavir (DCV) 10 mg or **(B)** DCV 60 mg and in previous non-responders receiving **(C)** DCV 10 mg or **(D)** DCV 60 mg. Solid lines indicate on-treatment data, dashed lines indicate off-treatment follow-up. The lower limit of quantitation (LLOQ; 15 IU/ml) and the cutoff for genotypic analysis (1,000 IU/ml) are also indicated. Patient numbers are indicated for patients with virological breakthrough (represented by a diamond) or post-treatment relapse (represented by a triangle). EVR, early virological response.

Possible pharmacokinetic reasons for virological failure were also investigated. Patient TN05, who failed to achieve EVR, and patients TN06 and TN17, who experienced virological breakthrough, all had DCV pharmacokinetic parameters lower than most other patients receiving the same 10 mg dose of DCV (Additional file 3). Pharmacokinetic parameters did not indicate any obvious deficiency in DCV exposure in patients TN04 or TN20, who both relapsed (Additional file 3).

Variants emerging on treatment

DCV-resistant variants emerged during treatment in all five treatment-naive patients with virological failure (one receiving alfa-2a, four receiving alfa-2b; Table 2).

Figure 1. Continued



In all cases, ≥ two DCV-resistant variants were detected with similar patterns in all five patients regardless of dose; L31V plus Y93H was the principal combination observed. All observed combinations of DCV-resistant variants were associated with >8,000-fold decrease in DCV susceptibility *in vitro* (50% effective concentration [EC_{50}]>25 nM) compared with the reference replicon (EC_{50}=0.003 nM). In all cases, DCV-resistant variants detected during breakthrough persisted post-treatment and DCV-resistant variants detected during early relapse persisted to the last post-treatment time point (post-treatment week 24 in this study).

Non-responders

Baseline analyses

As with treatment-naive patients, virological responses among non-responders did not appear to be influenced by baseline HCV RNA levels although it was unclear