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Original Article

Entecavir Reduces Hepatocarcinogenesis in Chronic Hepatitis B Patients

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Chronic hepatitis B (CHB) leads to cirrhosis and hepatocellular carcinoma (HCC). With a cohort of 1,206 CHB patients who visited Okayama University Hospital and related hospitals in 2011 and 2012, we compared the incidence rates of HCC among the patients grouped by age, hepatitis B virus (HBV) DNA, hepatitis B e antigen (HBeAg), and treatment. HCCs were observed in 115 patients with the median observation period of 1,687 days. Among the HCC patients aged ≥ 35 years, HBV DNA ≥ 4 log copies/mL and positive HBeAg at diagnosis (n = 184), the HCC incidence rate was 8.4% at 5 years in the entecavir (ETV)-treated patients, 21.8% in the lamivudine (LVD)-treated patients, and 26.4% among the patients not treated with drugs. The cumulative HCC incidence was significantly reduced in the ETV-treated patients compared to those treated with LVD or not treated ($p = 0.013$). Among the patients aged ≥ 35 years with HBV DNA ≥ 4 log copies/mL and negative HBeAg (n = 237), the cumulative HCC incidence was 14.6% in 5 years in ETV group and 13.9% among those not treated with a drug ($p > 0.05$). Only small numbers of HCCs occurred in other patients. In CHB patients aged ≥ 35 years with HBV DNA ≥ 4 log copies/mL and positive HBeAg, ETV treatment is recommended for the suppression of HCC development.

Key words: entecavir, hepatitis B virus, lamivudine, hepatocellular carcinoma

Approximately 350–400 million people worldwide have a chronic hepatitis B (CHB) infection, and the majority of them live in the Asia-Pacific region [1, 2]. CHB patients with active hepatitis are at risk of developing liver cirrhosis, liver failure, and hepato-

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cellular carcinoma (HCC). The incidence of HCC development among individuals with a CHB infection is 0.8% annually, approx. 100-fold higher than the rate among healthy people. In addition, every year approx. 500,000 patients around the world die from hepatitis B virus (HBV)-related liver disease [3].

Several studies have shown that persistent elevations of HBV-DNA and alanine aminotransferase (ALT) in serum and active hepatitis in laparoscopic findings are significantly associated with rapid disease progression and HCC development [4-6]. Antiviral treatment, including treatment with interferon (IFN) or nucleos(t)ide analogs (Nucs), is designed to counteract active hepatitis and to suppress the development of liver failure and HCC [7]. Nucs are now also used for post-liver transplantation antiviral prophylaxis [8-10].

In 2004, Liaw *et al.* reported the HCC-suppressing effect of Nucs in a randomized controlled trial. The lamivudine (LVD)-treated group showed a significantly lower HCC development rate than the placebo group, with the observation period of 32.4 months (3.9% vs. 7.4%, $p=0.047$) [11]. Several cohort studies have shown that LVD treatment significantly reduced mortality and prevented HCC development compared to patients not treated with Nucs [12-14].

After the introduction of LVD for the treatment of CHB in the late 1990s, 4 additional Nucs, *i.e.*, adefovir, telbivudine, entecavir, and tenofovir, were approved worldwide. Adefovir monotherapy lead to a high incidence of drug-resistant mutations and renal dysfunction, and thus nowadays adefovir is prescribed in combination with other Nucs [15].

Entecavir (ETV) and tenofovir are more potent than LVD in terms of suppressing HBV replication, with a very low risk of drug-resistant mutations [16]. Thus, ETV and tenofovir are currently recommended as first-line Nucs treatment for patients with active CHB [17]. However, it is not yet known whether ETV or tenofovir is as effective at reducing the risk of HCC development as LVD.

Entecavir (ETV) is a relatively new antiviral Nucs that efficiently suppressed the HBV DNA production and improved the hepatitis of nucleos(t)ide-naïve CHB patients [18]. In addition, drug-resistant mutations to long-term ETV treatment remained rare.

In the present study we examined whether long-term ETV treatment would reduce the HCC risk in HBV-infected patients compared with Nucs-naïve patients. We suspected that the risk of hepatocarcinogenesis would differ by patient age, hepatitis B envelope antigen (HBeAg) status, HBV DNA titer, and fibrosis stage in chronic HBV infection. We then investigated who should be treated with ETV by priority among CHB patients.

Patients and Methods

Patients and design. We enrolled 1,875 CHB patients who visited Okayama University Hospital and related hospitals in 2011 and 2012. These patients were chronically monoinfected with HBV and were confirmed as being hepatitis B surface antigen (HBsAg)-positive for at least 6 months. Within this patient group, we excluded 669 patients: those whose observation period among the non-treated patients or the treatment period in Nucs-treated patients was <180 days, those who had a co-infection with hepatitis C virus or human immunodeficiency virus, those who underwent a consultation for the treatment of HCC, and those who had been treated with an immunosuppressive drug or anticancer drug. The remaining 1,206 patients were included in the study.

Among the patients without Nucs treatment, the observation period was calculated from the patient's first visit to the development of HCC or to the end of the observation. Among the patients treated with Nucs, the observation period was calculated from the introduction of Nucs treatment to the development of HCC or the end of observation.

In accord with the Japanese treatment guidelines for HBV infection, nucleoside analogues were initiated in patients who had both an abnormal ALT level (defined as ALT ≥ 45) and an HBV DNA level of 4 log copies/mL or more. LVD was administered to patients who were treated with a nucleoside analogue before ETV was approved in Japan in 2006. Patients who developed LVD- or ETV-resistant HBV received adefovir (ADV) when their serum HBV DNA level increased by ≥ 1 log copies/mL over the minimal titer according to the guideline.

We defined the LVD group as patients treated with LVD monotherapy or LVD combined with ADV throughout their treatment history. The ETV group was defined as the patients treated with ETV monotherapy or ETV combined with ADV at the end of the

follow-up. All study protocols were approved by the Ethics Committee at the Okayama University Hospital and affiliated hospitals.

Clinical data collection and follow-up. All patients were followed at < 6-month intervals, during which biochemical and HBV virological markers, blood counts and tumor markers (*e.g.* alpha-fetoprotein [AFP] and des-c-carboxylprothrombin) were monitored. All patients also underwent ultrasonography, dynamic computed tomography (CT) or dynamic magnetic resonance imaging (MRI) every 3–6 months. A viral response in the ETV or LVD group was defined as a reduction in the HBV DNA level to below 400 copies/mL. In accord with the Japanese clinical practice guidelines for HCC, HCC was diagnosed predominantly via imaging, including dynamic CT, MRI, and/or digital subtraction angiography. When a hepatic nodule did not show typical imaging features, the diagnosis was confirmed by a fine-needle aspiration biopsy followed by histological examination.

HBV status. Each patient's HBV-DNA level was measured using a transcription-mediated amplification (TMA) assay (SRL, Tokyo, Japan), a polyerase chain reaction (PCR) assay (Amplicor HBV Monitor assay; Roche Diagnostics, Tokyo, Japan) or a real-time PCR assay (COBAS TaqMan HBV Test; Roche Diagnostics).

HBsAg, HBeAg, hepatitis B surface antibody (HBsAb), and hepatitis B envelope antibody (HBeAb) were routinely measured using a commercially available chemiluminescent enzyme immunoassay (CLEIA) system (Lumipulse System; Fujirebio, Tokyo, Japan) or an enzyme-linked immunosorbent assay (ELISA) (Sysmex, Kobe, Japan).

Statistical analysis. Statistical comparisons were performed using JMP version 7.0.1 software (SAS Institute, Cary, NC, USA). Categorical data were compared using chi-square or Fisher's exact tests. Continuous variables were compared using Student's t-test or the Wilcoxon rank sum test. We compared the cumulative incidence of HCC using the log-rank test, and we used a Cox proportional hazard regression analysis to assess the variables that were significantly associated with the development of HCC. Deaths before HCC development were censored.

The incidence rates of HCC were compared among the patients grouped by age, HBV DNA, HBe antigen, and treatment. The cumulative HCC incidences were analyzed with the Kaplan-Meier method and log rank test. Significance was defined as $p < 0.05$ for all two-tailed tests.

Results

Patient characteristics. The patient characteristics at the baseline are shown in Table 1. The median age of the 1,206 patients was 49 years among the 670 males (55%) and 536 females (45%). Nucs

Table 1 Patient characteristics and demographics (n = 1,206)

Age†	years	49	(11–88)
Sex	male/female	670/536	
Observation period†	days	1,687	(182–11,752)
Nuc.	ETV/LVD/Not treated	433/116/657	
HCC		115	
HBV genotype	A/B/C/D	21/30/483/2	
T. Bil†	mg/dL	0.8	(0.1–27.5)
Albumin†	g/dL	4.2	(2.0–5.7)
Platelet†	$\times 10^4/mm^3$	17.9	(1.7–46.3)
PT†	%	90	15 ～189
ALT†	IU/L	43	1 ～13,320
AST†	IU/L	36	1 ～6,325
GGT†	IU/L	31	4 ～5,213
AFP†	ng/mL	4	0 ～8,912
DCP†	mAU/mL	18	0 ～62,600
HBV DNA†	log copies/mL	5.4	– ～>9.1
HBeAg	+/–	308/580	

†median (range). Nucs., nucleoside analogue; ETV, entecavir; LVD, lamivudine; HCC, hepatocellular carcinoma; HBV, hepatitis B virus.

were administered to 549 patients: 433 patients were treated with a regimen that included ETV, and 116 patients were treated with a regimen that included LVD. HCCs were observed in 115 patients, with the mean observation period of 1,687 days.

Change of laboratory data 1 year after the initiation of Nucs treatment. The baseline parameters of the patients grouped by treatment (ETV regimen, LVD regimen, and no treatment) are shown in Table 2. Most of the parameters examined differed significantly among the treatment groups. In general, the patients in the 2 Nucs-treated groups were older and more likely to be male, and to have high serum HBV DNA, a high AFP titer and poor hepatic reserve compared to the patients in the non-treated group.

One year after the initiation of Nucs treatment, the patients' laboratory data and viral markers were significantly improved (Table 3). Because the ETV group's baseline total bilirubin (T. Bil), aspartate aminotransferase (AST), ALT, and AFP values were lower and their baseline albumin and platelet count values were higher compared to those of the LVD group, these parameters improved less in the ETV group. Of note, HBV DNA became undetectable after 1 year of ETV treatment in 85 of the 279 patients assessed; in the other 118 patients, HBV DNA was not measured after 1 year of ETV treat-

Table 2 Patient characteristics and demographics by treatment groups

		ETV	LVD	Not treated	*P*-value: (LVD vs. Not-treated)	*P*-value (ETV vs. Not- treated)
No. of patients		433	116	657		
Age†	years	52	50	46	<0.0005	<0.0001
Sex	male/female	269/164	70/46	331/326	<0.05	<0.0001
Observation period†	days	1,197	2,714.5	1,978	<0.05	<0.0001
HCC		32	39	44	<0.0001	n.s.
HBV DNA†	Log copies/mL	6.7	6.9	3.8	<0.0001	<0.0001
HBV genotype	B/C	9/245	1/60	20/178	<0.05	<0.0001
HBeAg	+/−	145/158	50/24	113/398	<0.0001	<0.0001
T. Bil†	mg/dL	0.8	0.9	0.7	<0.0001	0.07
Albumin†	g/dL	4.1	3.955	4.4	<0.0001	<0.0001
Platelets†	$\times 10^4/mm^3$	15.65	12.5	19.8	<0.0001	<0.0001
ALT†	U/L	71	82	27	<0.0001	<0.0001
AST†	U/L	57	68	25	<0.0001	<0.0001
AFP †	ng/mL	5.4	11.8	3.1	<0.0001	<0.0001

†median (range)

Table 3 Changes in laboratory data at 1 year after the start of Nucs treatment

		All Nucs (n = 510)		ETV (n = 397)		LVD (n = 113)	
			p		*p*		*p*
T. Bil†	mg/dL	0 (−26.7〜+1.3)	<0.005	0 (−20.4〜+1.3)	<0.01	−0.08 (−26.7〜+1.1)	n.s.
Albumin†	g/dL	+0.25 (−0.9〜+2.2)	<0.0001	+0.2 (−0.9〜+2.2)	<0.0001	+0.4 (−0.4〜+2.1)	<0.0001
Platelets†	$\times 10^4/mm^3$	+0.5 (−22〜+13.1)	n.s.	+0.6 (−22〜+13.1)	n.s.	+0.5 (−7.3〜+11.2)	n.s.
ALT†	U/L	−43 (−1,628〜+518)	<0.0001	−42 (−1,628〜+518)	<0.0001	−51 (−697〜+23)	<0.0001
AST†	U/L	−28 (−1,439〜+140)	<0.0001	−26 (−1,439〜+140)	<0.0001	−37 (−507〜+37)	<0.0001
AFP†	ng/mL	−1.3 (−989〜+240)	<0.005	−1 (−989〜+240)	<0.05	−7 (−650〜+0.8)	<0.05
HBV DNA undetectable	yes/no	85/262	<0.0001	85/194	<0.0001	0/68	<0.0001
HBsAg clearance	yes/no	1/77		1/65		0/7	
HBeAg clearance	yes/no	15/87		10/69		5/18	

This table shows the change from the baseline to 1 year after treatment.
†median (range). T. Bil, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B envelope antigen. Other abbreviations are explained in the Table 1 footnote.

ment. In contrast, no patient in the LVD group reached the undetectable HBV DNA level. Each Nucs showed effective suppression of HBV reproduction.

Risk factors associated with HCC. We next analyzed all patients to search for the risk factors of hepatocarcinogenesis. In the univariate analysis, older age, male sex, higher serum total bilirubin, lower serum albumin, lower platelet count, higher serum ALT, higher serum AFP, HBeAg positivity, and higher serum HBV DNA were the risk factors for HCC development. In the subsequent multivariate analysis, higher age, male sex, a regimen not including ETV, lower serum albumin, lower platelet count, HBeAg negativity, and higher HBV DNA were the risk factors for hepatocarcinogenesis (Table 4).

However, in the log-rank analysis, the ETV group showed significantly more HCC development compared to the Not-treated group (Fig. 1, $p < 0.005$). As shown in Table 2, the ETV group was significantly older, with a significantly higher proportion of males, and significantly higher HBV DNA, lower serum albumin, higher serum ALT and higher serum AFP at baseline compared to the Not-treated group. In this analysis, we could not confirm the effect of ETV for

Table 4 Risk factors for HCC in all patients (n = 1,206) in a proportional hazards model

		Univariate		Multivariate	
Factors		HR	*p*	HR	*p*
Age	35≧years	4.7	<0.001	–	<0.005
Sex	Male	1.4	n.s.	6.3	<0.001
ETV	+ (vs. Not-treated)	1.2	n.s.	0.19	<0.001
T. Bil	≧1 mg/dL	1.6	0.n.s.	0.8	n.s.
Albumin	<4 g/dL	3.4	<0.001	2.8	<0.05
Platelets	$<15 \times 10^4/mm^3$	6.3	<0.001	5.2	<0.001
PT	<90%	2.8	<0.005	1.2	n.s.
ALT	≧31 U/L	2.7	<0.001	0.26	n.s.
AFP	≧5 ng/mL	6.0	<0.001	2.2	n.s.
HBeAg	+	1.1	n.s.	0.32	<0.01
HBVDNA	≧5 log copies/mL	3.1	<0.001	6.5	<0.005

HCC, hepatocellular carcinoma; HR, hazard ratio; ETV, entecavir; HBV, hepatitis B virus; HR, hazard ratio.

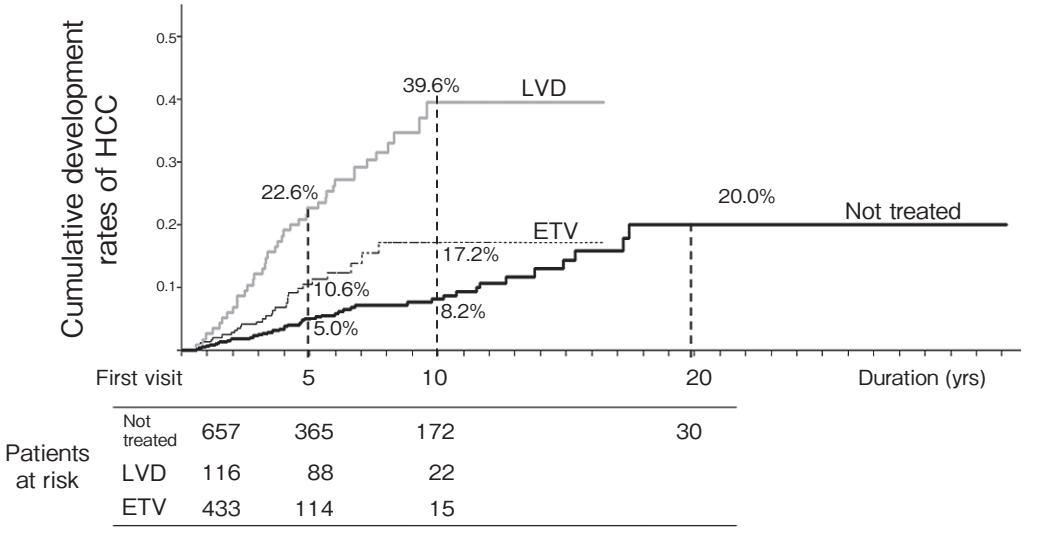

Fig. 1 Comparison of HCC cumulative incidence rates among the treatment groups in all patients (n = 1,206). The cumulative HCC incident rate was significantly lower in the Not-treated group compared to the ETV and LVD groups (log-rank test: $p < 0.005$ and $p < 0.0001$, respectively). Black line: not treated. Dotted line: ETV. Gray line: LVD.

the suppression of hepatocarcinogenesis.

Subgroup analysis categorized by age, HBV DNA and HBeAg. To decrease the bias and to identify who should be treated with ETV, we categorized our cohort by age, HBV DNA titer and HBeAg (Fig. 2). The patients categorized in Group 1 (n = 184) were characterized by age ≥ 35 years, HBV DNA ≥ 4 log copies/mL, and HBeAg positivity. The patients in Group 2 (n = 237) were characterized by age ≥ 35 years, HBV DNA ≥ 4 log copies/mL, and HBeAg negativity. The patients in Group 3 were characterized by age ≥ 35 years and HBV DNA < 4 log copies/mL. The patients in Group 4 were < 35 years old. As shown in Tables 5–8, most of the difference in each parameter (age, sex, observation period, HBV DNA, HBeAg, T. Bil, albumin, platelets count,

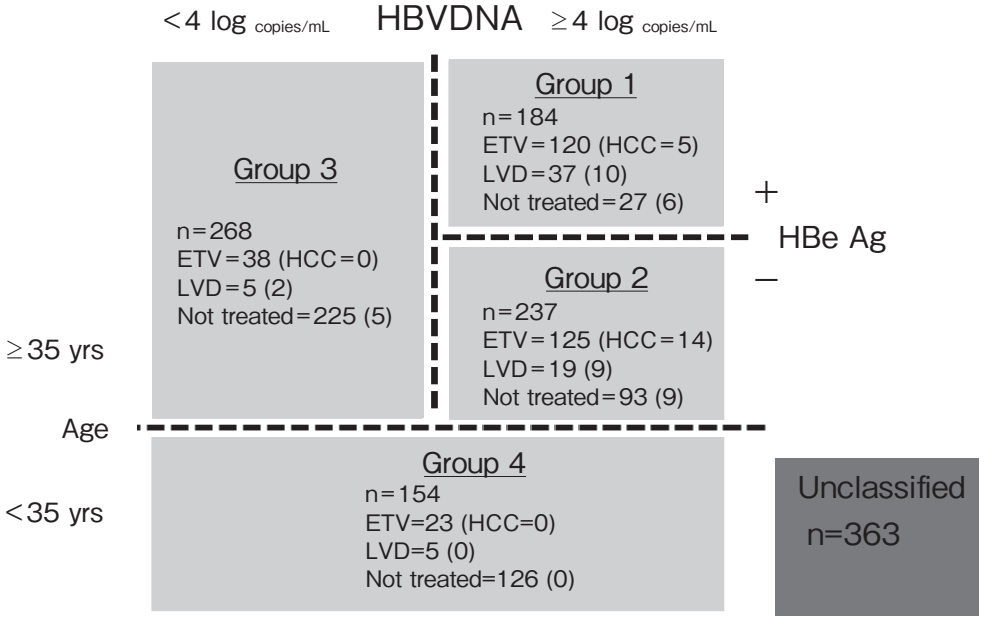

Fig. 2 Categorization by age, HBV DNA and HBeAg among all patients (n = 1,206).

Table 5 Baseline parameters by Nucs treatment, Group 1: age ≥ 35 years, HBV DNA 4 log copies/mL and more, and HBeAg positivity

		ETV	LVD	Not-treated	*P* (LVD vs. Not-treated)	*P* (ETV vs. Not-treated)
No. of patients		120	37	27		
Age†	years	49	51	49	n.s.	n.s.
Sex	male/female	81/39	19/18	15/12	n.s.	n.s.
Observation period†	days	1,331	2,670	1,683	n.s.	<0.05
HCC		5	10	6	n.s.	<0.0005
HBV DNA†	Log copies/mL	7.6	7.2	7.2	n.s.	n.s.
HBV genotype	B/C	2/85	0/19	0/10	n.s.	n.s.
HBeAg	+/−	120/0	37/0	27/0	−	−
T. Bil†	mg/dL	0.7	0.9	0.84	n.s.	n.s.
Albumin†	g/dL	4	3.8	4.2	<0.005	<0.05
Platelets†	$\times 10^4/mm^3$	15.3	11.8	15.3	<0.01	n.s.
ALT†	U/L	79.5	64	52	<0.005	<0.005
AST†	U/L	62	62	41	n.s.	<0.005
AFP†	ng/mL	9	13.2	5.2	<0.05	n.s.

†median (range). Abbreviations are explained in earlier tables' footnotes.

ALT, AST, AFP) between the 2 treatment groups was decreased after this categorization.

In Group 1, 120 patients were treated with ETV, 37 were treated with LVD, and 27 were non-treated patients. In this group, 85.3% (157 patients) were treated with Nucs. Age, sex, HBV DNA, AFP were similar among these 3 treatment groups (Table 5). We then compared the cumulative incidence of HCC. As shown in Fig. 3, HCC development was significantly decreased in the ETV group (ETV vs. Not-treated $p < 0.05$, ETV vs. LVD $p < 0.01$).

In Group 2, the numbers of patients in the ETV, LVD and non-treated subgroups were 125, 19 and 93, respectively. In this Group, 60.8% (n = 144) of the patients were treated with Nucs. There were still significant differences in age, HBV DNA, AFP and other biochemical parameters among the treatment groups (Table 6). The HCC development was comparable between the ETV group and the Not-treated group. The LVD group developed HCC significantly more frequently compared to the other groups (ETV vs. LVD, $p < 0.05$; LVD vs. Not-treated, $p < 0.005$).

In Group 3, the numbers of patients in the ETV, LVD and non-treated subgroups were 38, 5 and 225,

Table 6 Baseline parameters by Nucs treatment, Group 2: age ≥ 35 years, HBV DNA 4 log copies/mL and more, and HBeAg negativity

		ETV	LVD	Not-treated	*P* (LVD vs. Not-treated)	*P* (ETV vs. Not-treated)
No. of patients		125	19	93		
Age†	years	54	58	52	<0.005	<0.01
Sex	male/female	69/56	15/4	46/47	<0.05	n.s.
Observation period†	days	1,078	2,396	1,402	n.s.	n.s.
HCC		14	9	9	0	n.s.
HBV DNA†	Log copies/mL	6.3	6.6	4.9	<0.0001	<0.0001
HBV genotype	A/B/C	2/3/68	0/1/5	1/8/28	n.s.	<0.05
HBeAg	+/−	0/128	0/19	0/90	–	–
T. Bil†	mg/dL	0.8	1.01	0.8	<0.001	n.s.
Albumin†	g/dL	4.1	4.1	4.4	<0.001	<0.0001
Platelets†	$\times 10^4/mm^3$	14.8	13.4	18.9	<0.0001	<0.0001
ALT†	U/L	70	88	28	<0.0001	<0.0001
AST†	U/L	57	64.5	27	<0.0001	<0.0001
AFP†	ng/mL	4.9	11.45	3	<0.005	<0.0001

†median (range)

Table 7 Baseline parameters by Nucs treatment, Group 3: age ≥ 35 years and HBV DNA lower than 4 log copies/mL

		ETV	LVD	Not-treated	*P* (LVD vs. Not-treated)	*P* (ETV vs. Not-treated)
No. of patients		38	5	225		
Age†	Years	56	51	55	n.s.	n.s.
Sex	male/female	23/15	2/3	105/120	n.s.	n.s.
Observation period†	days	623	2,823	1,506	n.s.	<0.0001
HCC		0	2	5	<0.01	n.s.
HBV DNA†	Log copies/mL	3.15	2.6	3.3	n.s.	n.s.
HBV genotype	A/B/C	2/1/19	0/0/1	6/6/50	n.s.	n.s.
HBeAg	+/−	3/22	2/2	4/205	<0.05	<0.01
T. Bil†	mg/dL	0.62	1.01	0.7	n.s.	n.s.
Albumin†	g/dL	4.3	4.1	4.4	n.s.	<0.05
Platelets†	$\times 10^4/mm^3$	17.5	12.5	19.7	<0.05	n.s.
ALT†	U/L	28	57	21	<0.005	<0.005
AST†	U/L	34.5	53	23	<0.005	<0.0001
AFP†	ng/mL	3.4	10	3	n.s.	n.s.

†median (range)

Table 8 Baseline parameters by Nucs treatment, Group 4: <35 years old

		ETV	LVD	Not-treated	*P* (LVD vs. Not-treated)	*P* (ETV vs. Not-treated)
No. of patients		23	5	126		
Age†	Years	32	30	30	n.s.	n.s.
Sex	male/female	19/4	5/0	71/55	n.s.	<0.05
Observation period†	days	1,813	3,039	1,876.5	<0.05	n.s.
HCC		0	0	0	–	
HBV DNA†	Log copies/mL	>7.6	>7.6	5.5	n.s.	<0.05
HBV genotype	A/B/C	0/0/0	0/0/3	5/1/43	n.s.	n.s.
HBeAg	+/−	15/7	5/0	61/62	<0.05	n.s.
T. Bil†	mg/dL	0.8	0.75	0.7	n.s.	n.s.
Albumin†	g/dL	4.2	4	4.4	n.s.	<0.005
Platelets†	$\times 10^4/mm^3$	17.1	17.7	20.5	n.s.	<0.05
ALT†	U/L	208	329	31	<0.001	<0.0001
AST†	U/L	102	99	25.5	<0.005	<0.0001
AFP†	ng/mL	5.6	76.9	3	<0.05	<0.005

† median (range)

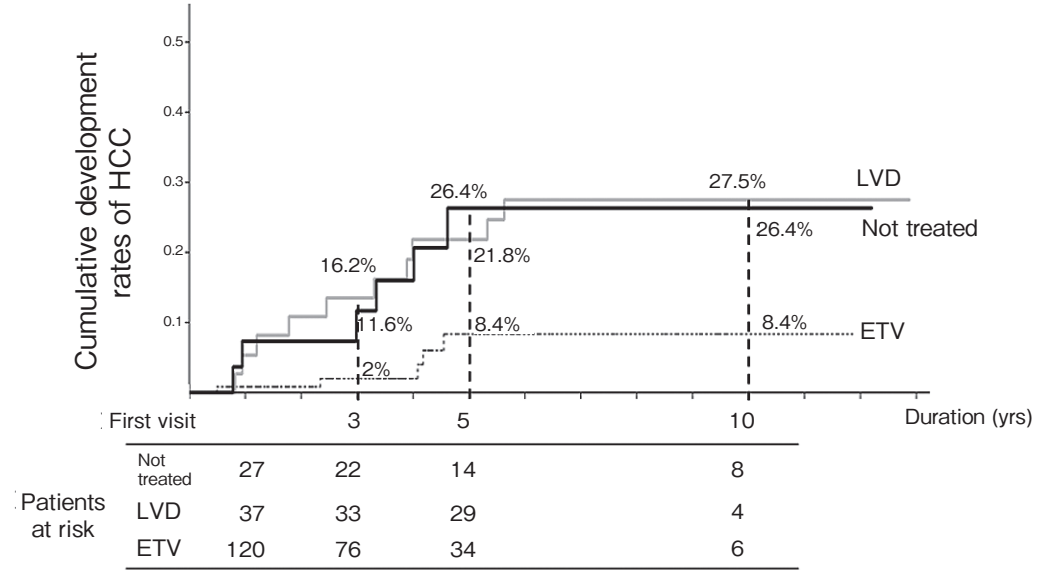

Fig. 3 Comparison of the HCC cumulative incidence rates among each treatment group in Group 1 (n = 184), characterized by age ≥ 35 years, HBV DNA ≥ 4 log copies/mL, and HBeAg positivity. The log-rank test revealed a significant difference between the ETV and Not-treated subgroups in the incidence of HCC (log-rank test: $p = 0.0131$).

respectively. In this group, only 16.0% (n = 43) of the patients were treated with Nucs and only seven patients developed HCC within the median observation period of 1,351.5 days. The patients' age, sex, HBV DNA, and AFP values were similar among the treatment groups (Table 7). The HCC development was comparable between the ETV group and the Not-treated group. Because of the small number of patients, the effect of LVD was difficult to assess.

In Group 4, the numbers of patients in the ETV, LVD and non-treated subgroups were 23, 5 and 126, respectively (Table 8). In this group, only 18.2% (n = 28) of the patients were treated with Nucs. We could not assess the HCC development in this group, because no patient developed HCC within the median observation period of 1,889.5 days. ETV significantly suppressed hepatocarcinogenesis only in Group 1.

Propensity matched analysis by age, sex,

platelet count, ALT and serum HBV DNA. We used propensity score matching in the ETV group and the Not-treated group by age, sex, platelet count, ALT and serum HBV DNA to eliminate the baseline bias, resulting in a sample size of 148 patients per cohort (Table 9). This operation also diminished the differences in AFP and HBeAg between these groups, but could not remove them because the patients who were treated with ETV were essentially at high risk for liver failure and HCC. As shown in Fig. 4, HCC development was significantly decreased in the ETV group ($p < 0.05$).

We conducted the same analysis for the LVD group and the Not-treated group. No significant difference was observed between these groups regarding hepatocarcinogenesis (Table 10, Fig. 5, $p > 0.05$).

Discussion

Our investigation revealed that long-term ETV

Table 9 Baseline parameters by Nucs treatment (ETV group and Not-treated group), propensity score matched by age, sex, ALT, Platelets count and HBV DNA

		ETV	Not-treated	*P*
Propensity score†		0.96	0.96	n.s.
No. of patients		148	148	
Age†	years	53	48	n.s.
Sex	male/female	91/57	91/57	n.s.
Observation period†	days	1,144.5	1,439.5	n.s.
HCC		7	20	<0.01
T. Bil†	mg/dL	0.7	0.8	<0.05
Albumin†	g/dL	4.2	4.4	<0.0001
Platelets†	$\times 10^4/mm^3$	17.5	17.8	n.s.
ALT†	IU/L	46	42	n.s.
AFP†	ng/mL	4.05	3.25	<0.05
HBV DNA†	log copies/mL	5.5	5	n.s.
HBeAg	+/−	51/64	37/104	<0.005

†median (range)

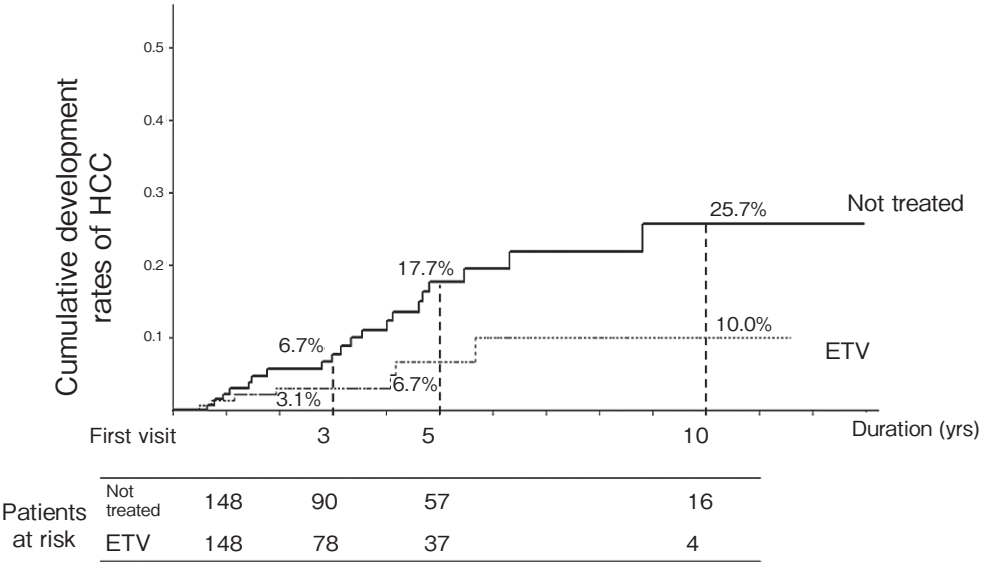

Fig. 4 Comparison of HCC cumulative incidence rates between the ETV group and the Not-treated patients, with propensity scores matched by age, sex, ALT, Platelets count and HBV DNA. HCC development was significantly decreased in the ETV group ($p < 0.05$).

Table 10 Baseline parameters by Nucs treatment (LVD group and Not-treated group), Propensity score matched by age, sex, ALT, Platelets count and HBV DNA

		LVD	Not-treated	*P*
Propensity score†		0.79	0.79	n.s.
No. of patients		48	48	
Age†	years	51	52.5	n.s.
Sex	male/female	26/22	26/22	n.s.
Observation period†	days	2,829.5	1,333.5	<0.0005
HCC		12	14	n.s.
T. Bil†	mg/dL	0.9	0.9	n.s.
Albumin†	g/dL	4	4.3	<0.01
Platelets	$\times 10^4/mm^3$	13.9	14	n.s.
ALT†	IU/L	86	61	n.s.
AFP†	ng/mL	11.5	5.1	<0.01
HBV DNA†	log copies/mL	7.1	6.3	n.s.
HBeAg	+/−	29/12	20/24	<0.05

†median (range)

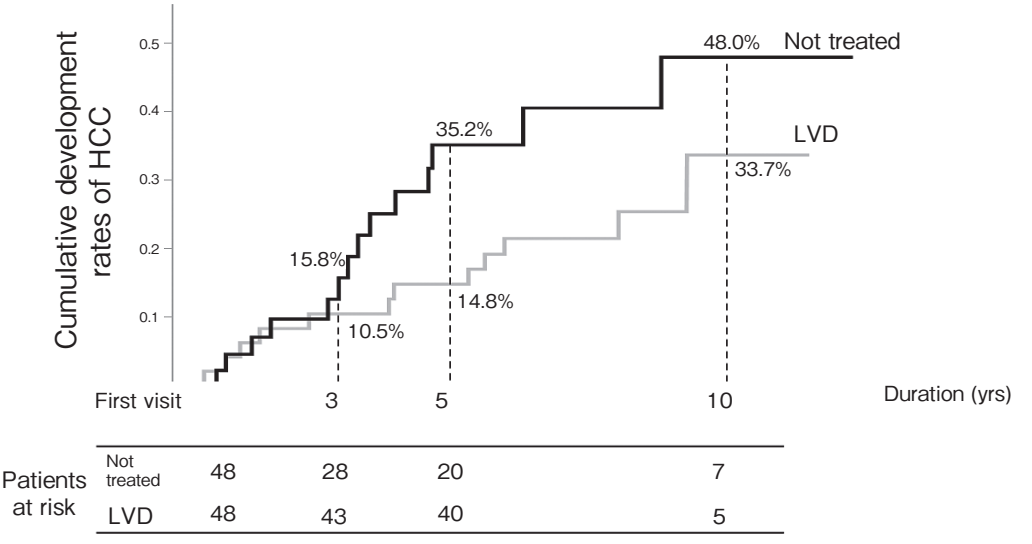

Fig. 5 Comparison of HCC cumulative incidence rates between the LVD group and the Not-treated patients, with propensity scores matched by age, sex, ALT, Platelets count and HBV DNA. No significant difference was observed between these groups for hepatocarcinogenesis ($p > 0.05$).

therapy significantly suppressed the hepatocarcinogenesis in CHB patients compared to CHB patients without Nucs treatment, in a propensity matched analysis. The effect of ETV for HCC suppression was more evident among the patients who were ≥35 years old, with HBV DNA ≥4 log copies/mL and positive HBe antigen at diagnosis.

Individuals who are chronically infected with HBV have a high risk of the development of HCC. There are many reports about the HCC incidence rate in CHB patients. The cumulative HCC incidence rates among Japanese Nucs-naïve HBV-infected patients were 2.1% at 5 years, 4.9% at 10 years, and 18.8% at 15 years [19]. In other studies, the 5-year cumulative HCC incidence rate of CHB patients was 3.3%, and in patients with HBV-related cirrhosis, the 5-year cumulative HCC incidence rate was 21.2–59% [20, 21].

In the present study, the cumulative HCC incidence rates in the Nucs-naïve patients were 5% at 5 years, 8.2% at 10 years, and 20% at 20 years. This result was comparable to the previous reports, because our cohort contained cirrhosis patients.

In our cohort, the multivariate analysis and the log-rank test resulted in different conclusions. This is due to the baseline differences between the ETV group and the Not-treated group. The ETV group was older and had a higher proportion of males, high serum HBV DNA, and poor hepatic reserve compared to the non-treated patients.

We analyzed all patients to search for the risk factors of HCC development. ETV treatment did not show a reduction of hepatocarcinogenesis. Our study was retrospective, and this finding is thus the result of strong selection bias. We then categorized our cohort by age, HBV DNA titer and HBeAg to remove the bias and to identify who should be treated with ETV.

Hosaka *et al.* reported that the effect of ETV on HCC development was more prominent among patients with cirrhosis or at high risk by some scoring systems [22]. The diagnosis of cirrhosis is sometimes difficult by less-invasive examinations (especially in chronic HBV infection), though laparoscopic examination is useful for diagnosing cirrhosis and estimations of the risk of HCC development [6]. Such scoring systems would be complex.

Our categorization by age, HBV DNA and HBeAg was easier and more objective to guide each treatment strategy. As a result, in Group 1, the cumulative HCC incidence was significantly reduced in the ETV-treated patients compared to the LVD-treated patients and the non-treated patients ($p < 0.05$). The patients in Group 1 had a high risk of HCC incidence, and they should thus be treated with ETV. In Group 2, however, the HCC incidence rate was lower than that of the Group 1 patients, and the cumulative HCC incidence was comparable among the ETV patients and the non-treated patients. The patients who were younger or had lower HBV DNA levels (*i.e.*, Groups 3 and 4) developed HCC very rarely. Longer observation would be needed to assess these groups.

ETV showed a significant beneficial effect in the present study's focus on hepatocarcinogenesis, especially in patients aged ≥ 35 years with HBV DNA levels ≥ 4 log copies/mL and positive HBe antigen at diagnosis. The Nucs also increased the hepatic reserve (as shown in Table 3). Further research is needed to assess the effects associated with hepatic failure with HBV infection.

In conclusion, in CHB patients aged 35 or older with an HBV DNA level ≥ 4 log copies/mL and positive HBe antigen at diagnosis, ETV treatment is recommended for the suppression of HCC development.

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