Sustained viral response was defined as an undetectable HCV RNA level at 24 weeks after the end of treatment. Relapse was defined as the reappearance of serum HCV RNA during the follow-up period from the state of undetectable serum HCV RNA at the end of treatment. Breakthrough was defined as the state when the viral level increased by 2 $\log_{10}$ IU/mL from nadir or a level of more than 3 $\log_{10}$ IU/mL after reaching undetectable levels during treatment. Partial responders were subjects whose HCV RNA level dropped by at least 2 $\log_{10}$ IU/mL during treatment but was still detected at the end of treatment.

Sequence analysis at HCV NS3 protease domain

HCV RNA was isolated from serum samples collected on the same day for the measurement of HCV RNA levels. A DNA fragment of 543 bases long (181 amino acids) from the NS3 protease domain was amplified by nested RT-PCR and cloned. At least 39 clones per specimen were sequenced bi-directionally. The limit of detection for the sequencing analysis was 3.0 $\log_{10}$ IU/mL.

Safety assessments

Safety of telaprevir was assessed by clinical laboratory tests, vital signs, abdominal ultrasonography and AEs. Twelve-lead electrocardiogram (ECG) examinations were performed once during the screening period. These safety parameters were reported at regular intervals from 4 weeks before the first dosing to the end of the follow-up period.

Statistical analysis

Statistical analyses were performed using the statistical software SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Reported AEs were classified according to MedDRA/J version 12.0 (MedDRA Japanese Maintenance Organization, Tokyo, Japan).

RESULTS

Baseline characteristics

Fifteen treatment-naïve patients infected with HCV subtype 1b were enrolled in this study. Baseline characteristics of patients are shown in Table 1. All patients were Japanese whose median age was 58.0 years (range: 45–68); 6 (40.0%) patients were men. Patients over 54 years of age accounted for 66.7% (10 of 15). Median baseline HCV RNA level was 6.80 $\log_{10}$ IU/mL (range: 3.55–7.10). The median BMI was 20.9 kg/m^2 (range: 16.2–27.5).

Virological response

Telaprevir alone caused a rapid decrease in HCV RNA levels after the initiation of treatment in all patients. The average changes were −3.24 $\log_{10}$ IU/mL on Day 3 and −4.24 $\log_{10}$ IU/mL on Week 1 (Fig. 1). The average of maximum reduction in each patient was 5.01 $\log_{10}$ IU/mL. The HCV RNA levels became undetectable in 1, 3, 3 and 5 patients at Weeks 1, 4, 6 and 8, respectively. Three patients with negative HCV RNA after 4 weeks achieved end of treatment response (ETR), of whom one patient achieved a SVR. The patient who achieved SVR had the lowest baseline viral load (3.55 $\log_{10}$ IU/mL) among all the patients.

Ten of 15 patients discontinued the telaprevir treatment because of the following reasons: six patients because of viral breakthrough, two patients because of AEs, one patient because of own drug discontinuation and one patient who met the exclusion criteria after administration.

Safety

AEs observed in two or more patients in this study are shown in Table 2. During the study, 14 of 15 patients experienced 80 AEs in total and 62 events were judged as adverse drug reactions. The common AEs that occurred in

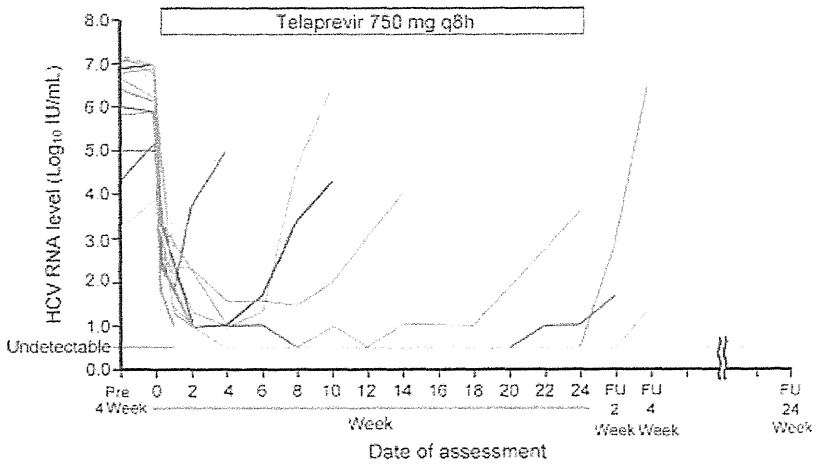

Fig. 1 HCV RNA kinetics during and after treatment with telaprevir monotherapy.

Table 2 Incidence of adverse events that occurred in two or more patients

	N = 15			
	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Rash	5 (33.3)	3 (20.0)	0 (0.0)	8 (53.3)
Anaemia	7 (46.7)	0 (0.0)	0 (0.0)	7 (46.7)
Low-density lipoprotein increased	6 (40.0)	0 (0.0)	0 (0.0)	6 (40.0)
Blood uric acid increased	4 (26.7)	0 (0.0)	0 (0.0)	4 (26.7)
Pruritus	3 (20.0)	1 (6.7)	0 (0.0)	4 (26.7)
Anorexia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Dysgeusia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Headache	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Diarrhoea	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Pyrexia	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Thirst	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Nasopharyngitis	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood creatinine increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood triglycerides increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Platelet count decreased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Dizziness	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)

MedDRA (Ver.12.0).

more than 25% of patients were rash (53.5%), anaemia (46.7%), low-density lipoprotein (LDL) increases (40.0%), blood uric acid increase (26.7%) and pruritus (26.7%). Two patients discontinued telaprevir treatment because of AEs (herpes zoster or rash pruritic). Except for the herpes zoster whose severity was judged as severe and serious, all the events were mild to moderate. Fifty of the 80 AEs were observed within the first 4 weeks.

In relation to skin AEs, rash, pruritus and rash pruritic were observed in 8, 4 and 1 patients, respectively. The onset day of these events is described in Fig. 2. The range of the onset day was Day 1 to Day 113, and the median was Day 15. Rash in three patients, pruritus in one patient and rash pruritic in one patient were moderate, and the others were mild. One patient discontinued telaprevir at Week 6 because of moderate rash pruritic. Most of the skin AEs were treated with oral antihistamines or topical steroids.

A decrease in haemoglobin levels was observed in all patients (Fig. 3a). Seven of 15 patients developed anaemia during and after the treatment. All anaemia events were mild and no patient needed discontinuation of telaprevir. Uric acid and LDL cholesterol increased during the treatment (Fig. 3b,c), but these changes were mild and no patient needed any medication for these AEs. There were no substantial increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-bil).

Sequence analysis at HCV NS3 protease domain

Amino acid substitutions in the NS3 protease domain were examined in 39 clones or more in each sample. Before Week 8, V36A/G, T54A and A156T/V as single substitutions, and T54A + R155K and A156T/V + V158I as multiple substitutions were observed. Among two patients who discontinued telaprevir within 2 weeks, all clones but three in one patient were wild-type variants after withdrawal of telaprevir. In three patients who discontinued at Weeks 5–7 because of viral breakthrough, predominant clones possessed A156V/T substitutions after the nadir of viral load. Predominant variants observed during and after telaprevir monotherapy in the eight patients who received telaprevir beyond 8 weeks are shown in Fig. 4 together with HCV RNA levels. In the two patients who showed the lowest HCV RNA level of on Week 4, the predominant clones detected after

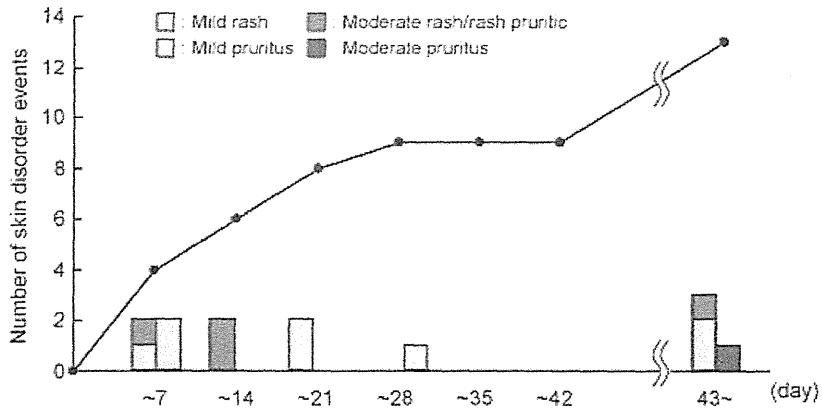

Fig. 2 Rash and pruritus occurrence.

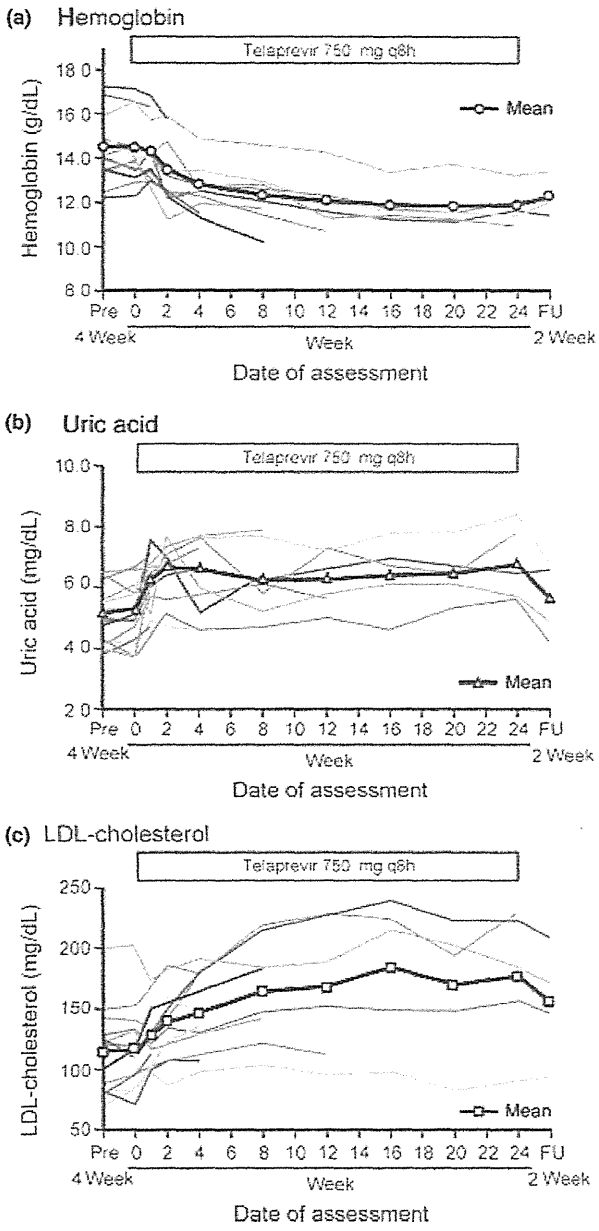

Fig. 3 Changes in (a) hemoglobin, (b) uric acid, (c) LDL-cholesterol.

viral breakthrough were A156F and T54A. One other patient with nadir HCV RNA level on Week 8 had a predominant clone of T54A + I132L after viral breakthrough. Among the five patients who completed the telaprevir treatment for 24 weeks as scheduled, two patients were HCV RNA positive at the end of treatment. One of these two patients had an A156F substitution at the end of treatment, and a A156Y substitution was also detected on Week 1 of the follow-up period. In the two patients who relapsed during the follow-up period, the predominant clone was T54A which shifted to the wild-type variant in one patient.

DISCUSSION

Although higher SVR rates and shorter duration of treatment were achieved by telaprevir in combination with PEG-IFN and RBV in US, EU and Japan [2–6], the DAA combination regimens also increased the frequency and severity of side effects usually observed in the PEG-IFN and RBV therapy. As most patients in Japan are aged people, IFN-free regimens are in urgent need because these patients are intolerant to IFN-based therapies [12–14].

In this exploratory study, one of 15 patients on telaprevir monotherapy was able to achieve SVR. A low viral load of <4 $\log_{10}$ IU/mL in this patient probably contributed to the achievement of SVR, and Suzuki *et al.* [15] published this case report in detail. Although the SVR rate obtained in the study was not beneficial enough, the telaprevir monotherapy could decrease HCV RNA levels dramatically in all cases. The severity of skin-related AEs during telaprevir monotherapy was milder than those of cases developing in the co-administration with PEG-IFN and RBV [5,6,16–18]. All the events were mild to moderate and manageable with antihistamines or topical steroids. Similarly to the skin-related events, decreases in haemoglobin levels were mild, and the incidence of anaemia was 46.7%. As all the anaemia events were mild, there was no need for discontinuation of telaprevir or use of any medications. Severe skin rash and anaemia observed in the therapy with telaprevir in combination with PEG-IFN and RBV are probably ascribable to the synergistic effect of these three drugs. Although the mechanism of uric acid and LDL cholesterol elevation during treatment with telaprevir has been established, these changes disappeared at the end of telaprevir dosing. Telaprevir was generally well tolerated in all the patients.

Amino acid substitutions in the HCV NS3 protease domain were monitored during the study. The relationship between these substitutions and resistance to NS3-4A protease inhibitors has been well documented by *in vitro*, *in vivo* and clinical studies [19–22]. In the eight patients who received the telaprevir monotherapy beyond 8 weeks, the predominant breakthrough variants were T54A and A156F, which were not observed at the earlier time points (Fig. 4). Furthermore, in the clones accounting for more than 10% of each specimen, the secondary substitution of V158I and I132L was identified along with the primary resistant-associated substitution of A156T/V and T54A, respectively, and a novel substitution of A156Y was also observed. This study confirms the higher genetic barrier of HCV subtype 1b against the V36M ± R155K substitutions. Our results clearly indicate that the prolonged telaprevir monotherapy leads to the development of various variants. As the replication fitness of drug-resistant variants tends to be lower than that of wild type, the former are likely to be overtaken by the wild-type virus under drug-free conditions within 3–7 months [11,23,24]. As Ozeki *et al.* [25] reported that four patients with favourable IL28B SNP who failed to eradicate HCV with telaprevir monotherapy were

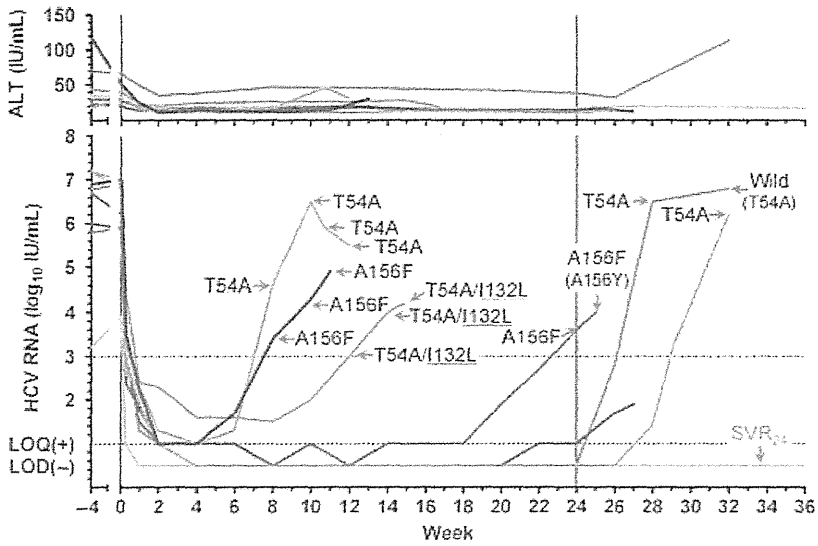

Fig. 4 Viral kinetics and predominant variants during and after telaprevir monotherapy beyond 8 weeks. Besides predominant clones, minority clones which account for 10% and more in a specimen are also summarized by brace notation. Putative secondary resistant-associated mutation is indicated by underline.

responsive to sequential therapy with PEG-IFN and RBV, the substitutions in the NS3 protease domain by the telaprevir treatment are not correlated with resistance to PEG-IFN and/or RBV directly as described previously [23,24]. Sequential therapy with PEG-IFN and RBV after relapse or viral breakthrough on telaprevir monotherapy might be a therapeutic option in some cases, including the case of low haemoglobin. By taking the error-prone nature of HCV replication into account, successful eradication with IFN-free DAA(s) regimens probably depends on how efficiently DAA can suppress various DAA-resistant variants that pre-exist and are selected under DAA pressure. The telaprevir-based combination therapy with other DAA(s) such as NS5A or NS5B polymerase inhibitors may be useful for successful treatment. Using a human chimeric liver mouse model for HCV infection, Ohara *et al.* [26] reported that the combination of telaprevir with a high-dose nucleoside analogue could successfully eradicate HCV infection. Recently, it was reported that the dual therapy with daclatasvir, an NS5A replication complex inhibitor, and asunaprevir, NS3-4A protease inhibitor, had high SVR rates in difficult-to-treat patients with subtype 1b and null responders [27,28]. These successful results are also helpful for us to consider telaprevir-based IFN-free regimens in combination with other DAAs against HCV.

In conclusion, telaprevir monotherapy was well tolerated and provided potent but temporary antiviral activity in Japanese patients with subtype 1b HCV, with an SVR rate of 7%. Most AEs were mild to moderate and much milder than those recorded in patients on combinations with PEG-IFN and RBV. As the essential characteristics of DAAs including telaprevir are substantially masked in the co-administration with other antivirals, the knowledge obtained from the long-term telaprevir monotherapy is most likely to contribute to the future HCV treatment with DAA-based regimens.

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Original Article

Exploratory study on telaprevir given every 8 h at 500 mg or 750 mg with peginterferon-alpha-2b and ribavirin in hepatitis C patients

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Aim: The aims of this study are to assess the antiviral effects, safety and telaprevir (TVR) pharmacokinetics in two cohorts given TVR every 8 h (q8h) at doses of 500 mg and 750 mg with peginterferon-α-2b and ribavirin in chronic hepatitis C patients.

Methods: Twenty chronic hepatitis C (HCV) patients with genotype 1b in high viral loads were randomly assigned to two TVR-based regimens of 750 mg q8h (group A) and 500 mg q8h (group B) in combination with peginterferon-α-2b and ribavirin for 12 weeks.

Results: Although the difference was not statistically significant other than trough concentration (C_{trough}) at week 4, the parameters of maximum concentration (C_{max}), the area under the concentration time curve ($AUC_{0-\infty}$) and C_{trough} tended to be higher in group A than those in group B. The antiviral effects were similar in the two groups (sustained virological response rates [SVR], 40% in group A, 50% in group B). The discontinuation rates by anemia were 30% in group A and 20% in group B. Serum creatinine concentrations were lower in group B than those in group A.

Conclusion: Although the exposure to TVR tended to be lower in 500 mg q8h than that in 750 mg q8h, the SVR rates in both groups were similar. The result suggests that the 500 mg q8h dose may be one option for treatment. In addition, the present findings indicate that the development of adverse events which increase with a TVR-based regimen, specifically anemia and creatinine, could be avoided by dose adjustment of TVR.

Key words: anemia, chronic hepatitis C, creatinine increase, pharmacokinetics, telaprevir

INTRODUCTION

THE WORLD HEALTH organization (WHO) estimates that approximately 170 million people are infected with hepatitis C virus (HCV).[1] Decompensated cirrhosis and hepatocellular carcinoma (HCC) develop in approximately 30% of individuals infected with HCV and result in a fatal outcome.[2,3] In Japan, it is estimated that more than 1.5 million people are chronically infected with hepatitis C. Telaprevir (TVR), a potent HCV protease inhibitor, has recently been approved for the treatment of people suffering from chronic genotype 1 HCV infection in the USA, European Union (EU) and Japan. The overseas phase 3 studies demonstrate that patients who received TVR in combination with peginterferon (PEG IFN)-α-2b and ribavirin (RBV) achieved significantly higher rates of sustained virological response (SVR) than those who received only PEG IFN and RBV, regardless of their prior treatment experience with the anti-HCV agents.[4–6] The high SVR rates were also observed in the Japanese phase 3 studies of the TVR-based triple regimen.[7,8] In Japanese patients, anemia was the most common side-effect in the TVR-based triple regimen. The epidemiology of chronic hepatitis C (CHC) in Japan takes on a different aspect

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from that of the USA and EU; thus, the age of the majority of Japanese patients is high and their bodyweights are low in comparison with those in Caucasians.[4–8] As a result, the RBV dose-reduction rates and the discontinuation rates of TVR treatment due to adverse events are higher in Japan than those in the USA and EU,[4–8] though the addition of RBV increased the SVR rates in patients receiving TVR-based regimens.[9] These backgrounds call for more efficient treatment of the aged and/or lower bodyweight in patients with CHC in Japan.

The antiviral activity at different doses of TVR was examined after administration of TVR alone for 14 days at 450 mg every 8 h (q8h), 750 mg q8h or 1250 mg q12h,[10] and the greatest HCV RNA reduction and the highest plasma trough concentrations (C_{trough}) were achieved in the 750 mg q8h cohort. On the basis of this result, the TVR 750 mg q8h regimen was selected in the TVR-based triple therapy thereafter. Indeed, TVR 750 mg q8h co-administrated with PEG IFN or PEG IFN/RBV resulted in greater HCV RNA reduction than that after the administration of TVR alone. The Advisory Committee Briefing Document for NDA prepared by the TVR Review Team reports that the higher exposure to TVR was significantly associated with the increased risk of anemia and grade 2 or higher hemoglobin toxicity, defined as hemoglobin of less than 10 g/dL or any decrease from baseline of more than 3.5 g/dL.[11] In addition, the comparison of individual exposure estimated from population pharmacokinetic analysis demonstrated that age, race, sex or weight/body mass index (BMI) of subjects had no clinically relevant effects on TVR exposure.[12]

We previously reported the dynamics of HCV RNA during 12 weeks of triple therapy of TVR (q8h at two doses of 500 mg and 750 mg) with PEG IFN and RBV in Japanese CHC patients.[13] From this perspective, in this study, we explored the antiviral effects, safety and TVR pharmacokinetics in the above Japanese CHC patients.

METHODS

Study design and organization

THIS DOUBLE-ARM, RANDOMIZED, open-label study was conducted between April 2008 and March 2009 at the Department of Hepatology in the Toranomon Hospital in compliance with Good Clinical Practice Guidelines and the Declaration of Helsinki. Before the study, the protocol and informed consent forms were approved by the Institutional Review Board. All patients had given informed consent in writing after sufficient explanation before they participated in this trial.

Patients

This study was conducted using 20 CHC patients who were selected according to the following inclusion and exclusion criteria.[13] Inclusion criteria: (i) diagnosed with CHC; (ii) infected with HCV-1b confirmed by the sequence analysis in the NS5B region; (iii) HCV RNA levels of 5.0 $\log_{10}$ IU/mL or higher determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) Japanese race (Mongoloid), aged 20–65 years at the entry; and (v) bodyweight of 35 kg or more but 120 kg or less at the time of registration. Exclusion criteria were the same as previously described.[13]

Study design

The 20 patients were randomly allocated to two groups with different doses of TVR by a third party institute, Bellsystem24 (Tokyo, Japan). TVR was administrated at a dose of 750 mg (group A) or 500 mg (group B) q8h intervals after meal. PEG IFN-α-2b (PegIntron; MSD, Tokyo, Japan) was injected s.c. to them at a median dose of 1.50 μg/kg (range, 1.250–1.739 μg/kg) once a week. RBV (Rebetol; MSD) was administrated at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose, 600–1000 mg). These three drugs were administrated for 12 weeks. After completion or discontinuation of the triple therapy, a follow-up observation was performed for 24 weeks. Doses of PEG IFN and RBV were reduced or their administration was discontinued, as required, based on the reduction of hemoglobin levels, white blood cell count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG IFN was reduced to half, when either leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm^3 or platelet count below 80×10^3/mm^3. PEG IFN was withdrawn when they decreased below 1000/mm^3, 500/mm^3 or 50×10^3/mm^3, respectively. When hemoglobin decreased below 10 g/dL, the daily dose of RBV was reduced from 600 to 400 mg, from 800 to 600 mg and from 1000 to 600 mg, depending on the initial dose of each patient. RBV was withdrawn when hemoglobin decreased below 8.5 g/dL. The decrease of TVR dose was not permitted, and its administration was stopped when the discontinuation was appropriate due to the development of adverse events.

In cases where the administration of TVR stopped, the administration of PEG IFN-α-2b and RBV was terminated also.

This study was registered at Clinical Trials (no. NCT00630058).

NS5A interferon-sensitivity determining region (ISDR) and core amino acid (a.a.) substitutions

Amino acid substitutions in the HCV core and NS5A ISDR regions were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. A core a.a. substitution at positions 70 and 91 (core 70 and core 91, respectively) was determined according to the procedure of Akuta *et al.*,[14,15] and the number of ISDR substitutions was determined using the methods of Enomoto *et al.*[16,17]

Single-nucleotide polymorphism (SNP) genotyping

Interleukin (IL)-28B (rs8099917 and rs12979860) and inosine triphosphate pyrophosphatase (rs1127354) were genotyped by the Invader assay, TaqMan assay or direct sequencing, as described elsewhere.[18–20]

HCV RNA measurements

Antiviral effects of TVR on HCV were assessed by measuring plasma HCV RNA levels. Blood samples were obtained on day 1 before dosing and at 2.5, 4, 8, 16 and 24 h after the first dose (the 8- and 16-h samples were collected before administration of the second and third administration, respectively). Pre-dose samples were obtained on days 2, 3, 8, 14, 29, 43, 57, 86, 92, 99, 113, 141, 169, 197, 225 and 253. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL.

Pharmacokinetic assessments

Blood samples were collected immediately before the first dose in the morning, and at 1, 2.5, 4, 6, 8, 12, 16 and 24 h after the first dose on days 1, 14 and 85 to determine the concentrations of TVR in the plasma. Samples were also taken before the first dose in the morning on days 3, 8, 29, 43, 57 and 99 for evaluation of trough concentrations of TVR.

Plasma concentrations of TVR were determined using a high-performance liquid chromatographic apparatus fitted with a mass spectrometer. Plasma concentrations and actual plasma-sampling times were used to calculate the area under the plasma concentration time curve from 0–8 h (AUC_{0-8h}) and terminal half-life ($t_{1/2}$) by the non-compartmental method using WinNonlin software Version 5.2.1. The maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were directly determined from the observed values on days 1, 14 and 85.

Safety assessments

During the on-study period, patients were monitored for safety at regular intervals from the start of dosing through every hospital visit. Safety assessments included physical examinations, clinical laboratory tests and check of adverse events. After the treatment was completed or aborted, patients were monitored for safety by the standard practice of investigators.

Statistical analysis

Hepatitis C virus RNA values in $\log_{10}$ IU/mL were summarized using descriptive statistics for each treatment group and at scheduled time points. From the plasma concentrations of TVR and clinical laboratory data, the descriptive statistics were calculated. Continuous variables between groups were compared by Student's *t*-test or Mann–Whitney *U*-test. The number of patients with adverse events was summarized by MedDRA (ver. 12.0) system organ class, preferred term and relationship to study drug. All statistical analyses were performed using the validated ver. 9.1.3 of the SAS System (SAS Institute, Cary, NC, USA) or SPSS software (ver. 19.0.0; IBM, Armonk, NY, USA).

RESULTS

Baseline demographic and virological characteristics of the 20 patients with CHC who received the triple treatment

TABLE 1 LISTS the baseline demographic and virological characteristics of the 20 patients who received the triple therapy with TVR, PEG IFN and RBV for 12 weeks. All of them were infected with HCV-1b in high viral loads with a median of 6.48 $\log_{10}$ IU/mL in group A and 6.80 $\log_{10}$ IU/mL in group B. Of the 20 patients in the study, 12 (60%) were older than 50 years. The bodyweights of 10 (50%) patients were lower than 60 kg. Of the 20 patients, 10 (50%) did not receive antiviral treatments previously, six (30%) did not respond to previous monotherapy with the standard IFN and four (20%) failed to respond to PEG IFN and RBV (non-responder) previously.

Table 1 Baseline characteristics of patients with chronic hepatitis C who received a telaprevir-based triple therapy

No. of patients	Group A (750 mg q8h) $n = 10$	Group B (500 mg q8h) $n = 10$	Total $n = 20$
Sex (male/female)	6/4	4/6	10/10
Age (years) (median [range])	47.0 (42–62)	55.0 (36–65)	53.5 (36–65)
Height (cm) (median [range])	163.00 (147.3–178.5)	160.25 (148.7–175.8)	160.75 (147.3–178.5)
Weight (kg) (median [range])	61.95 (38.0–72.6)	61.00 (44.3–79.0)	61.95 (38.0–79.0)
HCV RNA (log_{10} IU/mL) (median [range])	6.48 (5.6–7.2)	6.80 (5.5–7.2)	6.78 (5.5–7.2)
rs8099917 (TT/TG/GG)	8/2/0	5/5/0	13/7/0
rs12979860 (CC/CT/TT)	8/2/0	5/5/0	13/7/0
rs1127354 (CC/CA/AA)	8/2/0	9/1/0	17/3/0
Core a.a. 70 (W/M)	6/4	6/4	12/8
Core a.a. 91 (W/M)	9/1	6/4	15/5
ISDR (0–1/≥2)	10/0	9/1	19/1
WBC (/mm^3) (median [range])	4900 (3600–6300)	5200 (4100–7800)	4900 (3600–7800)
Plt ($\times 10^4/mm^3$) (median [range])	164 (95–248)	160 (129–243)	163 (95–248)
Hb (g/dL) (median [range])	14.20 (12.8–16.0)	14.00 (11.7–16.8)	14.20 (11.7–16.8)
ALT (IU/L) (median [range])	57.0 (36–94)	43.0 (26–167)	49.5 (26–167)
GGT (IU/L) (median [range])	45.0 (15–85)	35.0 (7–142)	38.5 (7–142)
Creatinine (g/dL) (median [range])	0.765 (0.49-0.93)	0.725 (0.45-0.89)	0.755 (0.45-0.93)
History of IFN-based therapy			
Treatment naïve	6 (60.0)	4 (40.0)	10 (50.0)
IFN monotherapy	3 (30.0)	3 (30.0)	6 (60.0)
PEG IFN/RBV	1 (10.0)	3 (30.0)	4 (40.0)

ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; Hb, hemoglobin; IFN, interferon; ISDR, interferon sensitivity-determining region; M, mutant; PEG, pegylated; Plt, platelets; RBV, ribavirin; W, wild type; WBC, white blood cell.

Pharmacokinetics

The pharmacokinetic parameters of TVR in group A (750 mg q8h) and group B (500 mg q8h) on days 1, 14 and 85 are given in Table 2. The TVR C_{trough} on days 1 and 3, and weeks 1, 2, 4, 6, 8 and 12 in both groups are shown in Figure 1(a). Because the C_{trough} did not reach the steady state until day 2 in group A and group B as shown in Figure 1(a), the parameters relating to exposure (C_{max}, AUC_{0-8h} and C_{trough}) on day 1 were lower than those on days 14 and 85 in both groups (Table 2). The mean value of $t_{1/2}$ on day 1 (4.87 and 4.03 h in groups A and B, respectively) was shorter than those on the other days (6.22 to 10.00 h), while mean t_{max} were approximately the same on these 3 days. The values of $t_{1/2}$ and t_{max} were not different between the two groups. Although the difference was not statistically significant other than the C_{trough} at week 4, the parameters of C_{max}, $AUC_{0-\infty}$ and C_{trough} tended to be higher in group A than those in group B.

Virological response and SVR

Figure 1(b) illustrates a comparison of the serum HCV RNA levels (mean ± standard deviation [SD]) in patients between group A and group B during the TVR triple therapy. Similar decreases were observed in both groups. Characteristics and clinical outcomes of the individual patients are shown in Table 3. The SVR rates were 40% (4/10 patients) in group A and 50% (5/10) in group B. The SVR rates in the naïve patients were 67% (4/6) in group A and 75% (3/4) in group B, while the SVR rates in non-responders to the IFN monotherapy were 0% (0/3) in group A and 67% (2/3) in group B, and those in non-responders to the PEG IFN and RBV therapy were 0% in both groups (0/1 vs 0/3). At week 2, the percentage of subjects with undetectable HCV RNA was 40% in group A and 60% in group B. The percentage of subjects with undetectable HCV RNA at week 4 (rapid viral response: RVR) in group A was similar to that in group B (80% vs 70%). Eight (80%) of the 10 patients with undetectable HCV RNA at week 2 achieved SVR. One patient (undetectable HCV RNA at week 2) who stopped the treatment at week 4 achieved transient response (TR).

Four of five naïve patients with IL-28B rs8099917 TT and wild-type core a.a. 70 achieved SVR. Two of four naïve patients with rs8099917 TT and mutant-type core a.a. 70 achieved SVR, and the other naïve patient with

Table 2 Pharmacokinetic parameters of plasma telaprevir

	n	C_{max} (μg/mL)	t_{max}† (h)	AUC_{0-8h} (μg·h/mL)	C_{trough}‡ (μg/mL)	$t_{1/2}$ (h)
(a) Group A (750 mg q8h)						
Day 1	10	1.62 ± 0.43	2.51 (2.25–6.00)	7.53 ± 1.93	0.846 ± 0.500	4.87 ± 2.12§,¶
Day 14	10	3.96 ± 1.10	2.50 (2.42–5.75)	26.00 ± 6.77††	2.639 ± 0.556††	9.99 ± 4.37§,‡‡
Day 85	6	3.67 ± 0.87	3.24 (2.35–7.75)	25.00 ± 5.23	2.679 ± 0.355	9.06 ± 3.98§§
(b) Group B (500 mg q8h)						
Day 1	10	1.45 ± 0.83	2.54 (2.33–8.02)	6.55 ± 3.73	0.681 ± 0.412	4.03 ± 1.63§,‡‡
Day 14	10	3.06 ± 0.90	2.45 (2.33–6.00)	19.94 ± 5.97	1.914 ± 0.717	10.00 ± 6.97§,††
Day 85	7	3.16 ± 1.10	2.43 (2.33–4.00)	21.35 ± 6.88	2.105 ± 0.819	6.22 ± 3.64¶¶

Mean values ± standard deviations.
†Medians (minimum value to maximum value).
‡C_{trough} at 8 h after the first administration.
§Calculated from measured values at 8 h after the first administration.
¶$n = 7$.
††$n = 9$.
‡‡$n = 8$.
§§Calculated from measured values at 24 h after the first administration.
¶¶Calculated from measured values at 24 h after the first administration.
AUC_{0-8h}, area under the plasma concentration time curve from 0–8 h; C_{max}, maximum plasma concentration; C_{trough}, plasma trough concentrations; $t_{1/2}$, terminal half-life; t_{max}, time to reach C_{max}.

rs8099917 TG and wild-type core a.a. 70 achieved SVR. Two of four non-responders receiving the IFN monotherapy with rs8099917 TT and wild-type core a.a. 70 achieved SVR. The other two non-responders receiving the IFN monotherapy with rs8099917 TG and wild-type core a.a. 70 achieved TR. All four non-responders receiving the PEG IFN and RBV therapy with rs8099917 TG achieved TR. However, none of the pharmacokinetic parameters (C_{trough}, C_{max}, t_{max}, $AUC_{0-\infty}$ and $t_{1/2}$) of TVR were different between patients with and without SVR. Moreover, the adherence of PEG IFN and RBV did not affect SVR (Table 3).

Safety

Adverse events were observed in all patients in groups A and B. Adverse events with a frequency of more than 20% in total patients are listed in Table 4. The overall safety profile was similar in both groups. The ratios of discontinuation of all the study drugs because of adverse events were 40% (three cases of anemia, one case of malaise and vertigo) in group A and 30% (two cases of anemia, one case of severe skin disorder) in group B. Despite the modification of RBV dose, five patients (one man and four women) developed low hemoglobinemia (<8.5 g/dL) on days 22, 31, 39, 78 and 85 after the start of triple therapy. One patient (female, aged 53 years) developed IFN-related symptoms including general malaise and vertigo, and another (female, aged 56 years) developed severe skin disorder that was unable to be treated with topical steroid ointments. There was no dose-dependent trend for adverse events. During the triple therapy for 12 weeks, the amounts of hemoglobin tended to be the same or low in group A in comparison with those in group B (Fig. 2a), while serum creatinine increased more eminently in group A than in group B, with the statistical significance at weeks 4 and 8 ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 2(b). The serum creatinine recovered to the baseline level at the end of the follow-up period.

We analyzed the relationship between the above adverse events and the pharmacokinetic parameters of TVR. The AUC_{0-8h} on day 1 of patients developing low hemoglobinemia (<8.5 g/dL) was significantly higher than that of the other patients ($P = 0.040$; 9.70 ± 3.29 vs 6.15 ± 2.28). There was no correlation of creatinine elevation (>0.3 or 0.5 mg/dL from baseline) or rush with the pharmacokinetic parameters of TVR. Moreover, there was no correlation between creatinine elevation and clinical factors (age, sex, bodyweight and BMI).

DISCUSSION

THE DOSE OF TVR in the triple therapy was determined based on the TVR monotherapy study[10] as described above, in which the highest TVR C_{trough} (1054 ng/mL) and the greatest reduction of HCV RNA

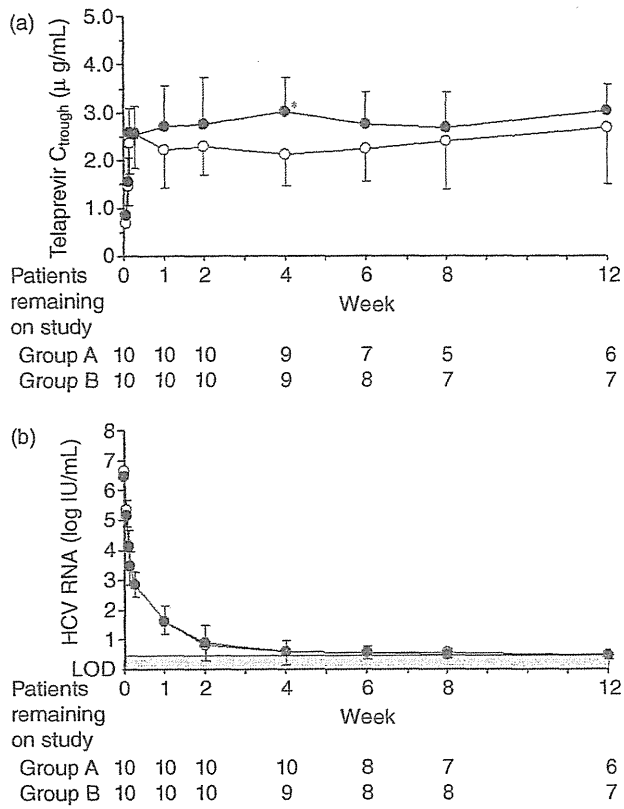

Figure 1 (a) Telaprevir C_{trough} levels and (b) change from baseline of hepatitis C virus (HCV) RNA in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. Statistical tests were performed at each point. *$P < 0.05$ difference. The linear dynamic range of this assay was 1.2–7.8 $\log_{10}$ IU/mL, and samples with no HCV RNA detected were reported as less than 1.2 $\log_{10}$ IU/mL (no HCV RNA detectable.). The areas below the sensitivity of detection are indicated by a shaded bar (<1.2 $\log_{10}$ IU/mL, LOD: limit of detection). —●—, Group (telaprevir 750 mg q8h); —○—, group B (telaprevir 500 mg q8h).

were achieved by a 750 mg q8h regimen. Thus, no dose-finding study of TVR was conducted based on the TVR-based triple regimen. This was the first exploratory study to evaluate the antiviral response, safety and pharmacokinetics of TVR after administration at doses of 750 mg q8h and 500 mg q8h with PEG IFN and RBV. The $t_{1/2}$ of TVR on days 14 and 85 were longer than those on day 1 in both groups, probably due to the saturation of CYP3A4 activity by the repeated administration, because CYP3A4 is the major isozyme involved in the metabolism of TVR and, in addition, TVR acts as the inhibitor of this isozyme. The mean C_{max}, $AUC_{0\text{-}8h}$ and C_{trough} of TVR at steady state increased in an approximately dose-dependent manner, and those at week 2 were 3.96 μg/mL, 26.00 μg·h/mL and 2.639 μg/mL in group A, and 3.06 μg/mL, 19.94 μg·h/mL and 1.914 μg/mL in group B, respectively. The steady state pharmacokinetic parameters of TVR were similar to those obtained in the C208 study.[21] The optimum TVR dose regimen, 750 mg q8h, in Japanese CHC patients was justified based on the overseas dose-finding study and the studies on TVR-based triple therapy, because: (i) no race-related pharmacokinetic difference has been noticed in TVR between Japanese and European patients; and (ii) co-administration with PEG IFN and RBV did not notably change the exposure to TVR.

The change of mean (±SD) $\log_{10}$ HCV RNA and viral response (HCV RNA undetectable) in group A were similar to those in group B (Fig. 1b). The SVR and TR rates were 40% and 60% in group A, and 50% and 40% in group B, respectively. Although the SVR rates of all patients in this study were lower than those in the previous reports,[7,8] the rates of naïve patients (67% in group A and 75% in group B) were similar. The SVR rate of difficult to treat patients, who had not achieved SVR in the prior IFN-based therapy, was lower (20%, 2/10) in this study; the result indicating that these patients will require the TVR-based triple therapy for 24 weeks (PEG IFN, RBV and TVR were administrated for 12 weeks followed by switching to PEG IFN and RBV therapy for an additional 12 weeks).[8] Moreover, the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 were likely to achieve higher SVR than the patients with other genotypes, regardless of TVR dose (Table 3). Recent reports identify IL-28B genotype and a.a. substitution of the core region as predictors of SVR to TVR-based triple therapy.[22,23] Although these results indicate that the optimum regimen for the patients possessing the IL-28B SNP rs8099917 TT and wild-type core a.a. 70 may be 500 mg q8h, the number of patients in this study was too small to reach a definitive conclusion on this point and a large-scale clinical study will be required.

The overall safety profiles of the triple regimen were similar in the two groups, and the ratios of TVR discontinuation due to anemia were 30% in group A and 20% in group B. We examined concentrations of hemoglobin and serum creatinine as the indicator of anemia and renal function, respectively (Fig. 2). The concentrations of hemoglobin were the same or higher in group B than those in group A during the dosing period, but there was no significant difference in this indicator. On the

Table 3 Individual characteristics and outcomes

	Patient									
	1	2	3	4	5	6	7	8	9	10
Group A (750 mg q8h)										
Baseline characteristics										
Age/sex	60/F	42/M	53/F	47/M	46/M	47/M	54/M	46/F	62/F	44/M
Height (cm)	154.6	171.6	147.3	168.0	178.5	165.0	169.0	154.0	159.0	161.0
Weight (kg)	54.0	58.3	65.1	64.9	72.6	72.0	65.0	38.0	54.0	59.0
IL-28B SNP (rs8099917)	TT	TG	TT	TG	TT	TT	TT	TT	TT	TT
IL-28B SNP (rs12979860)	CC	CT	CC	CT	CC	CC	CC	CC	CC	CC
ITPA SNP (rs1127354)	CC	CC	CA	CC	CC	CC	CA	CC	CC	CC
Core a.a. 70 (W/M)	W	M	W	W	M	W	M	M	W	W
Core a.a. 91 (W/M)	W	W	W	W	W	W	W	W	W	M
ISDR substituted a.a. sites	0	0	1	0	0	0	0	1	1	1
History of IFN-based therapy†	IFN	IFN	Naïve	PR	Naïve	IFN	Naïve	Naïve	Naïve	Naïve
Baseline laboratory data										
HCV RNA (log_{10} IU/mL)	6.10	6.85	7.10	7.15	6.85	6.55	6.40	5.60	6.00	6.00
Hb (g/dL)	13.1	14.3	16.0	14.2	14.9	13.8	14.2	13.9	12.8	15.8
Creatinine (g/dL)	0.93	0.77	0.66	0.77	0.76	0.85	0.73	0.49	0.51	**0.83**
Dose										
RBV, max/min (mg)	600/400	600/200	800/200	800/200	800/400	800/200	800/200	600/200	600/200	600/200
Duration of treatment (weeks)	4	12	7	12	12	12	12	12	6	12
Telaprevir, adherence (%)	36.1	99.2	44.7	99.2	98.0	99.2	97.6	98.8	45.1	**101.6**
PEG IFN, adherence (%)	41.7	100	41.7	100	100	75.0	66.7	100	41.7	**100**
RBV, Adherence (%)	32.2	59.6	28.2	51.2	67.4	64.7	51.5	42.7	21.6	**45.1**
Pharmacokinetic parameter‡										
C_{trough} (μg/mL)	3.102	2.485	3.408	2.662	3.807	2.947	1.294	3.396	3.164	1.932
Outcome										
HCV RNA negativity (weeks)	2	6	2	4	4	4	4	6	2	2
Effect of therapy (SVR/BT/TR/NR)§	TR	TR	SVR	TR	SVR	TR	TR	TR	SVR	SVR

Table 3 *Continued*

	Patient									
	1	2	3	4	5	6	7	8	9	10
Group B (500 mg q8h)										
Baseline characteristics										
Age/sex	64/M	54/F	36/F	60/F	52/M	46/F	56/F	65/M	56/F	54/M
Height (cm)	173.2	151.0	148.7	160.5	175.8	160.0	160.0	167.0	158.0	170.0
Weight (kg)	75.0	47.6	44.3	67.9	71.8	52.0	57.0	79.0	65.0	55.0
IL-28B SNP (rs8099917)	TT	TG	TG	TT	TG	TT	TG	TT	TT	TG
IL-28B SNP (rs12979860)	CC	CT	CT	CC	CT	CC	CT	CC	CC	CT
ITPA SNP (rs1127354)	CC	CC	CC	CC	CC	CC	CC	CC	CC	CA
Core a.a. 70 (W/M)	M	W	W	W	M	W	M	W	W	M
Core a.a. 91 (W/M)	W	W	W	W	M	W	M	W	M	M
ISDR substituted a.a. sites	6	0	1	0	0	0	0	0	0	0
History of IFN-based therapy†	Naïve	IFN	Naïve	Naïve	PR	Naïve	PR	IFN	IFN	PR
Baseline laboratory data										
HCV RNA (log_{10} IU/mL)	5.50	7.15	6.15	6.80	6.80	7.00	6.10	7.20	6.85	6.75
Hb (g/dL)	16.1	11.7	12.1	13.6	14.5	12.3	16.8	14.3	13.7	14.8
Creatinine (g/dL)	0.78	0.50	0.45	0.56	0.87	0.58	0.80	0.89	0.75	0.70
Dose										
RBV, max/min (mg)	800/400	600/200	600/200	800/400	800/200	600/200	600/600	800/200	800/200	600/200
Duration of treatment (weeks)	12	12	11	12	12	3	12	12	5	12
Telaprevir, adherence (%)	98.0	99.2	91.0	99.2	101.6	25.5	98.8	99.2	43.1	98.4
PEG IFN, adherence (%)	98.3	66.7	87.5	100	91.7	25.0	100	100	41.7	100
RBV, adherence (%)	68.5	44.7	39.2	54.4	48.8	24.3	99.2	36.5	28.2	64.3
Pharmacokinetic parameter‡										
C_{trough} (μg/mL)	1.950	2.763	3.276	1.690	1.478	1.939	2.955	4.065	1.962	1.846
Outcome										
HCV RNA negativity (weeks)	2	6	2	2	4	-	2	2	2	8
Effect of therapy (SVR/BT/TR/NR)	SVR	TR	SVR	SVR	TR	NR	TR	SVR	SVR	TR

†Naïve, treatment naïve, IFN, IFN monotherapy, PR, PEG IFN/RBV.

‡Pharmacokinetic parameters of the patients who received triple therapy at weeks 2.

a.a., amino acid; ALT, alanine aminotransferase; C_{trough}, plasma trough concentrations; GGT, γ-glutamyltransferase; Hb, hemoglobin; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, interferon sensitivity-determining region; M, mutant; PEG, pegylated; Plt, platelets; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response, BT, breakthrough, TR, transient response, NR, non-response; W, wild type; WBC, white blood cell.

Table 4 Adverse events developing in more than 20% of patients in total

MedDRA/J (ver. 12.0) PT	Group A (750 mg q8h) $n = 10$ n (%)	Group B (500 mg q8h) $n = 10$ n (%)	Total $n = 20$ n (%)
Platelet count decreased	10 (100.0)	10 (100.0)	20 (100.0)
Anemia	10 (100.0)	9 (90.0)	19 (95.0)
White blood cell count decreased	9 (90.0)	10 (100.0)	19 (95.0)
Rash	7 (70.0)	7 (70.0)	14 (70.0)
Pyrexia	6 (60.0)	8 (80.0)	14 (70.0)
Malaise	6 (60.0)	5 (50.0)	11 (55.0)
Blood triglycerides increased	6 (60.0)	5 (50.0)	11 (55.0)
Headache	3 (30.0)	7 (70.0)	10 (50.0)
Blood lactate dehydrogenase increased	3 (30.0)	7 (70.0)	10 (50.0)
Anorexia	3 (30.0)	6 (60.0)	9 (45.0)
Blood uric acid increased	4 (40.0)	4 (40.0)	8 (40.0)
Nausea	3 (30.0)	5 (50.0)	8 (40.0)
Pruritus	3 (30.0)	5 (50.0)	8 (40.0)
Protein total decreased	0 (0.0)	8 (80.0)	8 (40.0)
Hyperuricaemia	5 (50.0)	2 (20.0)	7 (35.0)
Blood creatinine increased	5 (50.0)	2 (20.0)	7 (35.0)
Nasopharyngitis	3 (30.0)	4 (40.0)	7 (35.0)
Neutrophil percentage decreased	3 (30.0)	4 (40.0)	7 (35.0)
Influenza-like illness	4 (40.0)	2 (20.0)	6 (30.0)
Abdominal discomfort	2 (20.0)	3 (30.0)	5 (25.0)
Vomiting	2 (20.0)	3 (30.0)	5 (25.0)
Dizziness	0 (0.0)	5 (50.0)	5 (25.0)
Dysgeusia	3 (30.0)	1 (10.0)	4 (20.0)
Stomatitis	3 (30.0)	1 (10.0)	4 (20.0)
Lymphocyte percentage increased	2 (20.0)	2 (20.0)	4 (20.0)
Diarrhea	1 (10.0)	3 (30.0)	4 (20.0)
Alopecia	1 (10.0)	3 (30.0)	4 (20.0)

contrary, there was observed a difference in serum creatinine concentrations between group A and group B; thus, the serum creatinine concentrations in group A were higher than those in group B at all of the time points examined with a statistical significance at weeks 4 and 8 ($P < 0.01$ and $P < 0.05$, respectively) as shown in Figure 2(b). The TVR Review Team confirms that higher exposure of TVR and RBV was significantly associated with increased risk of anemia and grade 2 or higher hemoglobin toxicity.[11] The behaviors of hemoglobin and creatinine in the triple therapy shown in Figure 2 are of interest from the viewpoints of development of anemia with TVR-based regimen and could be explained by the following possibilities: (i) the increase of plasma concentration of TVR may directly affect the renal function to cause the increase of creatinine especially in group A and the decrease of hemoglobin; (ii) TVR first caused the increase of systemic exposure to RBV which in turn additively or synergistically resulted in renal dysfunction. The decrease of renal function reportedly leads to the increase of RBV concentration in plasma, because RBV is mainly excreted via the renal route.[24,25] In this study, the AUC_{0-8h} on day 1 of patients who developed low hemoglobinemia (<8.5 g/dL) were significantly higher than those of the other patients. The pharmacokinetic parameters of TVR on day 14, at which plasma concentrations of TVR were in the steady state, did not affect low hemoglobinemia. The timing of reducing RBV dose may cause development of low hemoglobinemia, because the RBV dose reduction set in the protocol of this study was less strict than that in the previous reports.[7,8]

Because the present data show that the TVR exposure tended to be increased in a dose-dependent manner, there is a possibility that the triple therapy with TVR 500 mg q8h is advantageous in aged patients whose renal function, body water content or both are lower than those of younger patients. It should be noted,

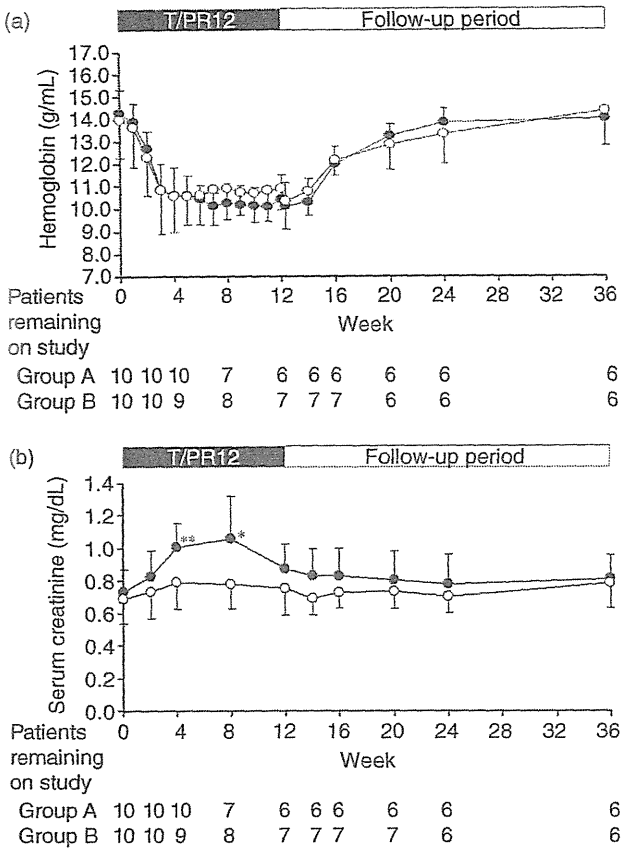

Figure 2 (a) Change from baseline of hemoglobin and (b) serum creatinine levels in Japanese patients with chronic hepatitis C during the telaprevir-based triple therapy. Each circle and bar represent mean values ± standard deviations, respectively. Number of patients at each time point is indicated below. Statistical tests were performed at each point. $*P < 0.05$ and $**P < 0.01$ difference. —●—, Group A (telaprevir 750 mg q8h); —○—, group B (telaprevir 500 mg q8h). T/PR12, triple therapy of telaprevir with peginterferon and ribavirin for 12 weeks.

however, that the small number of patients per arm in this study limits conclusions that can be drawn, and a future larger study is essential.

In conclusion, although the exposure to TVR tended to be lower in 500 mg q8h than that in 750 mg q8h in the TVR-based triple therapy, relatively high exposure of TVR was observed in Japanese CHC patients given TVR at the lower dose. The result suggests that the lower dose regimen may be one of the options for the treatment of Japanese patients. In addition, in the view of antiviral effects, TVR pharmacokinetics and safety profiles, the present findings indicate that development of adverse events, specifically anemia and creatinine increase in the treatment with TVR-based regimen, could be avoided by dose adjustment of TVR as well as RBV.

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Long-Term Entecavir Treatment Reduces Hepatocellular Carcinoma Incidence in Patients With Hepatitis B Virus Infection

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Chronic hepatitis B virus (HBV) infection leads to cirrhosis and hepatocellular carcinoma (HCC). Antiviral agents are thought to reduce HCC development, but agents such as lamivudine (LAM) have a high rate of drug resistance. We compared the incidence of HCC in 472 entecavir (ETV)-treated patients and 1,143 nontreated HBV patients (control group). Propensity score matching eliminated the baseline differences, resulting in a sample size of 316 patients per cohort. The drug mutation resistance was 0.8% (4/472) in the ETV group. The cumulative HCC incidence rates at 5 years were 3.7% and 13.7% for the ETV and control groups, respectively ($P < 0.001$). Cox proportional hazard regression analysis, adjusted for a number of known HCC risk factors, showed that patients in the ETV group were less likely to develop HCC than those in the control group (hazard ratio: 0.37; 95% confidence interval: 0.15-0.91; $P = 0.030$). Both cohorts were applied in three previously reported risk scales and risk scores were generated based on age, gender, cirrhosis status, levels of alanine aminotransferase, hepatitis B e antigen, baseline HBV DNA, albumin, and bilirubin. The greatest HCC risk reduction occurred in high-risk patients who scored higher on respective risk scales. In sub analyses, we compared treatment effect between nucleos(t)ide analogs, which included matched LAM-treated patients without rescue therapy (n = 182). We found HCC suppression effect greater in ETV-treated ($P < 0.001$) than nonrescued LAM-treated ($P = 0.019$) cirrhosis patients when they were compared with the control group. *Conclusion*: Long-term ETV treatment may reduce the incidence of HCC in HBV-infected patients. The treatment effect was greater in patients at higher risk of HCC. (HEPATOLOGY 2013;58:98-107)

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More than 2 billion people worldwide have been exposed to hepatitis B virus (HBV) and about 350 million people are chronically infected, the majority of whom are in Asia (75%). The prevalence of HBV in Japan is 0.8%, which is lower than other Asian countries such as Taiwan (>10%) and China.[1-3] As chronic HBV infection leads to cirrhosis and hepatocellular carcinoma (HCC), published studies have shown that up to 25% of chronically infected patients eventually die of liver cirrhosis or HCC.[4]

A large-scale longitudinal epidemiologic study has shown that a patient's baseline HBV DNA level is an independent predictor for the development of HCC.[5] Studies have begun to show that treatment to decrease HBV DNA reduces the risk of HCC development in HBV patients with cirrhosis or advanced fibrosis or in chronic HBV patients.[6,7]

Within the past 10 years, new antiviral therapies, including nucleos(t)ide analogs (NAs), have been approved and were successful in suppressing circulating serum viral loads. Studies that have examined the relationship between NA therapy and HCC almost exclusively used older drugs such as lamivudine and/or adefovir. Although results of long-term studies showed the importance of antiviral suppression, HCC risk among patients treated by newer NAs remains inconclusive. Entecavir (ETV) is a relatively new antiviral NA that has proved effective in suppressing HBV

Abbreviations: ALT, alanine aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; ETV, entecavir; HBeAg, hepatitis B e antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HR, hazard ratio; NA, nucleos(t)ide analogs; PS, propensity score; ROC, receiver operating characteristic curve.

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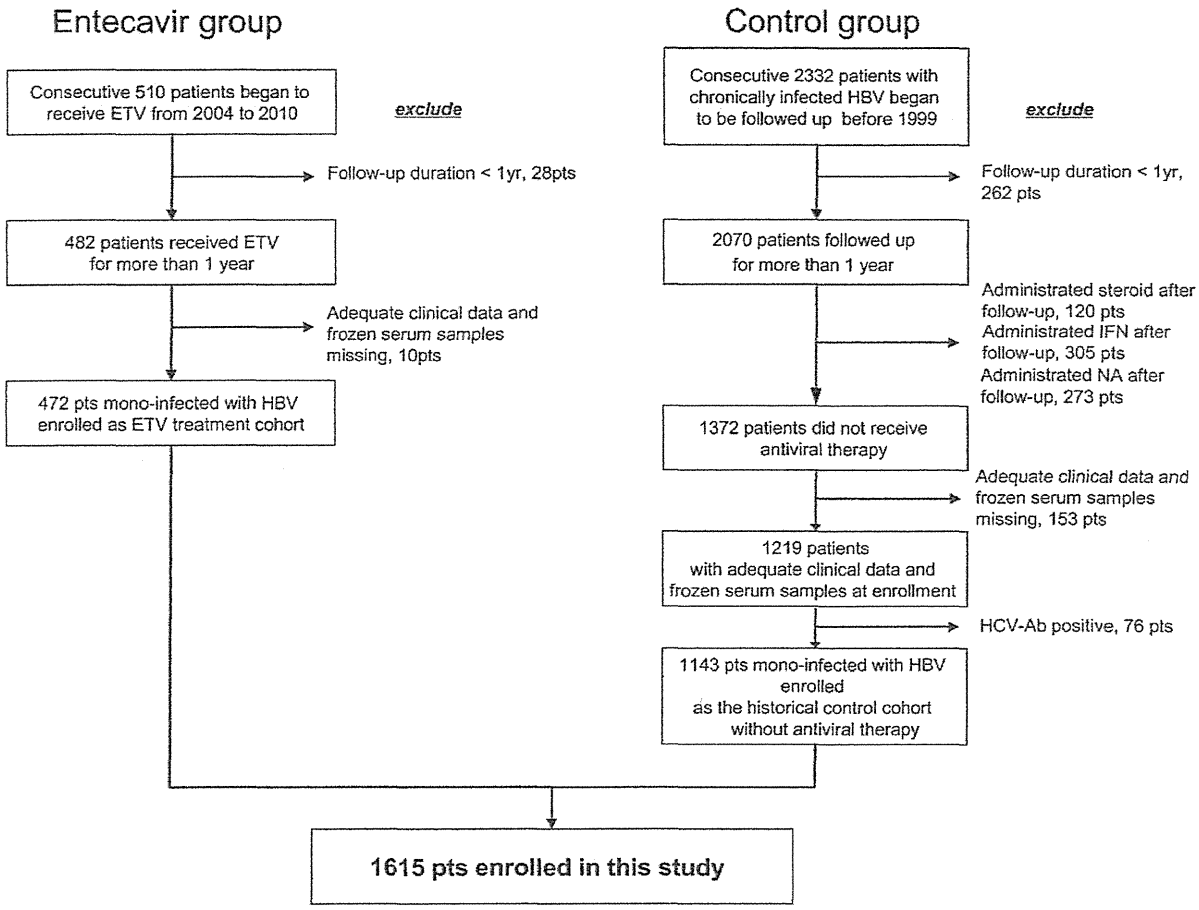

Fig. 1. Entecavir-treated and nontreated cohorts. ETV, entecavir; HBV, hepatitis B virus; IFN, interferon; NA, nucleos(t)ide; HCV-Ab, anti-hepatitis C virus antibody.

DNA replications with minimal drug resistance.[8,9] In this study we examined whether long-term ETV treatment would reduce HCC risk in HBV-infected patients when compared with NA-naïve patients.

Patients and Methods

Patients and Design. From 2004 to 2010, we consecutively recruited 510 patients treated with 0.5 mg ETV (ETV group); the ETV group was compared with a retrospective cohort of 2,332 NA-naïve, HBV-infected patients (control group).

These patients were chronically monoinfected with HBV and were confirmed as hepatitis B s antigen (HBsAg)-positive for at least 6 months. As a general rule, ETV was initiated in a patient who had both abnormal alanine aminotransferase (ALT) levels (defined as ALT ≥45) and elevated HBV DNA levels of ≥4 log copies/mL. A patient with advanced fibrosis would be treated with ETV if the ALT level was normal; however, a patient without fibrosis or with a normal HBV DNA/ALT level would not be treated with ETV. Among the treated patients, 38 were excluded from the ETV group either because their follow-up period was less than 1 year (n = 28) or because the clinical data or serum samples were incomplete (n = 10). The remaining 472 ETV-treated patients were included in the analysis (Fig. 1). No patient in the ETV group received other NAs before ETV treatment.

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Additional Supporting Information may be found in the online version of this article.

The control group patients were recruited from 1973 to 1999. These patients were NA-naïve at baseline, as no NA therapy had yet been approved. Patients were excluded from the control group if (1) their follow-up duration was less than 1 year (n = 262); (2) corticosteroid withdrawal therapy (n = 120), IFN treatment (n = 305) or NA treatment (n = 273) was initiated during follow-up; (3) clinical data or serum samples were incomplete (n = 153); or (4) patients were found to be positive for anti-hepatitis C virus antibodies (HCV-Ab) (n = 76). The remaining 1,143 patients served as the control population (Fig. 1).

We also made subanalyses to examine the difference of HCC suppression effect between NAs. To make this comparison, we recruited a cohort of 949 consecutive patients from our hospital who were treated with lamivudine (LAM) (September 1995 to September 2007). LAM-treated patients who met the same inclusion criteria as the ETV group, who had no rescue therapy (LAM group, n = 492), were used in the comparison.

We received informed consent from each patient at their entry into the study. Informed consent for the clinical data collection and storage of serum samples were obtained from each patient in the historical control group. The study protocol was in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the Toranomon Hospital Ethics Committee.

Clinical Data Collection and Follow-up. All ETV-treated and untreated patients were followed at 1- to 3-month intervals, during which biochemical and HBV virological markers, blood counts, tumor markers (e.g., alpha-fetoprotein and des-γ-carboxylprothrombin), and cirrhosis and HCC status were monitored. Viral response in the ETV group was defined as a reduction in HBV DNA levels to below 400 copies/mL. Cirrhosis was determined by laparoscopy, liver biopsy, imaging modalities, or portal hypertension. HCC was diagnosed predominantly via imaging, including dynamic computed tomography, magnetic resonance imaging, and/or digital subtraction angiography. When the hepatic nodule did not show typical imaging features, diagnosis was confirmed by fine-needle aspiration biopsy followed by histological examination. Patients were followed until any confirmed HCC diagnosis 1 year after the start of observation (primary outcome) or until the last visit before December 2011. All patients also underwent ultrasonography or helical dynamic computed tomography every 3 to 6 months (cirrhosis patients) or every 6 to 12 months (noncirrhosis patients).

HBV Infection Markers. HBV DNA levels were quantified using the COBAS Amplicor HBV Monitor Test (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.6 to 7.6 log copies/mL, or COBAS TaqMan HBV Test v2.0 (Roche Diagnostics) which has a dynamic range of over 2.1 to 9.0 log copies/mL. HBV DNA of the control group was measured from their stored frozen serum (−80°C) using COBAS TaqMan HBV v.2.0 once at the start of observation. Previous measurements were taken using the old DNA polymerase assay in the control group and thus were not used for comparisons. For the ETV group, drug-resistant mutations were determined from a nested polymerase chain reaction, using a primer specific at the polymerase region in patients who had an HBV DNA relapse of ≥1 log copies from nadir. Hepatitis B e antigen; (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to serologically determine HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the eight major genotypes (A to H).

HCC Incidence by Risk Scores. To examine HCC incidence by risk scores, we applied published HCC risk scales, which are based on the natural course of HCC among HBV-positive patients, to our cohorts. We first searched Medline/PubMed using "hepatitis B," "cancer," and "risk score" as keywords and found four publications in English that used risk-score estimations.[10-13] One article was rejected because we were unable to compute the risk scores with our variables, and therefore we used only the scales indicated by the remaining three publications to generate the risk scores.[13] The risk scales were based on parameters such as age, gender, cirrhosis, levels of ALT, HBeAg, baseline HBV DNA, albumin, and bilirubin. The original risk score formula and the risk score distributions for our two cohorts derived from these formulas are shown in Supporting Table 1. The risk score cutoff points were determined from the following original articles. In Yang et al.'s article,[10] the risk score was derived from 17-point categories. When we applied the scores to our control group, we found that the 12-point scale was at best in detecting a difference in HCC incidence. With that, we examined the HCC suppression treatment effect by dividing the patients into equal halves with 12 points as the cutoff. Yuen et al.[11] divided their cohort in half and found risk scores of 82 as the optimal cutoff point. We also applied the same cutoff point to our cohorts. Wong et al.[12] used their risk scores to categorize their cohort into low-risk, medium-risk, and high-risk groups with respective cutoff points at <4, 4-19, ≥20. We also applied the same cutoff points to our cohorts to examine the treatment effect. Cumulative

HCC incidence rates were compared by these risk scores between the ETV and control groups.

Statistical Analysis. Categorical data were compared using chi-square or Fisher's exact tests. Continuous variables with normal distributions were compared using Student's *t* test, and those without normal distributions were compared using the Mann-Whitney *U* test. Cumulative HCC incidence rates were analyzed using the Kaplan-Meier method; patients followed beyond 5 years were censored to better compare the two cohorts because the ETV group had a shorter follow-up period when compared with the historical control group. We compared the cumulative incidence of HCC using the log-rank test, and Cox proportional hazard regression analysis, which was used to assess the variables that were significantly associated with the development of HCC. Deaths before HCC development were censored. Significance was defined as $P < 0.05$ for all two-tailed tests.

We used the propensity score (PS) matching method to reduce significant differences in demographics between the ETV and control groups.[14,15] Using multiple logistic regression analysis, a PS was estimated for all patients treated with ETV.[14] Variables used in the model included age, sex, presence of cirrhosis, HBeAg, HBV DNA< aspartate aminotransferase (AST), ALT, γ-glutamyl transpeptidase; (γ-GTP), bilirubin, albumin, and platelet counts. We performed caliper matching on the PS (nearest available matching). Pairs (ETV and the control group) on the PS logit were matched to within a range of 0.2 standard deviation (SD).[16,17] The PS logit distributions for each cohort showing the overlaps and SD ranges are shown in Supporting Fig. 1. The balance of covariates was measured by their standardized differences. A difference >10% of the absolute value was considered significantly imbalanced.[17] The cohorts were divided into five PS quintiles (Supporting Table 2). We also made subanalyses to examine the difference of HCC suppression effect between NAs by comparing the HCC incidence between propensity score matched ETV- and lamivudine (LAM)-treated patients without a rescue therapy. The LAM-treated patients were derived from consecutive sampling at our institution and were PS matched with ETV group according to the same method described above. Interaction of the subgroups by preexisting cirrhosis or risk scores and ETV treatment were evaluated. $P < 0.10$ was considered statistically significant. Data analysis was performed using IBM SPSS v. 19.0 software (Armonk, NY) and R software v. 2.13 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

Results

Patient Characteristics. The patient characteristics at the baseline, before PS matching are shown in Table 1. The ETV group was followed for an average of 3.2 years (1,561 person-years), whereas the control group was followed for an average of 9.5 years (12,381 person-years). Before matching, patients in the ETV group and the control group differed significantly in age, gender, genotype, baseline HBV DNA level, and other clinical data. In the ETV group, 421 patients (89%) had HBV DNA (<400 copies/mL) at year 1. Not all patients in the control group were tested for HBV DNA level during follow-up. The drug mutation resistance was 0.8% (4/472). The four patients who had drug mutation did not develop HCC. During follow-up, 12 patients (2.54%) in the ETV group and 144 patients (12.60%) in the control group developed HCC. The incidence rates of HCC for the ETV and the control groups were 76/10,000 patient-years and 116/10,000 patient-years, respectively. During this period, 21 patients in the control group developed liver cirrhosis while no patient developed liver cirrhosis in the ETV group. During the same observation period, there were four deaths in the ETV group and 10 deaths in the control group. We took competing risk into account[18,19] and compared incidence of non-HCC deaths between the cohorts and the results were not different. However, because there were only four patients in the non-HCC deaths in the ETV group (two patients in the PS matched cohort) and 10 patients in the control group (six patients in the PS matched cohort), we considered that it was not meaningful to apply competing risk analysis in our cohorts.

Factors Associated with HCC and Effect of ETV Treatment on HCC Development. To allow a common ground for comparison between the two cohorts, we used PS matching with selected key characteristics and compared the two groups within the same time period of 5 years. The PS matching process resulted in a matched sample size that consisted of 316 patients in each group (Table 1). The PS matching reduced the significant variability of the two cohorts. While five (42%) of the 12 covariates varied by >10% before matching, all covariates differed by <10% of the absolute value after matching (Supporting Fig. 2). In the PS score matched cohort, 10 out of the 231 noncirrhosis patients progressed to liver cirrhosis within the 5 years of observation. The cumulative incidence rates of HCC in the matched ETV groups were 0.7% at year 2, 1.2% at year 3, 2.5% at year 4, and 3.7% at year 5. The cumulative incidence rates of HCC in the