

## HEPATOBIILIARY

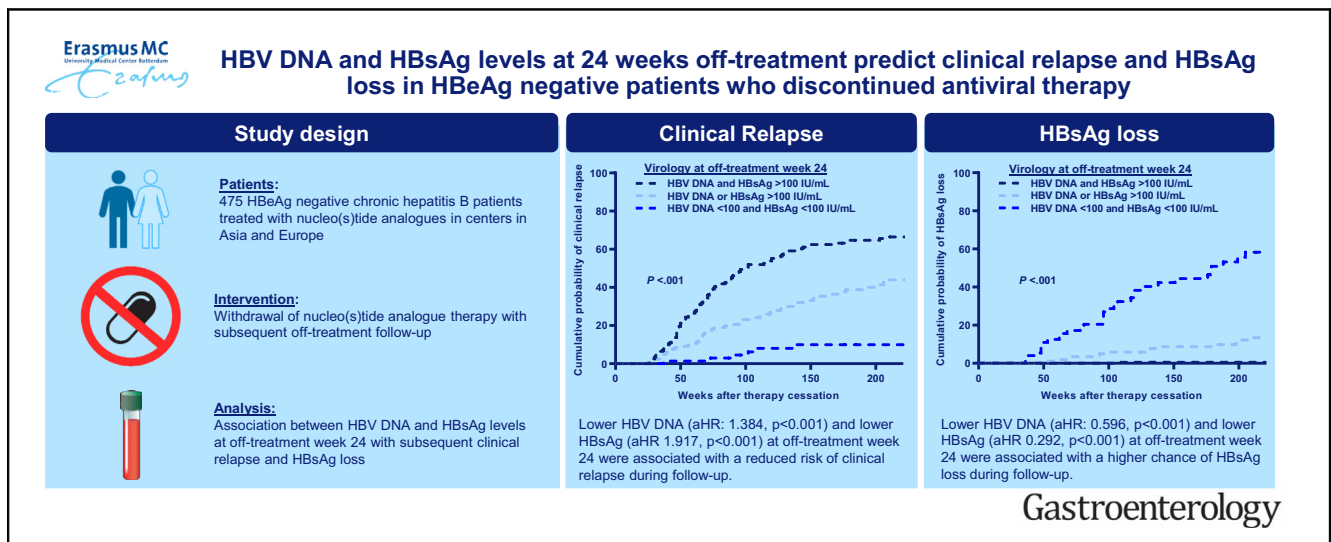
## HBV DNA and HBsAg Levels at 24 Weeks Off-Treatment Predict Clinical Relapse and HBsAg Loss in HBeAg-Negative Patients Who Discontinued Antiviral Therapy



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**BACKGROUND & AIMS:** Patients who discontinue nucleo(s)lide analogue therapy are at risk of viral rebound and severe hepatitis flares, necessitating intensive off-treatment follow-up. **METHODS:** We studied the association between hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA levels

at off-treatment follow-up week 24 (FU W24), with subsequent clinical relapse, and HBsAg loss in a multicenter cohort of hepatitis B e antigen (HBeAg)-negative patients with chronic hepatitis B who discontinued nucleo(s)lide analogue therapy. **RESULTS:** We studied 475 patients, 82% Asian, and 55% treated with entecavir. Patients with higher HBV DNA levels at FU W24 had a higher risk of clinical relapse (hazard ratio [HR], 1.576;  $P < .001$ ) and a lower chance of HBsAg loss (HR, 0.454;  $P < .001$ ). Similarly, patients with higher HBsAg levels at FU

W24 had a higher risk of clinical relapse (HR, 1.579;  $P < .001$ ) and a lower chance of HBsAg loss (HR, 0.263;  $P < .001$ ). A combination of both HBsAg  $<100$  IU/mL and HBV DNA  $<100$  IU/mL at FU W24 identified patients with excellent outcomes (9.9% clinical relapse and 58% HBsAg loss at 216 weeks of follow-up). Conversely, relapse rates were high and HBsAg loss rates negligible among patients with both HBsAg  $>100$  IU/mL and HBV DNA  $>100$  IU/mL ( $P < .001$ ). **CONCLUSIONS:** Among HBeAg-negative patients with chronic hepatitis B who discontinued antiviral therapy and who did not experience clinical relapse before FU W24, serum levels of HBV DNA and HBsAg at FU W24 can be used to predict subsequent clinical relapse and HBsAg clearance. A combination of HBsAg  $<100$  IU/mL with HBV DNA  $<100$  IU/mL identifies patients with a low risk of relapse and excellent chances of HBsAg loss and could potentially be used as an early surrogate end point for studies aiming at finite therapy in HBV.

**Keywords:** HBsAg; HBV DNA; Clinical Relapse; HBsAg Loss.

**D**iscontinuation of nucleo(s)tide analogue (NUC) therapy in patients positive for hepatitis B surface antigen (HBsAg) has recently been shown to be feasible and to result in sustained remission and even HBsAg loss in a subset of patients.<sup>1,2</sup> Caucasian ethnicity and low end-of-treatment viral antigen levels have been identified as important predictors of favorable outcomes after therapy cessation.<sup>2,3</sup> Hepatitis B virus (HBV) genotype C may also be associated with a higher chance of HBsAg clearance after treatment cessation among Asian patients, whereas no definitive data are available on the influence of HBV genotype on outcomes in non-Asians.<sup>3</sup>

Unfortunately, even patients with multiple favorable prognostic indicators remain at risk of relapse and hepatitis flares after therapy withdrawal. Previous studies have shown that whereas reappearance of HBV DNA is observed in most patients after therapy cessation, the timing and severity of viral rebound may vary significantly, necessitating prolonged and intensive follow-up in all patients who discontinue therapy.<sup>4</sup> Recent studies indicate that off-treatment viral antigen kinetics may correlate poorly with HBV DNA kinetics and may therefore offer important additional information over HBV DNA monitoring alone.<sup>5,6</sup> We hypothesized, based on experienced gained with finite interferon-based therapies, where remission at 6 months after therapy withdrawal is associated with excellent long-term outcomes<sup>7,8</sup> and the observation that the highest risk of virologic relapse occurs during the first months after NUC withdrawal,<sup>2,9</sup> that serum levels of HBV DNA and HBsAg at 6 months after therapy withdrawal could be used to predict subsequent relapse risk and HBsAg loss.

## Patients and Methods

### Patients

The current study used data from a pooled data set comprising patients with chronic hepatitis B who discontinued NUC therapy as part of studies or clinical practice

## WHAT YOU NEED TO KNOW

### BACKGROUND AND CONTEXT

Patients who discontinue nucleo(s)tide analogue therapy are at risk of viral rebound and severe hepatitis flares, necessitating intensive off-treatment follow-up. Early off-treatment predictors of longer-term outcomes are required to guide patient management.

### NEW FINDINGS

Higher hepatitis B virus DNA and hepatitis B surface antigen levels at 24 weeks after therapy withdrawal are associated with an increased risk of subsequent clinical relapse and a lower chance of hepatitis B surface antigen loss during long-term follow-up. Patients with both hepatitis B virus DNA  $<100$  IU/mL and hepatitis B surface antigen  $<100$  IU/mL at off-treatment week 24 have a very limited risk of subsequent clinical relapse and a very high chance of hepatitis B surface antigen loss.

### LIMITATIONS

The current study included only patients with negative hepatitis B e antigen at the start of antiviral therapy, and the findings may therefore not be applicable to patients with positive baseline hepatitis B e antigen. Most of the cohort comprised Asian patients, and further validation of the findings is therefore required in other populations.

### CLINICAL RESEARCH RELEVANCE

Hepatitis B virus DNA and hepatitis B surface antigen levels at off-treatment week 24 can be used to guide patient management. Retreatment should be considered in patients with high hepatitis B virus DNA and hepatitis B surface antigen levels, whereas careful follow-up may be considered for patients with both low hepatitis B virus DNA and hepatitis B surface antigen levels.

### BASIC RESEARCH RELEVANCE

The combination of low HBV DNA and hepatitis B surface antigen levels at off-treatment week 24 could be used as a surrogate for long-term favorable outcomes and could be used as a response parameter in translational studies.

in centers in Europe and Asia (the CREATE cohort).<sup>2,10</sup> Patients were eligible for the current analysis if they had been treated with only NUCs (no history of interferon add-on was allowed) and if they were (1) hepatitis B e antigen; (HBeAg) negative at the time of therapy initiation, (2) had undetectable HBV DNA at the time of therapy cessation, (3) had available information on HBV DNA and HBsAg levels at follow-up week 24 (FU W24), and (4) were still HBsAg

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**Abbreviations used in this paper:** aHR, adjusted hazard ratio; CI, confidence interval; FU W24, follow-up week 24; HBcrAg, hepatitis B core related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HR, hazard ratio; NUC, nucleo(s)tide analogue.

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0016-5085

<https://doi.org/10.1053/j.gastro.2023.09.033>

positive and did not meet criteria for clinical relapse before or at FU W24 (Supplementary Figure 1).

### Laboratory Testing

HBV DNA was measured using local laboratory methods. HBsAg was measured using various standardized methods. Hepatitis B core-related antigen (HBcrAg) was quantified with the Lumipulse G HBcrAg assay (Fujirebio Inc) on a Lumipulse G1200 analyser (Fujirebio Inc). The assay's lower limit of quantification is 3 log U/mL. HBV genotyping was performed using various methods, including line-probe assays, restriction fragment length polymorphism, or sequencing. Other biochemical tests were performed using local laboratory facilities.

### End Points and Statistical Analysis

The association between HBV DNA and HBsAg levels at FU W24 was assessed using Pearson's correlation. Next, we explored the association between HBV DNA levels (continuous and categorized as <100, 100–1000, 1,000–10,000, and >10,000 IU/mL), and HBsAg levels (continuous and categorized as <100, 100–1000 and >1,000 IU/mL), with clinical relapse and HBsAg loss during subsequent follow-up. Analyses were performed in the overall population, and after stratification, by end-of-treatment HBsAg (<100 and >100 IU/mL) and HBcrAg (<3 log and >3 log IU/mL) levels. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason. Patients achieving HBsAg loss were considered to remain free from clinical relapse. HBsAg loss was defined as undetectable HBsAg at any time during off-treatment follow-up. Re-treated patients were considered persistently HBsAg positive. Associations were explored using the Kaplan-Meier methods and Cox's regression. Analyses were performed using SPSS 28 software (IBM). All statistical tests were 2-sided and were evaluated at the 0.05 level of significance.

## Results

### Cohort Characteristics

The CREATE database comprised data of 1241 patients who discontinued antiviral therapy; of these, 300 patients were excluded because of a positive HBeAg at baseline, 289 were excluded for missing data at FU W24, 126 were excluded because of clinical relapse or HBsAg loss before FU W24, and 51 patients did not have sufficient follow-up beyond W24 (Supplementary Figure 1). Characteristics of the patients included in this analysis and of those who were excluded for clinical relapse in the first 24 weeks after therapy cessation are shown in Supplementary Tables 1 and 2. This left 475 patients for further analysis, most of whom were previously treated with entecavir (54.9%) and of Asian ethnicity (82%). HBV DNA levels at FU W24 were <100 IU/mL in 177 (37%) and >10,000 IU/mL in 82 (17%). HBsAg levels at FU W24 were <100 IU/mL in 139 (29%) and >1000 in 121 (26%). Additional cohort characteristics are summarized in Table 1 and Supplementary

**Table 1.** Characteristics of the Patients Included in This Cohort

Characteristics	Values (N = 475)
<b>Demographics</b>	
Age, y	52 (45–59)
Male sex	358 (75)
Asian ethnicity	391 (82)
Cirrhosis	18 (3.8)
Duration of treatment, wk	157 (156–211)
<b>Treatment</b>	
Entecavir	261 (54.9)
Tenofovir disoproxil fumarate	169 (35.6)
Other/combination	45 (9.5)
<b>Characteristics at end of treatment</b>	
Alanine aminotransferase, U/L	22 (17–32)
HBV DNA undetectable	475 (100)
HBsAg, log IU/mL	2.67 (2.1–3.1)
HBsAg <100 IU/mL	112 (23.6)
HBcrAg, log U/mL <sup>a</sup>	3.0 (3.0–3.5)
HBcrAg <3 log U/mL	286 (60.2)
<b>Characteristics at FU W24</b>	
Alanine aminotransferase, U/L	27 (19–38)
HBV DNA, log IU/mL	2.6 (1.3–3.6)
HBV DNA <100 IU/mL	177 (37.3%)
HBsAg, log IU/mL	2.6 (1.9–3.0)
HBsAg <100 IU/mL	139 (29.3%)

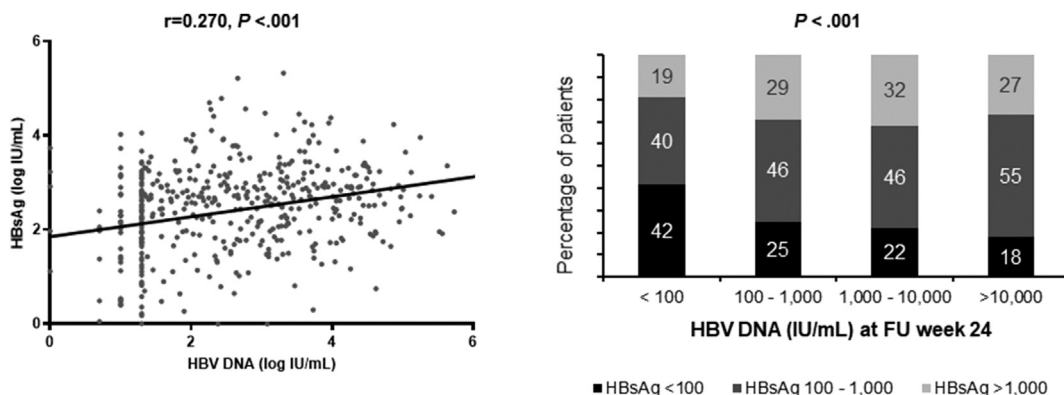
NOTE. Data are presented as n (%) or median (interquartile range).

<sup>a</sup>The lower limit of quantification for HBcrAg is 3 log U/mL, the lower limit of detection is 2 log U/mL.

**Table 3.** At FU W24 we observed a limited correlation between HBV DNA and HBsAg levels (Pearson's  $r = 0.270$ ,  $P < .001$ ) (Figure 1). In the overall study population, the cumulative probability of clinical relapse and HBsAg loss at 216 weeks of follow-up were 48.9% and 13.6%. Among the 193 patients who experienced clinical relapse during FU >W24, 3 (1.9%) experienced decompensation of liver disease, 1 of whom died.

### HBV DNA Levels at Follow-up Week 24 Predict Clinical Relapse and HBsAg Loss

Patients with higher HBV DNA levels at FU W24 had a higher risk of clinical relapse (hazard ratio [HR], 1.576; 95% confidence interval [CI], 1.423–1.747;  $P < .001$ ) and a lower chance of HBsAg loss (HR, 0.454; 95% CI, 0.360–0.573;  $P < .001$ ). The cumulative probability of clinical relapse at 216 weeks was 27.8% among patients with HBV DNA <100 IU/mL vs 83% among patients with HBV DNA >10,000 IU/mL ( $P < .001$ ) (Figure 2A). Conversely, patients with HBV DNA levels <100 IU/mL had the highest chance of subsequent HBsAg loss; the cumulative probability of HBsAg loss at 216 weeks was 31.3% vs 2.8% among patients with HBV DNA >10,000 IU/mL at off-treatment W24 ( $P < .001$ ) (Figure 2B). Findings were consistent among patients with low and high viral antigen levels at the end of treatment (Supplementary Figures 2 and 3).



**Figure 1.** Association between HBV DNA and HBsAg levels at FU W24. There was a modest correlation between HBV DNA and HBsAg levels in this cohort.

### HBsAg Levels at Follow-up Week 24 Predict Subsequent Clinical Relapse and HBsAg Loss

Patients with higher HBsAg levels at FU W24 had a higher risk of clinical relapse (HR, 1.579; 95% CI, 1.364–1.828;  $P < .001$ ) and a lower chance of HBsAg loss (HR, 0.263; 95% CI, 0.212–0.328;  $P < .001$ ). The cumulative probability of clinical relapse at 216 weeks was 24.9% among patients with HBsAg levels  $<100$  IU/mL vs 60.3% among patients with HBsAg  $>1000$  IU/mL ( $P < .001$ ) (Figure 3A). Conversely, patients with HBsAg levels  $<100$  IU/mL had the highest chance of subsequent HBsAg loss. The cumulative probability of HBsAg loss at 216 weeks was 40.2% vs 1.4% among patients with HBsAg  $>1000$  IU/mL ( $P < .001$ ) (Figure 3B). Findings were consistent among patients with low and high HBcrAg levels at the end of treatment and among patients with HBsAg levels  $>100$  IU/mL at the end of treatment (Supplementary Figures 4 and 5).

Interestingly, outcomes were similar for patients who had an HBsAg level  $>100$  IU/mL at the end of treatment but who experienced a decline to  $<100$  at FU W24 compared with patients who had HBsAg levels  $<100$  IU/mL at both end of treatment and FU W24 ( $P > .424$ ) (Supplementary Figure 6).

### A Combination of HBsAg $<100$ IU/mL With HBV DNA $<100$ IU/mL at Follow-up Week 24 Identifies Patients With Excellent Long-term Outcomes

Among the 475 patients in this cohort, HBsAg and HBV DNA levels at FU W24 were both  $>100$  IU/mL in 233 (49%), 168 (35%) had HBsAg  $>100$  IU/mL with HBV DNA  $<100$  IU/mL or HBsAg  $<100$  IU/mL with HBV DNA  $>100$  IU/mL, and 74 (16%) had both HBsAg and HBV DNA levels  $<100$  IU/mL. A combination of both HBsAg  $<100$  IU/mL and HBV DNA  $<100$  IU/mL at FU W24 identified patients with the best outcomes: at 216 weeks of follow-up, the cumulative risk of clinical relapse was only 9.9%, and the cumulative incidence of HBsAg loss was 58.3% in this subset of the cohort ( $P < .001$ ) (Figure 4). Conversely, patients with both HBV DNA

$>100$  IU/mL and HBsAg  $>100$  IU/mL had a very high risk of clinical relapse (66.5% at 216 weeks) and virtually no chance of HBsAg loss ( $<1\%$  at 216 weeks). Importantly, among the patients who had an end-of-treatment HBsAg level  $<100$  IU/mL, only 57 (50.9%) had both HBsAg  $<100$  IU/mL and HBV DNA  $<100$  IU/mL at off-treatment W24.

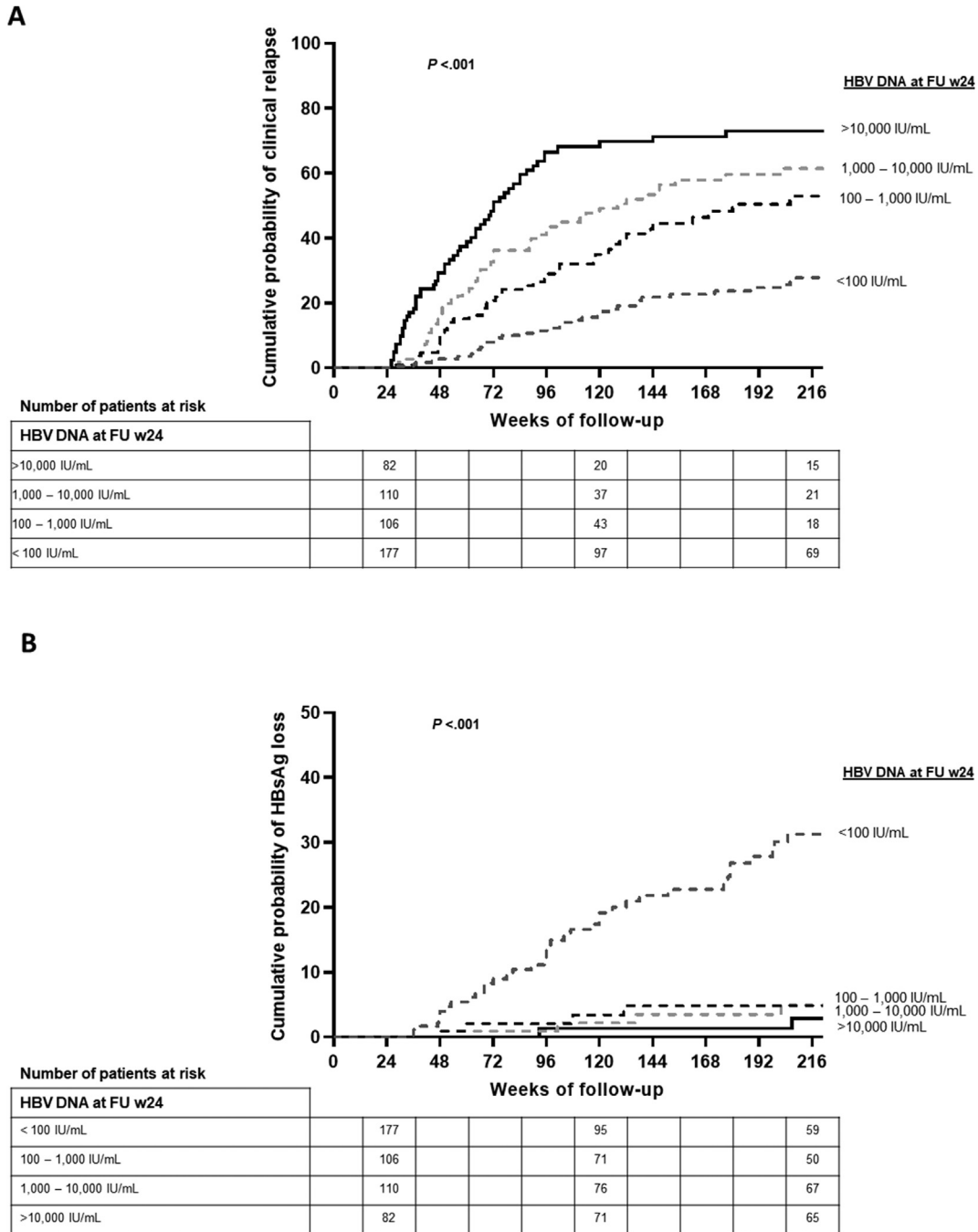
### HBV DNA and HBsAg Levels at Follow-up Week 24 Predict Subsequent Relapse and HBsAg Loss: Multivariable Analysis

In multivariable Cox’s regression analysis, adjusting for other established predictors of outcomes after therapy cessation, higher HBV DNA (adjusted HR [aHR], 1.384;  $P < .001$ ) and HBsAg (aHR, 1.917;  $P < .001$ ) levels at FU W24 were independently associated with an increased risk of clinical relapse (Table 2). Similarly, lower HBV DNA (aHR, 0.596;  $P < .001$ ) and HBsAg levels (aHR, 0.292;  $P < .001$ ) were independently associated with an increased chance of HBsAg loss (Table 3). In similar models, the combined presence of both HBV DNA  $<100$  IU/mL with HBsAg  $<100$  IU/mL was associated with a very low risk of clinical relapse (aHR, 0.158;  $P < .001$ ) (Supplementary Table 4) and a very high chance of HBsAg loss (aHR, 13.874;  $P < .001$ ) (Supplementary Table 5).

## Discussion

Most patients who discontinue NUC therapy will experience virologic relapse during follow-up and will not achieve HBsAg clearance. In this multicenter study, we show that serum levels of HBV DNA and HBsAg at FU W24 can be used to stratify future relapse risk and to predict which patients are (un)likely to achieve HBsAg clearance. These findings can be used to guide patient management and suggest that FU W24 HBV DNA and HBsAg levels could be used as surrogate end points for trials aiming for finite treatment.

Withdrawal of NUCs has gained major clinical interest because a substantial proportion of patients may achieve sustained disease remission and even HBsAg loss during

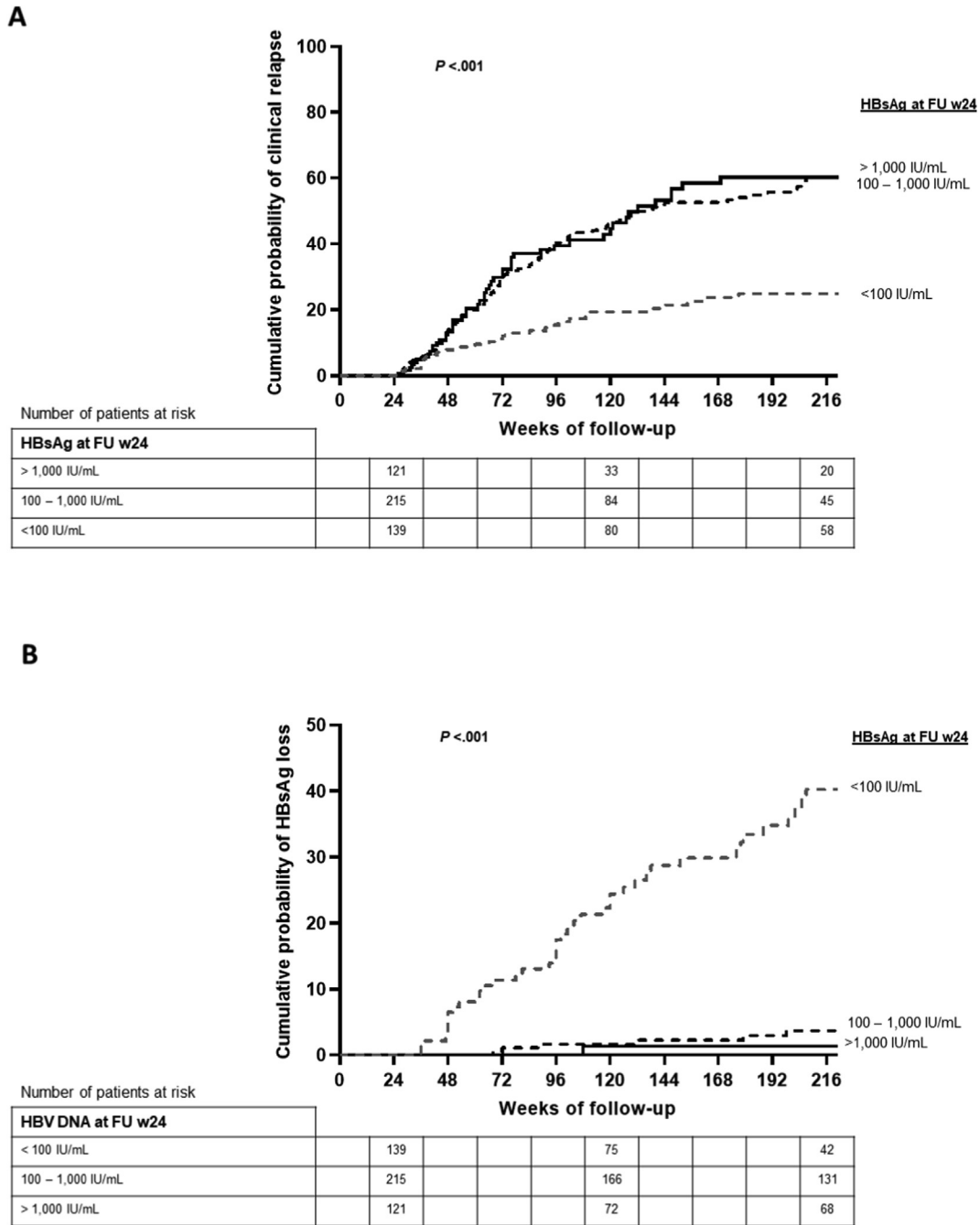


**Figure 2.** Association between HBV DNA levels at FU W24 with subsequent outcomes. Association between (A) HBV DNA levels at FU W24 with clinical relapse and (B) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at  $\leq 24$  weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA  $>2000$  IU/mL with alanine aminotransferase  $>2$  times the upper limit of normal or reinitiation of antiviral therapy for any reason.

off-treatment follow-up. However, most patients will not achieve these outcomes and will remain at risk for virologic relapse and severe hepatitis flares during long-term follow-up. These findings have prompted various studies to focus on identifying predictors of successful therapy withdrawal. Potential predictors of off-treatment outcomes are patient age, ethnicity, HBV genotype, antiviral agent, and lower end-of-treatment levels of alanine aminotransferase, HBsAg, HBcrAg, and HBV RNA.<sup>1,2,10-12</sup> Importantly, even

combinations of these factors cannot guarantee successful therapy withdrawal, and careful follow-up remains essential in all patients who discontinue therapy.

Prospective studies have shown that although relapse may occur at any time during off-treatment follow-up, the risk is highest during the first months after therapy withdrawal.<sup>9</sup> We therefore hypothesized that HBV DNA and HBsAg levels at 6 months after therapy withdrawal could potentially be used as predictors of longer-term outcomes.



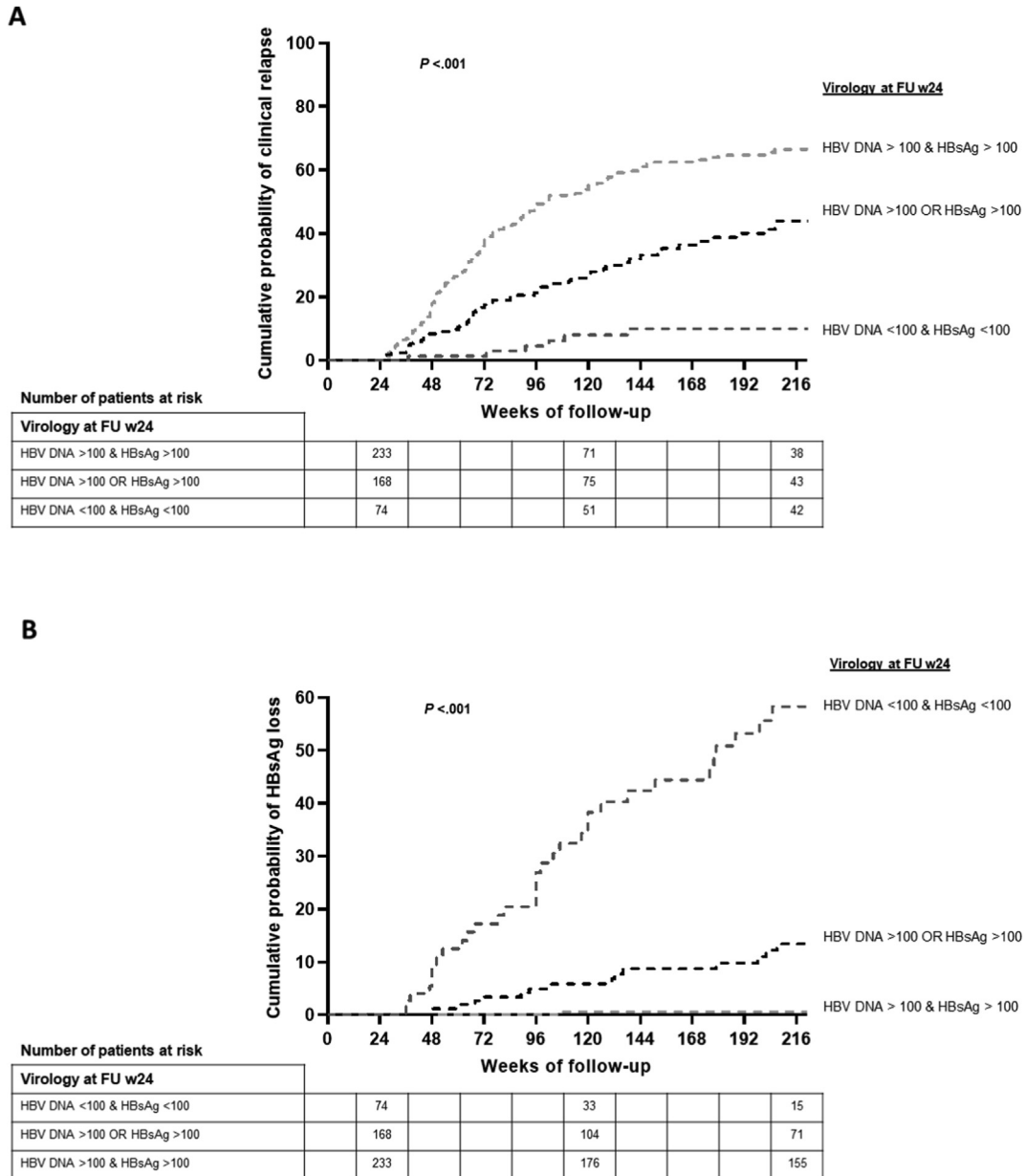
**Figure 3.** Association between HBsAg levels at FU W24 with subsequent outcomes. Association between (A) HBsAg levels at FU W24 with clinical relapse and (B) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at  $\leq 24$  weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA  $>2000$  IU/mL with alanine aminotransferase  $>2$  times the upper limit of normal or reinitiation of antiviral therapy for any reason.

This approach is analogous to that which has been applied to interferon-based therapies, where responses observed at 6 months after therapy withdrawal have been shown to be durable.<sup>7,8</sup>

In the current study, we were able to analyze data from 475 patients who were still off-treatment at FU W24. Interestingly, we observed a wide range of HBV DNA and HBsAg levels at off-treatment FU W24 in these patients, with only limited correlations observed between HBV DNA

and HBsAg levels. This finding supports previous observations that these factors may be complimentary.<sup>5,6</sup>

In our cohort, lower levels of HBV DNA and HBsAg at FU W24 were associated with a lower risk of clinical relapse and a higher chance of HBsAg loss. Importantly, patients with both HBV DNA  $<100$  IU/mL and HBsAg  $<100$  IU/mL had a very low risk of clinical relapse during prolonged follow-up and a very high chance of HBsAg loss. However, patients with both HBsAg  $>100$  IU/mL and HBV DNA



**Figure 4.** A combination of HBV DNA and HBsAg levels at FU W24 predicts clinical relapse and HBsAg loss. Association between (A) a combination of HBV DNA and HBsAg levels at FU W24 with clinical relapse and (B) HBsAg loss. The study excluded patients who experienced clinical relapse or HBsAg loss at  $\leq 24$  weeks of follow-up. Clinical relapse was defined as the occurrence of HBV DNA  $>2000$  IU/mL with alanine aminotransferase  $>2$  times the upper limit of normal or reinstitution of antiviral therapy for any reason.

$>100$  IU/mL had a very high risk of clinical relapse and virtually no chance of HBsAg loss. These findings were consistent across several clinically relevant subgroups, including those with high or low end-of-treatment viral antigen levels, and were also robust in multivariable analysis.

Our observations may have important clinical applications. Patients with both low HBsAg and HBV DNA levels at FU W24 could potentially enter a less restrictive follow-up protocol, given their limited risk of relapse. Furthermore, our findings suggest that patients with both high HBsAg and high HBV DNA levels may be better off restarting antiviral therapy, because they are at very high risk of

clinical relapse, with virtually no chance of achieving HBsAg clearance. These patients are therefore unlikely to benefit from further off-treatment follow-up and are potentially at risk of developing severe hepatitis flares, as highlighted by a recent case report and the cases of hepatic decompensation after therapy withdrawal observed in this cohort.<sup>13</sup>

In addition to the potential clinical application of our findings in patients who discontinue NUC therapy, our results also suggest that low off-treatment HBsAg and HBV DNA levels could have utility as a potential surrogate end point for future studies investigating finite treatment

**Table 2.** Factors Associated With Clinical Relapse

Variable	aHR (95% CI)	P
HBV DNA (log IU/mL) FU W24	1.384 (1.238–1.548)	<.001
HBsAg (log IU/mL) FU W24	1.917 (1.568–2.342)	<.001
Alanine aminotransferase (U/L) FU W24	1.011 (1.001–1.021)	.026
Age	1.040 (1.026–1.056)	<.001
Male sex	1.271 (0.890–1.816)	.188
Asian ethnicity	5.976 (2.560–13.95)	<.001
Tenofovir therapy	1.257 (0.907–1.742)	.169

NOTE. Factors associated with clinical relapse after NUC withdrawal in multivariable Cox's regression analysis. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

approaches. At present, such studies are challenging to perform because HBsAg loss is infrequently achieved. There is therefore a major unmet need for other more easily achievable intermediate end points, a so-called partial cure, to evaluate the efficacy of novel compounds and treatment strategies. Based on our findings, we propose that a combination of low HBsAg and HBV DNA levels at 6 months after therapy withdrawal could potentially be used as such a surrogate end point, although this should still be validated for novel antiviral agents.

Although our study is relatively large and studied patients from centers in Europe and Asia, it does have several limitations. First, because we excluded patients re-treated before week 24, only a limited subset of the cohort was eligible for enrollment. Furthermore, other off-treatment factors, such as HBV RNA and HBcrAg, were not available to us, and future studies should focus on assessment of the additive value of these biomarkers.

**Table 3.** Factors Associated With Hepatitis B Surface Antigen Loss

Variable	aHR (95% CI)	P
HBV DNA (log IU/mL) FU W24	0.596 (0.466–0.761)	<.001
HBsAg (log IU/mL) FU W24	0.292 (0.232–0.369)	<.001
Alanine aminotransferase (U/L) FU W24	1.023 (1.007–1.038)	.004
Age	0.988 (0.963–1.013)	0.348
Male sex	0.725 (0.424–1.239)	0.239
Asian ethnicity	0.206 (0.096–0.440)	<0.001
Tenofovir therapy	1.303 (0.774–2.193)	0.319

NOTE. Factors associated with HBsAg loss after NUC withdrawal in multivariable Cox's regression analysis.

Additionally, we studied a relatively small number of non-Asian patients. Although findings were consistent across Asian and non-Asian patients (Supplementary Figure 7), further validation in non-Asian patients is required.

It is also important to note that the type of antiviral therapy was not associated with clinical relapse in our analyses, which appears to contradict other reports.<sup>14,15</sup> This is likely due to exclusion of patients with early clinical relapse, which is more likely to be observed in patients previously treated with tenofovir.<sup>14,15</sup>

## Conclusion

Our multicenter study shows that, among patients with chronic hepatitis B who are HBeAg negative and who discontinued antiviral therapy and who did not experience clinical relapse before FU W24, serum levels of HBV DNA and HBsAg at FU W24 can be used to predict subsequent clinical relapse and HBsAg clearance. A combination of low HBsAg levels and low HBV DNA levels identifies patients with very low risk of clinical relapse and excellent chances of HBsAg loss and could potentially be used as an early surrogate end point for studies aiming at finite therapy in HBV. Patients with both high HBsAg and HBV DNA levels have a very high risk of relapse and virtually no chance of HBsAg loss and may benefit from reinitiation of antiviral therapy.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org), and at <https://doi.org/10.1053/j.gastro.2023.09.033>.

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 Fabien Zoulim, MD (Conceptualization: Supporting; Methodology: Supporting; Writing–review & editing: Supporting)  
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 Benjamin Maasoumy, MD (Conceptualization: Equal; Data curation: Equal; Investigation: Equal; Methodology: Equal; Writing–original draft: Equal; Writing–review & editing: Equal)

#### Conflicts of interest

These authors disclose the following: Milan J. Sonneveld has received speaker's fees and research support from Roche, Gilead, Bristol-Myers Squibb, and Fujirebio. Jun Yong Park is an investigator in clinical trials sponsored by AbbVie, Gilead Sciences, Hanmi, and Novartis. Wai-Kay Seto has received speaker's fees from AstraZeneca, provided consultancy for Abbott, received research funding from Pfizer, Boehringer Ingelheim, Ribo Life Science, and Alexion Pharmaceuticals, and received speaker's fees, provided consultancy, and received research funding from Gilead Sciences. Yasuhito Tanaka reports lecture fees from Fujirebio, GlaxoSmithKline Pharmaceuticals Ltd, and Gilead Sciences, research fees from Fujifilm Corp, Janssen Pharmaceutical K.K., Gilead Sciences, GlaxoSmithKline Pharmaceuticals Ltd, and Sysmex. Florian van Bömmel has received grants from Gilead Sciences, Roche, Janssen, VIR, and Ipsen, received consulting fees from Gilead, Janssen, Ipsen, Roche, AstraZeneca, and Esai, and lecture fees from Gilead, Janssen, Ipsen, Roche, AstraZeneca, Esai, and ADVANZ Pharma. Harry L. Janssen has received grants from Gilead Sciences, GlaxoSmithKline, Janssen, Roche, Vir, and Biotechnology Inc, and is a consultant for Aligos, Gilead Sciences, GlaxoSmithKline, Janssen, Roche, Vir Biotechnology Inc, and Precision Biosciences. Thomas Berg currently acts as an advisor to AbbVie, Alexion, Bayer, Bristol-Myers Squibb, Gilead, Intercept, Janssen, MSD/Merck, Merz, Novartis, and Sequana Medical, and has received speaking honoraria from AbbVie, Alexion, Bayer, Bristol-Myers Squibb, Eisai, Gilead, Intercept, Ipsen, Janssen, MSD/Merck, Merz, Novartis, Sirtex, and Sequana Medical in the past 2 years, and has received grant support from AbbVie, Bristol-Myers Squibb, Gilead, Humedics, Intercept, Janssen, MSD/Merck, Merz, Novartis, Aligos, Antios, Assembly, Blue Jay, Evotec, Gilead, GlaxoSmithKline Pharmaceuticals Ltd, and discloses research grants from Assembly, Beam, Janssen, and Viravaxx. Sang Hoon

Received March 21, 2023. Accepted September 13, 2023.

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#### Acknowledgments

The authors would like to thank Laura Vernoux (Fujirebio) for supporting the CREATE project.

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Ahn has acted as advisor and investigator for Gilead, Janssen, AbbVie, Roche, Assembly Biosciences, Arbutus, Bria, Vaccitech, GlaxoSmithKline Pharmaceuticals Ltd, Inovio, Aligos, Vir Biotechnology, SL Vaxigen, GeneOne Life Science, GreenCross, Yuhan, Samil, and Ildong. George N. Dalekos is an advisor or lecturer for Pfizer, Roche, Sanofi, and Sobi, received research grants from Gilead, and has served as principal investigator in studies for Gilead, Novo Nordisk, Genkyotex, Regulus Therapeutics Inc, Tiziana Life Sciences, Bayer, Astellas, Pfizer, Amyndas Pharmaceuticals, CymaBay Therapeutics Inc, Sobi, and Intercept Pharmaceuticals. Maurizia Brunetto discloses consultancy/speakers bureau for AbbVie, Gilead, Janssen, Eisai, MSD, and Roche. Heiner Wedemeyer has received research grants from Abbott, AbbVie, Bristol-Myers Squibb, Gilead, Merck, Novartis, Roche, Roche Diagnostics, Siemens, consultant fees from Abbott, AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead, Janssen-Cilag, Merck/Schering-Plough, Novartis, Roche, Roche Diagnostics, Siemens, Transgene, and ViiV, and speaker fees from Abbott, AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim, Gilead, Janssen-Cilag, Merck/Schering-Plough, Novartis, Roche, Roche Diagnostics, Siemens, Transgene, and ViiV. Markus Cornberg has received personal fees from AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen-Cilag, Merck (MSD), Biogen, Falk Foundation, Boehringer Ingelheim, Siemens, Spring Bank, and grants and personal fees from Roche. Man-Fung Yuen has provided consultancy or received research funding from AbbVie, Arbutus Biopharma, Assembly Biosciences, Bristol-Myers Squibb, Dicerna Pharmaceuticals, GlaxoSmithKline Pharmaceuticals Ltd, Gilead Sciences, Janssen, Merck Sharp and Dohme, Clear B Therapeutics, and Springbank Pharmaceuticals, and received research funding from Arrowhead Pharmaceuticals, Fujirebio, and Sysmex Corp. Kosh Agarwal discloses consultancy/speakers bureau for Assembly, Aligos,

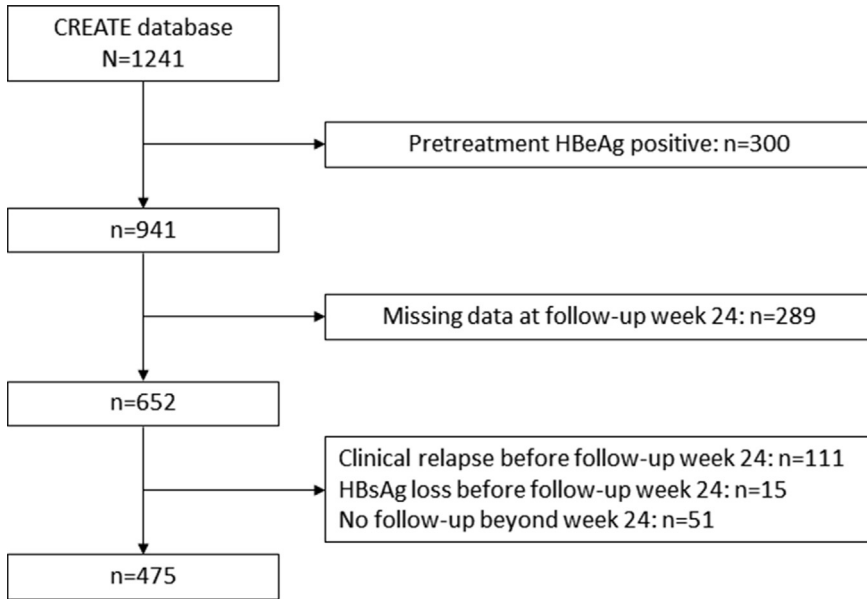
Arbutus, Gilead, Immunocore, Janssen, Roche, Sobi, Springbank, and Vir Biotechnology, and research support from Abbott, Gilead, and MSD. Andre Boonstra reports research fees from Fujirebio, GlaxoSmithKline Pharmaceuticals Ltd, Gilead Sciences, and Janssen Pharma. Maria Buti reports consultancy and lecture honoraria from AbbVie, Arbutus, Gilead, Janssen, Merck/MSD, and Spring-Bank. Teerha Piratvisuth has received research grants from Gilead Sciences, Roche Diagnostic, Janssen, Fibrogen, and Vir Biotechnology, and speaker honoraria from Bristol-Myers Squibb, Gilead Sciences, Bayer, Abbott, Eisai, Mylan, Ferring, and MSD. George Papatheodoridis has served as advisor/lecturer for AbbVie, Albireo, Dicerna, Gilead, GlaxoSmithKline Pharmaceuticals Ltd, Janssen, Ipsen, MSD, Novo Nordisk, Roche, and Takeda, and reports research grants from AbbVie and Gilead. Benjamin Maasoumy received speaker or consulting fees, or both, from Abbott Molecular, Astellas, Intercept, Falk, AbbVie, Norgine, Bristol-Myers Squibb, Fujirebio, Janssen-Cilag, Merck (MSD), and Roche, and has received research support from Abbott Molecular, Altona Diagnostics, Fujirebio, and Roche. The remaining authors disclose no conflicts.

#### Funding

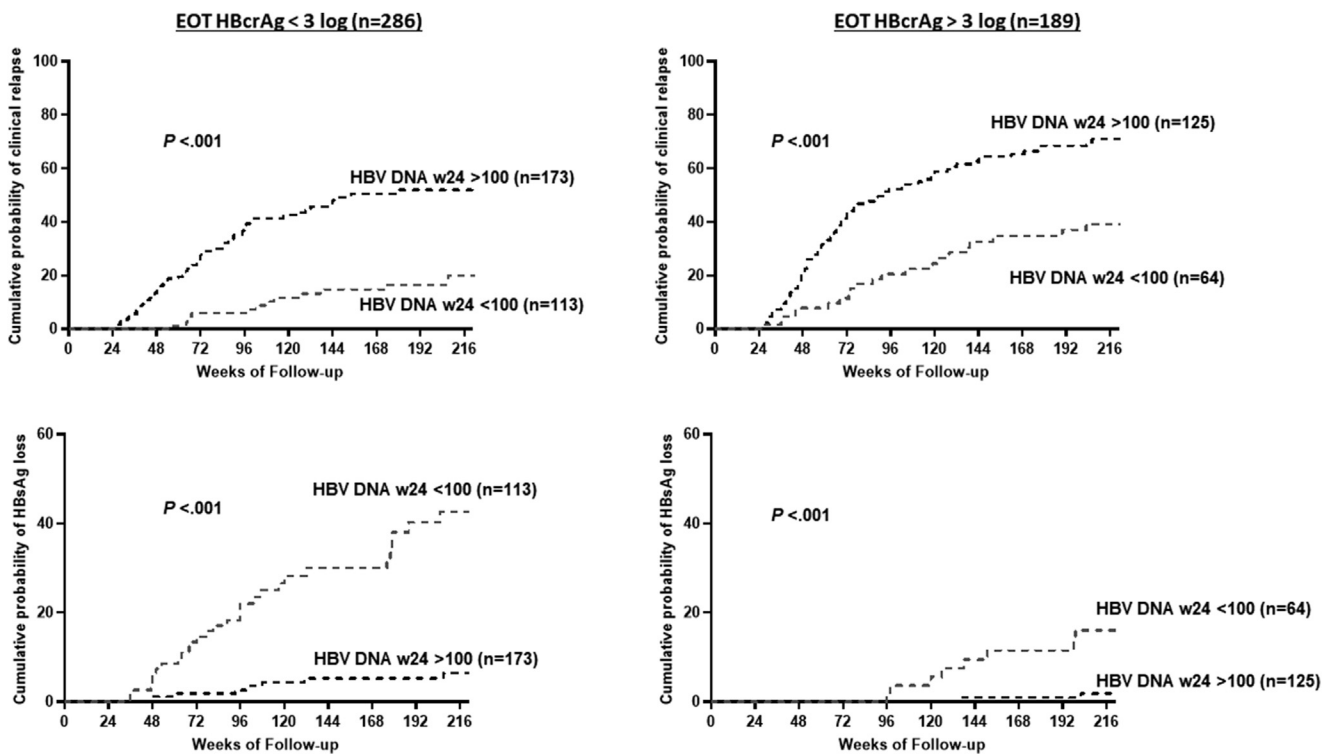
The CREATE study was supported by Fujirebio. Materials for HBcrAg testing were provided free of charge to several participating centers. Fujirebio had no influence on CREATE study design, data collection, data analysis, writing of the current manuscript, or the decision to submit for publication.

#### Data Availability

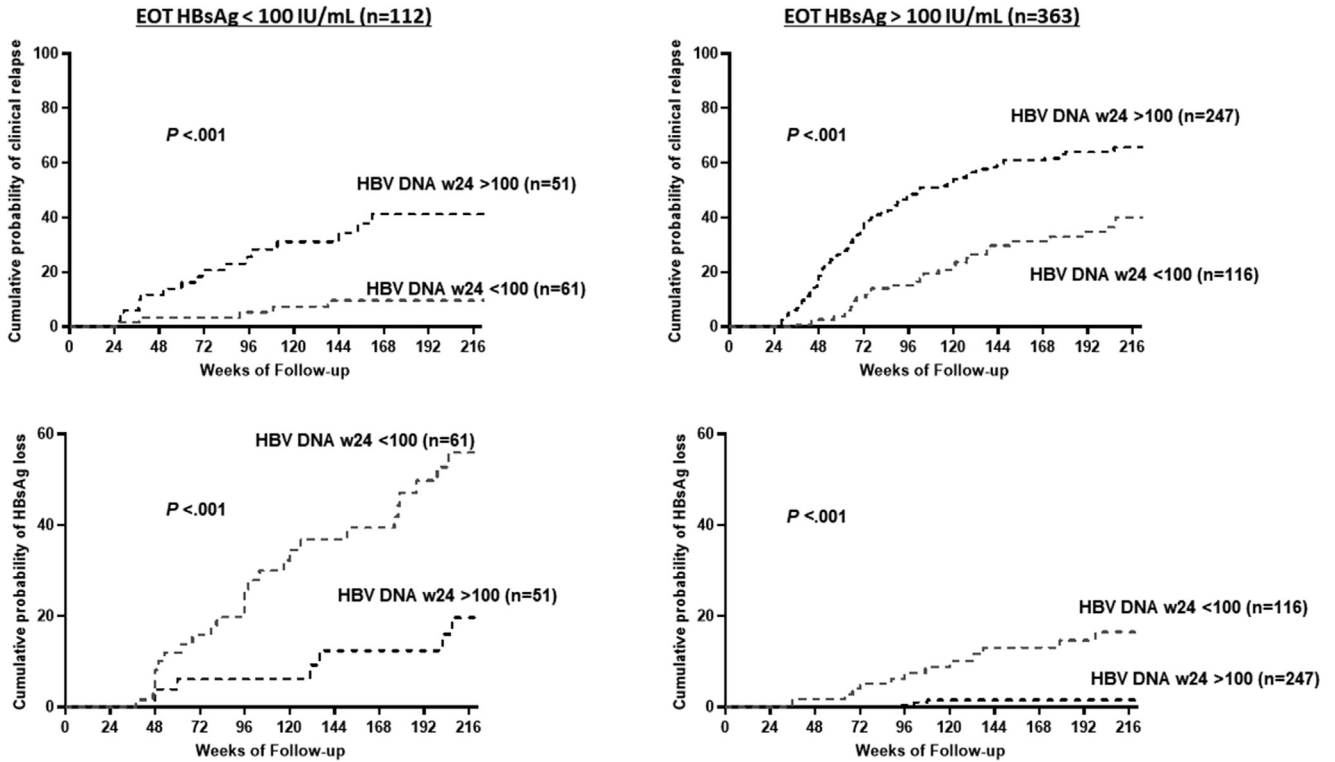
The data used for the current analysis were derived from previously published cohorts and clinical data sets. The data cannot be shared.



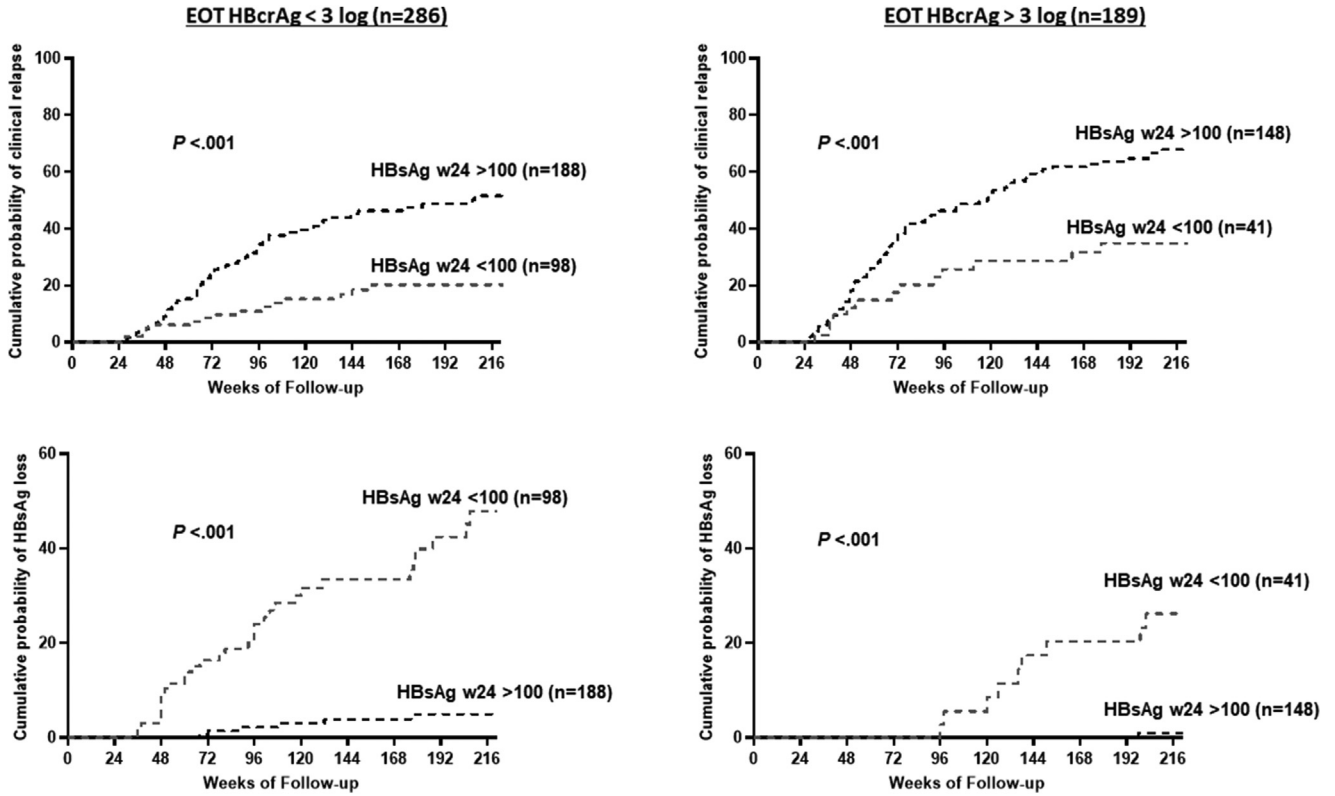
**Supplementary Figure 1.** Patient flow chart. Selection of patients for the current analysis. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



**Supplementary Figure 2.** Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBcrAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal, or reinitiation of antiviral therapy for any reason.

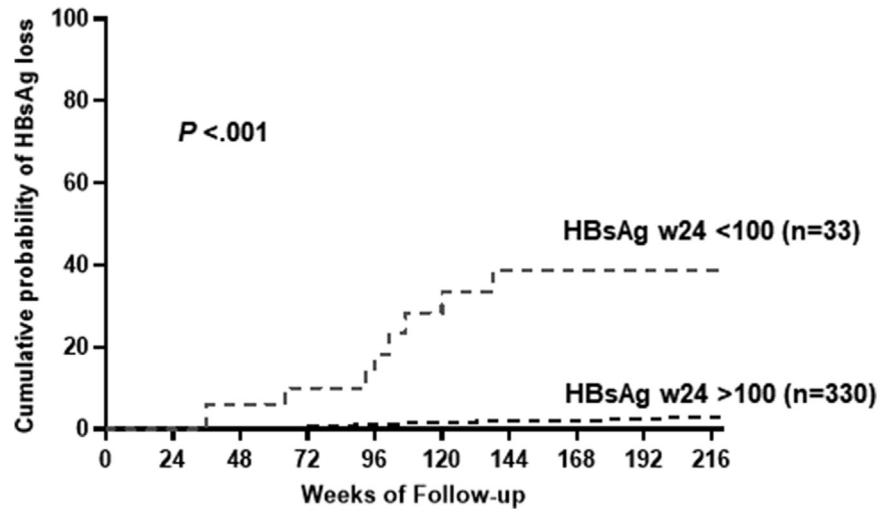
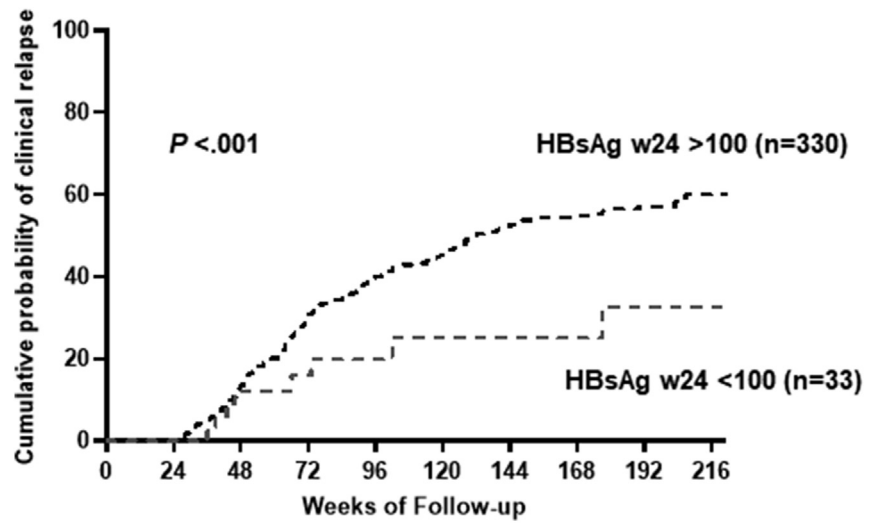


**Supplementary Figure 3.** Association between HBV DNA levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBsAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

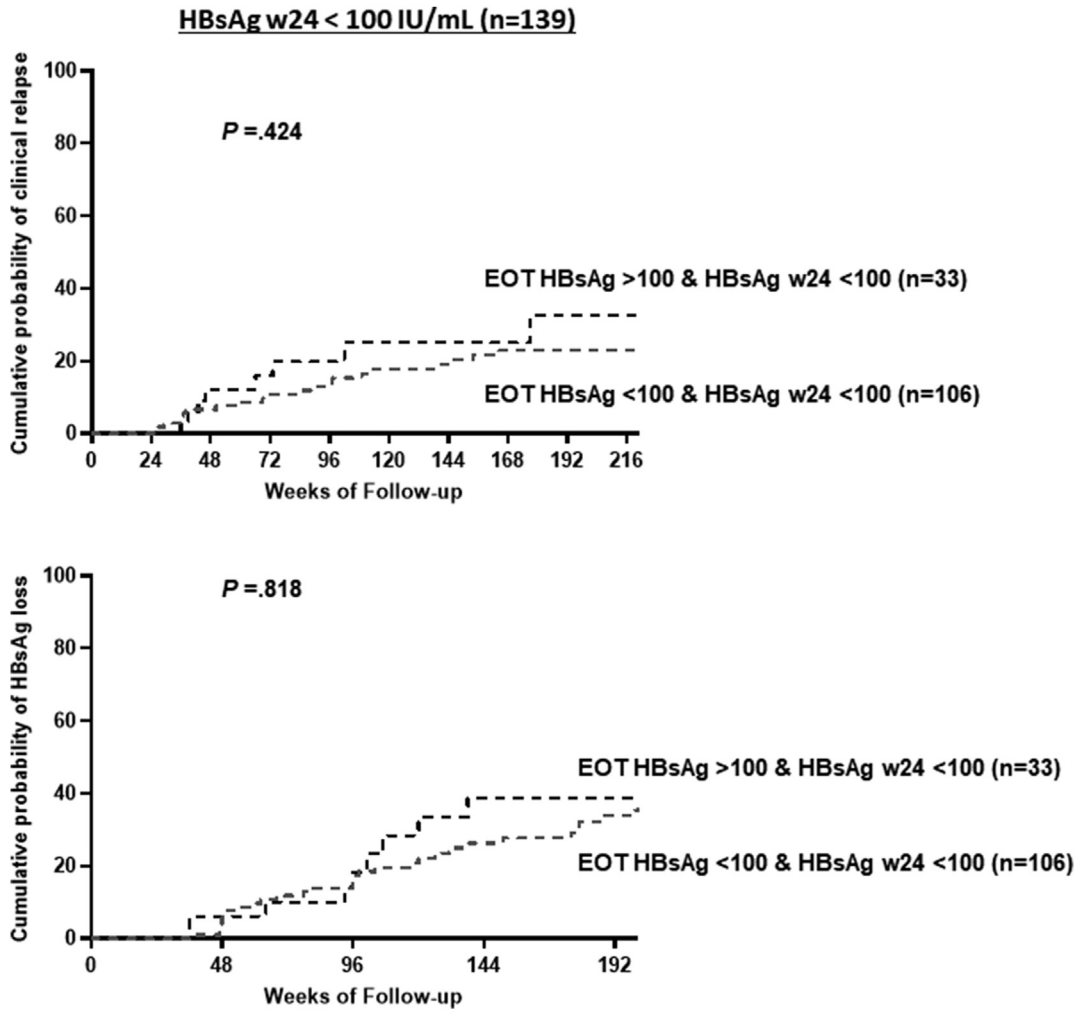


**Supplementary Figure 4.** Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss for patients with low and high HBcrAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

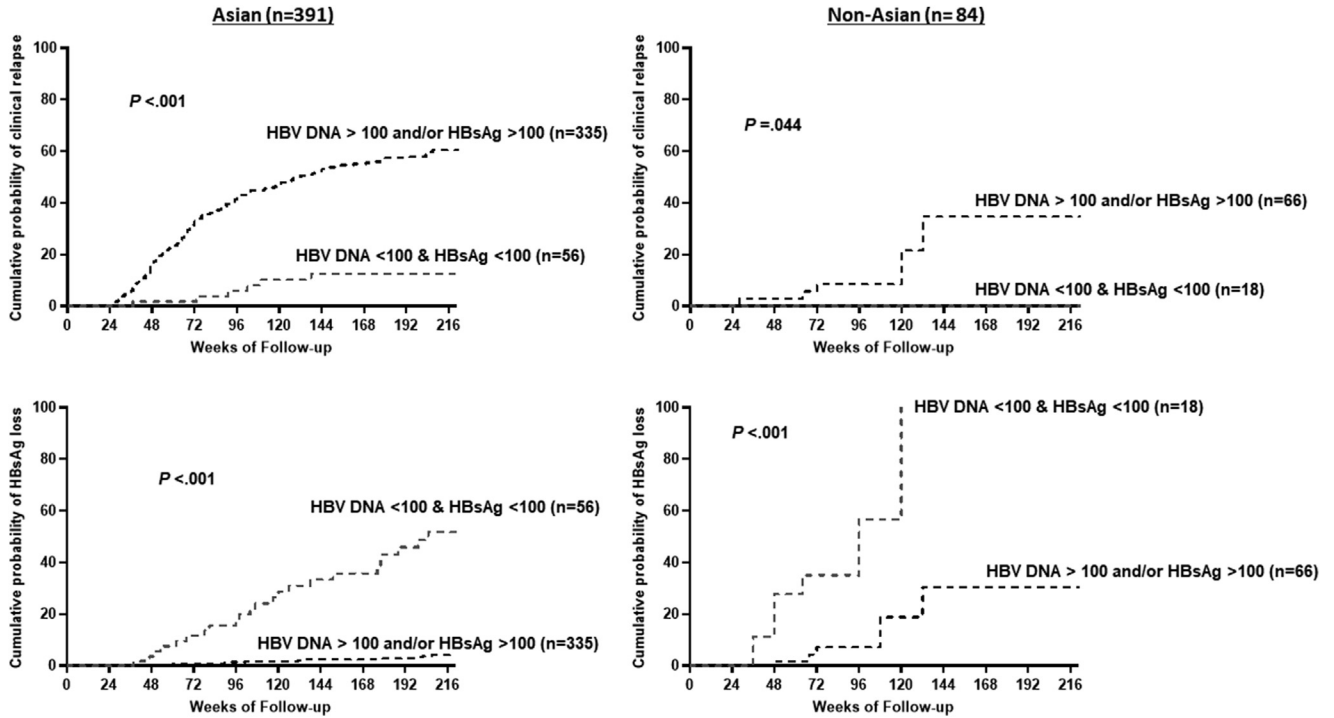
**EOT HBsAg > 100 IU/mL (n=363)**



**Supplementary Figure 5.** Association between HBsAg levels at FU W24 with clinical relapse and HBsAg loss in the subset of patients with high HBsAg levels at end of treatment (EOT). Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



**Supplementary Figure 6.** Cumulative incidence of clinical relapse and HBsAg loss for patients with an HBsAg level <100 IU/mL at both end of treatment (EOT) and FU W24 and for patients with an HBsAg level >100 IU/mL at end of treatment who achieved a decline to <100 IU/mL by FU W24. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



**Supplementary Figure 7.** Cumulative incidence of clinical relapse and HBsAg loss for patients with both HBV DNA <100 IU/mL and HBsAg <100 IU/mL at FU W24 compared with patients with HBV DNA >100 IU/mL or HBsAg >100 IU/mL, or both, stratified by ethnicity. Clinical relapse was defined as the occurrence of HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

**Supplementary Table 1.** Comparison of the Characteristics of Patients Included in the Study vs Those Who Were Excluded for Clinical Relapse Within 24 Weeks After Therapy Cessation

Characteristics	Included in study (n = 475)	Clinical relapse <24 weeks (n = 111)	P
<b>Demographics</b>			
Age, y	52 (45–59)	53 (44–60)	.053
Male sex	358 (75)	87 (78.4)	.504
Asian ethnicity	391 (82)	97 (87.4)	.197
Cirrhosis	18 (3.8)	2 (1.8)	.289
Duration of treatment, wk	157 (156–211)	157 (156–200)	.083
Alanine aminotransferase, U/L	22 (17–32)	27 (19–40)	<.001
<b>Treatment</b>			
Entecavir	261 (54.9)	48 (43.2)	.003
Tenofovir disoproxil fumarate	169 (35.6)	58 (52.3)	
Other/combination	45 (9.5)	5 (4.5)	
<b>Virology at end of treatment</b>			
HBsAg <100 IU/mL	112 (23.6)	17 (15.3)	.059
HBcrAg <3 log U/mL	286 (60.2)	59 (53.2)	.174

NOTE. Data are presented as n (%) or median (interquartile range). Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.



**Supplementary Table 2.** Comparison of the Characteristics of Patients With Hepatitis B Virus DNA <100 IU/mL and Hepatitis B Surface Antigen <100 IU/mL at Follow-up Week 24 vs Those Who Experienced Clinical Relapse Within 24 Weeks After Therapy Cessation

Characteristics	HBV DNA <100 and HBsAg <100 IU/mL at FU W24	Clinical relapse <24 weeks	P
	(n = 74)	(n = 111)	
<b>Demographics</b>			
Age, y	52 (45–58)	53 (44–60)	.182
Male sex	55 (74.3)	87 (78.4)	.522
Asian ethnicity	56 (75.7)	97 (87.4)	.039
Cirrhosis	9 (12.5)	2 (1.8)	.003
Duration of treatment, wk	157 (156–283)	157 (156–200)	.718
Alanine aminotransferase, U/L	24 (19–39)	27 (19–40)	.112
<b>Treatment</b>			
Entecavir	35 (47.3)	48 (43.2)	.862
Tenofovir disoproxil fumarate	36 (48.6)	58 (52.3)	
Other/combination	3 (4.1)	5 (4.5)	
<b>Virology at end of treatment</b>			
HBsAg <100 IU/mL	57 (77.0)	17 (15.3)	<.001
HBcrAg <3 log U/mL	51 (68.9)	59 (53.2)	.032

NOTE. Data are presented as n (%) or median (interquartile range). Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or re-initiation of antiviral therapy for any reason.

**Supplementary Table 3.** Comparison of the Characteristics of Patients With Hepatitis B Virus DNA <100 IU/mL and Hepatitis B Surface Antigen <100 IU/mL at Follow-Up Week 24 vs Those Who Had Hepatitis B Virus DNA or Hepatitis B Surface Antigen Levels >100 IU/mL, or Both

Characteristics	HBV DNA <100 IU/mL and HBsAg <100 IU/mL at FU W24	HBV DNA >100 IU/mL or HBsAg >100 IU/mL at FU W24, or both	P
	(n = 74)	(n = 401)	
<b>Demographics</b>			
Age, y	52 (45–58)	52 (45–59)	.973
Male sex	55 (74.3)	303 (75.6)	.821
Asian ethnicity	56 (75.7)	335 (83.5)	.103
Cirrhosis	9 (12.5)	9 (2.2)	<.001
Duration of treatment, wk	157 (156–283)	157 (156–208)	.933
Alanine aminotransferase, U/L	24 (19–39)	22 (17–31)	.460
<b>Treatment</b>			
Entecavir	35 (47.3)	226 (56.4)	.020
Tenofovir disoproxil fumarate	36 (48.6)	133 (33.2)	
Other/combination	3 (4.1)	42 (10.5)	
<b>Virology at end of treatment</b>			
HBsAg <100 IU/mL	57 (77.0)	55 (13.7)	<.001
HBcrAg undetectable	51 (68.9)	235 (58.6)	.096

NOTE. Data are presented as n (%) or median (interquartile range).

**Supplementary Table 4.** Factors Associated With Clinical Relapse

Variable	aHR (95% CI)	<i>P</i>
HBsAg <100 and HBV DNA <100 IU/mL at FU W24	0.158 (0.078–0.323)	<.001
Alanine aminotransferase (U/L) at FU W24	1.010 (1.001–1.020)	.033
Age	1.022 (1.008–1.036)	.002
Male sex	1.152 (0.808–1.642)	.433
Asian ethnicity	4.880 (2.138–11.137)	<.001
Tenofovir therapy	1.024 (0.746–1.406)	.884

NOTE. Factors associated with clinical relapse after NUC withdrawal in multivariable Cox's regression analysis. Clinical relapse was defined as the occurrence of either HBV DNA >2000 IU/mL with alanine aminotransferase >2 times the upper limit of normal or reinitiation of antiviral therapy for any reason.

**Supplementary Table 5.** Factors Associated With Hepatitis B Surface Antigen Loss

Variable	aHR (95% CI)	<i>P</i>
HBsAg <100 and HBV DNA <100 IU/mL at FU W24	13.874 (8.405–22.902)	<.001
Alanine aminotransferase (U/L) at FU W24	1.013 (0.998–1.029)	.088
Age	1.011 (0.988–1.035)	.343
Male sex	0.719 (0.427–1.210)	.214
Asian ethnicity	0.229 (0.116–0.455)	<.001
Tenofovir therapy	0.942 (0.566–1.568)	.818

NOTE. Factors associated with HBsAg loss after NUC withdrawal in multivariable Cox's regression analysis.