


Virological breakthrough after immune checkpoint inhibitor and nucleos(t)ide analog treatment in patients with hepatitis B surface antigen positive hepatocellular carcinoma: a real-world study

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ABSTRACT

Background Immune checkpoint inhibitors (ICIs) have been shown to be a promising and effective treatment for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). However, there is a lack of evidence-based data demonstrating the impact of ICIs on HBV DNA level in HBV-HCC patients undergoing nucleos(t)ide analog (NA) therapy and of HBV DNA variation on patient survival. In this study, we aimed to investigate this issue in the real world.

Methods In this single-center retrospective study, we reviewed 182 baseline hepatitis B surface antigen (HBsAg)-positive HBV-HCC patients who were treated with ICIs and pre-emptive NAs. The demographic characteristics, tumor status, treatments, HBV DNA, HBsAg, liver function, antitumor response, and patient survival were investigated. The primary endpoints were the virological breakthrough (VB) rate, HBV reactivation (HBVr) rate, and long-term HBV DNA control; the secondary endpoints were the overall survival (OS) and progression-free survival (PFS).

Results (1) VB and HBVr occurred in 18.1% (33/182) and 4.4% (8/182) of patients with a median occurrence time of 3.9 months (range, 0.7–16.0) and 8.0 months (range, 3.0–16.0), respectively. The HBV DNA negative rates were 26.1% and 0 at 24 and 48 weeks in the VB group and 12.5% and 0 in the HBVr group, respectively. A baseline HBsAg level ≥ 200 IU/mL was the only risk factor for VB (OR 9.9, 95% CI 2.2 to 45.2, $p=0.003$); (2) patients with VB had much shorter median OS and median PFS than those without (12.3 months vs 18.1 months, $p=0.035$; 4.5 months vs 7.5 months, $p=0.011$).

Conclusions There was a high risk of VB and a moderate risk of HBVr in HBsAg-positive HBV-HCC patients (with poor long-term HBV DNA control) undergoing ICI and pre-emptive NA therapies. The only risk factor for VB was the pretreatment HBsAg level. Further, VB might be considered as a clinical biomarker predicting inferior OS and PFS in the patients.

BACKGROUND

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second-leading

cause of cancer-related death worldwide.¹ Of Chinese patients with HCC, 73.5% are at an intermediate to advanced stage at diagnosis (China Primary Liver Cancer Clinical Registry Survey), for which systemic therapy is the main treatment.^{2,3} Sorafenib and lenvatinib have been approved by the US Food and Drug Administration (FDA) for the first-line systemic treatment of HCC, and regorafenib, cabozantinib and ramucirumab have been approved as the second-line treatments. In recent years, immune checkpoint inhibitors (ICIs), such as anti-programmed cell death-1 (PD-1) antibody, anti-programmed cell death ligand-1 (PD-L1) antibody, and anti-cytotoxic T lymphocyte-associated antigen-4 antibody, have emerged as new systemic therapies for HCC after targeted drugs.⁴ Pembrolizumab (second line), nivolumab (second line), ipilimumab (second line combined with nivolumab) and atezolizumab (first line combined with bevacizumab) have been shown to be effective in previous studies and have been approved by the FDA.^{5–7} In addition, camrelizumab has been approved by the National Medical Products Administration of China for the second-line treatment of advanced HCC.⁸

HCC is mainly associated with hepatitis B virus (HBV) infection in China. However, the impact of ICIs on HBV remains unclear. Although previous studies have shown that blocking the PD-1/PD-L1 pathway restores anti-HBV T-cell responses and enhances the control of HBV,⁹ the impact of ICIs on HBV in patients with HBV-related HCC, as reported in previous studies, has been inconsistent. Apart from several case reports,^{10,11} no identified cases of HBV flare were reported

in Keynote-224 and Keynote-240 studies with pembrolizumab^{5,12}; further, no HBV reactivation (HBVr) cases were recorded in the Checkmate-040 study with nivolumab,⁶ and in the IMbrave-150 study with atezolizumab, no safety information about HBV DNA variation was mentioned.⁷ However, it is noteworthy that a 9% HBV virological breakthrough (VB) rate (defined as a 1 log increase in HBV DNA from baseline) and a 10% hepatitis C virus VB rate were reported in a phase I/II clinical trial with nivolumab plus ipilimumab.¹³ In a phase II clinical trial which used camrelizumab monotherapy, 25.5% of patients had increased HBV DNA;⁸ however, this was not mentioned in the report of the subsequent phase III study in combination with apatinib.¹⁴ Recently, two real-world studies from Asia reported HBVr rates of 3.6% and 1.7% in patients with HBV-HCC treated with ICIs.^{15,16}

Therefore, at present, there are no consistent conclusions on the impact of ICIs on HBV DNA level as studies use varying indicators and have found different results. Moreover, evidence-based data indicating the impact of HBV DNA variation on prognosis in ICI treated HBV-HCC patients is extremely rare. In this study, we aimed to investigate the variation in HBV DNA in hepatitis B surface antigen (HBsAg)-positive patients with HBV-HCC undergoing ICI and prophylactic NA therapy and the impact of HBV DNA variation on patient survival in the real world.

MATERIALS AND METHODS

Study design

In this retrospective study, 298 patients with HCC undergoing ICI treatment who were referred to Nanfang Hospital, Southern Medical University, Guangzhou, China between June 2018 and October 2020 were screened. The inclusion criteria were as follows: (1) baseline HBsAg positivity, diagnosis of HCC according to the American Association for the Study of Liver Diseases (AASLD) treatment guidelines of HCC; (2) received at least one cycle of ICI therapy at our center; (3) had HBV DNA, quantitative HBsAg, hepatitis B e antigen (HBeAg), and other relevant laboratory test results at our center within 2 months prior to the initiation of ICIs, and had a post-treatment HBV DNA test which was administered within 21 days after the last dose of ICIs. The exclusion criteria were as follows: (1) patients who were antihepatitis C antibody positive; (2) patients with combined HCC and intrahepatic cholangiocarcinoma; (3) patients with a concurrent second tumor; and (4) patients who had received any previous ICIs or who were undergoing steroid or interferon treatment. A total of 182 patients were included in this study. Sex, age, type of ICI, ICI duration, ICI cycles, combined therapies, baseline nucleos(t)ide analogs (NAs), stage of HCC, liver function, liver cirrhosis, HBV DNA, HBsAg, HBeAg, post-treatment HBV DNA and liver function, replacement of NAs, radiological imaging, and patient survival were reviewed. We followed up with all patients until June 1, 2021. The primary endpoints were the VB

rate, HBVr rate, and long-term HBV DNA control and the secondary endpoints were the overall survival (OS) and progression-free survival (PFS).

Patients and treatments

The patient characteristics and treatments are summarized in table 1. The patients in the study were predominantly male (87.9%, 160/182), and the median age was 50 years (range, 17–75). Most of the patients were at BCLC stage C (69.2%, 126/182) and had evidence of liver cirrhosis (65.4%, 119/182). The majority (64.8%, 118/182) were classified beyond ALBI grade 1. The median cycle and duration of ICIs were five cycles (range, 1–35) and 4.5 months (range, 0.7–24.7). A total of 89.0% (162/182) patients underwent combined therapies in which a targeted drug was the most common choice (80.8%, 147/182). The median baseline HBV DNA level was 150.5 IU/mL (range, 0–5,310,000) and 11.5% (21/182) of the patients were HBeAg-positive. A total of 98.9% (180/182) patients enrolled were naturally treated with preferred NAs¹⁷ before or at the initiation of ICIs, such as entecavir (ETV), tenofovir alafenamide fumarate (TAF), tenofovir disoproxil fumarate (TDF); the rest of the patients who had insufficient renal function (1.1%, 2/182) underwent telbivudine therapy. No patients discontinued NAs according to the records.

ICIs were prescribed according to the recommended dosage and safety information every 2–3 weeks. The ICIs involved in the study included ICIs prescribed by physicians in the real world that are approved for other tumor types, such as durvalumab, sintilimab, toripalimab, and tislelizumab, all of which have been shown to be efficacious in phase I/II clinical trials in HCC patients; phase III clinical trials are ongoing (NCT03951597, NCT02519348, NCT03794440, ChiCTR1900028295, NCT03412773). ICI therapy cycles were determined by the treating physicians based on the antitumor response, adverse effects, and personal will of the patient. All challenging cases were discussed by a multidisciplinary team for liver tumors at Nanfang Hospital, Southern Medical University.

Virological tests and antitumour response evaluation

Serum HBsAg levels were measured quantitatively using the ARCHITECT i2000SR platform (either with an upper limit of 250 IU/mL or an upper limit of 125 000 IU/mL), and HBV DNA levels were measured using plasma with the Roche (Basel, Switzerland) COBAS TaqMan HBV Test V.2.0, with a lower limit of 20 IU/mL and Light-Cycler 480 Instrument II system (Roche, Mannheim, Germany) with a lower limit of 100 IU/mL. Based on the definition of undetectable HBV DNA (<10 IU/mL) in the AASLD Guidelines for the treatment of chronic hepatitis B,¹⁷ we recorded ‘not targeted’ results for the COBAS assay as 0 IU/mL (assessed as undetectable in this study); results for <20 IU/mL as 10 IU/mL; and results for <100 IU/mL as 50 IU/mL; log values were measured separately. Serum HBV DNA levels were monitored every 3–9 weeks, while other laboratory tests were performed

Table 1 Baseline characteristics of all 182 HBsAg positive HBV-HCC patients treated with ICIs and pre-emptive NAs

Characteristic	N=182
Age, years	50 (17–75)
Sex, N (%)	
Male/female	160 (87.9)/22 (12.1)
Combined treatments with ICIs, N (%)	162 (89.0)
Surgery/ablation/radiation/transarterial intervention/targeted drugs	2 (1.1)/5 (2.7)/4 (2.2)/64 (35.2)/147 (80.8)
Type of targeted drugs, N (%)	
Sorafenib/lenvatinib/apatinib/regorafenib/bevacizumab	21 (11.5)/65 (35.7)/33 (18.1)/5 (2.7)/26 (14.3)
Type of ICIs, N (%)	
Camrelizumab/sintilimab/toripalimab/tislelizumab/nivolumab/pembrolizumab/durvalumab/atezolizumab	57 (31.3)/52 (28.6)/37 (20.3)/20 (11.0)/3 (1.6)/8 (4.4)/2 (1.1)/3 (1.6)
ICIs treatment cycles	5 (1–35)
ICIs treatment duration, months	4.5 (0.7–24.7)
Baseline NAs, N (%)	
ETV/TAF/TDF/LdT	146 (80.2)/14 (7.7)/20 (11.0)/2 (1.1)
BCLC stage, N (%)	
0/A/B/C	1 (0.5)/8 (4.4)/47 (25.8)/126 (69.2)
Liver cirrhosis, N (%)	119 (65.4)
HBsAg positive, N (%)	21 (11.5)
ALT, U/L	35.5 (8–302)
Albumin, g/L	37.3 (23.4–50.8)
Total bilirubin, mg/dL	15.3 (4.4–121.6)
Platelet count, K/cumm	142 (26–605)
AFP, ng/mL	250 (0.5–187 560)
ALBI grade, N (%)	
1/2/3	64 (35.2)/112 (61.5)/6 (3.3)
Baseline serum HBV DNA level, IU/mL	150.5 (0–5 310 000)
Baseline serum HBsAg level, IU/mL	
<200 IU/mL	53 (29.1)
≥200 IU/mL	129 (70.9)

Continuous variables are presented as median (range).

Normal range: ALT: 0–50 U/L (male), 0–40 U/L (female); albumin: 40.0–50.0 g/L; total bilirubin: 0–26.0 mg/dL; platelet count: 125–350 K/cumm; AFP: 0–7.0 ng/mL.

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; ETV, entecavir; ALBI grade, albumin-bilirubin grade; HBsAg, hepatitis B e antigen; HBV-HCC, hepatitis B virus-related hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; LDT, telbivudine; NAs, nucleos(t)ide analogs; BCLC stage, Barcelona Clinic Liver Cancer stage; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate.

on demand. Contrast-enhanced CT or contrast-enhanced MRI examination were administrated approximately every 6–8 weeks after enrolment, and tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors V.1.1. The time window for the adoption of the results was ± 2 weeks.

Definitions

The AASLD guidelines refer to varying criteria for VB and HBVr in patients under certain conditions, especially in those already undergoing NAs therapy.¹⁷ Given that most patients in this cohort were already on continuous

NAs therapy at baseline, and that natural resistance to preferred NAs is very rare,¹⁷ this study defined:

(1) A VB as a ≥ 1 log (10-fold) increase in serum HBV DNA compared with baseline or post-baseline nadir, or HBV DNA ≥ 100 IU/mL in a patient with previously undetectable levels. (2) an HBVr as a ≥ 2 log (100-fold) increase in serum HBV DNA compared with baseline or post-baseline nadir, or HBV DNA ≥ 1000 IU/mL in a patient with previously undetectable levels. (3) HBV DNA negative if the serum HBV DNA level was below the lower limit of the local laboratory assay. (4) The ICI duration was

conservatively defined as the period from the initiation of ICIs to 21 days after the last dose, as 21 days is the dosing interval for most ICIs. The duration in this study refers to the exposure to first-line ICIs and does not include the duration after changing to a second ICI. (5) Time to VB or time to HBVr was defined as the time from the initiation of ICIs to the first occurrence of VB or HBVr within the ICI duration. (6) The ALBI score, liver cirrhosis status, and Barcelona Clinic Liver Cancer (BCLC) staging system were based on previous reports.^{18,19} (7) A risk of <1% is considered low, a risk of 1%–10% is moderate, a risk of 11%–20% is high, and a risk of >20% is very high.²⁰ (8) OS and PFS were calculated from the date of first dose of ICIs until disease progression or death or censored at the date of last follow-up. (9) Objective response rate (ORR) includes complete response (CR) and partial response (PR) rates; disease control rate (DCR) includes CR, PR, and stable disease (SD) rates.

Statistical analysis

The χ^2 test, Fisher's exact test, Student's t-test, or Mann-Whitney test were used to compare differences between categorical or continuous variables, as appropriate. Logistic regression and Cox regression were employed to determine the potential predictors of a VB and OS, respectively. OS and PFS curves were analyzed via the Kaplan-Meier method and compared using the log-rank test. A two-tailed $p < 0.05$ was considered statistically significant for all analyses. All statistical analyses were performed using the Statistical Package for Social Sciences V.25.0 (IBM).

Patient and public involvement

Neither patients nor the public were involved in the research.

RESULTS

Viral kinetics in ICIs duration

In the ICIs duration, a total of 18.1% (33/182) patients underwent VB and 4.4% (8/182) patients underwent HBVr with a median occurrence time of 3.9 months (range, 0.7–16.0) and 8.0 months (range, 3.0–16.0), respectively (table 2). The median HBV DNA levels were 669 IU/mL (range, 108–72,200) and 5690 IU/mL (range, 1050–45,100) at the time of VB and HBVr, respectively.

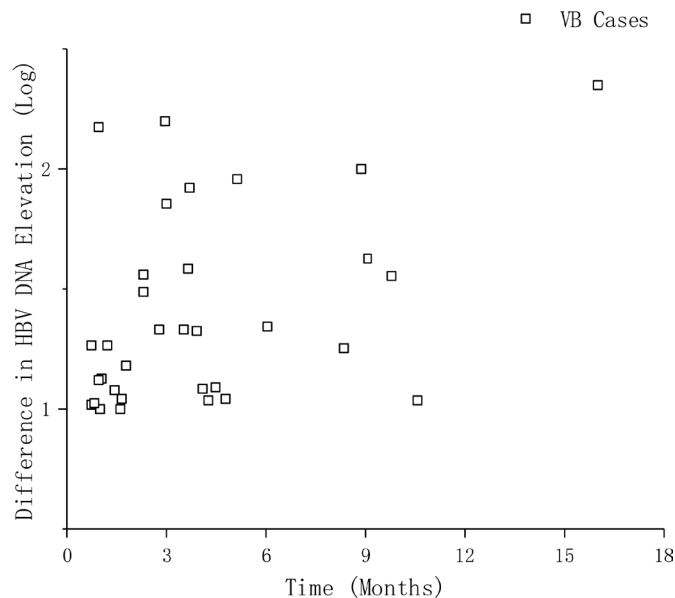


Figure 1 Differences in HBV DNA elevation and time to occur in 33 VB patients. The difference in HBV DNA elevation (measured by log values) was between the nadir and VB in each patient. HBV, hepatitis B virus; VB, virological breakthrough.

The variations in the HBV DNA level and time to occur in the VB and HBVr groups are shown in figures 1 and 2 and the details of the eight HBVr patients are listed in online supplemental table 1. In patients with VB and HBVr, the HBV DNA level either elevated suddenly after a period of ICI treatment and fluctuated afterwards or fluctuated repeatedly since the beginning of ICI treatment (figure 3). In the VB group (n=33), a total of 23 patients had records of HBV DNA at 24 weeks, and the HBV DNA negative rate was 26.1% (6/23). Moreover, six patients had records of HBV DNA at 48 weeks with an HBV DNA negative rate of 0. Analogously, HBV DNA negative rates were 12.5% (1/8) and 0 (0/4) at 24 and 48 weeks in the HBVr group, respectively (table 2). In addition, no disruption of ICIs due to HBV DNA elevation was observed.

All patients were classified into the VB and VB-free groups, and baseline factors were analyzed. Age ($p=0.012$), albumin level ($p=0.034$), ALBI grade ($p=0.033$), and the HBsAg level ≥ 200 IU/mL ($p=0.001$) were significant

Table 2 Primary endpoints

	N (%)	Time to occur, months	HBV DNA negative rate at 24 weeks*	P value	HBV DNA negative rate at 48 weeks*	P value
VB-free	149 (81.9)		69.6		70.5	
VB	33 (18.1)	3.9 (0.7–16.0)	26.1	<0.001	0	<0.001
HBVr	8 (4.4)	8.0 (3.0–16.0)	12.5	<0.001	0	0.002

Continuous variables are presented as median (range).

*The HBV DNA negative rate for available cases.

HBVr, hepatitis B virus reactivation; VB, virological breakthrough.

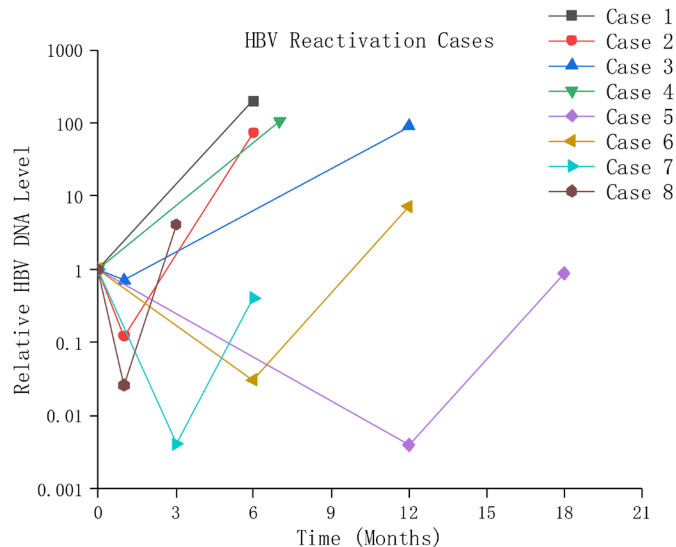


Figure 2 HBV DNA variations in eight HBVr patients. The relative HBV DNA levels at baseline, post-baseline nadir, and HBVr are displayed in the figure; for cases 1 and 4, the baseline HBV DNA levels were their nadirs. HBVr, hepatitis B virus reactivation.

risk factors for VB in the single factor analysis (online supplemental table 2), whereas the multifactor analysis indicated that an HBsAg level ≥ 200 IU/mL was the only risk factor for VB (OR 9.9, 95% CI, 2.2 to 45.2, $p=0.003$) (online supplemental table 3).

Switch of NAs and outcomes in ICIs duration

Among the 60 patients whose HBV DNA control was deemed unsatisfactory (ie, HBV DNA elevation or continuous HBV DNA positivity after NA therapy) by the treating physicians, 26.7% (16/60) of them replaced NAs, and only three experienced a decrease in HBV DNA (defined as a 1 log decrease) after replacement. Of the 44 patients who did not switch NAs, decreased HBV DNA was observed in nine patients. However, HBV DNA fluctuations may have reappeared under both conditions (online supplemental figure 1). The most common strategy for switching was replacing ETV with TAF in this cohort, and the remaining patients usually switched from ETV to TDF or ETV plus TDF.

Impact of VB on liver function

The comparison of main laboratory parameters between baseline and VB in the VB patients is shown in online supplemental table 4. When VB occurred, three of them (9.1%) were assessed as Child-Pugh C liver function. Significantly elevated ALT (greater than twice the upper limit of normal) occurred in only two cases (6.1%). One of which may have been associated with rapid tumor progression during the same period, and the other was diagnosed with immune-related hepatitis and pneumonia with a concurrent HBV DNA level of 1530 IU/mL. Neither of these two patients could be definitively diagnosed with a hepatitis flare due to HBV activity. Detailed data regarding the liver function of the eight HBVr

patients is shown in online supplemental table 1. Additionally, two fatal cases of immune-related hepatitis with multi-organ functional disturbances were observed in the entire cohort.

Impact of VB on antitumor response

A total of 157 patients out of the entire cohort who had both baseline and at least one postbaseline radiological imaging at our hospital were analyzed as a subgroup (baseline characteristics are listed in online supplemental table 5), and 31 patients among them experienced VB in ICIs duration. None of the VB patients were evaluated as CR, 6.5% (2/31) of them presented with PR and 61.3% (19/31) of them had SD. In the VB-free patients, 0.8% (1/126) of them was evaluated as CR, 16.7% (21/126) of them presented with PR and 59.5% (75/126) had SD. There was a trend of inferior antitumor response with lower ORR and DCR in patients with VB compared with those without (6.5% vs 17.5%, $p=0.168$; 67.7% vs 77.0%, $p=0.353$) (online supplemental figure 2).

Additionally, the intercomparison of radiological imaging before and after VB or HBVr showed that 50.0% of the VB and 100.0% of the HBVr patients (with available radiological imagings) were assessed as PD when VB and HBVr occurred, respectively. Radiological imaging of three HBVr patients shows this phenomenon in online supplemental figure 3.

Impact of VB on patient OS and PFS

In the subgroup, the patients with VB had significantly shorter median OS and median PFS than those without (12.3 months vs 18.1 months, $p=0.035$; 4.5 months vs 7.5 months, $p=0.011$) (table 3, figure 4), a single-factor analysis showed female ($p=0.016$), AFP >400 ng/mL ($p=0.005$), HBV DNA >500 IU/mL ($p=0.015$), VB ($p=0.035$), and albumin <35 g/L ($p=0.010$) were risk factors for OS and a multi-factor analysis indicated that VB was an independent risk factor (OR 1.875, 95% CI 1.134 to 3.100, $p=0.014$) (online supplemental table 6). Furthermore, the patients with 24 weeks HBV DNA positivity had significantly shorter median OS and median PFS than those without (11.5 months vs not reached, $p<0.001$; 6.3 months vs 16.7 months, $p<0.001$) (table 3, figure 4).

DISCUSSION

To the best of our knowledge, this is a relatively large study that has used a real-world cohort to investigate HBV DNA variation in ICI-treated HBV-HCC patients. All patients were baseline HBsAg positive according to the quantitative assay and 98.9% of them were given the preferred NAs. In this study, VB was primarily investigated because (1) not only HBVr, but rather all types of elevation in HBV DNA are noteworthy in HBV-HCC patients, and (2) because HBsAg-positive patients are generally at a high risk of HBVr if their HBV DNA level starts to increase.^{21 22}

Currently, it is recognized that in HBV-infected patients with tumors, HBVr is associated with immunosuppressive

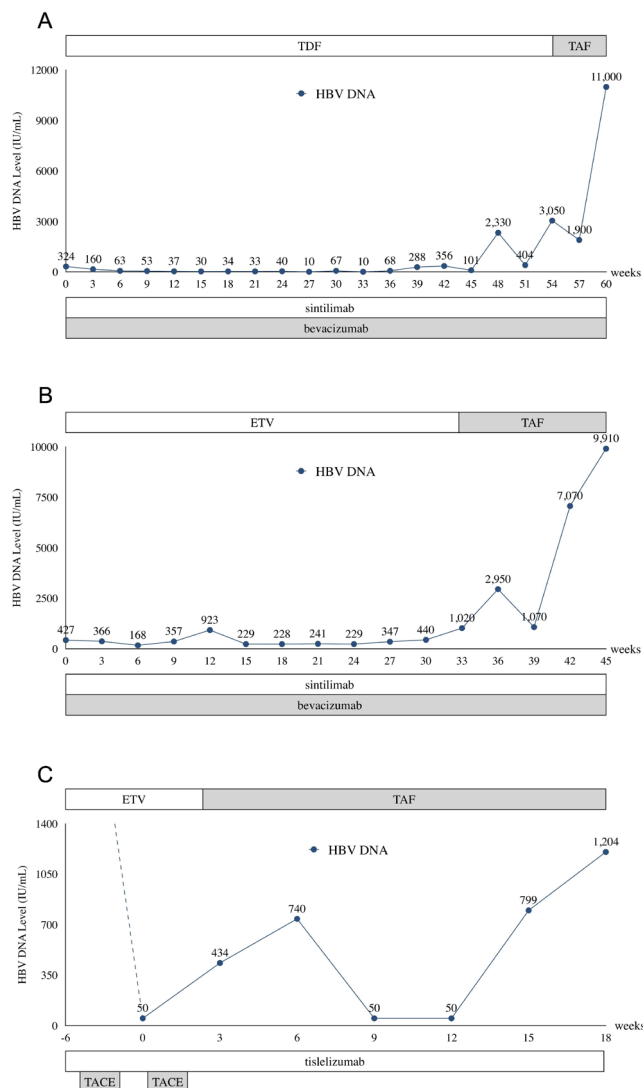


Figure 3 Details of HBV DNA variations in three VB or HBVr cases. Case (A) was a patient diagnosed with HBV-HCC, BCLC stage C, with baseline liver cirrhosis, HBsAg >250 IU/mL, HBeAg negative, ALT 49 IU/L, HBV DNA 324 IU/mL. The patient received no previous treatment with NAs and received 22 cycles of sintilimab plus bevacizumab every 3 weeks, without locoregional treatment, which was started simultaneously. The HBV DNA level decreased to a minimum of <20 IU/mL at 27 weeks, but increased to 288 IU/mL at 39 weeks, assessed as VB, and to 2330 IU/mL at 48 weeks, assessed as HBVr. At the first instance of HBVr, the patient did not receive NAs replacement. However, the HBV DNA level decreased spontaneously at 51 weeks and fluctuated thereafter. TAF was used as a substitute for TDF at 54 weeks, though finally the HBV DNA at 60 weeks increased to 11 000 IU/mL. Throughout the follow-up period, the patient had a maximum ALT level of 67 IU/mL. Case (B) was a patient diagnosed with HBV-HCC with combined bone and pulmonary metastases that were treated with sintilimab plus bevacizumab every 3 weeks for a total of 16 cycles, during which external radiation therapy was administered to the bone metastases with no other combined locoregional therapy. This patient had a baseline HBsAg level >250 IU/mL, was HBeAg negative, had no liver cirrhosis, no prior NA therapy, a baseline HBV DNA level of 427 IU/mL, and the patient started ETV therapy at the initiation of sintilimab, though no significant decrease in the serum HBV DNA level was observed; TAF was used as a substitute for ETV at 33 weeks, but at 36 weeks the HBV DNA increased to 2950 IU/mL, assessed as VB, and elevated rapidly afterwards. The serum ALT level of the patient was below 1 ULN throughout the follow-up period. Case (C) was a patient diagnosed with HBV-HCC, BCLC stage C, and a ruptured tumor with bleeding before baseline. The patient first received transarterial chemoembolization (TACE) in and concurrently started ETV therapy with a good initial response to ETV before enrolment (HBV DNA decreased from 1 870 000 IU/mL to <100 IU/mL in 6 weeks). The patient, with a baseline HBsAg level of 34.9 IU/mL (HBeAg-positive), was treated with seven cycles of tislelizumab and a second round of TACE. The HBV DNA level fluctuated repeatedly at low levels since the initiation of the ICI, even when the ETV was changed to TAF. VB occurred at six weeks. Throughout the follow-up period, except for transient elevation caused by TACE treatment, the serum ALT level of the patient was less than 2 ULN. ALT, alanine aminotransferase; BCLC stage, Barcelona Clinic Liver Cancer stage; ETV, entecavir; HBV-HCC, hepatitis B virus-related hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBVr, hepatitis B virus reactivation; NAs, nucleos(t)ide analogs; TACE, transarterial chemoembolization; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal; VB, virological breakthrough.

Table 3 Comparison of median OS and median PFS between VB and VB-free patients in a subgroup and that between 24 weeks HBV DNA negative and positive patients in a subgroup

	VB-free, N=126	VB, N=31	P value	HBV DNA negative at 24 weeks*, N=62	HBV DNA positive at 24 weeks*, N=29	P value
Median OS, months (95% CI)	18.1 (13.5 to 22.8)	12.3 (7.8 to 16.8)	0.035	Not reached	11.5 (9.5 to 13.5)	<0.001
Median PFS, months (95% CI)	7.5 (5.5 to 9.6)	4.5 (3.4 to 5.7)	0.011	16.7 (7.7 to 25.7)	6.3 (3.3 to 9.3)	<0.001

*For available cases.

HBV, hepatitis B virus; OS, overall survival; PFS, progression-free survival; VB, virological breakthrough.

therapy and cytotoxic therapy.¹⁷ A meta-analysis showed that the risk of HBVr in HBV-infected patients with solid tumors is 4%–68% in the absence of anti-HBV prophylaxis, indicating that most cases of HBVr are caused by chemotherapy (cyclophosphamide in particular).^{23 24} An early randomized controlled clinical trial demonstrated that pre-emptive anti-HBV therapy with lamivudine would significantly reduce the risk of HBVr from 29.7% to 2.8% in HBV-HCC patients undergoing transarterial

intervention therapy.²⁵ Further, with regard to targeted drugs, most HBVr cases are caused by anti-CD 20 monoclonal antibody; HBVr cases caused by erlotinib have also been reported; and a previous study showed that the viral reactivation rate was 0 in HBV-HCC patients undergoing sorafenib and NA therapy.^{24 26–29} However, in terms of ICIs, only approximately 10 cases of HBVr had been reported in various cancers until 2020.²⁰ A cohort of 114 patients with various cancers was retrospectively studied

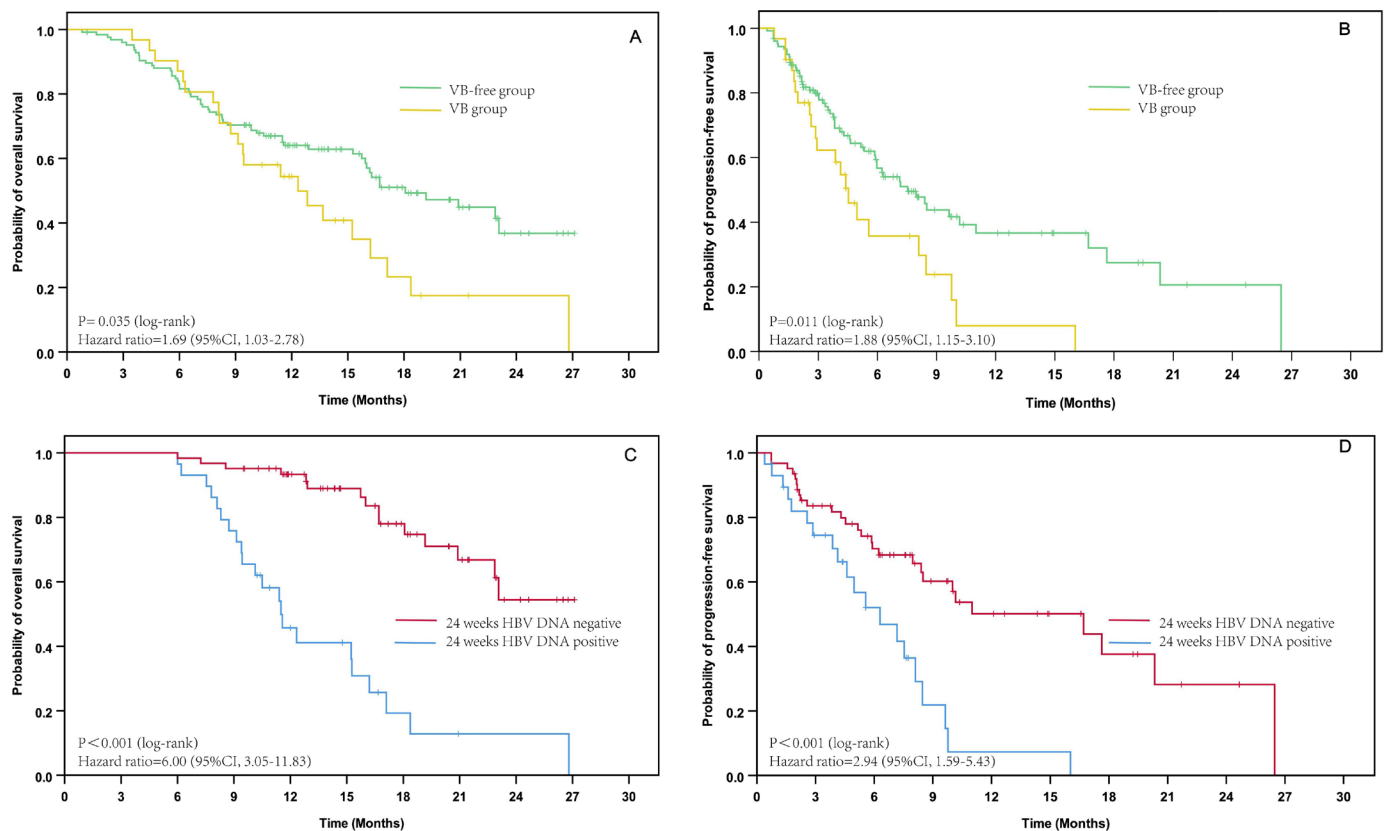


Figure 4 Kaplan-Meier curves for OS and PFS in VB and VB-free patients in a subgroup. Kaplan-Meier curves for OS and PFS in 24 weeks HBV DNA positive and negative patients in a subgroup. (A) OS between VB and VB-free patients. Patients with VB had a shorter median OS than those without (12.3 months vs 18.1 months, $p=0.035$). (B) PFS between VB and VB-free patients. Patients with VB had a shorter median PFS than those without (4.5 months vs 7.5 months, $p=0.011$). (C) OS between 24 weeks HBV DNA positive and negative patients. Patients with 24 weeks HBV DNA positivity had a shorter median OS than those with 24 weeks HBV DNA negativity (11.5 months vs not reached, $p<0.001$). (D) PFS between 24 weeks HBV DNA positive and negative patients. Patients with 24 weeks HBV DNA positivity had a shorter median PFS than those with 24 weeks HBV DNA negativity (6.3 months vs 16.7 months, $p<0.001$). OS, overall survival; PFS, progression-free survival; VB, virological breakthrough.

by Chinese scholars in 2019, and a total HBVr risk of 5.3% (6/114) was reported. Five of the six HBVr patients had other tumor types and did not receive prophylactic anti-HBV therapy; the other one HBVr patient with HBV-HCC (28 patients enrolled) was treated with nivolumab and prophylactic ETV.¹⁶ In 2020, another retrospective study from Asia revealed that the risk of HBVr was 1.7% (1/60) in patients with HBV-HCC undergoing ICI therapy, and one patient who underwent HBVr was treated with nivolumab without pre-emptive anti-HBV treatment.¹⁵ However, previous studies have usually included a non-antiviral population. The outcomes of this study revealed that there remains a high risk of VB (18.1%) and a moderate risk of HBVr (4.4%) in ICI-treated HBV-HCC patients, even when pre-emptive NAs were used. Furthermore, long-term HBV DNA control was investigated for the first time in this specific population. Our study indicated that the long-term HBV DNA negative rates seemed to be poor in VB or HBVr groups compared with those in the VB-free group, in which the rates were likely consistent with the previous data of HBV-infected patients treated with preferred NAs (64%–67%).^{30 31}

In terms of the risk factors of HBV DNA elevation, a previous study indicated that the lack of prophylactic anti-HBV therapy was the only risk factor for HBVr in patients undergoing ICI therapy.¹⁶ The HBV DNA level has also been reported to be the most important risk factor for chemotherapy-induced HBVr in patients positive for HBsAg undergoing autologous hematopoietic cell transplantation.³² However, in HBV-HCC patients, relatively few HBVr cases in ICIs duration have been reported in former studies, and quantitative baseline HBsAg was not analyzed; thus, further research on risk factors is still needed. In this study, for the first time, the baseline HBsAg level was found to be the only risk factor for VB with a cut-off value of 200 IU/mL, and the risk was nearly nine times higher in patients with a baseline HBsAg level ≥ 200 IU/mL than in those with a HBsAg level < 200 IU/mL (24.0% vs 3.8%; OR 9.9; $p=0.003$). Thus, this might provide further evidence for the alert of patients at high-risk of HBV DNA elevation before initiation of ICIs. In early clinical trials with ICIs, a baseline HBV-DNA level < 100 IU/mL was required, and in more recent trials, the cut-off value was raised to < 500 IU/mL. In addition, the protocols of several trials do not require regular HBV DNA assays after enrolment, likely because (1) patients undergo NA treatment after enrolment; (2) the low HBVr rates reported in the past; and (3) researchers have confidence in NAs. However, the outcome of this study does not support either of these practices, and instead suggests that the baseline HBV DNA level is not likely to be related to HBV DNA elevation during ICI therapy if NA therapy is administered and that regular monitoring of HBV DNA is necessary.

There does not seem to be any evidence supporting the idea that ICIs could directly interfere with NAs, therefore, we speculate that VB and HBVr may be indirect signals of the host immune system response to ICIs.

Although previous studies have shown that blockade of the PD-1/PD-L1 pathway restores the function of tumor-specific T-cells and virus-specific T-cells and thus aids antiviral therapy,^{33–35} a recent study has also shown that serum HBsAg is related to inhibitory receptor expression and that checkpoint blockade with anti-PD-1 antibody only improves HBV-specific CD4⁺T cell function in patients with low serum HBsAg levels.³⁶ Since the special immune regulatory functions of the liver are mediated by the local expression of inhibitory receptors, including PD-1, which help to prevent overwhelming hepatocyte damage, another hypothesis is that the blockade of the PD-1/PD-L1 pathway may break the previous balance between immunity and tolerance, causing hepatocyte damage and releasing latent virus under certain conditions.^{16 37} Notably, the serum HBsAg level is an indicator that partially reflects the covalently closed circular DNA inside hepatocytes and the intrahepatic HBV DNA level.³⁸ However, the mechanism of VB or HBVr in ICIs duration is still unclear, and if VB or HBVr should be assessed as an immune-related adverse event (irAE) requires further discussion.

In this study, physicians empirically switched NAs in 26.7% (16/60) of patients with unsatisfactory HBV DNA control. One to two strategies for switching in case of virological failure during preferred NA therapy are recommended in the AASLD guidelines, but the level of recommendation is very low¹⁷ because of the similarly high effectiveness and low-drug resistance rates of preferred NAs; however, there are no evidence-based switching strategies for ICI-treated patients to date. Observations from this cohort showed that switching of NAs may not effectively reduce HBV DNA levels long term, whereas some of the patients who did not switch could have declining HBV DNA spontaneously. This suggests that the effect of replacement of NAs may not be satisfactory under these conditions, if the preferred NA is already used.

In the current study, there was a moderate risk (6.1%) of significant ALT elevation accompanied by VB. This might be attributed to the relatively low HBV DNA level at the time of VB or HBVr (the median HBV DNA levels were 669 IU/mL and 5690 IU/mL, respectively), which was likely suppressed by NAs as well as the empirical use of drugs that reduce serum ALT during anti-HCC therapy.

In terms of prediction of ICIs efficacy, previous studies suggested that tumor mutational burden-high (TMB-H) and microsatellite instability-high (MSI-H) may be valuable,^{39–41} however, two former studies revealed that only 0.8% (6/755) of HCC specimens presented as TMB-H and 0 of 122 HCC patients showed a MSI-H phenotype, respectively.^{42 43} Additionally, the PD-L1 expression has been reported to be associated with ICI efficacy in various cancers,^{44–46} however, the predictive value of PD-L1 expression in HCC patients has not been clearly shown in previous clinical trials.^{5–7} The value and feasibility of PD-L1 status test remains unclear in clinical practice of HCC patients with some limitations including general requirement of tissue samples, extra charge, and unclear

predictive role in particular. To date, the examinations of the biomarkers mentioned above have not been recommended to HCC patients prior to ICI initiation by the guidelines.²⁴ Therefore, clinical biomarkers such as irAEs were investigated instead. A cohort of 101 HBV-HCC patients demonstrated that patients with irAEs such as rash had a much prolonged median PFS and better ORR and DCR than those without.⁴⁷ In this study, for the first time, we found that VB may be related to OS and PFS in this population. Notably, unlike the prognostic role of irAEs reported in HCC patients,^{47,48} VB was an indicator of poor outcome. The median occurrence time of VB was 3.9 months, which is close to the second time of evaluation of antitumor response in HCC patients treated with ICIs (commonly at a 6–8 weeks interval). Thus, the onset of VB might help predict a less beneficial subgroup at an early stage. Nonetheless, the patients with VB may still benefit from ICIs and the relationship between HBV DNA variation and ICI efficacy still needs further research.

Furthermore, we collected a population of 17 HCC patients with HBsAg-negativity and hepatitis B core antibody-positivity at the time of enrolment, of which VB was observed in two patients undergoing ICIs and ETV therapy (one of which was HBV DNA positive at baseline); however, no cases of HBVr were observed.

This study has several limitations. First, this was a single-center retrospective cohort study that was conducted in Asia. Second, to avoid the impact of changing ICIs on the multifactor analysis, we did not record VB or HBVr that occurred during treatment with secondary ICIs (although more endpoints might have been observed). Third, most patients did not undergo a regular post-treatment HBsAg assay, which enabled us to assess the impact of ICIs on HBsAg. Forth, in our clinical practice, a biopsy was not commonly applied to the patients who can be clinically diagnosed with HCC and a PD-L1 status test was not a routine according to the current guidelines, this and the retrospective nature of this study resulted in our inability to obtain tissues or blood samples for investigational testing. Only 6.6% (12/182) of the patients in our cohort had undergone a PD-L1 status test and none of them presented with positivity, thus the relationship between PD-L1 expression and VB and prognosis could not be analyzed.

In conclusion, in the real world, when HBsAg-positive patients with HBV-HCC receiving preferred NAs were treated with ICIs, they remained at a moderate risk of HBVr and at a high risk of VB with poor long-term HBV DNA control. Second, only the pretreatment HBsAg level with a cut-off value of 200 IU/mL was found to be a risk factor for VB. Third, VB might be considered as a clinical predictive biomarker of inferior OS and PFS in this population.

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