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Withdrawal of Long-Term Nucleotide Analog Therapy in Chronic Hepatitis B: Outcomes From the Withdrawal Phase of the HBRN Immune Active Treatment Trial

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INTRODUCTION: **Withdrawal of nucleos(t)ide analog therapy is increasingly being evaluated in chronic hepatitis B infection as a strategy to induce hepatitis B surface antigen (HBsAg) loss. The Hepatitis B Research Network Immune-Active Trial evaluated treatment with tenofovir (TDF) for 4 years ± an initial 6 months of peginterferon-α (PegIFN) (NCT01369212) after which treatment was withdrawn.**

METHODS: **Eligible participants (hepatitis B e antigen [HBeAg]−/anti-HBe+, hepatitis B virus [HBV] DNA <10^3 IU/mL, no cirrhosis) who discontinued TDF were followed for at least 1 year with optional follow-up thereafter. Retreatment was based on predefined criteria.**

RESULTS: **Among 201 participants who received 4 years of treatment, 97 participants (45 TDF and 52 TDF + PegIFN arm, 79 Asian) discontinued TDF. HBsAg loss occurred in 5 participants, 2 within 25 weeks and 3 within 89–119 weeks postwithdrawal (cumulative rate 4.3% by 2 years). Alanine aminotransferase (ALT) flares (>5× upper limit of normal) after TDF withdrawal occurred in 36 (37.1%) participants and occurred more frequently and earlier in those HBeAg− compared with HBeAg+ at treatment initiation. ALT flares were associated with older age and higher HBV DNA pretreatment and at the visit before the flare. ALT flares were not significantly associated with HBsAg decline or loss but were associated with immune active disease at 1 year (70.6% vs 11.9%, $P < 0.0001$) and 2 years (66.7% vs 25.9%, $P = 0.03$) postwithdrawal. Treatment reinitiation was required in 13 (13.4%) participants, and 13 others remained in a sustained inactive carrier state by the end of the study follow-up. No criteria reliably predicted safe treatment withdrawal.**

DISCUSSION: **Results from this trial do not support TDF withdrawal as a therapeutic strategy. HBsAg loss was infrequent within 2 years of stopping long-term TDF. If withdrawal is considered, HBV DNA should be carefully monitored with reinitiation of therapy if levels rise above 4 $\log_{10}$IU/mL to reduce the risk of ALT flares, as they were not associated with subsequent HBsAg decline or loss.**

KEYWORDS: ALT flare; treatment withdrawal; HBsAg loss; inactive carrier

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/C856, http://links.lww.com/AJG/C857, http://links.lww.com/AJG/C858, and http://links.lww.com/AJG/C859

Am J Gastroenterol 2023;118:1226–1236. https://doi.org/10.14309/ajg.0000000000002176

INTRODUCTION

The mainstay of treatment for chronic hepatitis B virus (HBV) infection is long-term treatment with nucleos(t)ide analogs (NAs). NAs are well tolerated and lead to potent suppression of viral replication, resulting in undetectable levels of HBV DNA in most treated patients, which is associated with fewer clinical outcomes. However, long-term

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Received September 15, 2022; accepted November 21, 2022; published online January 13, 2023

NA treatment rarely leads to clearance of hepatitis B surface antigen (HBsAg), which is referred to as functional cure and is considered the primary goal of therapy because of its association with excellent long-term prognosis (1). A small, uncontrolled cohort study reported a high rate of HBsAg loss after withdrawal of long-term NA therapy leading to interest in this as an approach to induce HBsAg loss (2).

Studies of NA withdrawal have consisted of large retrospective cohorts (3–5) and relatively small prospective studies (6,7). Outcomes have varied with differing rates of HBsAg loss and variable incidence of virological and clinical relapse, often necessitating reinstitution of NA treatment. Variability in outcomes of NA withdrawal likely relates to differences in the treated populations in terms of age, ethnicity, HBV genotype, hepatitis B e antigen (HBeAg) status pretreatment, indications for retreatment, and possibly other factors (3,8–10). Understanding the outcomes after NA withdrawal is particularly important both for current clinical management and to guide development of finite curative strategies for chronic HBV infection. We evaluated the outcome of NA withdrawal in the Hepatitis B Research Network (HBRN) Immune Active (IA) Trial that randomized participants in the immune active phase of chronic HBV to 4 years of tenofovir disoproxil fumarate (TDF) therapy with or without an initial 6 months of weekly peginterferon-α 2a (PegIFN).

METHODS

Participants

The HBRN is a clinical research network funded by the National Institute of Diabetes, Digestive and Kidney Diseases with 21 clinical sites throughout the US and in Toronto, Canada, that enrolled HBsAg-positive adult (≥18 years old) patients not on antiviral therapy into a prospective cohort study or 1 of 2 clinical trials between 2012 and 2017 (11). The IA trial enrolled participants meeting the following criteria: HBeAg positive (HBeAg+) or negative (HBeAg−) with compensated liver disease who had not previously received antiviral therapy for >24 weeks with alanine aminotransferase (ALT) levels >1.5× upper limit of normal (ULN) and HBV DNA >1,000 IU/mL. ALT ULN was defined as 30 U/L for men and 20 U/L for women (12). Participants were randomized to treatment with TDF 300 mg daily for 192 weeks with or without an initial 24 weeks of once-weekly PegIFN 180 μg subcutaneously (NCT01369212). Outcomes at the end of 192 weeks of therapy have been described separately (13).

A major objective of the trial was to assess outcomes after discontinuing TDF at week 192 in eligible participants. Initially, criteria for TDF discontinuation at 192 weeks were the absence of cirrhosis at study entry and HBV DNA <1,000 IU/mL from weeks 168–192 of TDF treatment, irrespective of HBeAg status. However, after 2 participants who were HBeAg positive at the time of TDF withdrawal had severe flares accompanied by jaundice, the protocol was amended to require HBeAg negativity at and after week 144 to qualify for withdrawal. The protocol was further amended to require the presence of anti-HBe at or after week 144 to meet the criteria for treatment discontinuation. Participants who did not meet the withdrawal criteria continued TDF. Participants meeting the withdrawal criteria stopped TDF at week 192 and were evaluated every 4 weeks to week 216 and then every 12 weeks until week 240. Participants who completed week 240 follow-up were offered to continue follow-up in the HBRN observational cohort study with follow-up visits every 24 weeks.

Criteria for retreatment after withdrawal included any of the following: clinical decompensation, impaired liver synthetic function (international normalized ratio >1.3, total bilirubin >1.3 mg/dL, or direct bilirubin >1.0 mg/dL), ALT >1,000 U/L with HBV DNA ≥10,000 IU/mL, ALT >10× ULN with HBV DNA ≥10,000 IU/mL on 3 occasions in a 4-week period, ALT >5× ULN with HBV DNA ≥10,000 IU/mL on 3 occasions over a 12-week period, and HBeAg positivity. Participants meeting the retreatment criteria were offered retreatment with TDF until week 240 and then continued at the discretion of the treating provider. ALT elevations after withdrawal were defined as an increase of ALT >ULN and ALT flares as ALT >5× ULN.

The clinical phenotype was assessed at the end of the study at week 240 (48 weeks post-TDF withdrawal) and the end of follow-up for those with extended follow-up in the HBRN Cohort Study after trial completion. The Inactive Carrier (IC) phenotype was defined as HBeAg− with ALT < ULN and HBV DNA <1,000 IU/mL. The IA phenotype was defined as ALT >2× ULN with HBV DNA >2,000 IU/mL or reinitiation of antiviral therapy. Those with ALT and/or HBV DNA levels in between IC and IA definitions were deemed indeterminant (14).

A sustained IC phenotype at the end of the trial required meeting the IC criteria at both week 228 and week 240. If at least 1 of these 2 visit results met the IA definition, the phenotype was deemed IA; otherwise, if at least 1 of those 2 visit results met the indeterminant criteria, the phenotype was deemed indeterminant. For participants with follow-up beyond 1 year, the same criteria for sustained IC, IA, and indeterminant were used for phenotype determination using the results of the final 2 visits of follow-up. The study schematic is shown in Figure 1. The study protocols were approved by the institutional review boards or research ethics boards (Toronto) of participating institutions, and participants provided written informed consent.

Measures

Demographic (sex, age, self-reported race, and continent of birth), clinical (body mass index, liver enzymes, platelets, history of cirrhosis, presumed mode of HBV transmission, and estimated duration of infection), and virologic (quantitative HBsAg [qHBsAg], qHBeAg, HBV DNA levels, and HBV genotype) characteristics at treatment start and withdrawal were recorded. Quantitative HBeAg and HBsAg (Roche Elecsys) assays were performed at the HBRN central virology laboratory at the University of Washington, with lower limits of detection of 0.3 IU/mL for HBeAg and 0.05 IU/mL for HBsAg. HBV DNA testing was performed centrally by real-time PCR (COBAS Ampliprep/COBAS TaqMan Test, v.2.0; Roche Molecular Diagnostics, Branchburg, NJ) with a lower limit of quantification of 20 IU/mL and a lower limit of detection of 10 IU/mL. In rare instances when central labs were not available, local lab values were used. HBV genotyping was performed at the Molecular Epidemiology and Bioinformatics Laboratory in the Division of Viral Hepatitis at the Centers for Disease Control and Prevention using mass spectrometry as previously described (15,16).

Statistical analysis

Prerandomization (baseline) and prewithdrawal (week 192) participant demographic, clinical, and virological characteristics across the randomized arms were summarized with frequencies and percentages and compared using the χ^2 or Fisher exact test for categorical variables and were summarized with medians and quartiles and compared using the Wilcoxon rank-sum test for continuous variables. Time to HBsAg loss after TDF withdrawal was analyzed using the Kaplan-Meier (KM) method, and results

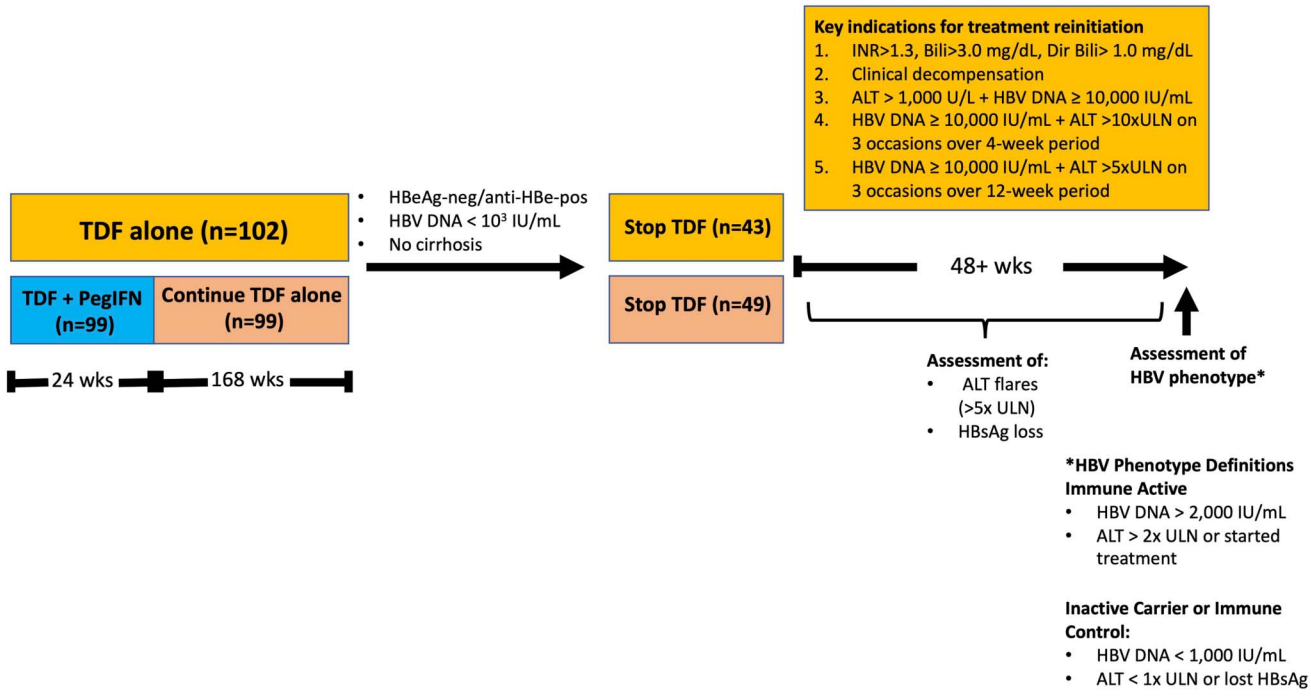

Figure 1. Study schematic. Participants in the Hepatitis B Research Network IA trial were randomized to tenofovir treatment for 4 years with or without an initial 6 months of PegIFN treatment. Those meeting eligibility criteria for treatment withdrawal stopped tenofovir and were followed off therapy. Retreatment criteria are listed, and participants were followed for evaluation of changes in quantitative HBsAg levels, ALT flares, and HBsAg loss off therapy. At the end of the trial (48 weeks after stopping therapy), the phenotype of chronic HBV infection was assessed using the criteria shown for IA and ICs. Participants who did not meet the criteria for IA or IC were deemed indeterminate. ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IA, immune active; IC, inactive carrier; PegIFN, peginterferon-α; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

are reported using estimated percent and corresponding 95% confidence intervals (CIs). Rates of decline in the qHBsAg level between weeks 144–192 (year preceding TDF withdrawal) and weeks 192–240 (1 year post-TDF withdrawal) were determined and compared between withdrawal and nonwithdrawal groups using 2-sample *t* tests.

Time to any ALT elevation and ALT flares were analyzed using KM curves and compared between treatment groups and by pretreatment HBeAg status by log-rank tests. Predictors of ALT flares over time were assessed using generalized linear mixed-effects models, and covariates included pretreatment and prewithdrawal characteristics, concurrent and prior visit HBV DNA and qHBsAg levels along with a subject-specific random intercept to account for the correlation between repeated measures. The results are presented as odds ratios (ORs) with corresponding 95% CIs and *P* values from the Wald test.

To evaluate the association between ALT elevations and subsequent qHBsAg decline, mean qHBsAg decline from the time of withdrawal was modeled using a linear mixed-effects model with ALT elevation at the prior visit as the only covariate along with a random intercept term. Results are reported as the least-square means, 95% CIs with *P* values. Time to treatment reinitiation was analyzed using KM curves, and the association between pretreatment and prewithdrawal characteristics was investigated using the Cox proportional hazard model, with results presented as hazard ratios (HRs) with 95% CIs and *P* values.

The association between sustained IC or IA phenotypes and prewithdrawal characteristics was ascertained using a relative risk regression model, with results reported as relative risks and adjusted relative risks with 95% CIs and *P* values. Variables in the multivariable model were those significant in the univariable regression plus treatment arm, which was included a priori. To analyze the binary outcome of safe withdrawal (no restart of treatment, no ALT flare, and no transition to immune active phase) within 1 year following TDF cessation, a logistic regression model was used with results expressed as odds ratios and 95% CIs.

Missing data were assumed missing at random, a valid approach for the parametric regression models used (linear mixed models, relative risk models, or generalized linear mixed models). KM, log-rank test, and the Cox proportional hazard models appropriately handle random censoring.

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC) and R (R Core Development Team, R Foundation for Statistical Computing, Vienna, Austria). All *P* values reported are 2 sided.

RESULTS

Initial results leading to protocol amendment to exclude TDF withdrawal in HBeAg+ or HBeAg−/anti-HBe− participants

Among 25 participants withdrawn from TDF based on the initial withdrawal criteria, severe, icteric hepatitis flares were observed in the first 2 participants who were HBeAg+ at the time of TDF withdrawal (see Supplementary Figure 2, Supplementary Digital Content 2, http://links.lww.com/AJG/C857). Both participants responded to reinitiation of TDF; however, one required admission to hospital. These severe outcomes in conjunction with results from another contemporary study outside of the HBRN led to the protocol amendment to require participants to be HBeAg−/anti-HBe+ before withdrawal. Accordingly, 6 HBeAg+ participants restarted treatment, and 8 HBeAg−/anti-HBe− participants were advised to restart treatment, of whom only 3 agreed to retreatment.

Table 1. Characteristics of participants before treatment and at treatment withdrawal

	TDF alone (n = 45) n (%)/median (IQR)	TDF + PegIFN (n = 52) n (%)/median (IQR)	All participants (n = 97) N (%)/median (IQR)	*P* value (TDF vs TDF + PegIFN)
Male sex, n (%)	31 (68.9)	33 (63.5)	64 (66.0)	0.66
Age (study entry), yr	45.4 (38.4–55.1)	42.7 (35.2–52.5)	44.0 (37.1–54.2)	0.13
Race/ethnicity				0.85
Asian	35 (79.5)	44 (84.6)	79 (82.3)	
White	4 (9.1)	4 (7.7)	8 (8.3)	
Black	5 (11.4)	4 (7.7)	9 (9.4)	
HBV genotype				0.24
A1	4 (8.9)	3 (5.8)	7 (7.2)	
A2	4 (8.9)	2 (3.8)	6 (6.2)	
B	19 (42.2)	25 (48.1)	44 (45.4)	
C	10 (22.2)	19 (36.5)	29 (29.9)	
D	5 (11.1)	1 (1.9)	6 (6.2)	
E	3 (6.7)	2 (3.8)	5 (5.2)	
At study entry (pretreatment)				
HBeAg+	10 (22.2)	15 (28.8)	25 (25.8)	0.50
HBV DNA (log_{10}IU/mL)	5.6 (4.9–6.6)	5.8 (4.6–6.6)	5.7 (4.7–6.6)	0.92
qHBsAg (log_{10}IU/mL)	3.3 (2.9–4.0)	3.2 (2.8–4.0)	3.2 (2.8–4.0)	0.24
qHBsAg <100 IU/mL	0	2 (3.8)	2 (2.1)	0.50
ALT × ULN	2.7 (2.0–4.8)	2.9 (1.9–4.4)	2.9 (2.0–4.5)	0.97
ALT × ULN				0.53
≤1	0 (0.0)	2 (3.8)	2 (2.1)	
>1–<3	25 (55.6)	25 (48.1)	50 (51.5)	
≥3–<5	9 (20.0)	14 (26.9)	23 (23.7)	
≥5–<10	9 (20.0)	7 (13.5)	16 (16.5)	
≥10	2 (4.4)	4 (7.7)	6 (6.2)	
At treatment withdrawal				
HBeAg+ or HBeAg−/anti-HBe−	4 (8.9)	5 (9.6)	9 (9.3)	>0.99
HBV DNA (log_{10}IU/mL)	0.8 (0.5–1.0)	0.9 (0.6–1.1)	0.8 (0.6–1.1)	0.19
qHBsAg (log_{10}IU/mL)	3.2 (2.6–3.6)	2.8 (2.0–3.4)	2.9 (2.4–3.5)	0.15
qHBsAg <100 IU/mL	5 (11.1)	14 (26.9)	19 (19–6)	0.07
ALT × ULN	1.1 (0.8–1.4)	1.0 (0.8–1.3)	1.1 (0.8–1.3)	0.38
ALT × ULN				0.21
≤1	18 (40.0)	27 (52.9)	45 (46.9)	
>1–<3	26 (57.8)	23 (45.1)	49 (51.0)	
≥3–<5	1 (2.2)	0 (0.0)	1 (1.0)	
≥5–<10	0 (0.0)	1 (2.0)	1 (1.0)	
≥10	0 (0.0)	0 (0.0)	0 (0.0)	

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IQR, interquartile range; PegIFN, peginterferon-α; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

Study population

Of 201 participants in the IA trial, 111 met the initial criteria for TDF withdrawal. The most common reasons for not meeting the withdrawal criteria were persistent HBeAg positivity (n = 33 TDF and n = 25 TDF + PegIFN arms) with a small number with cirrhosis (n = 3 TDF and n = 4 TDF + PegIFN) or who declined to withdraw treatment (n = 3 TDF and n = 1 TDF + PegIFN) (see Supplementary Figure 1, Supplementary Digital Content 1, http://links.lww.com/AJG/C856). Of these 111, 6 participants who were HBeAg+ and 3 who were HBeAg−/anti-HBe− restarted on

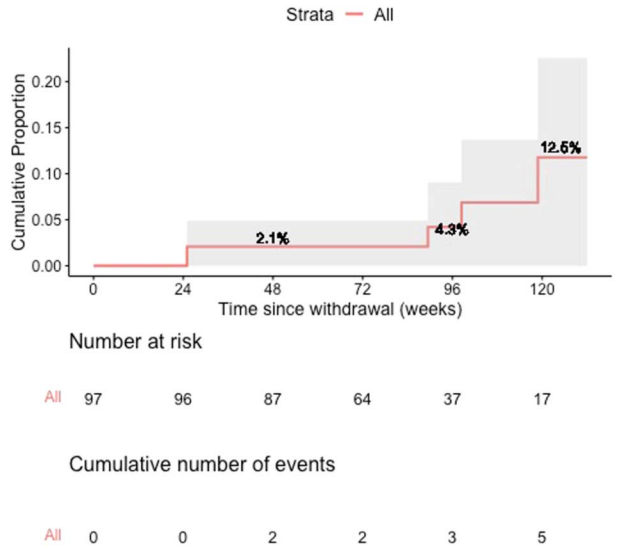

Figure 2. HBsAg loss after withdrawal. Participants were followed off treatment, and the proportion with HBsAg loss over time is indicated. HBsAg, hepatitis B surface antigen.

TDF after the protocol amendment. Of 102 participants who stopped treatment at week 192 and did not resume treatment due to protocol amendment, 5 had sustained HBsAg loss before treatment withdrawal, leaving 97 for this primary TDF withdrawal analysis.

Of these 97, 72 (74.2%) were HBeAg− and 25 (25.8%) were HBeAg+ before enrollment in the treatment trial; 45 (46.4%) had been randomized to TDF alone and 52 (53.6%) to TDF + pegIFN; 64 (66.0%) were male with a median age of 44.0 years, 79 (82.3%) were Asian, 9 (9.4%) were Black, and 8 (8.3%) were White. The predominant HBV genotypes were B and C. The median pretreatment HBV DNA, qHBsAg, and ALT levels were 5.7 $\log_{10}$IU/mL, 3.2 $\log_{10}$IU/mL, and 2.9× ULN, respectively. At TDF withdrawal (week 192), these levels were 0.8 $\log_{10}$IU/mL, 2.9 $\log_{10}$IU/mL, and 1.1× ULN, respectively. Most characteristics were similar between the TDF and the TDF + PegIFN groups; however, more participants in the TDF + PegIFN group (14, 27%) had HBsAg <100 IU/mL at the time of TDF withdrawal than those in the TDF group (5, 11%, $P = 0.07$) (Table 1).

HBsAg changes

HBsAg levels ranged from 0.18 to 5.28 $\log_{10}$IU/mL before treatment withdrawal (week 192). HBsAg loss occurred in 5 (5.2%) participants after TDF withdrawal, 3 from the TDF treatment group and 2 from the TDF + PegIFN treatment group. Two participants lost HBsAg in the first year off treatment, both at 25 weeks postwithdrawal, and had subgenotype A2 infection. Three other participants cleared HBsAg at weeks 89, 98, and 119 after TDF withdrawal, leading to a cumulative HBsAg loss rate of 2.1% (95% CI 0.0%–5.0%) at year 1 and 4.3% (95% CI 0.0%–9.5%) at year 2 (Figure 2). In the 2 participants with subgenotype A2, HBsAg loss was immediately preceded by an ALT elevation (4× ULN and 12× ULN), whereas milder ALT elevations were observed in 2 of the other 3 with HBsAg loss that occurred after year 1 (Table 2).

The mean qHBsAg level decrease after TDF withdrawal was not significantly different by initial treatment arm or baseline HBeAg status. Mean qHBsAg levels decreased by 0.09 $\log_{10}$ IU/mL in the final year of treatment, compared with an increase of 0.14 $\log_{10}$ IU/mL in the year after TDF withdrawal ($P = 0.18$). Excluding those who restarted treatment, the mean decrease in qHBsAg in the final year of treatment was 0.10 $\log_{10}$ IU/mL compared with 0.24 $\log_{10}$ IU/mL in the year post-TDF withdrawal ($P = 0.053$). In the cohort who continued treatment, the decrease in qHBsAg between weeks 144 and 192 and between weeks 192 and 240 was similar, 0.13 and 0.14 $\log_{10}$ IU/mL, respectively. Mean qHBsAg declined from week 192 to week 240 in those continuing treatment but increased in those with TDF withdrawal (−0.14 vs +0.14, $P = 0.07$), although the direction was reversed in the latter when participants who resumed treatment were excluded from that group (−0.14 vs −0.24, $P = 0.27$) (Table 3).

Excluding the 5 participants with HBsAg loss after treatment cessation, the reduction in qHBsAg level was ≥0.5 $\log_{10}$IU/mL in 13 (14.1%), including 2 (2.2%) with ≥2 and 3 (3.3%) with 1 to <2 $\log_{10}$IU/mL reduction by 1 year of postwithdrawal follow-up. Of 19 participants with qHBsAg levels <100 IU/mL at treatment withdrawal, 4 lost HBsAg after treatment discontinuation, 2 within the first year and 2 after the first year of off-treatment follow-up. The last participant who lost HBsAg (week 119) had qHBsAg level 123 IU/mL at TDF withdrawal. At the end of follow-up, 17 had HBsAg levels below 100 IU/mL including 5 with HBsAg loss.

Table 2. Characteristics of participants who lost HBsAg after treatment withdrawal

ID	Study arm	HBeAg at baseline	HBV genotype	qHBsAg ($\log_{10}$IU/mL)		Time of HBsAg loss since withdrawal (wk)	Any ALT elevation	Time of ALT elevation since withdrawal (wk)	Time since ALT elevation to HBsAg loss (wk)	ALT flare	Peak ALT (× ULN) after withdrawal
				Prestudy	TDF withdrawal						
1	TDF	Neg	E	3.02	2.09	119	No			No	1.27
2	TDF	Pos	A2	4.10	1.12	25	Yes	11	14	No	3.9
3	TDF	Pos	A2	4.91	1.17	25	Yes	11.7	13	Yes	12.27
4	TDF + PegIFN	Neg	B2	2.76	0.66	98	Yes	8.6	89	No	3.13
5	TDF + PegIFN	Neg	C5	2.54	1.79	89	Yes	8.4	81	No	4.33

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PegIFN, peginterferon-α; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

Table 3. Changes in quantitative HBsAg levels over time with treatment withdrawal or continuation

	Mean qHBsAg change per year (log 10 IU/mL)			
			Weeks 144–192 vs weeks 192–240	
Cohort	Weeks 144–192 (on TDF)	Weeks 192–240 (postwithdrawal)	Difference	*P* value
1. Withdrawal cohort include all TDF withdrawal and data before treatment restart (n = 97)[a]	−0.09	0.14	−0.19	0.18
2. Withdrawal cohort exclude those who restarted treatment (n = 84)[b]	−0.10	−0.24	0.14	0.053
3. Continued treatment cohort (n = 77)[c]	−0.13	−0.14	0.01	0.87
P value 1 vs 3	0.35	0.07	0.19	
P value 2 vs 3	0.51	0.27	0.18	

HBsAg, hepatitis B surface antigen; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate.
[a]Withdrawal but censoring at treatment restart. For those 13 who restarted treatment, qHBsAg change during weeks 192–240 was calculated as the change of qHBsAg from week 192 to the date before the treatment restart date (among 97, only 90 patients had all week 144, 192, and 240 (or last date before the treatment restart date) qHBsAg measured; therefore, the paired *t* test is based on these 90 patients).
[b]Thirteen of 97 withdrawals with retreatment excluded (among 84, only 78 have qHBsAg measured at week 240; therefore, the paired *t* test is based on these 78 patients).
[c]Pure continuation: all continued treatment throughout with no withdrawal (among 77, only 73 have qHBsAg measured at week 240; the paired *t* test is based on these 73 patients).

Virological relapse

Following treatment withdrawal, HBV DNA became detectable in 95 (97.9%) participants. Peak HBV DNA exceeded 2,000 IU/mL in 82 (84.5%) participants and 100,000 IU/mL in 21 (21.6%) participants. Among those with initial virological relapse (n = 82), 4 (4.9%) participants returned to persistently undetectable HBV DNA and 22 (26.8%) to persistent HBV DNA <2,000 IU/mL without treatment. The cumulative virological relapse rate (HBV DNA >2,000 IU/mL) at 1 year was 86% (see Supplementary Figure 3, Supplementary Digital Content 3, http://links.lww.com/AJG/C858).

Of the 24 participants who were HBeAg+ pretreatment and HBeAg− at withdrawal, 6 (25.0%) experienced HBeAg seroreversion after treatment withdrawal (see Supplementary Table 1, Supplementary Digital Content 4, http://links.lww.com/AJG/C859), which occurred 9–37 weeks post-TDF withdrawal and was associated with ALT elevations in all. In 2 participants, HBeAg seroreversion was transient and qHBeAg was undetectable at subsequent time points, and neither was retreated; the other 4 were retreated, of whom 2 lost HBeAg again. Notably, 4 of the 6 with HBeAg seroreversions were in the TDF-alone group.

ALT elevations, predictive factors, and impact on qHBsAg levels

ALT elevations occurred in 67 (69.1%) participants after TDF withdrawal, with most elevations occurring within 20 weeks postwithdrawal (median 11 weeks postwithdrawal). ALT flares were observed in 36 (37.1%) participants and most occurred between weeks 8 and 16 post-TDF withdrawal. After the protocol amendment to exclude HBeAg+ and HBeAg−/anti-HBe− participants, ALT elevations were not associated with jaundice or other evidence of clinical decompensation. As seen in Figure 3, ALT elevations were not affected by the initial treatment arm but were more frequent and occurred earlier in participants who were HBeAg− before the initial treatment.

To better understand clinically relevant postwithdrawal ALT elevations, factors associated with ALT flares were evaluated, controlling for time since TDF withdrawal. Among pretreatment factors, only older age (OR 1.1 per year 95% CI 1.01–1.1, $P = 0.01$) and higher HBV DNA (OR 1.4 per $\log_{10}$IU/mL, 95% CI 1.1–1.9, $P = 0.02$) were significantly associated. ALT, HBV DNA, and qHBsAg levels at the time of TDF withdrawal were not significantly associated with the likelihood of ALT flares; however, HBV DNA and qHBsAg levels were higher at the time of ALT flares, and higher HBV DNA levels at the visit prior were predictive of ALT flares (OR 3.0 per $\log_{10}$IU/mL 95% CI 2.3–4.0, $P < 0.0001$). HBV DNA >4 $\log_{10}$IU/mL at the prior visit was strongly associated with a hepatitis flare at the next visit with an OR of 32.7 (95% CI 13.5–79.2, $P < 0.0001$) (Table 4); of those with HBV DNA >4 $\log_{10}$IU/mL, 32.0% had an ALT flare at the next visit compared with 1.9% with lower HBV DNA levels.

To determine whether ALT elevations were associated with qHBsAg decline, the mean change in qHBsAg from week 192 to week 240 was compared between participants without ALT elevations, those with ALT elevations <5× ULN, and those with ALT flares. Participants without ALT elevations and with ALT <5× ULN had greater mean decrease in qHBsAg levels (0.23 and 0.38 $\log_{10}$ IU/mL) than those with ALT flares (0.17 $\log_{10}$ IU/mL); however, the differences were not statistically significant (see Supplementary Table 2, Supplementary Digital Content 4, http://links.lww.com/AJG/C859). The individual qHBsAg plots with and without ALT flares post-TDF withdrawal are shown in Figure 4.

Treatment reinitiation

Treatment was restarted in 13 participants, most frequently due to ALT >1,000 U/L with HBV DNA ≥10,000 IU/mL (n = 8) and/or HBeAg seroreversion (n = 6), with retreatment occurring within 19 weeks of TDF withdrawal in all but 1 participant. Two participants were retreated before meeting the protocol-defined retreatment criteria: 1 with a peak ALT of ~2× ULN and the other with a peak ALT of ~12× ULN. Pretreatment HBeAg+ (HR 3.6, 95% CI 1.2–10.7, $P = 0.02$) and higher pretreatment HBV DNA (per $\log_{10}$IU/mL) (HR 1.5, 95% CI 1.04–2.2, $P = 0.03$) were significantly associated with a higher likelihood of needing retreatment (see

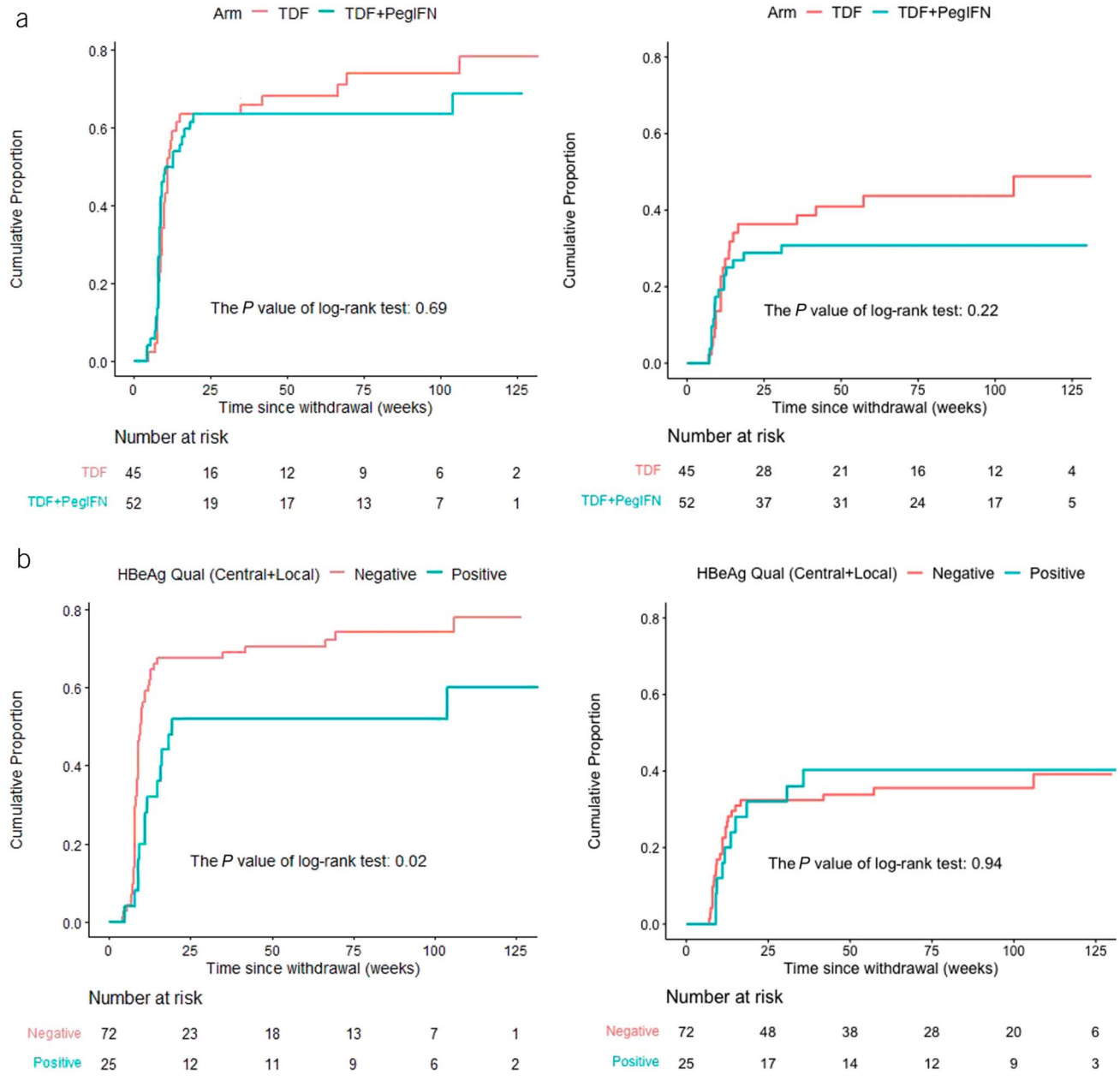

Figure 3. Cumulative proportion with ALT elevation (any) and ALT flare (>5× ULN) by subgroup. The cumulative proportion with any ALT elevation (A1/B1) and with ALT flares (A2/B2) is shown by A. Initial treatment group by B. Baseline HBeAg status. *P* values shown are based on the log-rank test. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; PegIFN, peginterferon-α; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

Supplementary Table 3, Supplementary Digital Content 4, http://links.lww.com/AJG/C859). After reinitiation of TDF therapy, HBV DNA was suppressed, ALT normalized, and qHBsAg levels declined to end-of-treatment levels with no HBsAg loss.

Clinical phenotype at the end of 48-week postwithdrawal follow-up

The clinical HBV phenotype was determined at the final 2 IA trial visits (weeks 228 and 240) in those with complete data (n = 93) and at the last 2 visits in those with extended follow-up. At the end of the trial (week 240), a sustained IC phenotype was observed in 13 of 93 (14.0%) participants, an IA phenotype in 31 (33.3%), and indeterminant phenotypes in 49 (52.7%). Of the 45 participants who completed 2 years of follow-up post-TDF withdrawal, 7 (15.6%) had a sustained IC phenotype, 19 (42.2%) had an IA phenotype, and 19 (42.2%) had an indeterminant phenotype. Features significantly associated with a sustained IC phenotype 1 year postwithdrawal were HBeAg positivity at baseline (risk ratio 2.4, 95% CI 1.02–5.6, $P = 0.04$) and qHBsAg <100 IU/mL at withdrawal (risk ratio 7.4, 95% CI 2.6–21.3, $P = 0.0002$) (Table 5).

The occurrence of any ALT elevation in the year following TDF withdrawal was associated with a higher likelihood of IA phenotype at the end of the study (48.4% vs 3.2%, $P < 0.0001$), as was the occurrence of an ALT flare with 70.6% vs 11.9% ($P < 0.0001$) ending in IA phenotype at the end of 1 year and 66.7% vs 25.9% ($P = 0.03$) at the end of 2 years post-TDF withdrawal among those with vs without flares. A total of 52 (53.6%) participants had no

Table 4. Association of pretreatment, prewithdrawal, and postwithdrawal factors with ALT flare after TDF withdrawal[a]

Factors	Odds ratio	95% confidence interval	*P* value
Week since withdrawal[a]	0.99	0.98–1.00	0.01
Study arm (TDF + PegIFN vs TDF)	0.54	0.24–1.25	0.15
Sex (female vs male)	1.31	0.55–3.14	0.54
Age at baseline (per year)	1.05	1.01–1.10	0.01
At baseline (pretreatment)			
HBeAg status (negative vs positive)	0.70	0.28–1.77	0.45
ALT (per ULN)	0.96	0.86–1.07	0.47
HBV DNA (per $\log_{10}$IU/mL)	1.40	1.06–1.85	0.02
qHBsAg (per $\log_{10}$IU/mL)	1.25	0.70–2.22	0.45
At treatment withdrawal			
ALT (per ULN)	1.26	0.68–2.31	0.46
HBV DNA (per $\log_{10}$IU/mL)	1.53	0.65–3.63	0.33
qHBsAg (per $\log_{10}$IU/mL)	1.01	0.61–1.66	0.98
At visit concurrent with ALT flare			
HBV DNA (per $\log_{10}$IU/mL)	2.74	2.20–3.40	<0.0001
qHBsAg (per $\log_{10}$IU/mL)	5.97	1.87–19.09	0.003
At visit before ALT flare			
HBV DNA (per $\log_{10}$IU/mL)	3.03	2.29–4.01	<0.0001
HBV DNA (>4 vs ≤4 $\log_{10}$IU/mL)	32.72	13.52–79.20	<0.0001
HBV DNA (>5 vs ≤5 $\log_{10}$IU/mL)	35.30	16.28–76.53	<0.0001
qHBsAg (per $\log_{10}$IU/mL)	1.38	0.89–2.13	0.15

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; PegIFN, peginterferon-α; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate.
[a]All estimates are from separate models adjusting for week since withdrawal except for the week since withdrawal variable.

clinical consequences to treatment withdrawal, defined as no need for retreatment, no ALT flare, and not in the IA phase at the end of the study (week 240). Factors significantly associated with safe withdrawal included younger age at baseline and lower qHBsAg at TDF withdrawal (see Supplementary Table 4, Supplementary Digital Content 4, http://links.lww.com/AJG/C859). However, no specific thresholds for age or qHBsAg could be identified that reliably allowed for safe TDF withdrawal.

DISCUSSION

NA withdrawal is increasingly being considered a therapeutic strategy for chronic HBV infection. In this prospective study of 97 predominantly Asian participants with close monitoring and strict criteria for treatment discontinuation and reinitiation, we evaluated clinical outcomes after withdrawal of TDF following 4 years of therapy, with or without an initial 6 months of PegIFN. HBsAg loss was infrequent, occurring in only 2.1% by 1 year and 4.3% by 2 years after TDF withdrawal. In addition, qHBsAg declines ≥0.5 $\log_{10}$IU/mL occurred in only 19% by 1 year after treatment withdrawal. ALT elevations were frequent, occurring in 69% of participants but were not associated with increased HBsAg decline or loss. ALT flares could be predicted by HBV DNA >4 $\log_{10}$IU/mL at the prior visit, potentially allowing for prevention of ALT flares with earlier retreatment.

As in previous studies (2,6,7,17), virological relapse following NA withdrawal was frequent and typically occurred 4–12 weeks after stopping therapy with HBV DNA increases usually preceding ALT elevations. Reports of HBsAg loss after long-term NA withdrawal in up to 20% of patients (7), sometimes preceded by ALT flares, have led guidelines to suggest NA withdrawal as a possible therapeutic strategy (12,18,19). It has been suggested that these flares represent immune clearance of HBV and might be viewed as beneficial. To test the hypothesis that ALT elevations may promote HBsAg loss, our retreatment criteria allowed for marked and relatively prolonged ALT elevations. We did not observe any significant association between ALT elevation and HBsAg decline or loss. In fact, greater declines in qHBsAg were seen in those with no or mild ALT elevations. Furthermore, participants with ALT flares were more likely to end up with active disease at the end of the study. We found that participants who had an HBV DNA above 4 $\log_{10}$IU/mL were very likely to have a subsequent ALT elevation. Reinitiation of antiviral therapy with HBV DNA levels above 4 $\log_{10}$IU/mL may prevent clinically significant ALT elevations given their lack of association with HBsAg decline or loss.

Notably, the initial study protocol allowed for treatment withdrawal in participants who remained HBeAg+ but met other withdrawal criteria at the end of treatment. We observed rapid and severe relapses in 2 HBeAg+ participants who withdrew therapy, 1 of which led to hospitalization. Following these events, the other 4 HBeAg+ participants who had withdrawn therapy were put back on TDF without complication. We also observed 18 flares in 6 participants who were HBeAg− but lacked anti-HBe at withdrawal. All participants recovered with retreatment, but these data highlight that if treatment withdrawal is considered, patients must be HBeAg− and anti-HBe+ without evidence of cirrhosis.

Results from previous treatment withdrawal studies have been varied. The initial report from Hadziyannis and colleagues showed that virological relapse was near universal but was usually followed by spontaneous viral control and sometimes HBsAg loss, leading to enthusiasm to evaluate NA withdrawal as a therapeutic approach (2). Treatment reimbursement restrictions in Asia allowed for evaluating treatment withdrawal in large numbers of patients with rates of HBsAg loss varying from <1% to 13% at 6 years (3,5,20–22). Recently, data from multiple treatment discontinuation cohorts have been compiled in the RETRACT-B collaboration (3). With 1,552 patients and a median of 18.4 months of follow-up, the authors found that HBsAg loss occurred in 3.2% by 1 year, increasing to 13% by 4 years, and was more common in Whites than Asians (subdistribution HR 6.8 95% CI 2.7–16.8, $P < 0.001$). Similar to other studies, the qHBsAg level at the time of withdrawal was a strong predictor of HBsAg loss, occurring in 43% with qHBsAg <100 IU/mL compared with 1.1% in those with qHBsAg levels above 1,000 IU/mL at NA withdrawal, with different thresholds for Whites and Asian patients. The authors suggested that NA withdrawal should be considered only in Asian patients with HBsAg levels <100 IU/mL and White patients with HBsAg levels <1,000 IU/mL. We observed only 5 participants with HBsAg loss after TDF withdrawal 2 in the first year and 3 between 89 and 119 weeks postwithdrawal. Because so few participants in our study

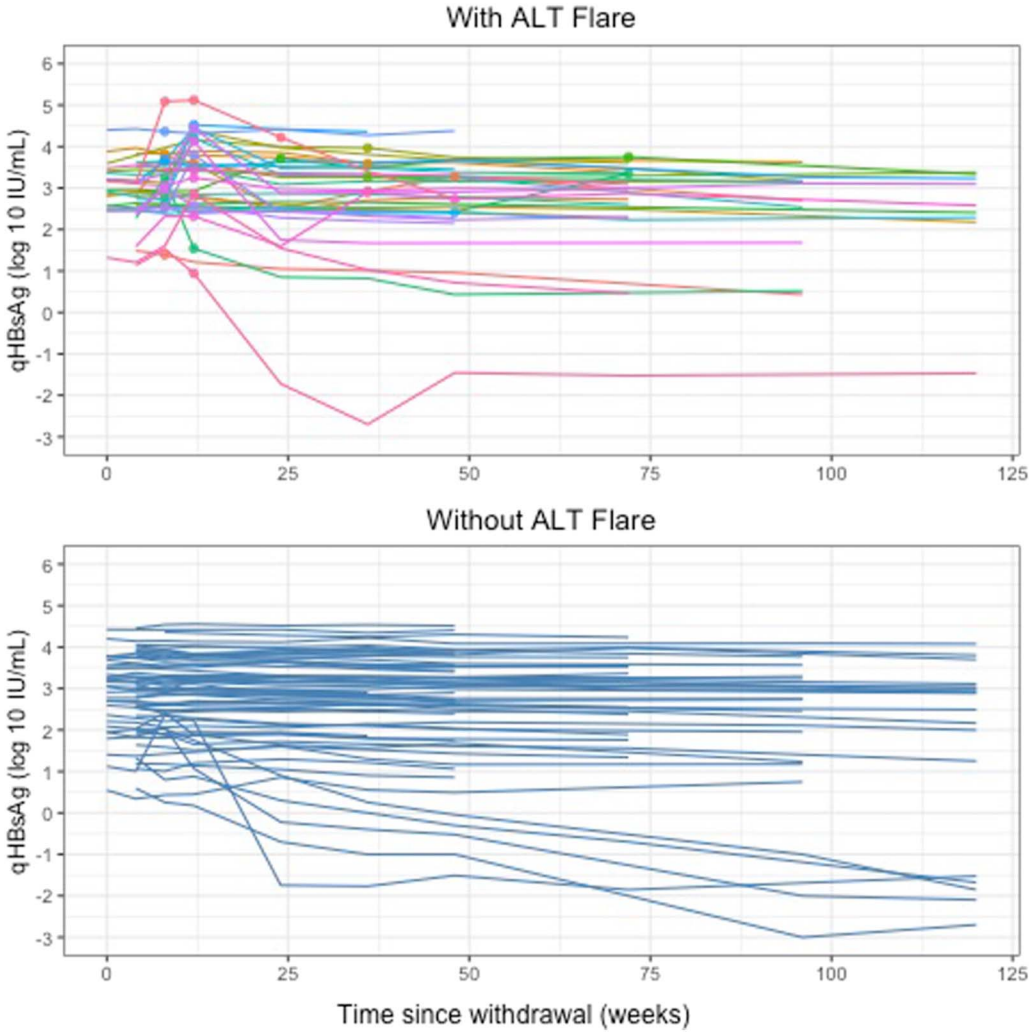

Figure 4. Individual trajectories of qHBsAg after TDF withdrawal in those with and without an ALT flare (>5× ULN). Each line represents the change in qHBsAg over time after treatment withdrawal. Those who experienced an ALT flare (>5× ULN) after treatment withdrawal are shown in the upper panel with the timing of ALT flares indicated by a solid dot. In the bottom panel, qHBsAg values over time are shown for participants who did not experience an ALT flare post-TDF withdrawal. ALT, alanine aminotransferase; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

cleared HBsAg, we were unable to evaluate predictors, although 4 of 5 who lost HBsAg after TDF withdrawal had qHBsAg <100 IU/mL at the time of withdrawal and qHBsAg was 123 IU/mL in the fifth patient. Notably, 3 of 17 (17.6%) non-Asian participants lost HBsAg compared with 2 of 79 (2.5%) Asian participants.

A major strength of our study was the standardized duration (4 years) and type of NA treatment (TDF), detailed pretreatment characterization, and prespecified, relatively stringent retreatment criteria allowing for potential virological benefits of significant ALT flares. Our prospective data contrast with results from 2 prospective trials in Europe (7,10) that included predominantly Whites but are similar to another Asian-predominant trial in Canada (6). Collectively, the data show that NA withdrawal rarely leads to HBsAg loss among Asian patients. Although younger age and lower qHBsAg levels were associated with lower risk of negative outcomes during follow-up, no specific thresholds could be identified to predict safe withdrawal or HBsAg loss. Furthermore, HBV DNA levels were higher in those who withdrew than in those who remained on therapy, suggesting that participants may have been better off remaining on TDF therapy.

Most studies in the literature focus on patients who were HBeAg− pretreatment. Our study is unique in that roughly 50% of the participants were HBeAg+ pretreatment. Among HBeAg+ participants who had seroconverted to anti-HBe at the time of NA withdrawal, HBsAg loss was more common, and ALT elevations were less frequent than in those who were HBeAg− pretreatment. Our findings are relevant not only for current NA monotherapy but also for HBV therapeutic development, particularly for strategies that rely on NA withdrawal to enhance functional cure rates.

In the overall treatment trial, HBsAg loss was much more common in those with subgenotype A2 infection (7 of 12, 58.3%) than with other genotypes (2 of 168, 1.2%). Two of the participants who cleared HBsAg after TDF withdrawal had genotype A2 infection, and both lost HBsAg by 25 weeks postwithdrawal compared with >89 weeks postwithdrawal in the 3 participants with other genotypes who cleared HBsAg. In both participants with subgenotype A2 infection, an ALT elevation (peak 4× ULN and 12× ULN) immediately preceded HBsAg loss, whereas no or mild (<2× ULN) ALT elevations were seen in the others who cleared HBsAg. Understanding why the

Table 5. Association of pretreatment, prewithdrawal, and postwithdrawal factors with a sustained inactive carrier phenotype at the end of the study (week 240 from randomization)

	Risk ratio (univariate)		Adjusted risk ratio[a]	
Factors	**Risk ratio (95% CI)**	***P* value**	**Risk ratio (95% CI)**	***P* value**
Arm (TDF vs TDF + PegIFN)	0.52 (0.17–1.56)	0.24	1.17 (0.36–3.75)	0.80
Female vs male	1.19 (0.42–3.34)	0.74		
Others vs Asian	1.85 (0.64–5.34)	0.25		
Age at baseline (per year)	0.98 (0.93–1.02)	0.30		
At baseline (pretreatment)				
HBeAg status (positive vs negative)	2.46 (0.92–6.61)	0.07	2.39 (1.02–5.58)	0.04
ALT (per ULN)	1.00 (0.94–1.06)	0.9995		
HBV DNA (per $\log_{10}$IU/mL)	1.26 (0.88–1.80)	0.20		
qHBsAg (per $\log_{10}$IU/mL)	1.02 (0.47–2.23)	0.95		
At treatment withdrawal (week 192)				
ALT (per ULN)	0.49 (0.13–1.79)	0.28		
HBV DNA (per $\log_{10}$IU/mL)	0.68 (0.26–1.74)	0.42		
qHBsAg (per $\log_{10}$IU/mL)	0.41 (0.25–0.69)	0.0007		
qHBsAg (<100 IU/mL vs ≥100 IU/mL)	7.15 (2.67–19.17)	<0.0001	7.37 (2.55–21.30)	0.0002
Change from baseline to week 192				
HBV DNA decline from baseline to week 192 (per log 10)	0.77 (0.54–1.09)	0.14		

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; PegIFN, peginterferon-α; qHBsAg, quantitative hepatitis B surface antigen; TDF, tenofovir disoproxil fumarate.
[a]Adjusted risk ratios are from multivariate regression with 3 selected variables: arm, HBeAg status at baseline, and qHBsAg (<100 IU/mL vs ≥100 IU/mL) at week 192.

frequency and pattern of HBsAg loss appear to differ with subgenotype A2 should be a focus of future investigation.

The study has some relevant limitations. The formal off-treatment follow-up was limited to 48 weeks; however, over half the participants were followed beyond 96 weeks. Whether additional HBsAg loss would have occurred with additional follow-up is unknown. The number of participants who lost HBsAg was very limited precluding assessment of predictors of functional cure with NA withdrawal. The low rate of HBsAg loss may have been due to the large proportion of Asian participants, based on the previous data showing significantly higher chance of HBsAg loss in non-Asian individuals (3). Because of the large predominance of Asians in the study population, we were not able to reliably assess the differences in outcome between Asian and non-Asian populations.

In conclusion, this prospective study showed that withdrawal of TDF after 4 years of therapy was associated with a low rate of HBsAg decline or loss. ALT elevations after treatment withdrawal were frequent but did not lead to HBsAg decline or loss and were associated with more active future disease. The results of this study do not support NA withdrawal as a therapeutic strategy in a predominantly Asian North American population, but if it is considered, restarting treatment when HBV DNA levels rise above 4 $\log_{10}$IU/mL might prevent subsequent futile ALT flares.

CONFLICTS OF INTEREST

Guarantor of the article: Jordan J. Feld, MD, MPH, Abdus S. Wahed, PhD, and Xue Yang, PhD had accepted full responsibility for the conduct of the study and the decision to publish.

Specific author contributions: J.J.F.: study concept and design; acquisition of data; interpretation of data; drafting of the manuscripts; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, or material support; and study supervision. A.S.W.: study concept and design; statistical analysis and interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. X.Y.: statistical analysis; drafting of the manuscript and critical revision of the manuscript for important intellectual content. S.H.B.: study concept and design; interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical of material support; and study supervision. M.G.G., M.F., A.D.B., N.T., R.P.P., M.K., H.L.A.J., and A.S.L.: study concept and design; acquisition of data; interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; administrative, technical, or material support; and study supervision.

Financial support: The HBRN was funded as a Cooperative Agreement between the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the following investigators: Lewis R. Roberts, MB, ChB, PhD (U01-DK082843), Anna Suk-Fong Lok, MD (U01-DK082863), Steven H. Belle, PhD, MScHyg (U01-DK082864), Kyong-Mi Chang, MD (U01-DK082866), Michael W. Fried, MD (U01-DK082867), Adrian M. Di Bisceglie, MD (U01-DK082871), William M. Lee, MD (U01-DK082872), Jordan J. Feld MD MPH (U01-DK082874), Daryl T-Y Lau, MD, MPH (U01-DK082919), Richard K. Sterling, MD, MSc (U01-DK082923), Steven-Huy B. Han, MD (U01-DK082927), Robert C. Carithers, MD (U01-DK082943), Mandana Khalili, MD (U01-DK082944), an interagency agreement with NIDDK: Lilia M. Ganova-Raeva, PhD (A-DK-3002-001), and support from the intramural program, NIDDK, NIH: Marc

G. Ghany, MD. Additional funding to support this study was provided to Kyong-Mi Chang, MD, the Immunology Center (NIH/NIDDK Center of Molecular Studies in Digestive and Liver Diseases P30DK50306, NIH Public Health Service Research Grant M01-RR00040), Richard K. Sterling, MD, MSc (UL1TR000058), NCATS (National Center for Advancing Translational Sciences, NIH), Mandana Khalili, MD (CTSA Grant Number UL1TR000004), Michael W. Fried, MD (CTSA Grant Number UL1TR001111), and Anna Suk-Fong Lok (CTSA Grant Number UL1RR024986 and U54TR001959.) Additional support was provided by Gilead Sciences and Roche Molecular Systems through Cooperative Research and Development Agreement (CRADA) with the NIDDK and Roche/Genentech through a Clinical Trials Agreement (CTA) with the NIDDK.

Potential competing interests: J.J.F.: research support from AbbVie, Eiger, Enanta, Gilead, and Janssen and consults for AbbVie, Antios, Arbutus, Eiger, Enanta, Gilead, GSK, Janssen, and Vir Biotechnology. A.S.W.: consulting support from Merck through the Department of Biostatistics, University of Pittsburgh. M.F. serves as Chief Medical Officer for Target RWE and receives personal compensation and is a stockholder in the company; he reports grants paid to the University of North Carolina from Gilead, AbbVie, National Institutes of Health, and Merck, outside the submitted work. M.G.G. and A.D.B.: nothing to disclose. R.P.P.: research support from Dynavax and served as a consultant for Assembly Biosciences. M.K.: research support from Gilead Sciences and Intercept pharmaceuticals and consults for Gilead Sciences. X.Y. and S.H.B.: none. H.L.A.J.: consultant fees and/or grant support from BMS, AbbVie, Gilead Sciences, Novartis, Roche, Janssen, Arbutus, VIR, and Merck. N.T.: research support from Gilead Sciences, GSK, and Roche Genentech and consulting for Exigo, Saol Therapeutics, and Moderna. A.S.L.: grants from Bristol Myers Squibb, Gilead Sciences, and TARGET and consultant/advisor for Ambys, Amgen, Arbutus, Chroma, Clear B, Eli Lilly, Enanta, Enochian, Gilead Sciences, GlaxoSmithKline, GNI, Huahui, Janssen, TARGET, and Virion.

Study Highlights

WHAT IS KNOWN

- ✓ Nucleos(t)ide analog (NA) therapy withdrawal is being increasingly evaluated as a therapeutic strategy in chronic hepatitis B virus (HBV) infection.
- ✓ Outcomes after long-term NA withdrawal are uncertain.

WHAT IS NEW HERE

- ✓ Hepatitis B surface antigen (HBsAg) loss or significant decline was rare after NA withdrawal.
- ✓ Alanine aminotransferase (ALT) flares occurred frequently after NA withdrawal but were not associated with subsequent HBsAg loss or decline.
- ✓ HBV DNA above 4 $\log_{10}$IU/mL after NA withdrawal was associated with ALT flares at the following visit, potentially allowing for reinitiation of therapy to prevent futile and potentially harmful flares.

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