Research Article
Viral Hepatitis

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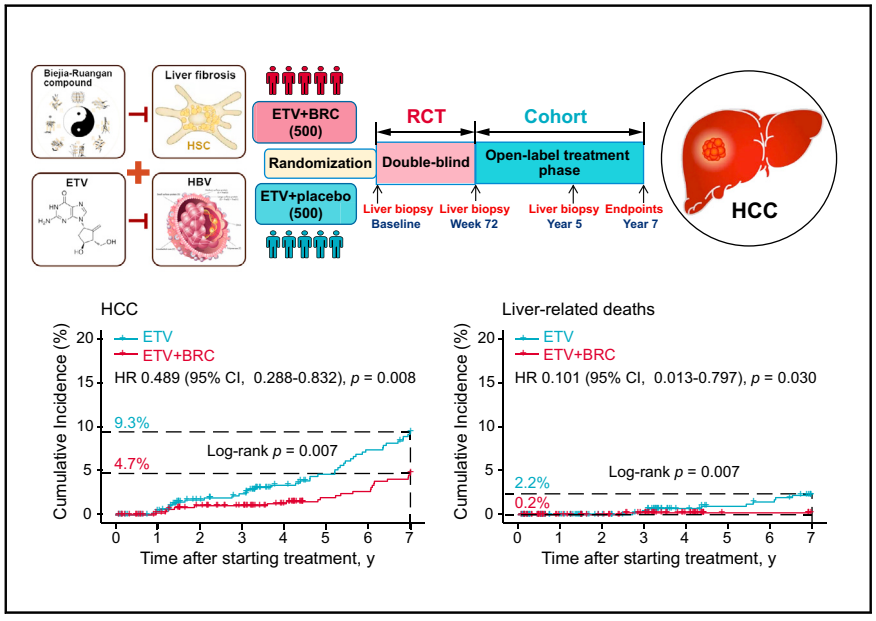
Entecavir plus Biejia-Ruangan compound reduces the risk of hepatocellular carcinoma in Chinese patients with chronic hepatitis B

Graphical abstract



Highlights

- This RCT-based prospective cohort study included 1,000 patients and lasted for 7 years.
- Compared with ETV treatment, the combination of ETV + Biejia-Ruangan compound could further reduce the incidence of HCC.
- Biejia-Ruangan compound in addition to ETV should be considered for patients with CHB and advanced fibrosis or cirrhosis.

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Lay summary

Patients with chronic hepatitis B virus infection are at an increased risk of developing liver cancer (specifically hepatocellular carcinoma [HCC]). While there are effective antiviral treatments that can suppress the virus in chronically infected patients, the risk of HCC remains. Herein, we show that adding a traditional Chinese medicine called Biejia-Ruangan compound to an antiviral reduced the risk of HCC in patients with chronic hepatitis B.

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Entecavir plus Biejia-Ruangan compound reduces the risk of hepatocellular carcinoma in Chinese patients with chronic hepatitis B

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Background & Aims: Chronic hepatitis B (CHB) and liver fibrosis are associated with a high risk of hepatocellular carcinoma (HCC) development. We assessed whether entecavir (ETV) plus Biejia-Ruangan compound (BRC), an anti-fibrotic traditional Chinese medicine, can further reduce the risk of HCC in treatment-naïve Chinese patients with CHB and an Ishak fibrosis score of ≥3 points derived from our parent double-blind randomized placebo-controlled trial.

Methods: After a 72-week comparison between ETV+BRC and ETV+placebo treatment, participants were eligible to enter an open-label treatment phase and were followed up every 6 months. The primary [secondary] endpoints were the incidence of HCC [liver-related deaths, non-HCC events, and non-liver-related deaths]. Modified intention-to-treat (mITT), intention-to-treat (ITT), and per-protocol (PP) populations were defined for the time-to-event analysis.

Results: A total of 1,000 patients were recruited; the median age was 42.0 years; 69.9% were male and 58.3% were HBeAg positive. In the mITT population, the 7-year cumulative incidence of HCC [liver-related deaths] was 4.7% [0.2%] for ETV+BRC, which was significantly lower than 9.3% [2.2%] for ETV monotherapy (p = 0.008 [p = 0.030]). Notably, ETV+BRC treatment yielded a lower incidence of HCC in those who did not achieve regression of fibrosis at week 72 than ETV monotherapy (p = 0.018). There were no differences in the other 2 secondary endpoints or safety profiles between the groups. Multivariable Cox proportional regression analysis, including the treatment allocation as a parameter, also demonstrated that ETV+BRC treatment was associated with a reduced incidence of HCC. The ITT and PP analyses showed consistent results.

Conclusions: ETV plus BRC combination treatment could further reduce the risk of HCC and liver-related deaths in patients with CHB and advanced fibrosis or cirrhosis, which may have important clinical implications for HCC prevention.

Lay summary: Patients with chronic hepatitis B virus infection are at an increased risk of developing liver cancer (specifically hepatocellular carcinoma [HCC]). While there are effective antiviral treatments that can suppress the virus in chronically infected patients, the risk of HCC remains. Herein, we show that adding a traditional Chinese medicine called Biejia-Ruangan compound to an antiviral reduced the risk of HCC in patients with chronic hepatitis B.

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies and the leading causes of cancer-related death worldwide. Although recent emerging molecular targeted therapy and immunotherapy have constituted a breakthrough, the overall survival of patients with advanced HCC has improved marginally. Effective measures to prevent or reduce the incidence of HCC in populations at high risk are urgently needed. Chronic hepatitis B (CHB) and liver fibrosis are the most prominent risk factors for HCC development.[1–3] However, current

Keywords: Hepatitis B virus; Long-term outcomes; Liver biopsy; Nucleot(s)ide analogs; Traditional Chinese medicine; Liver stiffness measurement.
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antiviral therapies, including nucleos(t)ide analogues (NAs) or pegylated interferons, cannot completely eradicate HBV, and their efficacy in reversing fibrosis/cirrhosis is limited. The risk of HCC in patients with CHB can only be reduced, not eliminated.[4,5]

Therefore, NA-based combination therapy represents a promising strategy to simultaneously inhibit HBV replication and arrest fibrogenesis. The Biejia-Ruangan compound (BRC) is a traditional Chinese medicine (TCM), which has been clinically used to treat liver fibrosis/cirrhosis caused by CHB in China for half a century. It consists of *Trionycis Carapax, Curcumae Rhizoma, Paeoniae Radix Rubra, Angelicae Sinensis Radix, Notoginseng Radix et Rhizoma, Codonopsis Radix, Astragali Radix, Cordyceps, Isatidis Radix* and *Forsythiae Fructus*. In 1999, it was approved by the China Food and Drug Administration (CFDA) as the first official registered oral prescription anti-fibrotic drug after a critical assessment of its toxicology and pharmacological effects. The BRC is produced in accordance with Good Manufacturing Practices and has complete quality control standards approved by the CFDA, including raw materials, intermediate products, and final preparation. The chemical constituents of the BRC, as well as their absorption and distribution *in vivo*, have been identified in a previous study.[6] We demonstrated that the BRC could significantly increase the rate of fibrosis regression in patients with CHB when combined with entecavir (ETV).[8] This finding challenged the conventional perspective that liver fibrosis regression can only be achieved with long-term antiviral therapy and suggested that an anti-fibrotic agent as a supplement to the current etiology-targeted treatment can further improve fibrosis regression. Herein, we aimed to assess the effects of ETV plus BRC combination treatment on the long-term clinical outcomes of Chinese patients with CHB derived from a well-monitored parent double-blind randomized placebo-controlled trial (RCT).

Patients and methods

Study design and participants

We performed a long-term extension of our parent prospective RCT (NCT01965418) involving 14 participating sites. The inclusion criteria were: age of ≥18 years, treatment-naïve CHB, and Ishak fibrosis score (IFS) of ≥3 points. The exclusion criteria were: chronic liver disease with any other etiologies, decompensated cirrhosis or any cancer, pregnancy, or breast-feeding. Originally, we had randomized 1,000 patients recruited from October 2013 to October 2014 in equal numbers (1:1) to receive either ETV (0.5 mg/day)+BRC (2.0 g/time, 3 times/day) or ETV+placebo treatment.[7–9] After the initial 72-week masked comparison, the patients transitioned to an open-label treatment phase. The study was conducted in accordance with the local regulatory requirements, Good Clinical Practice, and the Declaration of Helsinki. The study protocol was approved by the relevant institutional review board or ethics committee at each site. All the participants provided written informed consent.

Procedures

Clinical, laboratory, and adverse event assessments were performed every 3 months during the double-blind phase and every 6 months during the subsequent open-label phase. The serum HBV-DNA level was measured using the COBAS TaqMan assay (Roche, Branchburg, NJ, USA), with a lower limit of quantification of 20 IU/ml. The baseline HBsAg level was measured using chemiluminescent immunoassay (Abbott, Abbott Park, IL, USA). Treatment adherence was measured using the 8-Item Morisky Medication Adherence Scale. HCC surveillance was performed via ultrasonography and alpha-fetoprotein every 6 months. HCC was diagnosed using contrast-enhanced magnetic resonance imaging or computed tomography, confirmed using liver biopsy, and classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system.[10] Liver stiffness measurement (LSM) was performed using FibroScan (Echosens, France).[9] Aspartate aminotransferase (AST)-to-platelet ratio index (APRI) was calculated using the following formula: APRI = ([AST/upper limit of normal]/platelet count)×100, with an upper limit of normal for AST of 40 U/L.[11]

Ultrasound-guided liver biopsy was performed at baseline and 72 weeks after starting treatment according to the standard protocol.[12] When necessary, a third biopsy was conducted to confirm the diagnosis of HCC or to assess histological improvement at year 5. At least 2 specimens were collected from each patient to acquire an adequate sample, defined as ≥20 mm in length and/or the presence of ≥11 portal tracts. Samples were then evaluated independently by 2 pathologists who were blinded to the clinical information. Hepatic inflammation was graded using the modified histologic activity index (HAI) defined by Ishak, while liver fibrosis was staged using IFS.[13,14] Virological response (VR) was defined as the proportions of patients with serum HBV-DNA loads of <20 IU/ml at week 48 (the on-treatment prognostic factor) or year 7 (the virological efficacy). Regression of liver fibrosis (REG) was defined as a ≥1-point decrease in the IFS without worsening of the modified HAI.[15]

Outcomes

The primary endpoint was the incidence of HCC. The secondary endpoints were the incidence of liver-related deaths, non-HCC events (*e.g.*, non-HCC malignancies, decompensating events, *etc.*), and non-liver-related deaths. The date of starting treatment was defined as the zero time-point, the patients who were lost to follow-up were censored at the last date they were known to be alive; and the patients who remained alive were censored at year 7 after starting treatment. All adverse events (AEs), except for the primary and secondary outcomes, were graded according to the Common Terminology Criteria for Adverse Events (version 5.0).[16]

Statistical analyses

Three populations were defined for the time-to-event and *post hoc* analyses: modified intention-to-treat (mITT), ITT, and per-protocol (PP). The mITT population included all eligible patients who were randomly assigned to the parent study, however, those who switched to the other group were censored at the time of drug-switching; the mITT analysis was considered as the primary analysis. The ITT population included all eligible patients who were randomly assigned to the parent study. The PP population excluded patients who were randomized but discontinued the treatment and treatment-converted patients (Fig. 1).

Continuous variables are expressed as medians (IQRs) and were compared using the Mann-Whitney test. Categorical variables are presented as numbers (percentages) and were compared using the Chi-square or Fisher's exact test. No imputation was performed for the missing data. The cumulative incidence of endpoints was analyzed using the Kaplan-Meier method and compared by the log-rank test. Hazard ratios (HRs) and 95% CIs were estimated using a Cox proportional-hazard model. The consistency of the effect on the incidence of HCC was explored in 9

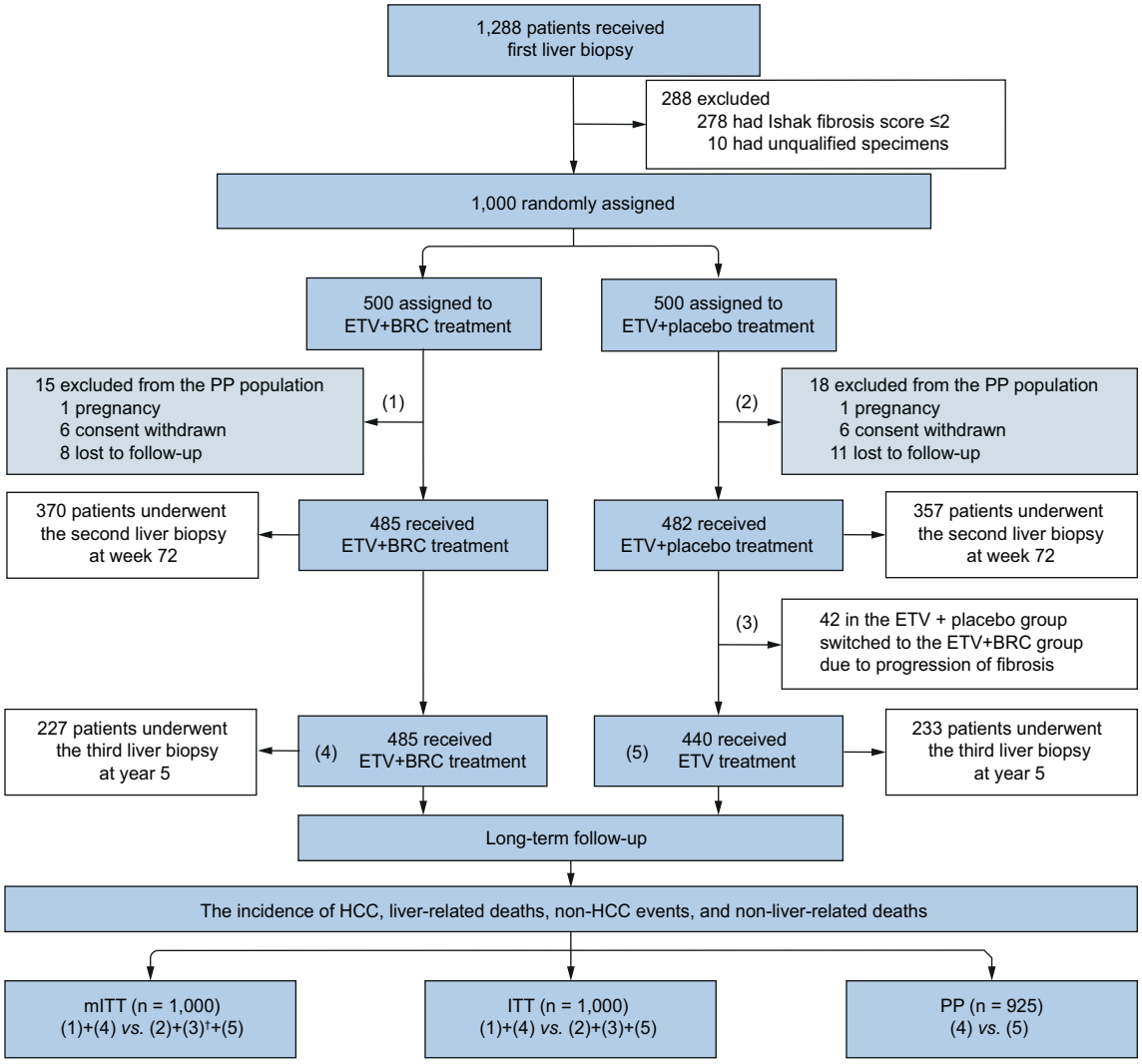

Fig. 1. The profile of participant flow through the parent and extension studies. BRC, Biejia-Ruangan compound; ETV, entecavir; HCC, hepatocellular carcinoma; ITT, intention-to-treat; mITT, modified intention-to-treat; PP, per protocol. †Censored at the time of drug-switching (week 72 after starting treatment).

post hoc subgroups without adjustment for multiple comparisons, and the interaction between subgroups was assessed using the Cochran-Mantel-Haenszel test. A linear mixed-effect model for repeated measures (LSM, APRI, estimated glomerular filtration rate [eGFR), and prothrombin time) was conducted to investigate the treatment × time interaction effects in the mITT population, corrected by epsilon (Greenhouse-Geisser) when the spherical hypothesis was not met; multiple Bonferroni-corrected *post hoc* comparisons were employed to test for differences. A 2-tailed *p* value of <0.05 was considered statistically significant. All statistical analyses were performed using the R software, version 4.1.1 (http://www.r-project.org/).

Results

Baseline characteristics

A total of 1,288 patients were screened by liver biopsy; 1,000 eligible patients were randomly assigned to receive either ETV+BRC (n = 500) or ETV+placebo (n = 500) treatment. After the 72-week double-blind phase, patients entered the open-label treatment phase. In the ETV+BRC group, the treatment of 485 patients remained unchanged; in the ETV+placebo group, 42 patients had to switch to ETV+BRC treatment owing to progression of fibrosis observed on liver biopsy at week 72, while the other 440 patients remained on ETV monotherapy (Fig. 1). In the mITT population, the median age was 42.0 (IQR 35.0-49.0) years; 69.9% were male and 58.3% were HBeAg positive. The baseline variables were comparable between the 2 groups (Table 1). These results were consistent in the PP populations (Table S1).

Clinical outcomes

In the mITT population, during the median 7-year treatment period, 33 patients discontinued treatment, 60 developed HCC with BCLC stage A, 10 died of liver-related causes (Table 2). The cumulative 1-, 3-, 5- and 7-year incidence of HCC were 0.2%, 1.0%, 1.9%, and 4.7% in the ETV+BRC group, which were significantly lower than 0.4%, 2.4%, 4.6% or 9.3% in the ETV group, respectively (HR 0.489; 95% CI 0.288-0.832; p = 0.008; Fig. 2A). The cumulative 1-, 3-, 5- and 7-year incidence of liver-related deaths were

Table 1. Baseline clinical characteristics of enrolled patients in the mITT population.

	Total	ETV+BRC	ETV	*p* value
n	1000	500	500	–
Age (years)	42.0 (35.0-49.0)	42.0 (35.0-49.0)	42.0 (35.0-49.0)	0.900
Male sex	699 (69.9)	346 (69.2)	353 (70.6)	0.679
Family history of HCC	238 (23.8)	114 (22.8)	124 (24.8)	0.504
Hypertension	99 (9.9)	57 (11.4)	42 (8.4)	0.138
Diabetes mellitus	43 (4.3)	22 (4.4)	21 (4.2)	1.000
BMI (kg/m^2)	22.9 (21.0-25.1)	22.9 (20.8-24.8)	22.9 (21.2-25.3)	0.238
HBsAg (log_{10} IU/ml)	3.5 (3.1-4.0)	3.5 (3.1-4.0)	3.5 (3.1-4.0)	0.289
HBeAg positive	583 (58.3)	287 (57.4)	296 (59.2)	0.608
HBV-DNA (log_{10} IU/ml)	6.1 (4.9-7.5)	6.1 (4.8-7.4)	6.1 (4.9-7.6)	0.908
PLT ($\times10^9$/L)	158 (120-199)	157 (121-200)	159 (120-198)	0.985
PT (s)	12.6 (11.5-13.8)	12.6 (11.4-13.8)	12.7 (11.6-13.7)	0.708
ALB (g/L)	42.0 (39.0-45.0)	42.2 (39.1-45.0)	42.0 (39.0-45.0)	0.943
ALT (IU/L)	53.0 (32.0-99.0)	51.5 (31.0-90.2)	55.0 (32.0-106.8)	0.157
AST (IU/L)	43.0 (29.0-74.0)	42.0 (29.0-69.0)	43.0 (29.0-78.0)	0.628
TBIL (μmol/L)	13.8 (10.8-18.7)	13.6 (10.5-18.6)	14.0 (11.0-19.0)	0.383
eGFR ($ml/min/1.73\ m^2$)	107 (90-125)	105 (88-127)	107 (93-124)	0.261
AFP (ng/ml)	5.0 (2.8-11.0)	5.0 (2.7-11.0)	5.0 (2.9-10.8)	0.695
APRI	0.7 (0.4-1.4)	0.7 (0.4-1.3)	0.7 (0.4-1.4)	0.591
LSM (kPa)	9.7 (6.8-16.1)	9.6 (6.7-16.2)	10.1 (6.8-16.1)	0.502
Cirrhosis	528 (52.8)	271 (54.2)	257 (51.4)	0.410
Modified HAI (points)				0.469
0-3	102 (10.2)	53 (10.6)	49 (9.8)	
4-8	661 (66.1)	329 (65.8)	332 (66.4)	
9-12	221 (22.1)	113 (22.6)	108 (21.6)	
13-18	16 (1.6)	5 (1.0)	11 (2.2)	
IFS (points)				0.256
3	257 (25.7)	115 (23.0)	142 (28.4)	
4	215 (21.5)	114 (22.8)	101 (20.2)	
5	204 (20.4)	106 (21.2)	98 (19.6)	
6	324 (32.4)	165 (33.0)	159 (31.8)	

Continuous variables are expressed as medians (IQRs) and were compared using the Mann-Whitney test. Categorical variables are presented as numbers (percentages) and were compared using the chi-square or Fisher's exact test, if appropriate. A two-tailed *p* value of <0.05 is considered statistically significant.
AFP, alpha-fetoprotein; ALB, albumin; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; HAI, histologic activity index; IFS, Ishak fibrosis score; LSM, liver stiffness measurement; mITT, modified intention-to-treatment; PLT, platelet; PT, prothrombin time; TBIL, total bilirubin.

0, 0.2%, 0.2%, and 0.2% in the ETV+BRC group, which were significantly lower than 0, 0.5%, 1.0%, and 2.2% in the ETV group, respectively (HR 0.101; 95% CI 0.013-0.797; $p = 0.030$; Fig. 2B). However, there were no differences in the incidence of non-HCC events (HR 0.628; 95% CI 0.239-1.651; $p = 0.346$; Fig. 2C) and non-liver-related deaths (HR 0.245; 95% CI 0.027-2.192; $p = 0.208$; Fig. 2D) between the 2 groups. These results were consistent in the ITT and PP populations (Table S2, Table S3, Figs. S1 and S2). The baseline characteristics of the 42 treatment-converted patients by HCC development are shown in Table S4.

Table 2. Cumulative clinical events occurred in the mITT population at year 7 after starting treatment.

	Total	ETV+BRC	ETV	*p* value
n	1000	500	500	–
Treatment discontinued	33 (3.3)	15 (3.0)	18 (3.6)	0.595
HBsAg loss	15 (1.5)	5 (1.0)	10 (2.0)	0.193
HBeAg seroconversion[†]	242 (41.5)	121 (42.2)	121 (40.9)	0.754
Virological response	916 (91.6)	461 (92.2)	455 (91.0)	0.494
Regression of liver fibrosis[‡]	296 (69.5)	174 (76.7)	122 (61.3)	<0.001
HCC	60 (6.0)	21[#] (4.2)	39 (7.8)	0.017
Liver-related deaths	10 (1.0)	1 (0.2)	9 (1.8)	0.026
Non-HCC events[§]	17 (1.7)	7 (1.4)	10 (2.0)	0.179
Non-liver-related deaths[¶]	5 (0.5)	1 (0.2)	4 (0.8)	0.370
All-cause deaths	15 (1.5)	2 (0.2)	13 (2.6)	0.009

Categorical variables are presented as numbers and percentages [n (%)]. The *p* value is investigated by chi-squared test or Fisher exact test if appropriate. A two-tailed *p* value of <0.05 is considered statistically significant.
BRC, Biejia-Ruangan compound; ETV, entecavir; HCC, hepatocellular carcinoma; mITT, modified intention-to-treat.
[†]The denominator is the number of patients who had a positive HBeAg at baseline (tatol: 583; ETV+BRC: 287; ETV: 296).
[‡]The denominator is the number of patients who underwent a third liver biopsy at year 5 after starting treatment (tatol: 426; ETV+BRC: 227; ETV: 199).
[#]One patient received liver transplantation.
[§]In the ETV+BRC group, 2 cases with hepatic encephalopathy, 1 case with esophageal variceal bleeding, 1 case with liver failure, 2 cases with gastric and colorectal cancers, 1 case with thyroid cancer; In the ETV group, 4 cases with esophageal variceal bleeding, 2 cases with gastric and colorectal cancers, 1 case with thyroid cancer, 1 case with pancreatic cancer, 1 case with acute kidney injury, and 1 case with lower extremity deep vein thrombosis.
[¶]In the ETV+BRC group, 1 case died of cardiovascular disease; In the ETV group, 2 cases died of cardiovascular disease, 1 case died of pancreatic cancer, and 1 case died from a traffic accident.

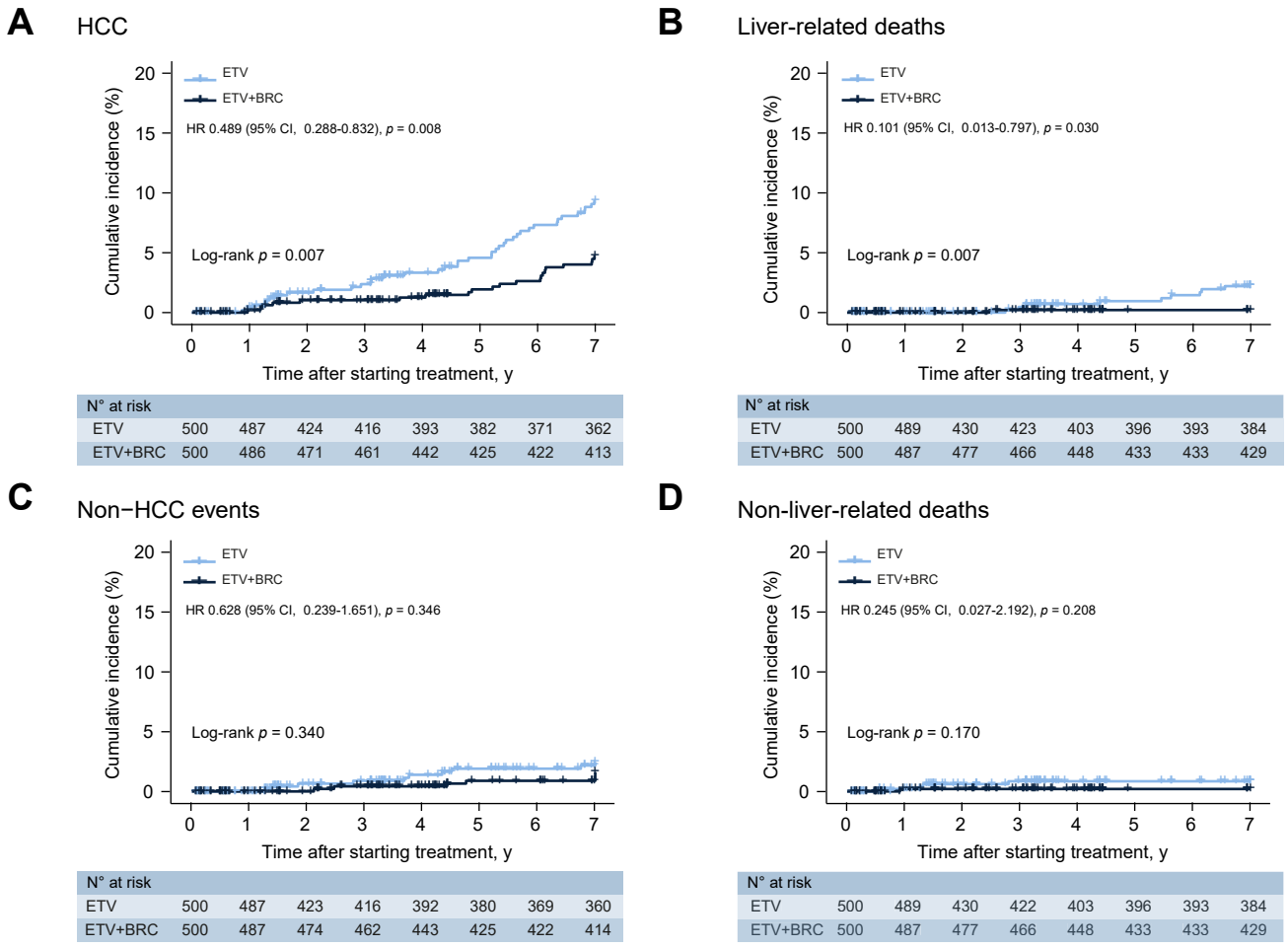

Fig. 2. Cumulative incidence of clinical outcomes in the mITT Population. (A) HCC. (B) Liver-related deaths. (C) Non-HCC events. (D) non-liver-related deaths. The HRs indicate the incidence of clinical outcomes in the ETV+BRC group compared with the ETV group, the *p* values are calculated using the Log-rank test and the Cox proportional regression. A 2-tailed *p* value of <0.05 is considered statistically significant. BRC, Biejia-Ruangan compound; CI, confidence interval; ETV, entecavir; HCC, hepatocellular carcinoma; HR, hazard ratio; mITT, modified intention-to-treat.

Baseline prognostic factors

The superiority of ETV+BRC treatment with respect to the incidence of HCC was revealed in an unplanned *post hoc* subgroup analysis. Baseline age (<44 or ≥44 years), BMI (<26.9 or ≥26.9 kg/m^2), and HBsAg level (<5,000 or ≥5,000 IU/ml) were classified into categories according to their relevant cut-off values defined on the basis of the Kaplan-Meier curve (Fig. S3). The cut-off values for HBV-DNA level (≤3.3, 3.3-7.0, or >7.0 $\log_{10}$ IU/ml) were based on the European Association for the Study of the Liver's 2017 guidelines on the management of HBV infection.[15] The cut-off value for LSM (<13.0 or ≥13.0 kPa) was based on a previous study, which defined subclinical cirrhosis as a non-clinical cirrhosis, but with an LSM value ≥13.0 kPa.[17]

The mITT analysis showed that patients with the following baseline factors could achieve favorable effects (a lower incidence of HCC) from ETV+BRC treatment compared with those from ETV monotherapy: male sex (HR 0.503; 95% CI 0.269-0.942; p = 0.032), age of ≥44 years (HR 0.460; 95% CI 0.238-0.893; p = 0.022), BMI of ≥26.9 kg/m^2 (HR 0.196; 95% CI 0.043-0.896; p = 0.036), HBV-DNA level between 3.3 and 7.0 $\log_{10}$ IU/ml (HR 0.478; 95% CI 0.274-0.836; p = 0.010), HBsAg level of <5,000 IU/ml (HR 0.443; 95% CI 0.248-0.790; p = 0.006), HBeAg positivity (HR 0.402; 95% CI 0.176-0.918; p = 0.031), and LSM value of ≥13.0 kPa (HR 0.486; 95% CI 0.256-0.922; p = 0.027). Notably, ETV+BRC treatment reduced the incidence of HCC regardless of the cirrhosis status (Fig. 3). These results were consistent in the ITT and PP populations (Figs. S4 and S5).

In addition, multivariable Cox proportional regression analyses including the treatment allocation as a parameter were performed, which also demonstrated that ETV+BRC treatment was associated with a reduced incidence of HCC in the mITT (HR 0.405; 95% CI 0.230-0.710; p = 0.002), ITT (HR 0.400; 95% CI 0.229-0.697; p = 0.001) and PP (HR 0.410; 95% CI 0.233-0.722; p = 0.002) populations (Tables S5-S8).

On-treatment prognostic factors

Virologically, 15 (1.5%), 242 (41.5%), and 916 (91.6%) patients achieved HBsAg loss, HBeAg seroconversion, and VR at year 7 in the mITT population, respectively. There were no differences in the virological parameters between the 2 groups (p >0.05; Table 2). The VR at week 48 (VR48) was selected as an on-treatment prognostic factor. The analysis showed that the patients who had achieved VR48 had a lower likelihood of developing HCC than those who had not achieved VR48 in the ETV+BRC

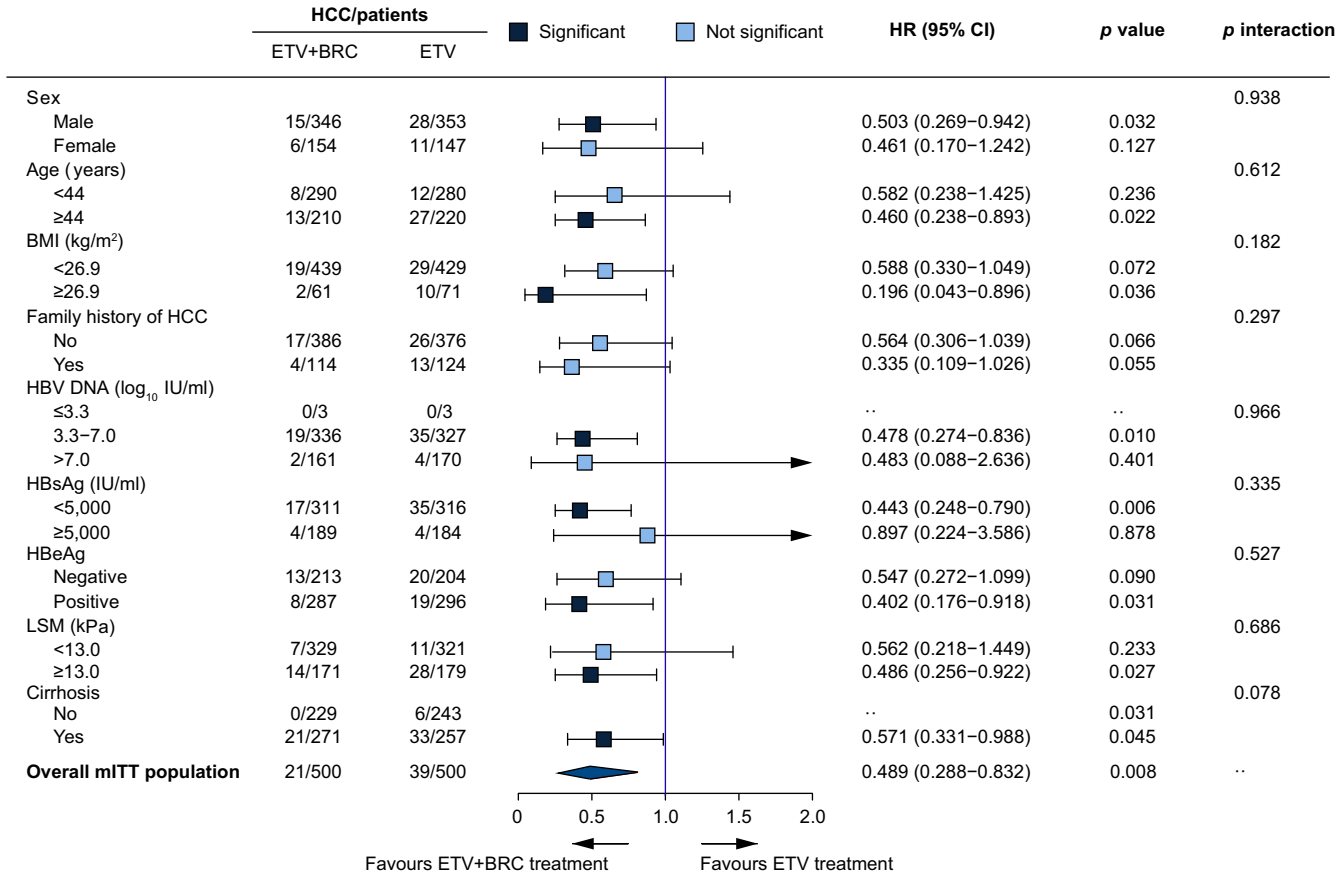

Fig. 3. Hazard ratios for the incidence of HCC by baseline prognostic factors in the mITT population. Each square represents the estimated HR, the horizontal lines represent the 95% CIs, and the diamond corresponds to the estimated HR and the 95% CI for the entire population. The *p* value is calculated using the Cox proportional regression or Fisher's exact test, if appropriate. The *p* value for interaction is analyzed by use of the Cochran-Mantel-Haenszel test. A two-tailed *p* value of <0.05 is considered statistically significant. BRC, Biejia-Ruangan compound; CI, confidence interval; ETV, entecavir; HCC, hepatocellular carcinoma; HR, hazard ratio; LSM, liver stiffness measurement; mITT, modified intention-to-treat.

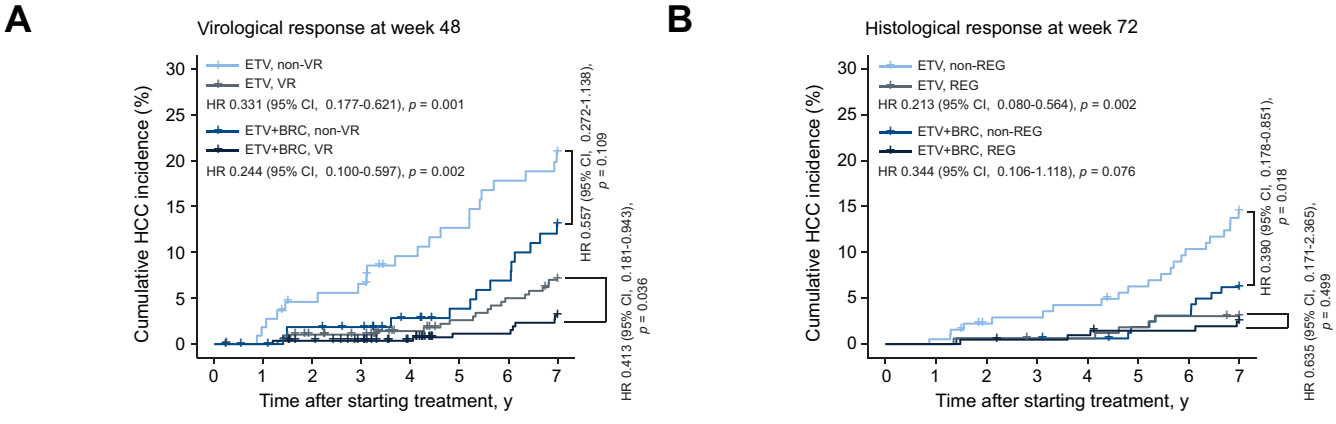

Fig. 4. Cumulative incidence of HCC by on-treatment prognostic factors in the mITT population. (A) Virological response at week 48 after randomization. (B) Histological response at week 72 after randomization. The HRs and *p* values are estimated with the use of the Cox proportional regression. A 2-tailed *p* value of <0.05 is considered statistically significant. BRC, Biejia-Ruangan compound; CI, confidence interval; ETV, entecavir; HCC, hepatocellular carcinoma; HR, hazard ratio; mITT, modified intention-to-treat; REG, regression of fibrosis; VR, virological response.

(HR 0.244; 95% CI 0.100-0.597; p = 0.002) and ETV group (HR 0.331; 95% CI 0.177-0.621; p = 0.001). Interestingly, compared with ETV treatment, ETV+BRC treatment had a greater effect on HCC risk in the patients who achieved VR48 (HR 0.413; 95% CI 0.181-0.943; p = 0.036) and a similar effect in those who did not achieve VR48 (HR 0.557; 95% CI 0.272-1.138; p = 0.109; Fig. 4A).

Histologically, 1,000, 727, and 426 patients underwent first (prior to treatment), second (at week 72), and third (at year 5) liver biopsies in the mITT population, respectively. The rate of REG in the ETV+BRC group was 55.9% at year 1.5 and 76.7% at year 5, which was significantly higher than that in the ETV group (46.2% and 61.3%, respectively, Fig. S6A). Representative liver biopsies from 2 patients are shown in Fig. S6B. REG at week 72 (REG72) was selected as another on-treatment prognostic factor. The cumulative incidence of HCC was significantly lower in the patients who had achieved REG72 than in those who had not after ETV treatment (HR 0.213; 95% CI 0.080-0.564; p = 0.002); whereas, the incidence was not significantly different between the patients who had and had not achieved REG72 after ETV+BRC treatment (HR 0.344; 95% CI 0.106-1.118; p = 0.076). In particular, although ETV+BRC treatment yielded a similar incidence of HCC in the patients who had achieved REG72 (HR 0.635; 95% CI 0.171-2.365; p = 0.499), it yielded a lower incidence of HCC (HR 0.390; 95% CI 0.178-0.851; p = 0.018) in those who did not achieve REG72 than ETV treatment. The results indicate that ETV+BRC treatment can lower the risk of HCC even in patients who have not achieved histological improvement (Fig. 4B). Such results were verified in the ITT and PP populations (Figs. S7 and S8).

Non-invasive measurements

Furthermore, the linear mixed model and multiple Bonferroni-corrected *post hoc* comparisons were performed to assess the trends in the non-invasive measurements over time in the mITT population. The LSM value significantly decreased in both groups during the 7-year treatment period (p <0.001), and the decline was much greater in the ETV+BRC group after 6 months (p <0.001;

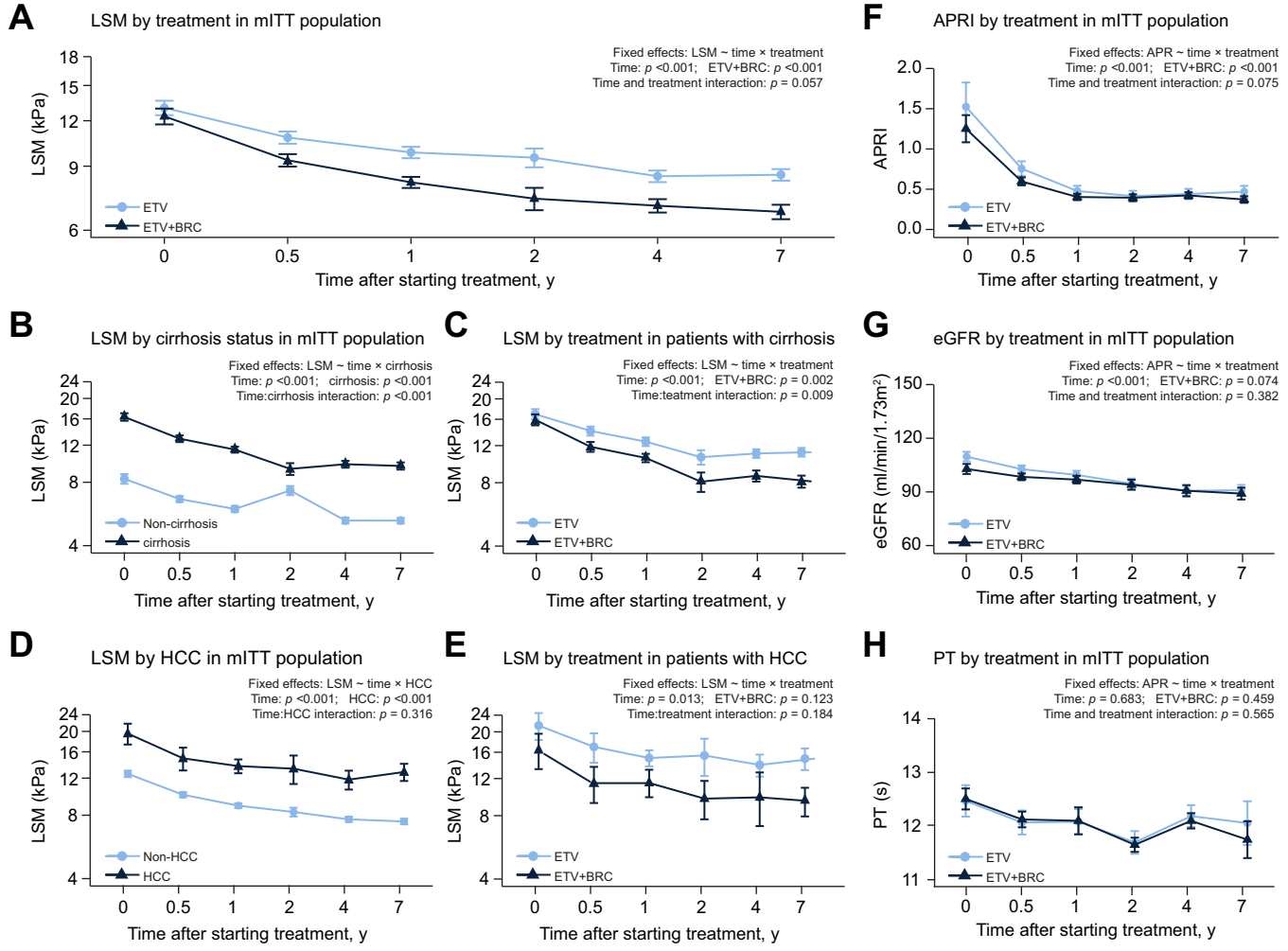

Fig. 5. Changes in LSM/APRI/eGFR/PT during the entire treatment in the mITT population. (A) LSM by treatment. (B) LSM by cirrhosis status. (C) LSM by treatment in patients with cirrhosis. (D) LSM by HCC. (E) LSM by treatment in patients with HCC. (F) APRI by treatment. (G) eGFR by treatment. (H) PT by treatment. Numbers of patients at each time-point were those with evaluable data, no imputation was performed for missing data. Solid boxes or triangles represent estimated marginal means and error bars represent standard error. The y-axis of LSM value is transformed as a log scale. The p values are analyzed by use of a linear mixed-effect model. A 2-tailed p value of <0.05 is considered statistically significant. APRI, AST-to-platelet ratio index; BRC, Biejia-Ruangan compound; eGFR, estimated glomerular filtration rate; ETV, entecavir; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; mITT, modified intention-to-treat; PT, prothrombin time.

Table 3. Adverse events and laboratory abnormalities reported in the mITT population.

	ETV+BRC	ETV	*p* value
n	500	500	–
Any AE	379 (75.8)	372 (74.4)	0.609
SAE	49 (9.8)	45 (9.0)	0.665
Discontinuation due to AEs	1 (0.2)	2 (0.4)	0.999
Most common AEs			
Upper respiratory tract infection	79 (15.8)	65 (13.0)	0.207
Pyrexia	65 (13.0)	52 (10.4)	0.201
Headache	61 (12.2)	60 (12.0)	0.923
Cough	56 (11.2)	57 (11.4)	0.920
Diarrhea	40 (8.0)	38 (7.6)	0.814
Laboratory abnormalities			
eGFR <50 ml/min/1.73 m^2	49 (9.8)	35 (7.0)	0.111
ALT flare[†]	19 (3.8)	16 (3.2)	0.606
ALB <25 g/L	0	0	–
PT prolong >3 s	0	0	–

Categorical variables are presented as numbers and percentages [n (%)]. The *p* value is investigated by chi-squared test or Fisher exact test if appropriate. A two-tailed *p* value of <0.05 is considered statistically significant.
AEs, adverse events; ALB, albumin; ALT, alanine aminotransferase; BRC, Biejia-Ruangan compound; eGFR, estimated glomerular filtration rate; ETV, entecavir; mITT, modified intention-to-treatment; PT, prothrombin time; SAEs, serious adverse events.
[†]Defined as ALT >2 × baseline and >10 × upper limit of normal.

Fig. 5A). The LSM value was consistently higher in the patients with baseline cirrhosis than in those without cirrhosis (p <0.001; Fig. 5B) and decreased more obviously after ETV+BRC treatment than after ETV treatment (p = 0.002; Fig. 5C). It was consistently higher in the patients who developed HCC than in those who did not (p <0.001; Fig. 5D) and showed no difference between the 2 treatment groups (p = 0.112; Fig. 5E). The APRI showed a similar declining trend to LSM (p <0.001; Fig. 5F). The eGFR also significantly declined during the 7-year period (p <0.001); however, there was no difference between the 2 groups (p = 0.074; Fig. 5G). The prothrombin time did not significantly change during the entire study period (p = 0.683), and no significant difference was observed between the 2 treatment groups (p = 0.459; Fig. 5H).

Safety

The spectrum of AEs observed throughout the study period was comparable between the 2 groups in the mITT population: 379 (75.8%) patients receiving ETV+BRC treatment and 372 (74.4%) patients receiving ETV treatment reported treatment-emergent AEs (p = 0.609). Most AEs (627/751, 83.5%) were classified by the investigators as unrelated or unlikely to be related to the study drug treatments (Table 3).

Discussion

To the best of our knowledge, this is the first large RCT-based prospective cohort study that included data from 3 liver biopsies, providing high-level evidence that BRC combination therapy could further reduce the incidence of HCC by 51.1% and liver-related deaths by 89.9% in Chinese patients with CHB receiving ETV treatment.

Previous studies have demonstrated that ETV-treated patients remain at a considerable risk of developing HCC despite long-term HBV suppression, with the annual incidence reaching up to 5.4%.[18] To data, knowledge on how to further reduce the risk of HCC when it is not possible to completely eliminate HBV (marked by HBsAg clearance) remains limited. Based on the fact that HBV infection and advanced liver fibrosis are the 2 most prominent risk factors for HCC, it can be inferred that the risk of HCC may be further reduced by inhibiting HBV replication with antiviral agents and simultaneously addressing liver fibrosis with anti-fibrotic drugs. The BRC has been shown to play an anti-fibrotic role in multiple pathogenic pathways of fibrogenesis. For example, it can reduce the expression of tissue inhibitor of metalloproteinase and type I and III procollagen, inhibit transforming growth factor-β/Smad-induced fibrogenesis, and suppress hepatic stellate cell proliferation.[19] Our results showed that the cumulative incidence of HCC after ETV+BRC treatment was significantly lower than that after ETV treatment, which is consistent with other retrospective studies.[20,21] Notably, owing to the regular follow-up, HCC was diagnosed on the basis of surveillance (early stage) in our study, which is more conducive to obtaining accurate time-to-event results than symptom-based diagnosis (advanced stage) or random check-up diagnosis (uncertain stage). In brief, our findings support the use of ETV+BRC combination treatment for patients with CHB and advanced liver fibrosis or cirrhosis, which may be beneficial in providing a more effective strategy to reduce the HCC risk.

It is also unclear which subgroup is more suitable for the combination therapy. Our *post hoc* subgroup analyses showed that ETV+BRC treatment had wide clinical applicability (HBV-DNA level between 3.3 and 7.0 $\log_{10}$ IU/ml, and HBsAg level of <5,000 IU/ml were the features of the current main treatment-targeting population), and could further reduce the incidence of HCC in specific subsets: males, older age (≥44 years), higher BMI (≥26.9 kg/m^2), HBeAg positivity, or higher LSM value (≥13.0 kPa). Interestingly, these factors have already been demonstrated to be associated with liver fibrosis progression or HCC development and have been used to establish HCC risk prediction models, such as the LSM-HCC,[22] modified Page-B,[23] CAMD,[24] and aMAP scores.[25] This result confirmed that the BRC can reduce the incidence of HCC by improving liver fibrosis. Nevertheless, owing to the absence of double-blind, randomized, placebo-controlled clinical trials, there is a lack of high-level evidence to support the clinical application of TCM.[19,20] Our study extended the treatment duration of the parent RCT and met such clinical needs, providing high-level evidence to support the application of TCM in clinical practice.

Furthermore, by evaluating the association between the on-treatment responses and incidence of HCC, we found that among the patients who did not achieve VR, ETV+BRC treatment could not further reduce the incidence of HCC, compared with ETV treatment. The results indicated that the BRC had no impact on the VR and could not resolve the problem of suboptimal viral suppression, which always led to the progression of the illness. A longitudinal study showed that detectable HBV-DNA at week 78 of ETV-based treatment was an independent risk factor for fibrosis progression (odds ratio 4.84; 95% CI 1.30-17.98; p = 0.019).[26] A retrospective cohort study showed that HCC developed more frequently in patients with low-level viremia (HBV-DNA <2,000 IU/ml) (HR 1.98; 95% CI 1.28-3.06; p = 0.002), especially in patients with cirrhosis (HR 2.20; 95% CI 1.34-3.60; p = 0.002).[27,28] Adjusting anti-HBV treatment may work better than continuing the original treatment.[29,30] Interestingly, ETV+BRC treatment could further lower the HCC risk in patients who had not achieved REG, indicating that at least 2 mechanisms might be involved in reducing the HCC risk with BRC treatment. One mechanism may be the improvement of liver fibrosis, and the other may be that the BRC can directly regulate the signaling

pathways involved in HCC development. Previous studies have shown that the BRC induces cell cycle arrest in patients with HCC by inhibiting PI3K/AKT/NF-κB activation,[31,32] and suppresses HCC growth by regulating the long non-coding RNA TUG1-microRNA-328-3p-SRSF9 mRNA axis.[33]

Another unclear issue is whether BRC combination treatment is safe enough for long-term use.[34,35] Concerning the repeated biochemical measurements, a significant decline in the eGFR was observed after long-term ETV treatment, which is consistent with a previous finding that each year of age was associated with a 0.86-unit decrease in eGFR in normal individuals and that ETV treatment resulted in an additional significant loss in the eGFR.[36] Actually, supplementation with the BRC did not exacerbate this decline. In addition, the coagulation function did not change significantly throughout the study period. The safety profiles were comparable between the 2 treatment groups, and the most commonly reported non-HCC malignancies were gastric and colorectal cancers, consistent with a previous finding.[37] These findings indicate that ETV+BRC combination treatment did not evidentially increase toxicity. Moreover, BRC has been used to treat liver fibrosis in China for over half a century, with seldom-reported treatment-related AEs, thus its safety and low toxicity have already been proven experimentally in long-term clinical practice.

Furthermore, as a promising non-invasive approach, LSM has been widely accepted for the evaluation of liver disease severity and prognosis,[38] However, there are still no recommendations or consensus regarding LSM cut-offs at which anti-fibrotic treatment is indicated. Hence, to further reduce the HCC risk, we proposed that among treatment-naïve patients with CHB, those with baseline cirrhosis (IFS = 5 or 6 points) or LSM value ≥13.0 kPa should receive ETV+BRC treatment with an individualized duration up to 5 years; those without cirrhosis (IFS = 3 or 4 points) or LSM value <13.0 kPa should receive ETV+BRC treatment for at least 72 weeks or until regression of fibrosis is achieved; those who cannot achieve VR should adjust anti-HBV treatment. For those with minimal fibrosis (IFS <3 points), further research is needed to address whether ETV+BRC treatment can reduce the risk of HCC. Additionally, the add-on strategy with anti-fibrotic agents for NA-experienced patients might still work even after liver fibrosis has progressed.

Although the findings of the present study have important clinical and research implications, 2 limitations should be noted. First, there were no patients of other races, which might limit the generalizability of this conclusion to broader populations. Further studies are required to confirm the results. Second, treatment was switched in 42 patients who experienced fibrosis progression, which seemed to break the random balance. However, as shown in Table 1 and Table S1, there was still no difference between the 2 treatment groups in the mITT, ITT and PP populations. Moreover, the time-to-event analyses from the above 3 populations were consistent, making the results more solid and the conclusion more convincing.

In conclusion, this 7-year cohort study derived from a multicenter RCT, provides strong evidence that ETV plus BRC combination treatment should be considered in treating CHB, owing to its high effectiveness (a lower HCC risk), extensive clinical applicability (eligible for most patients), and good tolerability (similar adverse events to ETV monotherapy and low toxicity).

Abbreviations

AEs, adverse events; AST, aspartate aminotransferase; BRC, Biejia-Ruangan compound; CHB, chronic hepatitis B; eGFR, estimated glomerular filtration rate; ETV, entecavir; HAI, histologic activity index; HCC, hepatocellular carcinoma; HR, hazard ratio; IFS, Ishak fibrosis score; ITT, intention-to-treat; LSM, liver stiffness measurement; mITT, modified intention-to-treat; NAs, nucleos(t)ide analogues; PP, per-protocol; PT, prothrombin time; RCT, randomized placebo-controlled trial; REG, regression of liver fibrosis; TCM, traditional Chinese medicine; VR, virological response.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors performed the study, provided the clinical data, and approved the manuscript. YY designed the study, revised the manuscript critically for important intellectual content, and supervised the study. DJ and JB did the statistical analyses. DJ wrote the manuscript. YY and YC were responsible for the execution of the study.

Data availability statement

All data relevant to the study are included in the article or provided as supplementary information. Additional supporting data are available from the corresponding author on request.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.07.018.

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Author names in bold designate shared co-first authors

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