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Clinical trial: An open-label, randomised trial of different re-start strategies after treatment withdrawal in HBeAg negative chronic hepatitis B

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Summary

Background: Stopping nucleos(t)ide analogue (NA) therapy in patients with chronic hepatitis B (CHB) may trigger a beneficial immune response leading to HBsAg loss, but clinical trials on re-start strategies are lacking.

Aim: To assess whether it is beneficial to undergo a prolonged flare after NA cessation.

Methods: One-hundred-and-twenty-seven patients with HBeAg negative, non-cirrhotic CHB with at least 24 months of viral suppression on NA therapy were included. All study participants stopped antiviral therapy and were randomised to either low-threshold (ALT > 80 U/L and HBV DNA > 2000 IU/mL) or high-threshold (ALT > 100 U/L for >4 months, or ALT > 400 U/L for >2 months) for the re-start of therapy. The primary endpoint was HBsAg loss within 36 months of stopping antiviral treatment. The primary analysis was based on intention-to-treat allocation with last observation carried forward.

Results: There was a numerical but not statistically significant difference in HBsAg loss between the low-threshold (3 of 64; 4.7%) and the high-threshold (8 of 63; 12.7%) group (risk difference: 8.0%, 95% CI: −2.3 to 19.6, $p = 0.123$). None of the patients with end-of-treatment HBsAg > 1000 IU/mL achieved HBsAg loss; among those with end-of-treatment HBsAg < 1000 IU/mL, 8 of 15 (53.3%) achieved

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HBsAg loss in the high-threshold group compared to 3 of 26 (11.5%) in the low-threshold group.

Conclusions: We could not confirm our hypothesis that a higher threshold for re-start of therapy after NA withdrawal improves the likelihood of HBsAg loss within 36 months in patients with HBeAg negative CHB. Further studies including only patients with HBsAg level <1000 IU/mL and/or larger sample size and longer follow-up duration are recommended.

1 | INTRODUCTION

Globally, an estimated 257.5 million people live with chronic hepatitis B virus (HBV) infection and 858,000 die each year of its complications, mainly decompensated cirrhosis and hepatocellular carcinoma (HCC).[1] Antiviral therapy with oral nucleo(t)side analogues (NA) effectively reduces the risk of these complications but does not eradicate HBV DNA from the hepatocytes.[2–4] Consequently, viral rebound occurs once NA therapy is stopped. Therefore, treatment is usually indefinite which implicates concerns about long-term toxicities, accumulated drug costs and patients' reluctance to adhere to lifelong medication.[5,6]

The ideal treatment outcome in chronic hepatitis B (CHB) is hepatitis B s-antigen (HBsAg) loss – considered a functional cure – in which disease progression is halted and prognosis is excellent even without antiviral therapy.[7–10] HBsAg loss, however, is rarely achieved with NA therapy; the reported incidence is 0.22% per year in hepatitis B e-antigen (HBeAg) negative patients treated with entecavir or tenofovir disoproxil fumarate.[11] Therefore, there is a need to explore new strategies to improve treatment outcome in patients with CHB.

In a landmark study, Hadziyannis and colleagues observed that 13 of 33 patients (39%) with CHB who stopped NA therapy after years of full virological suppression experienced HBsAg loss after a transient immunological 'flare' (i.e. increase in HBV DNA and liver transaminases).[12] Other studies have later confirmed that the chance of HBsAg loss increases when long-term NA therapy is interrupted, but the proportion who achieves HBsAg loss varies.[13–18]

International clinical practice guidelines provide a conditional recommendation to stop antiviral treatment in patients with HBeAg negative, non-cirrhotic CHB after years of full viral suppression.[19,20] However, it is still unclear which re-start strategy to employ after NA withdrawal and liver society guidelines do not provide clear guidance on this matter.[19–21] A retrospective study from Taiwan suggested that delaying re-treatment after clinical relapse increased the likelihood of HBsAg loss.[15] On the other hand, a vigorous acute flare after NA withdrawal carries the risk of hepatic decompensation and a long-standing low-grade flare might lead to liver fibrosis progression and increased HCC risk.[13,17]

In the present study, we aimed to compare two different re-start strategies (low-threshold vs. high-threshold for restart) after NA withdrawal in a prospective, randomised, controlled trial ('The Nuc-Stop Study'). The hypothesis was that delaying restart after NA withdrawal – and thereby allowing a more pronounced flare – would increase the likelihood of HBsAg loss.

2 | MATERIALS AND METHODS

2.1 | Trial design

This was a multi-centre, open-label, randomised, controlled, parallel group, clinical trial comparing two different re-start strategies after cessation of NA therapy in patients with HBeAg negative CHB and no history of advanced fibrosis or cirrhosis (EudraCT no.: 2018-000724-34; ClinicalTrials.gov id.: NCT03681132). The trial was monitored by an independent data safety and monitoring committee. The trial protocol was designed by AJ, DHR and OD and the statistical analysis plan (SAP) was written by CSR and ICO; both are available in Appendix S1. AJ, DHR and CSR had access to all of the data and can vouch for the integrity of the data analyses.

Study participants were randomised to either high-threshold or low-threshold for restart of NA therapy. Indication for restart in the two groups were:

- High-threshold: Alanine aminotransferase (ALT) >100 U/L persisting for more than 4 months without any spontaneous decline toward normal, or ALT > 400 U/L persisting for more than 2 months
- Low-threshold: HBV DNA > 2000 IU/mL and ALT > 80 U/L at one assessment

As a safety precaution, NA therapy was re-started regardless of randomised group in study participants who experienced any of the following:

- ALT > 800 U/L
- Bilirubin >38 mmol/L or the international normalised ratio (INR) ≥1.4, in two consecutive samples and ALT > 80 U/L at the confirmatory test
- Diagnosed with cirrhosis based on elastography (transient elastography (TE) >11.0 kPa; acoustic radiation force impulse (ARFI) elastography >1.8 m/s; two-dimensional shear wave elastography (2D-SWE) >11.5 kPa)

2.2 | Participants

The study was conducted at 10 sites in Scandinavia (Norway, Sweden and Denmark) and one site in Ethiopia (Table S1). Adults (18–70 years) with HBeAg negative CHB were eligible for inclusion if they had been treated with tenofovir or entecavir uninterruptedly for 2 years or more with full viral suppression and had liver fibrosis assessment performed within the past 12 months not showing advanced fibrosis (i.e. equivalent to Metavir score <F3; Table S2). For the (few) patients who lacked pre-treatment fibrosis assessment, a more conservative liver stiffness threshold was applied (i.e. equivalent to Metavir score <F2). Patients were excluded if they had a history of decompensated liver disease, previous evidence of cirrhosis or HCC, other active liver disease, co-infection with human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis D virus (HDV), current treatment with immunosuppressive medication, current alcohol consumption >14 (women) or >21 (men) standard units per week, or were pregnant.

2.3 | Interventions

After the initial screening consultation, regular study visits were conducted at inclusion (the time of NA cessation), 4, 8, 12 weeks and thereafter at 3 months intervals until 36 months after NA cessation (Table S3). Compliance with treatment cessation was ensured by the participants handing in all remaining medication to the study personnel at inclusion. In case of a flare, defined as a rise in ALT to >80 U/L from normal levels or a rise in ALT to >2× baseline ALT level, blood tests were repeated at 1–2 weeks intervals until the liver enzymes stabilised or declined. HBsAg, HBV DNA, ALT, aspartate aminotransferase (AST), bilirubin and INR were assessed at all regular study visits and the participants were evaluated according to their allocated re-start strategy and safety criteria.

2.3.1 | Laboratory analysis

HBV DNA and biochemistry analyses were performed locally according to standard laboratory procedures. An ALT level of 40 U/L was considered the upper limit of normal across all sites. Quantitative HBsAg (qHBsAg) analysis was performed at Oslo University Hospital in one batch for all study visits with the Elecsys HBsAg II Quant assay (Roche Diagnostics GmbH, Mannheim, Germany) on the Roche Cobas e801 platform, according to the manufacturer's instructions. The assay is a two-step sandwich chemiluminescent microparticle immunoassay with a limit of detection of 0.05 IU/mL.

2.3.2 | Elastography

Liver elastography was performed at 12, 24 and 36 months using TE (Fibroscan, Echosens, France), ARFI (Siemens AG, Erlangen, Germany), or 2D-SWE (GE Healthcare, Wauwatosa, USA). Established thresholds for fibrosis and cirrhosis were employed (Table S2). Elastography results with concomitant ALT elevations >5× upper limit of normal were discarded as falsely elevated liver stiffness measurements might be observed in this setting.[22]

2.4 | Outcomes

The primary outcome was occurrence of HBsAg loss, defined as qHBsAg below the detection limit, within 36 months after stopping NA therapy.

Secondary outcomes were: (i) time to HBsAg loss, (ii) time to restart of antiviral therapy, (iii) liver fibrosis progression, (iv) occurrence of cirrhosis, (v) occurrence of virological relapse, (vi) occurrence of clinical relapse and (vii) sustained off-therapy virological response, defined as HBV DNA < 2000 IU/mL and ALT < 40 U/L in the absence of NA therapy at all study visits during the third year (i.e. at 27, 30, 33 and 36 months after NA withdrawal). Post-hoc exploratory outcomes were time to first virological and clinical relapse.

2.4.1 | Safety evaluations

All patients were evaluated for unintended medical events (UME) or serious UME (SUME) at each visit. Detailed definitions of UME and SUME are given in the SAP.

2.5 | Sample size

Sample size calculation was done for the primary endpoint of the study (HBsAg loss). Based on previous studies,[12,23] we estimated that HBsAg loss would occur in 20% of those in the high-threshold group (intervention arm) and 1% in the low-threshold group (control arm). With a two-sided two-sample proportions Fisher's exact test the study required a sample size of 120 participants (60 per group) for 90% power and 5% type-I error probability.

2.6 | Randomisation and masking

Study participants were allocated to the two groups in a 1:1 ratio by a computer randomisation procedure stratified by region (Scandinavia vs. Ethiopia). The randomisation was blocked within each stratum.

The study design did not enable blinding of study participants or healthcare providers, but the laboratory staff performing the qHBsAg analysis were masked to group allocation. Furthermore, data review and pre-specification and programming of analyses were done without knowledge of group allocation. After signing of the SAP and

locking the trial database, the group allocation was included in the analysis.

2.7 | Statistical methods

There was only one identified primary analysis in this trial and no adjustment for multiple testing was performed. Trial populations included the full analysis set (FAS) defined as all patients randomly assigned to a treatment group, who met the study eligibility criteria, the safety analysis set (SAS) including all randomised patients who stopped taking antiviral treatment and the per protocol set (PPS) including all randomised patients meeting the study eligibility criteria and without major protocol deviations.

The primary null hypothesis was that there was no difference in the proportion with HBsAg loss between the high-threshold and the low-threshold group. The effect measure was the difference between the probability of having HBsAg loss in the two groups using Agresti-Min exact unconditional 95% confidence interval (CI).[24] The *p*-value was calculated with the Suissa-Shuster exact unconditional test.[25] Missing data were imputed with the last observation carried forward (LOCF, worst-case scenario). Sensitivity analyses of the primary endpoint included: (1) restricting the primary analysis to the PPS; (2) missing data imputed with best case scenario; (3) risk difference, 95% CI and *p*-value calculated based on the stratified Mantel–Haenszel estimate (stratified for the randomisation factor region) and Wald interval (with FAS and LOCF) and (4) exact logistic regression adjusted for the randomisation factor region, to report the odds ratio (OR) with 95% CI and *p*-value for the intervention effect between high and low-threshold group (with FAS and LOCF).

Post-hoc stratification of the primary outcome and sustained virological response by baseline HBV genotype (B/C vs. A/D/E/unknown) was performed. Post-hoc significance test of the primary outcome was performed in participants with baseline qHBsAg < 1000 IU/mL using the primary analysis method. Secondary analyses and descriptive statistics are detailed in the SAP.

2.8 | Ethical considerations

All study participants signed informed consent prior to any study-related assessments or procedures. The study was approved by the National Ethics Committees and the Medical Agencies of all participating countries and the Data Protection Officials of all participating study sites. The study was conducted according to the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice.

2.9 | Role of the funding source

The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

3 | RESULTS

3.1 | Study setting and participants

A total of 127 patients were included between November 1st, 2018 and January 31st, 2020. All study participants stopped taking antiviral therapy at inclusion and were randomised to either the low-threshold ($n = 64$) or the high-threshold ($n = 63$) group for restart of antiviral therapy (Figure 1).

Overall, 86 patients (67.7%) were men and the median age was 43 years (range: 25–66). The majority was taking tenofovir ($n = 97$; 76.4%) and the median duration of antiviral treatment prior to inclusion was 45 months (interquartile range: 32–76). There were no major differences between the two re-treatment groups (Table 1).

Three patients in the low-threshold group and four patients in the high-threshold group did not complete 36 months of follow-up due to patient withdrawal ($n = 3$), pregnancy ($n = 3$) or moving abroad ($n = 1$). In addition, six patients (three in each group) did not re-start antiviral therapy according to protocol and were excluded from the PPS (Figure 1).

3.2 | Primary outcome

Eleven patients (8.7%) reached the primary outcome (HBsAg loss) within 36 months of follow-up. The primary analysis showed no statistically significant difference in HBsAg loss between the low-threshold (3 of 64; 4.7%) and high-threshold (8 of 63; 12.7%) group in the full analysis set (risk difference: 8.0%; 95% CI: −2.3 to 19.6; $p = 0.123$; Figure 2A). This finding remained when restricting the analysis to the per protocol set (risk difference: 9.1%; 95% CI: −2.2 to 21.8; $p = 0.111$) and in the other predefined sensitivity analyses (Table 2). Compared to the low-threshold group, the high-threshold group had an odds ratio of 3.0 (95% CI: 0.7–18.2; $p = 0.193$) for HBsAg loss. None of the patients who restarted therapy achieved HBsAg loss in either of the groups.

The proportion with HBsAg loss appeared to be contingent on qHBsAg level at the time of NA withdrawal. In patients with end-of-treatment qHBsAg < 100, 100–999 and ≥1000 IU/mL the proportion with HBsAg loss was 67.7%, 10.3% and 0%, respectively. The corresponding proportion was 50.0%, 0% and 0% in the low-threshold group and 83.3%, 33.3% and 0% in the high-threshold group (Figure 2B). However, the absolute number of patients with qHBsAg < 100 IU/mL at the time of NA withdrawal was low (Table 2).

In a post-hoc analysis of study participants with end-of-treatment qHBsAg level < 1000 IU/mL, there seemed to be a higher chance of HBsAg loss in the high-threshold group compared to the low-threshold group: 8 of 15 (53.3%) lost HBsAg in the high-threshold group compared to 3 of 26 (11.5%) in the low-threshold group (risk difference: 41.8%; 95% CI: 12.7–66.8; $p = 0.004$).

HBV genotype also appeared to be associated with HBsAg loss. Only one of 35 patients (2.9%) with genotype B and C (the dominating strains in East Asia) achieved HBsAg loss, compared

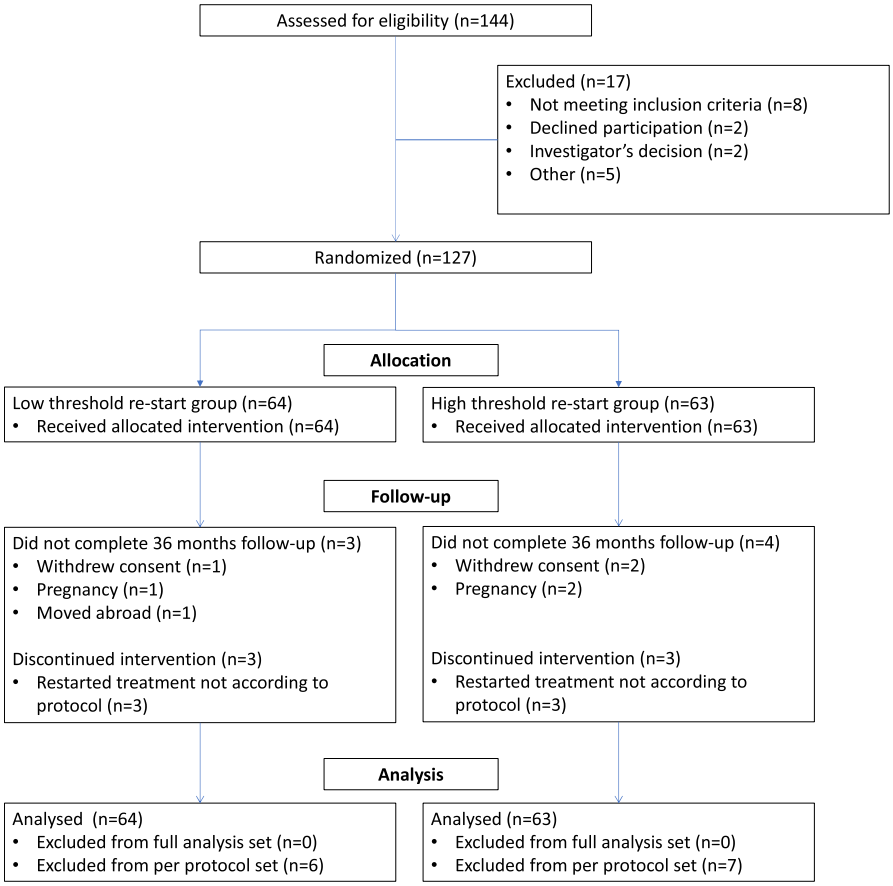

FIGURE 1 CONSORT diagram demonstrating enrolment, allocation, follow-up, and analysis of study patients.

to 10 of 92 (10.9%) with other genotypes (Figure 2C), although the mean end-of-treatment qHBsAg was comparable between the groups (genotype B/C: 3.0 log10 IU/mL vs. other genotypes: 3.2 log10 IU/mL). The type of antiviral medication was not associated with HBsAg loss: 8 of 97 (8.2%) who stopped tenofovir achieved HBsAg loss, compared to 3 of 30 (10.0%) who stopped entecavir (Figure 2D); the mean end-of-treatment qHBsAg was comparable between the groups (tenofovir: 3.1 log10 IU/mL vs. entecavir: 3.3 log10 IU/mL).

3.3 | Secondary outcomes

There was no significant difference in time from NA withdrawal to HBsAg loss between the groups (high-threshold vs. low-threshold group; hazard ratio: 2.87; 95% CI: 0.76–10.84; $p = 0.119$) (Figure 3).

As expected, restart of therapy was more frequent in the low-threshold group: 27 (43.5%) patients in the low-threshold group and 10 (16.1%) in the high-threshold group restarted treatment within 36 months of follow-up. Similarly, the time from NA withdrawal to restart of antiviral therapy was shorter in the low-threshold group and the majority restarted within the first year after treatment withdrawal (Figure S1).

Overall, 110 (86.6%) study participants had virological relapse (HBV DNA > 2000 IU/mL) within 36 months of follow-up; the median time to first virological relapse was 2.1 months (Figure S2A). A total of 51 (40.2%) study participants had clinical relapse (HBV DNA > 2000 IU/mL and ALT > 80 U/L) during follow-up (Figure 3A). Time to first virological and clinical relapse was similar in the low-threshold and high-threshold group. Patients who stopped tenofovir had earlier virological and clinical relapse compared to those who stopped entecavir (Figures S2B and S3B). The proportion of study participants with HBsAg loss, restart of therapy, virological relapse and clinical relapse at each follow-up visit is shown in Figure 4.

Sustained off-therapy virological response was assessed in 120 patients who completed 36 months of follow-up. Overall, 37 patients (30.8%) had sustained off-therapy virological response, which included 10 of the 11 patients with HBsAg loss (one patient had ALT > 40 U/L and thus did not meet the pre-specified definition). No difference was observed between the two groups: 20 of 61 (32.8%) achieved sustained off-therapy response in the low-threshold group and 17 of 59 (28.8%) in the high-threshold group (odds ratio for high-threshold group: 0.81; 95% CI: 0.38–1.75; $p = 0.593$) (Table S4).

There was no difference between the groups with respect to liver fibrosis progression or occurrence of cirrhosis (Table S5).

3.4 | Safety and adverse events

In the low-threshold group, 40 patients (62.5%) reported a total of 87 UMEs, of whom nine (14.1%) were classified as SUMEs. In the

TABLE 1 Baseline characteristics of study participants ($N = 127$).

	Low threshold restart group ($N = 64$)	High threshold restart group ($N = 63$)
Age (years)		
Mean (SD)	45.7 (9.6)	42.7 (9.4)
Body mass index (kg/m^2]		
Mean (SD)	24.7 (3.7)	24.8 (4.5)
Quantitative HBsAg ($\log_{10}$ IU/mL)		
Mean (SD)	3.2 (0.9)	3.2 (1.0)
Quantitative HBsAg level, *n* (%)		
<100 IU/mL	6 (9.4%)	6 (9.5%)
100–999 IU/mL	20 (31.3%)	9 (14.3%)
≥1000 IU/mL	38 (59.4%)	48 (76.2%)
Study region, *n* (%)		
Scandinavia	50 (78.1%)	49 (77.8%)
Ethiopia	14 (21.9%)	14 (22.2%)
Sex, *n* (%)		
Male	41 (64.1%)	45 (71.4%)
Female	23 (35.9%)	18 (28.6%)
Country of birth, *n* (%)		
Africa	23 (35.9%)	29 (46.0%)
Asia	29 (45.3%)	24 (38.1%)
Europe/North America	12 (18.8%)	10 (15.9%)
HBV genotype, *n* (%)		
A	13 (22.0%)	16 (28.6%)
B	11 (18.6%)	6 (10.7%)
C	10 (16.9%)	8 (14.3%)
D	21 (35.6%)	20 (35.7%)
E	4 (6.8%)	6 (10.7%)
Missing	5	7
Antiviral medication, *n* (%)		
Tenofovir[a]	51 (79.7%)	46 (73.0%)
Entecavir	13 (20.3%)	17 (27.0%)
Harmful alcohol consumption, *n* (%)		
No	64 (100.0%)	63 (100.0%)
Diabetes, *n* (%)		
No	60 (93.8%)	59 (93.7%)
Yes	4 (6.3%)	4 (6.3%)
Liver fibrosis stage at NA withdrawal, *n* (%)		
F0/F1	58 (96.7%)	55 (91.7%)
F2	2 (3.3%)	5 (8.3%)
Missing	4	3
Liver fibrosis stage prior to starting NA therapy, *n* (%)		
F0/F1	37 (66.1%)	34 (68.0%)
F2	11 (19.6%)	9 (18.0%)
F3	8 (14.3%)	7 (14.0%)
Missing	8	13

Abbreviations: NA, nucleos(t)ide analogue; SD, standard deviation.

[a]Tenofovir disoproxil fumarate ($N = 90$), tenofovir alafenamide ($N = 6$), tenofovir disoproxil fumarate/emtricitabine ($N = 1$).

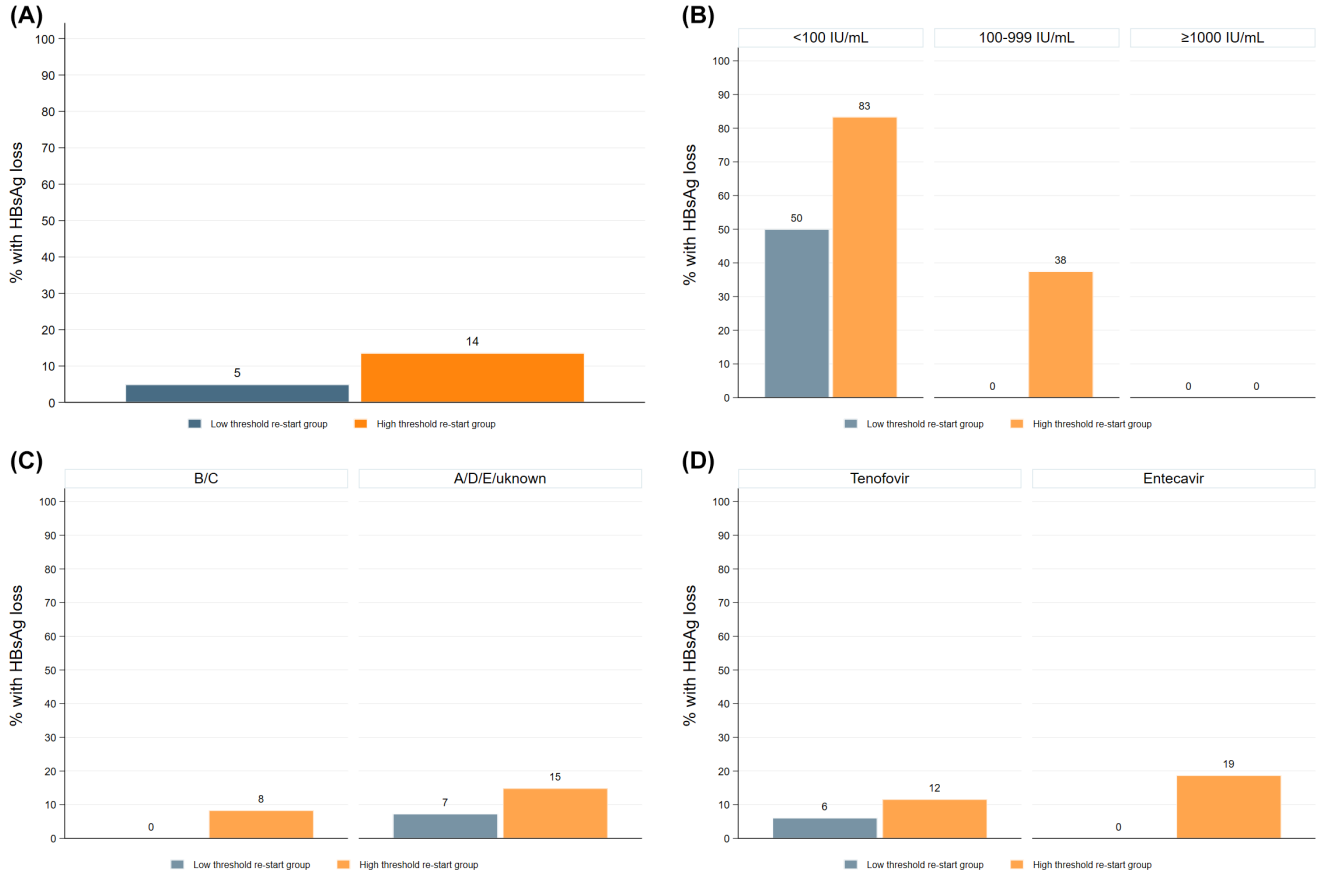

FIGURE 2 HBsAg loss at 36 months. Proportion of patients with HBsAg loss at 36 months according to restart group: (A) Total study population ($n = 127$). (B) Stratified by HBsAg level at end-of-treatment grouped into <100 IU/mL ($n = 12$), 100–1000 IU/mL ($n = 29$), and >1000 IU/mL ($n = 86$). (C) Stratified by HBV genotype (B/C [$n = 35$] vs. A/D/E/unknown [$n = 92$]). (D) Stratified by antiviral medication prior to treatment cessation (tenofovir [$n = 90$] vs. entecavir [$n = 30$]).

high-threshold group, 34 patients (54.0%) reported 89 UMEs, 16 (25.4%) of whom were classified as SUMEs. Tables S6–S11 summarise all UMEs and SUMEs reported.

Twelve patients (9.4%) restarted therapy after meeting the absolute safety criteria defined in the protocol, six in the low-threshold group and six in the high-threshold group. Ten of these patients restarted because of ALT > 800 U/L (maximum ALT: 2600 U/L), one because of elevated bilirubin >38 mmol/L in two consecutive samples and ALT > 80 U/L at the confirmatory test and one because of high INR ≥ 1.4 in two consecutive samples and ALT > 80 U/L at the confirmatory test. Table S12 summarises the characteristics of patients with an ALT flare >800 U/L. Notably, all severe flares occurred in patients who stopped tenofovir and all but one occurred within 4 months of treatment withdrawal. In all patients, ALT declined to normal levels and HBV DNA was fully suppressed after restart of NA therapy (Figure S4) and none developed signs or symptoms of decompensated liver disease.

INR ≥ 1.4 was defined *a priori* as a SUME and was reported in 8 (12.5%) and 12 (19.0%) patients in the low-threshold and high-threshold group, respectively. However, most ($N = 17$) were observed at the Ethiopian study site where the average INR was higher, both at the baseline and follow-up and thus not a manifestation of liver dysfunction; only one of these events was accompanied by elevated ALT and seen by the investigator as possibly linked to liver disease.

One patient in the high-threshold arm was diagnosed with HCC during follow-up. This was an Asian man in his late 50s with a family history of HCC, who restarted treatment 2 months after NA withdrawal because of an ALT flare to >800 U/L. His liver enzymes quickly normalised but 17 months later a liver mass (diameter: 2.8 cm) was detected on routine imaging. He underwent liver resection and immunotherapy without signs of recurrence to date, 39 months after resection.

3.5 | Sub-group analyses

No formal sub-group analysis was performed for the primary outcome because of low number of participants experiencing the outcome, in agreement with the SAP.

Sub-group analysis for sustained off-therapy virological response showed an association with end-of-treatment qHBsAg level: 67.7%, 44.8% and 18.6% achieved sustained off-therapy virological response in patients with qHBsAg < 100, 100–999 and ≥1000 IU/mL, respectively. The corresponding proportion was 67.7%, 50.0% and 15.8% in the low-threshold group and 67.7%, 33.3% and 20.8% in the high-threshold group. Sustained off-therapy virological response was seen in 11.4% of patients with HBV genotype B and C

TABLE 2 Effect of low-threshold vs. high-threshold for treatment restart on HBsAg loss.

	N (%) with HBsAg loss			
	Low threshold group (*N* = 64)	**High threshold group (*N* = 63)**	**Risk difference (95% CI)**	***p*-value**
Primary analysis[a]	3 (4.7%)	8 (12.7%)	8.0% (−2.3 to 19.6)	0.123
Analysis restricted to the PPS[b]	3 (5.2%)	8 (14.3%)	9.1% (−2.2 to 21.8)	0.111
Best case imputation[c]	6 (9.4%)	12 (19.1%)	9.7% (−0.3 to 22.8)	0.127
Stratified analysis[d]				
Scandinavia	3 (6.0%)	7 (14.3%)	8.0% (−1.6 to 17.7)	0.103
Ethiopia	0 (0%)	1 (7.1%)		
			OR (95% CI)	*p* value
Exact logistic regression[e]				0.193
Low-threshold group			1.0	
High-threshold group			3.0 (0.7 to 18.2)	

Abbreviations: CI, confidence interval; OR, odds ratio; PPS, per protocol set.

[a]Difference between the probability of having HBsAg loss in the two groups using Agresti-Min exact unconditional 95% CI and *p*-value calculated with the Suissa-Shuster exact unconditional test. Missing values imputed with last observation carried forward.

[b]Same as primary analysis but restricted to the per protocol set ($n = 114$).

[c]Same as primary analysis but missing imputed with having HBsAg loss.

[d]Stratified Mantel–Haenszel estimate and Wald interval. Missing values imputed with last observation carried forward.

[e]Odds ratio, 95% CI and *p*-value calculated from exact logistic regression adjusted for region. Missing values imputed with last observation carried forward.

compared to 35.9% with other genotypes; and in 32.0% of patients who stopped tenofovir compared to 20.0% of those who stopped entecavir (Figure S5). No significant interaction was observed in the subgroup analyses by qHBsAg level, HBV genotype or type of antiviral therapy (Table S4).

4 | DISCUSSION

In this investigator-initiated, multicentre, randomised controlled trial, we investigated whether a high-threshold versus a low-threshold for restarting treatment after NA withdrawal in patients with HBeAg negative CHB was associated with an increased probability of functional cure, defined as achieving HBsAg loss. Although our results strengthen this hypothesis, we were unable to confirm it at the prespecified significance level.

Overall, HBsAg loss was less common in our study (8.7% after 36 months) than we anticipated. We based our sample size calculation on the much-cited study by Hadziyannis and colleagues where 8 of 33 patients (24.2%) lost HBsAg after 36 months[12] and the prospective FINITE study where 4 of 21 patients (19.0%) lost HBsAg after 36 months.[23] Recent studies, however, report more modest rates of HBsAg loss. The German STOP-NUC trial reported 10.1% HBsAg loss after 24 months[26] and a retrospective multicentre study with 1552 participants reported that 10.4% had HBsAg loss after 36 months of follow-up – in line with our findings.[17] Further investigations of the hypothesis that a prolonged flare promotes HBsAg loss should take recent information (including our findings) into consideration when calculating the required sample size.

We found that all cases of HBsAg loss occurred in those with end-of-treatment qHBsAg <1000 IU/mL. Among those with HBsAg levels <1000 IU/mL, 8 of 15 (53%) lost HBsAg in the high-threshold group compared to 3 of 26 (12%) in the low-threshold group. This resonates with the retrospective RETRACT-B study, where low end-of-treatment qHBsAg was the strongest predictor of HBsAg loss, with a 50 times higher chance of HBsAg loss in those with qHBsAg < 100 IU/mL compared to >1000 IU/mL.[17] Furthermore, in a systematic review which included 11 observational studies with 1716 patients from Asia, end-of-treatment qHBsAg < 100 IU/mL was associated with 21.1%–58.8% chance of subsequent HBsAg loss compared to 0.0% in those with qHBsAg > 1000 IU/mL.[27] Further studies of optimal stop and restart strategies, therefore, should consider including only patients with end-of-treatment HBsAg level <1000 IU/mL.

Although only 8.7% achieved HBsAg loss in our study, it is worth noting that almost one third of patients who stopped NA therapy had sustained off-therapy response, defined as viral load <2000 IU/

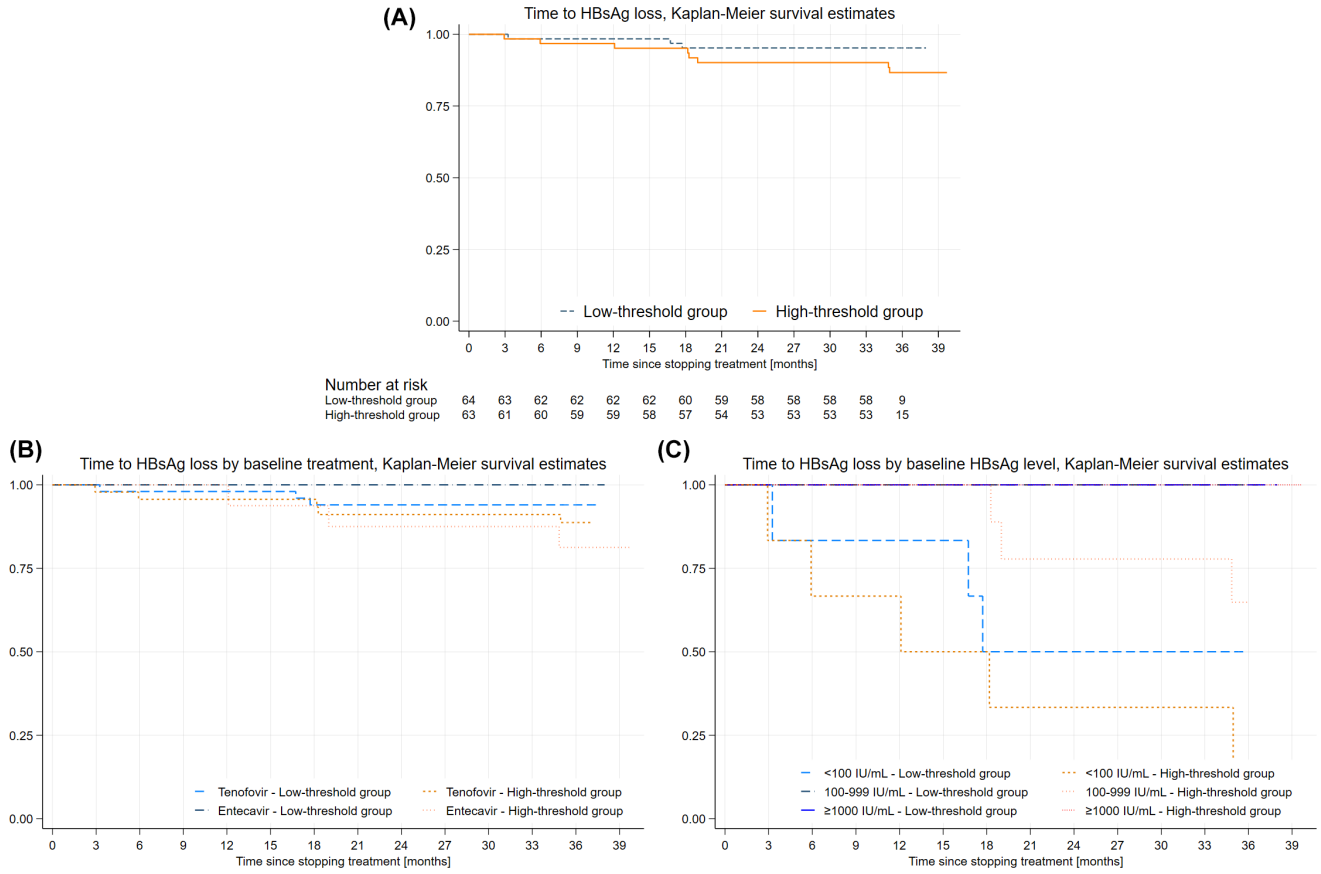

FIGURE 3 Time to HBsAg loss. Kaplan-Meier curve showing time to HBsAg loss for the two restart groups. (A) Total study population ($n = 127$). (B) Stratified by antiviral medication prior to treatment cessation (tenofovir [$n = 90$] vs. entecavir [$n = 30$]). (C) Stratified by HBsAg level at end-of-treatment grouped into <100 IU/mL ($n = 12$), 100–1000 IU/mL ($n = 29$) and >1000 IU/mL ($n = 86$).

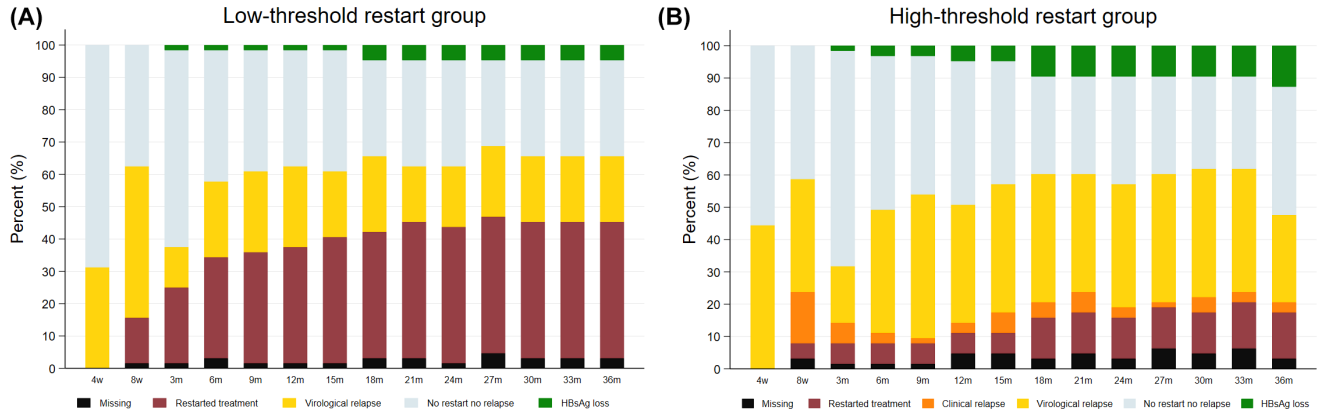

FIGURE 4 HBsAg loss, virological relapse, clinical relapse and restart of treatment during 36 months follow-up for low-threshold (A) and high-threshold (B) restart groups. Diagrams showing the different outcomes: HBsAg loss (green); virological relapse defined as HBV DNA > 2000 IU/mL and ALT < 80 U/L (yellow); clinical relapse defined as HBV DNA > 2000 IU/mL and ALT > 80 U/L (orange); restarted treatment (burgundy); off-therapy HBV DNA < 2000 IU/mL (grey); and missing data (black) at each time point.

mL and normal ALT at all measurements during the third study year. This compares well with the German STOP-NUC trial where clinical remission was reported in 41% of study participants.[26] These patients can be classified as HBeAg negative HBV infection (previously 'inactive carriers'), a group considered to have a beneficial prognosis without antiviral therapy.[28] Thus, even patients without HBsAg loss might benefit from NA withdrawal in terms of avoiding lifelong NA therapy.

In the current trial 7.1% experienced a severe flare, defined as ALT increase to >800 U/L. The peak ALT activity observed was 2600 U/L; however, bilirubin increased slightly only in one patient. All patients with a flare responded quickly to restart of NA therapy with normalisation of ALT and bilirubin and full viral suppression. Although we took measures not to enrol patients with cirrhosis – who are at increased risk of hepatic flares and decompensation following treatment withdrawal – it should be noted that 21 patients lacked pre-treatment fibrosis assessment and we cannot rule out that some had subclinical cirrhosis even though liver stiffness at enrolment in the trial was normal.[29] Notably, with the monitoring and retreatment strategy employed in the present study, none developed hepatic decompensation. The frequency of severe flares in our study is in line with a recent systematic review and meta-analysis reporting severe hepatic flares or hepatic decompensation after NA withdrawal in 0.89% of non-cirrhotic HBV patients.[30] Of concern, the same meta-analysis reported liver related death or liver transplantation in 0.3% of non-cirrhotic HBV patients following NA withdrawal. Taken together with the recent report of subacute liver failure shortly after NA withdrawal in the REEF-2 trial,[31] this calls for caution and underscores the need for reliable predictors of outcome rather than seeing NA withdrawal as a 'one-size-fits-all' intervention.

We observed one case of HCC during the follow-up period of the present study, in a person without past or current evidence of cirrhosis. At the time of the detection, 17 months after a brief stop of NA, the tumour diameter was 2.8 cm. Considering that the doubling time of HCC lesions is on average 4–5 months,[32] it was deemed unlikely that the development of cancer was related to NA withdrawal. Instead, the tumour detection was considered a result of closer monitoring within the framework of a clinical trial.

There were some weaknesses of the present study. First, our *a priori* estimate of HBsAg loss was higher than observed and higher than reported in studies published after the initiation of our trial and therefore the study might have been underpowered. Second, the study was open-label and conducted with a hypothesis that a high-threshold for restarting NA therapy was beneficial. Thus, we cannot exclude that knowing the allocation might have influenced the clinical decisions and caused deviations from the study protocol. Third, we did not have data on qHBsAg evolution prior to stopping NA therapy and thus cannot exclude that some patients already had a decline towards HBsAg loss; however, previous studies have shown that HBsAg decline is very slow and spontaneous HBsAg loss rarely occurs in persons on stable NA therapy.[11,26]

The main strength of our study was that it was the first randomised, controlled trial testing the hypothesis that a powerful flare after NA withdrawal may be beneficial. Moreover, we had few drop-outs and we included patients from different geographic locations and of various ethnic origin with a corresponding spread in HBV genotypes, making our findings more generalisable.

In conclusion, we could not confirm our hypothesis that a higher threshold for restart of therapy after NA withdrawal increases the likelihood of HBsAg loss in non-cirrhotic patients with HBeAg negative CHB. There was, however, a numerical difference between the two arms and further studies including only patients with end-of-treatment qHBsAg < 1000 IU/mL and/or larger sample size and longer follow-up duration are recommended.

AUTHOR CONTRIBUTIONS

Asgeir Johannessen: Conceptualization; investigation; funding acquisition; writing – original draft; validation; visualization; project administration; methodology; data curation; supervision; resources. **Dag Henrik Reikvam:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; project administration; data curation; supervision; resources. **Soo Aleman:** Writing – review and editing; investigation. **Nega Berhe:** Investigation; writing – review and editing. **Nina Weis:** Investigation; writing – review and editing. **Hailemichael Desalegn:** Investigation; writing – review and editing. **Tore Stenstad:** Investigation; writing – review and editing. **Lars Heggelund:** Investigation; writing – review and editing. **Ellen Samuelsen:** Investigation; writing – review and editing. **Lars Normann Karlsen:** Investigation; writing – review and editing. **Karin Lindahl:** Investigation; writing – review and editing. **Frank Olav Pettersen:** Investigation; writing – review and editing. **Jonas Iversen:** Investigation; writing – review and editing. **Elisabeth Kleppa:** Investigation; writing – review and editing. **Signe Bollerup:** Investigation; writing – review and editing. **Anni Assing Winckelmann:** Investigation; writing – review and editing. **Pascal Brugger-Synnes:** Investigation; writing – review and editing. **Hans Erling Simonsen:** Investigation; writing – review and editing. **Jan Svendsen:** Investigation; writing – review and editing. **Anne-Marte Bakken Kran:** Methodology; writing – review and editing. **Marte Holmberg:** Investigation; writing – review and editing. **Inge Christoffer Olsen:** Methodology; formal analysis; writing – review and editing. **Corina Silvia Rueegg:** Methodology; formal analysis; writing – review and editing; software. **Olav Dalgard:** Conceptualization; methodology; writing – original draft; investigation; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

DHR has received a grant from Gilead, unrelated to this work. SA has received honoraria for lectures and educational events from Gilead, AbbVie, MSD and Biogen and reports grants from Gilead and AbbVie, not related to this work. OD has received honoraria for lectures and educational events from Gilead, AbbVie and MSD and reports grants from Gilead not related to this work. All other authors declare that no competing interests exist.

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REFERENCES

1. Polaris Observatory Collaborators. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modelling study. Lancet Gastroenterol Hepatol. 2023;8(10):879–907.
2. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013;381(9865):468–75.
3. Kim WR, Loomba R, Berg T, Aguilar Schall RE, Yee LJ, Dinh PV, et al. Impact of long-term tenofovir disoproxil fumarate on incidence of hepatocellular carcinoma in patients with chronic hepatitis B. Cancer. 2015;121(20):3631–8.
4. Dandri M, Petersen J. cccDNA maintenance in chronic hepatitis B – Targeting the matrix of viral replication. Infect Drug Resist. 2020;13:3873–86.
5. Wong GL, Seto WK, Wong VW, Yuen MF, Chan HL. Review article: long-term safety of oral anti-viral treatment for chronic hepatitis B. Aliment Pharmacol Ther. 2018;47(6):730–7.
6. Grossi G, Vigano M, Loglio A, Lampertico P. Hepatitis B virus long-term impact of antiviral therapy nucleot(s)ide analogues (NUCs). Liver Int. 2017;37(Suppl 1):45–51.
7. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006;119:71.e9–71.e16.
8. Yip TC, Wong GL, Chan HL, Tse YK, Lam KL, Lui GC, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol. 2019;70(3):361–70.
9. Ghany MG, Buti M, Lampertico P, Lee HM, 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference Faculty. Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in chronic hepatitis B and D: report from the 2022 AASLD-EASL HBV-HDV Treatment Endpoints Conference. J Hepatol. 2023;79(5):1254–69.
10. Kim GA, Lim YS, An J, Lee D, Shim JH, Kim KM, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut. 2014;63(8):1325–32.
11. Hsu YC, Yeh ML, Wong GL, Chen CH, Peng CY, Buti M, et al. Incidences and determinants of functional cure during entecavir or tenofovir disoproxil fumarate for chronic hepatitis B. J Infect Dis. 2021;224(11):1890–9.
12. Hadziyannis SJ, Sevastianos V, Rapti I, Vassilopoulos D, Hadziyannis E. Sustained responses and loss of HBsAg in HBeAg-negative patients with chronic hepatitis B who stop long-term treatment with adefovir. Gastroenterology. 2012;143(3):629–636.e1.
13. Hall SAL, Vogrin S, Wawryk O, Burns GS, Visvanathan K, Sundararajan V, et al. Discontinuation of nucleot(s)ide analogue therapy in HBeAg-negative chronic hepatitis B: a meta-analysis. Gut. 2022;71(8):1629–41.
14. Buti M, Wong DK, Gane E, Flisiak R, Manns M, Kaita K, et al. Safety and efficacy of stopping tenofovir disoproxil fumarate in patients with chronic hepatitis B following at least 8 years of therapy: a prespecified follow-up analysis of two randomised trials. Lancet Gastroenterol Hepatol. 2019;4(4):296–304.
15. Jeng WJ, Chen YC, Chien RN, Sheen IS, Liaw YF. Incidence and predictors of hepatitis B surface antigen seroclearance after cessation of nucleos(t)ide analogue therapy in hepatitis B e antigen-negative chronic hepatitis B. Hepatology. 2018;68(2):425–34.
16. Garcia-Lopez M, Lens S, Pallett LJ, Testoni B, Rodríguez-Tajes S, Mariño Z, et al. Viral and immune factors associated with successful treatment withdrawal in HBeAg-negative chronic hepatitis B patients. J Hepatol. 2021;74(5):1064–74.
17. Hirode G, Choi HSJ, Chen CH, Su TH, Seto WK, Van Hees S, et al. Off-therapy response after nucleos(t)ide analogue withdrawal in patients with chronic hepatitis B: an international, multicenter, multiethnic cohort (RETRACT-B Study). Gastroenterology. 2022;162(3):757–771 e754.
18. Sonneveld MJ, Chiu SM, Park JY, Brakenhoff SM, Kaewdech A, Seto WK, et al. Probability of HBsAg loss after nucleo(s)tide analogue withdrawal depends on HBV genotype and viral antigen levels. J Hepatol. 2022;76(5):1042–50.
19. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67(2):370–98.
20. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10(1):1–98.
21. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology. 2016;63(1):261–83.

22. Huang LL, Yu XP, Li JL, et al. Effect of liver inflammation on accuracy of FibroScan device in assessing liver fibrosis stage in patients with chronic hepatitis B virus infection. World J Gastroenterol. 2021;27(7):641–53.
23. Berg T, Simon KG, Mauss S, Schott E, Heyne R, Klass DM, et al. Long-term response after stopping tenofovir disoproxil fumarate in non-cirrhotic HBeAg-negative patients - FINITE study. J Hepatol. 2017;67(5):918–24.
24. Agresti A, Min Y. On small-sample confidence intervals for parameters in discrete distributions. Biometrics. 2001;57(3):963–71.
25. Suissa S, Shuster JJ. Exact unconditional sample sizes for the 2 ×2 binomial trial. J R Stat Soc Ser A. 1985;148(4):317–27.
26. van Bommel F, Stein K, Heyne R, Petersen J, Buggisch P, Berg C, et al. A multicenter randomized-controlled trial of nucleos(t)ide analogue cessation in HBeAg-negative chronic hepatitis B. J Hepatol. 2023;78(5):926–36.
27. Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos(t)ide analogues cessation among asian patients with chronic hepatitis B: a systematic review. Hepatology. 2019;70(3):1045–55.
28. Kumada T, Toyoda H, Yasuda S, Ito T, Tanaka J. Mortality of inactive hepatitis B virus carriers in Japan is similar to that of the general population. Hepatol Res. 2022;52(1):81–92.
29. Dong XQ, Wu Z, Li J, Wang GQ, Zhao H, China HepB-Related Fibrosis Assessment Research Group. Declining in liver stiffness cannot indicate fibrosis regression in patients with chronic hepatitis B: a 78-week prospective study. J Gastroenterol Hepatol. 2019;34(4):755–63.
30. Tseng CH, Chen TH, Wu JL, Lee TY, Borghi JA, Lin JT, et al. Serious adverse events after cessation of nucleos(t)ide analogues in individuals with chronic hepatitis B: a systematic review and meta-analysis. JHEP Rep. 2023;5(1):100617.
31. Agarwal K, Lok J, Carey I, Shivkar Y, Biermer M, Berg T, et al. A case of HBV-induced liver failure in the REEF-2 phase II trial: implications for finite treatment strategies in HBV 'cure'. J Hepatol. 2022;77(1):245–8.
32. Nathani P, Gopal P, Rich N, Yopp A, Yokoo T, John B, et al. Hepatocellular carcinoma tumour volume doubling time: a systematic review and meta-analysis. Gut. 2021;70(2):401–7.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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