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Viral Hepatitis

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A multicenter randomized-controlled trial of nucleos(t)ide analogue cessation in HBeAg-negative chronic hepatitis B

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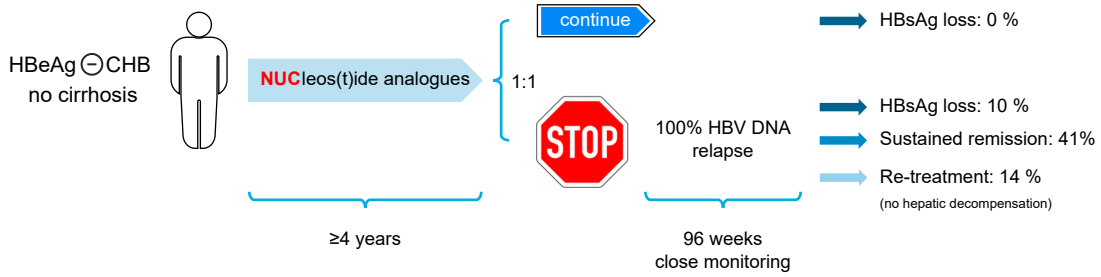
Graphical abstract



Highlights

- Stopping long-term NUC treatment can induce functional cure in HBeAg-negative patients.
- Functional cure is associated with HBsAg levels <1,000 IU/ml at the time point of NUC treatment cessation.
- All patients showed relapses in HBV DNA levels after NUC treatment discontinuation. But re-treatment with NUCs was only required in 14% of patients over a 96-week follow-up and no patient suffered hepatic decompensation.

Impact and implications

As HBeAg-negative patients with chronic hepatitis B on nucleos(t)ide analogues (NUCs) rarely achieve functional cure, treatment is almost always lifelong. The STOP-NUC trial was conducted to investigate whether discontinuing long-term NUC treatment can increase the cure rate. We found that some patients achieved functional cure after stopping NUCs, which was especially pronounced in patients with HBsAg levels <1,000 at the end of NUC treatment, and that many did not need to resume therapy. The results of the Stop-NUC trial provide evidence for the concept of stopping NUC treatment as a therapeutic option that can induce functional cure.

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A multicenter randomized-controlled trial of nucleos(t)ide analogue cessation in HBeAg-negative chronic hepatitis B

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Background & Aims: Nucleos(t)ide analogues (NUCs) are the standard and mostly lifelong treatment for chronic HBeAg-negative hepatitis B, as functional cure (loss of HBsAg) is rarely achieved. Discontinuation of NUC treatment may lead to functional cure; however, to date, the evidence for this has been based on small or non-randomized clinical trials. The STOP-NUC trial was designed with the aim of increasing the HBsAg loss rate using a NUC treatment interruption approach.
Methods: In this multicenter, randomized-controlled trial, 166 HBeAg-negative patients with chronic hepatitis B on continuous long-term NUC treatment, with HBV DNA <172 IU/ml (1,000 copies/ml) for ≥4 years, were randomized to either stop (Arm A) or continue NUC treatment (Arm B) for a 96-week observation period. In total, 158 patients were available for final analysis, 79 per arm. The primary endpoint was sustained HBsAg loss up to week 96.
Results: Our study met its primary objective by demonstrating HBsAg loss in eight patients (10.1%, 95% CI 4.8%–19.5%) in Arm A and in no patient in Arm B ($p = 0.006$). Among patients with baseline HBsAg levels <1,000 IU/ml, seven (28%) achieved HBsAg loss. In Arm A, re-therapy was initiated in 11 (13.9%) patients, whereas 32 (40.5%) patients achieved sustained remission. A decrease of HBsAg >1 log IU/ml was observed in 16 patients (20.3%) in Arm A and in one patient (1.3%) in Arm B. No serious adverse events related to treatment cessation occurred.
Conclusions: Cessation of NUC treatment was associated with a significantly higher rate of HBsAg loss than continued NUC treatment, which was largely restricted to patients with end of treatment HBsAg levels <1,000 IU/ml.

Introduction

An estimated 250 million people are chronically infected with the HBV, putting them at risk of cirrhosis, hepatic decompensation, and hepatocellular carcinoma.[1] Nucleos(t)ide analogues (NUCs) – the standard treatment option for chronic hepatitis B worldwide – reliably suppress HBV replication and slow down or prevent disease progression.[1–3] The optimal treatment endpoint proposed by international guidelines is loss of HBsAg.[4–6] However, even long-term NUC treatment leads to HBsAg loss in only 1% of HBeAg-negative patients with chronic hepatitis B, resulting in a majority of them requiring lifelong treatment with possible consequences including bone density loss, kidney function deterioration, and potentially other drug-related side effects, as well as the development of drug resistance, compliance problems and costs.[7,8]

In 2012, a European study triggered a debate about lifelong NUC treatment in HBeAg-negative patients.[9] In this study, 55% (n = 18/33) of patients who discontinued NUC therapy (adefovir) after a 4-year period achieved sustained response and, of those, 72% (n = 13/18) cleared HBsAg. This study inspired numerous retrospective and prospective studies to evaluate the controlled termination of NUCs.[10–21] However, results are heterogeneous, and conclusive evidence for the efficacy of NUC treatment discontinuation from controlled randomized trials is lacking. International treatment guidelines have tried to incorporate this approach into their recommendations.[4–6] We present here the results of the prospective, randomized multicentric STOP-NUC trial on controlled NUC cessation as a therapeutic intervention in HBeAg-negative patients with chronic hepatitis B.

Keywords: Hepatitis B surface antigen; HBsAg seroconversion; Treatment cessation; HBV treatment; Functional cure; Carrier state; Tenofovir EudraCT 2013-004882-15.
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Patients and methods

Trial design

The STOP-NUC trial was a prospective, multicenter, randomized, controlled trial (EudraCT 2013-004882-15, German Clinical Trial Register DRKS00006240) (Fig. 1) funded by the German Federal Ministry of Education and Research (BMBF, 01KG1308) that was conducted across 25 German sites. The STOP-NUC trial was conducted in accordance with Good Clinical Practice guidelines, local regulations, and the ethical principles described in the Declaration of Helsinki.[22] The trial protocol and amendments (supplementary trial protocol) were approved by the leading ethics committee (Leipzig University) and by the German competent authority (Bundesinstitut für Arzneimittel und Medizinprodukte). The trial was monitored by an independent data and safety monitoring board. The trial protocol and statistical analysis plan are provided online as supplementary files.

Participants

Patients with chronic hepatitis B were eligible for inclusion if they were older than 18 years, had received continuous treatment with licensed NUCs for at least 4 years, had HBV DNA levels ≥2,000 IU/ml at the initial start of NUC treatment, were continuously HBeAg-negative from the initial start of NUC treatment until inclusion, were showing suppression of HBV DNA below the level of 172 IU/ml (1,000 copies/ml) for at least 4 years (a cut-off chosen to reflect the different methods available at the time), and had alanine aminotransferase (ALT) levels within normal ranges, as well as undetectable HBV DNA (measured centrally, detection level 10 IU/ml) at inclusion. Any treatments for HBV infections performed prior to the currently ongoing line of therapy were allowed. Patients with a history of decompensated liver function or signs of advanced liver fibrosis defined either histologically by Scheuer score ≥ stage 3 (within last year before screening) and/or liver stiffness ≥10 kPa by elastography (FibroScan) at screening, with evidence of hepatocellular carcinoma, concomitant human immunodeficiency, hepatitis delta or hepatitis C virus infections, alcohol consumption >30 g/day for women and >50 g/day for men, extrahepatic manifestations of HBV infections, or pregnancy were excluded. All participants provided written informed consent.

Randomization and trial interventions

Patients were randomly assigned in a 1:1 ratio to either stop (Arm A) or continue NUC treatment according to current guidelines (Arm B) and presented for clinical assessment and

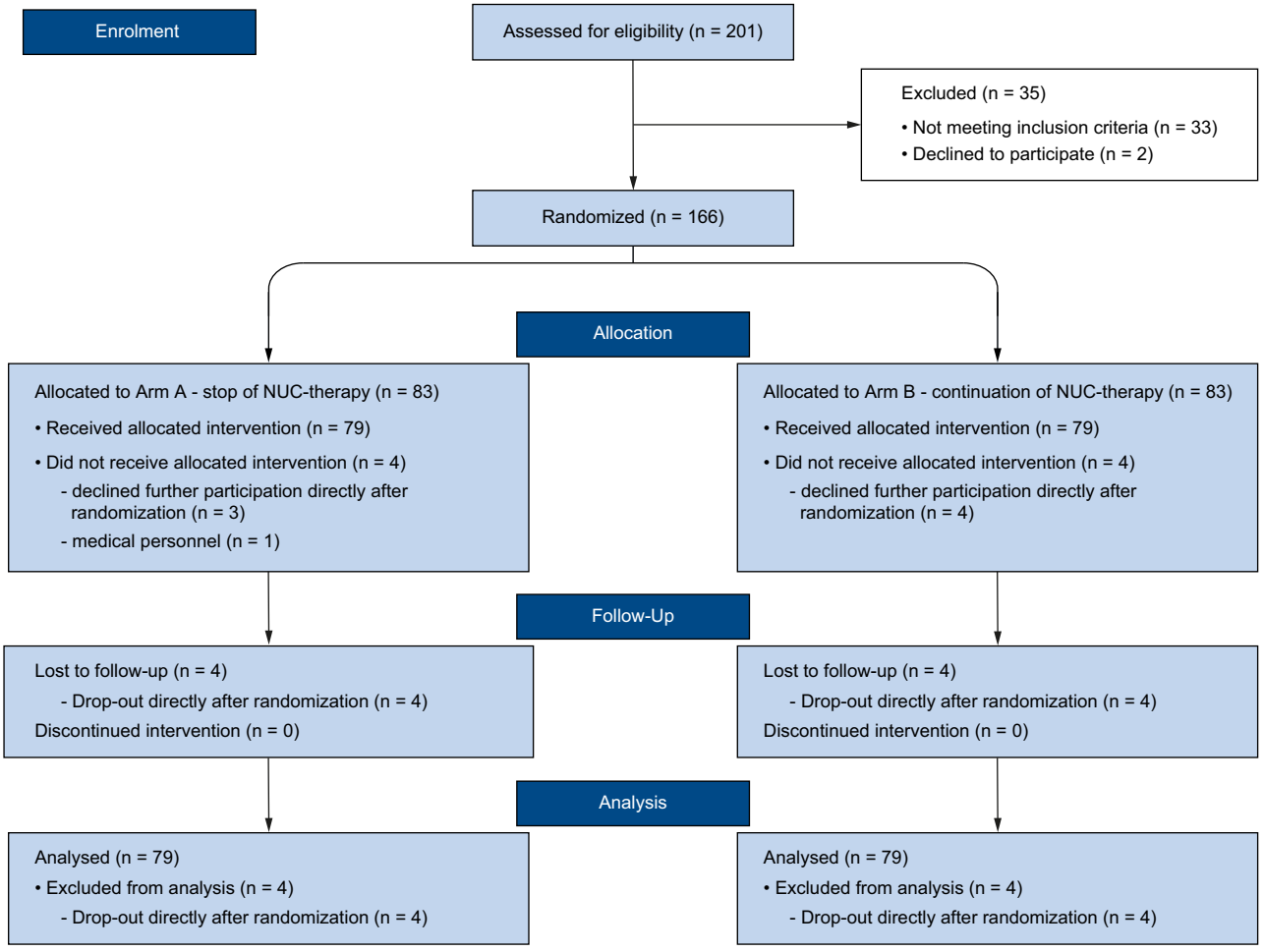

Fig. 1. CONSORT diagram showing enrolment and randomization of patients.

laboratory tests regularly over 96 weeks (Visit schedule provided as Table S1). Patients were centrally randomized with a web-based system using a minimization algorithm with random component. Patients were stratified by center, HBsAg levels <1,000 IU/ml or ≥1,000 IU/ml at inclusion, and by substances with high (entecavir or tenofovir) and low or intermediated antiviral potency (lamivudine, adefovir or telbivudine).

Safety measures and re-treatment in Arm A

In case of suspected potential severe HBV reactivation (ALT >2x upper limit of normal [ULN] at any visit) patients were switched into a bi-weekly visit schedule until normalization of ALT levels. Criteria for immediate NUC re-treatment were either:

1. ALT >10x ULN confirmed by two independent consecutive local laboratory results within 7 days.
2. ALT >5x ULN and ≤10x ULN persisting for ≥28 days (*i.e.*, 3 consecutive measurements within 28 days).
3. ALT >2x ULN and ≤5x ULN persisting for ≥112 days (*i.e.*, 8 consecutive measurements within 112 days) and viral relapse (HBV DNA >20,000 IU/ml) (*i.e.*, 3 consecutive measurements within the last 28 days of the 112-day time window)
4. Confirmed (*i.e.*, two independent consecutive local laboratory results within 1 week) increase of total bilirubin by >1.5x ULN from baseline (independent from HBV DNA level).

Investigators were encouraged to use either entecavir or tenofovir for re-treatment, which are the recommended drugs according to recent treatment guidelines.[4–6]

Outcomes

The primary endpoint was sustained HBsAg loss up to week 96, confirmed during at least one follow-up visit and remaining undetectable until end of follow-up. Secondary endpoints were HBsAg seroconversion (*i.e.* anti-HBs ≥10 U/L), time to HBsAg loss and HBsAg seroconversion, and changes in liver stiffness. The following secondary endpoints applied for the non-treatment arm only (Arm A): sustained remission up to week 96 defined by HBV DNA <2,000 IU/ml and ALT <ULN in all subsequent assessments after first occurrence of remission, virologic (HBV DNA ≤20 IU/ml) and biochemical response (ALT ≤ ULN) at week 96, number of ALT flares (*i.e.*, ALT >3x ULN) up to week 96 and time to fulfil re-treatment criteria. We assessed factors possibly associated with HBsAg loss in an exploratory analysis.

For the endpoints virologic response and biochemical response, the main interest is to describe the status of patients continuously off NUC therapy up to week 96. Therefore, patients who received re-treatment are reported as "not informative" in these descriptive analyses. HBsAg (Roche Elecsys HBsAg II, lower detection limit 0.05 IU/ml), anti-HBs (Abbott CMIA assay, lower detection limit 10 U/L) and HBV DNA (Roche COBAS 6800 HBV test, lower detection limit 10 IU/ml) were measured in a central laboratory (Labor Limbach, Heidelberg, Germany).

Sample size calculation

The planned sample size was 160 patients, assuming a rate of HBsAg loss of 15% after stopping treatment and 1% in the treatment arm (Fisher's exact test, two-sided, $\alpha = 0.05$, power 90%, drop-out rate 10%).

Statistical analysis

All comparative analyzes were based on a modified intention-to-treat principle, *i.e.* the full analysis set comprised all randomized patients who had attended at least one regular trial visit. The primary endpoint was compared by Fisher's exact test. Wilson's score interval method was used to provide 95% CIs for the efficacy rates and their difference. Binary endpoints were analyzed analogously to the primary endpoint. Time to event endpoints were described with the Kaplan-Meier estimator. Changes in liver stiffness from baseline were analyzed by ANCOVA. Exploratory subgroup analyzes were predefined for the type of NUC before enrolment, and prior HBsAg levels. ALT levels were measured in local laboratories and adjusted to local upper limits of normal ranges.

Results

Participants

Between 18 November 2014 and 11 January 2018, 201 HBeAg-negative adults with chronic hepatitis B from 25 German trial sites were screened for eligibility. A total of 166 patients were randomized to either stop (Arm A, n = 83) or continue NUC treatment (Arm B, n = 83). Recruitment was stopped per protocol at the time the target sample size was reached. Eight patients dropped out shortly after randomization; thus, the full analysis set comprises 158 patients, 79 each in Arms A and B (Fig. 1). Patient characteristics were similar in both arms (demographics and baseline characteristics are summarized in Table 1). In Arm A and Arm B, the rates of patients presenting with HBsAg levels below 1,000 IU/ml were similar (32% and 33% of patients, p = n.s.), as well as the rate of patients treated with highly potent NUCs (90% and 91% of patients, p = n.s.) (Table 1).

A total of 2,042 visits were performed, 1,182 in Arm A and 860 in Arm B. The visits in Arm A included 233 pre-planned extra visits (see visit schedule in Table S1), and 83 safety visits (performed to closely monitor patients with ALT >2x ULN). Central lab measurements were performed in 1,791 visits, 932 in Arm A and 860 in Arm B.

Primary outcome

The primary endpoint (HBsAg loss up to week 96) was observed in eight patients in Arm A (10.1%, 95% CI 4.8%–19.5%) and in no patient in Arm B (0%, 95% CI 0%–5.8%) (difference 10.1%, 95% CI 2.2–18, $p = 0.006$) (Fig. 2A, Table 2).

Secondary outcomes

Six patients in Arm A (7.6%, 95% CI 3.1%–16.4%) experienced HBsAg seroconversion to anti-HBs (Fig. 2B). The time to HBsAg loss ranged from 9-74 weeks, with a median of 37 weeks. A decrease of HBsAg levels >1 log IU/ml was found in 16 (20.3%, 95% CI 12.4%–31.1%) patients in Arm A and only one (1.3%, 95% CI 0.1%–7.8%) patient in Arm B (p <0.001) (Fig. 3). Sustained remission up to week 96 was achieved by 32 (41.0%, 95% CI 30.2%–52.7%) patients in Arm A. In those Arm A patients who did not require re-treatment, virologic response and biochemical response at week 96 were achieved in 14

Table 1. Patient characteristics at baseline.

Characteristic	Arm A (n = 79)	Arm B (n = 79)	*p* value[5]
Age, years[1]	53 (28-66)	51 (28-75)	0.964
Female sex, n (%)	29 (36.7)	28 (35.4)	1
Body mass index[1]	26.7 (18.6-36.8)	25.4 (18.3-41.7)	0.205
Ethnicity, n (%)			
Caucasian	62 (78.5)	65 (82.3)	
Asian	9 (11.4)	6 (7.6)	
African	2 (2.5)	5 (6.3)	
Other	6 (7.6)	3 (3.8)	0.398
Duration of HBV infection, years[1]	13.0 (4-52)	13.5 (5-57)	0.744
NUC treatment, n (%)			
Entecavir	27 (34.2)	35 (44.3)	0.428
Tenofovir disoproxil fumarate	44 (55.7)	37 (46.8)	
Lamivudine	2 (2.5)	4 (5.1)	
Telbivudine	6 (7.6)	3 (3.8)	
Log_{10} (HBsAg, IU/ml)[1]	3.2 (-0.9 to 4.4)	3.3 (-0.1 to 4.5)	0.523
HBsAg, n (%)			
<10 IU/ml	3 (3.8)	1 (1.3)	0.733
≥10 IU/ml – <100 IU/ml	7 (8.9)	7 (8.9)	
≥100 IU/ml – <1,000 IU/ml	15 (19.0)	18 (22.8)	
≥1,000 IU/ml	54 (68.4)	53 (67.1)	
ALT, xULN[1,2]	0.6 (0.2-1.3)	0.6 (0.2-1.1)	0.727
Liver elastography, kPa[1,3,4]	5.7 (2.4-10.4)	5.7 (2.3-14.4)	0.644

ALT, alanine aminotransferase; NUC, nucleos(t)ide analogue; ULN, upper limit of normal.
[1]Median (range).
[2]Seven patients had baseline ALT slightly above ULN due to protocol violation at inclusion.
[3]Available for n = 135 patients (Arm A, n = 70; Arm B n = 65).
[4]Three patients had liver elastography result >10 kPa (10.4, 11.8, 14.4) due to protocol violation at inclusion.
[5]Mann-Whitney *U* test for differences in medians, Chi-squared test for frequencies.

(17.9%, 95% CI 11.0%–27.9%) and 61 (77.2%, 95% CI 66.8%–85.1%) patients, respectively (Table 2). In Arm A, 28 patients (35.4%) experienced an ALT flare. From these, eight (10.1%) patients experienced an ALT flare with an ALT maximum >10x ULN, 10 (12.7%) had an ALT maximum >5x and ≤10x ULN, and 10 (12.7%) had an ALT maximum >3x and ≤5x ULN. In 20 of these patients, the ALT flares resolved spontaneously, while in eight patients, criteria for re-therapy were met and re-therapy was initiated. In two patients, ALT flares were associated with an increase in bilirubin levels, which was reversible in both patients (Fig. S2D,H). One of these patients experienced transient jaundice, which was reported as an adverse event. The status of the patients in Arm A with regard to re-treatment, virologic and biochemical outcomes at the different study visits is shown in Fig. 4. Changes in liver stiffness from baseline to week 96 did not differ between the randomization arms (Table 2).

Exploratory outcomes

In patients in Arm A with baseline HBsAg levels <100 IU/ml, 5/10 (50%) achieved HBsAg loss, while 2/15 (13.3%) patients with baseline HBsAg levels ≥100 and <1,000 IU/ml and only 1/54 (1.9%) patients with baseline HBsAg levels ≥1,000 IU/ml experienced HBsAg loss ($p = 0.001$). Indeed, patients in Arm A with HBsAg loss showed lower median HBsAg levels at the end of NUC therapy than those without HBsAg loss (72 *vs*. 2,046 IU/ml, $p < 0.001$). HBsAg loss in Arm A was not associated with the use of NUCs with high antiviral potency compared to NUCs with lower antiviral potency, and there was no difference across patients who were treated with TDF or ETV. Thus, HBsAg loss was observed in 4/44 (9%) patients receiving prior TDF, in 3/27 (11%) receiving prior ETV and in 1/8 (12.5%) receiving a different NUC.

After cessation of NUC treatment, all patients in Arm A experienced a relapse in HBV DNA levels (Fig. 3C) compared to six patients in Arm B, who showed only minimal (<1,000 IU/ml) and transient increases of HBV DNA (Fig. 3D). No patient who received re-treatment with NUCs achieved HBsAg loss. Median maximal HBV DNA elevations were higher in patients requiring re-treatment compared to patients without re-treatment including those with and without HBsAg loss, who showed similar maximal HBV DNA elevations (median maximal HBV DNA 2,120,000 IU/ml *vs*. 2,420 IU/ml and 10,400 IU/ml, $p < 0.001$).

From the 28 patients in Arm A who experienced ALT flares, three (11%) achieved HBsAg loss, compared to five (10%) of the patients without ALT flares ($p = 0.9$) (Fig. 3E). ALT flares were higher in patients without HBsAg loss requiring re-treatment than in patients without HBsAg loss not requiring re-treatment and patients with HBsAg loss (median maximal ALT 4.5x ULN, 1.2x ULN and 1.8x ULN, $p = 0.001$), respectively. In Arm B, ALT elevations occurred only transiently and were below 2x ULN in all cases (Fig. 3F). *Post hoc* analyzes of the baseline factors age, sex, ethnicity, body mass index, alcohol consumption, gamma-glutamyltransferase level, liver elastography results, and duration of HBV infection showed no significant association with HBsAg loss.

After discontinuation of NUC treatment, patients treated with either TDF (n = 44) or ETV (n = 27) showed differences in relapses in HBV DNA and ALT, respectively. Thus, the median maximum levels of HBV DNA after NUC treatment cessation were 27.8 (range, 0.7–170,000) kIU/ml in the TDF group and 5.4 (range, 0.1–89,300) kIU/ml in the ETV group ($p = 0.035$). HBV DNA relapses occurred after a median duration of 9.2 (range, 4–103.6; 95% CI 8.1–11.0) weeks in the TDF group compared to 40.9 (range, 12.1–103.7; 95% CI 36.1–73.7) weeks in the ETV

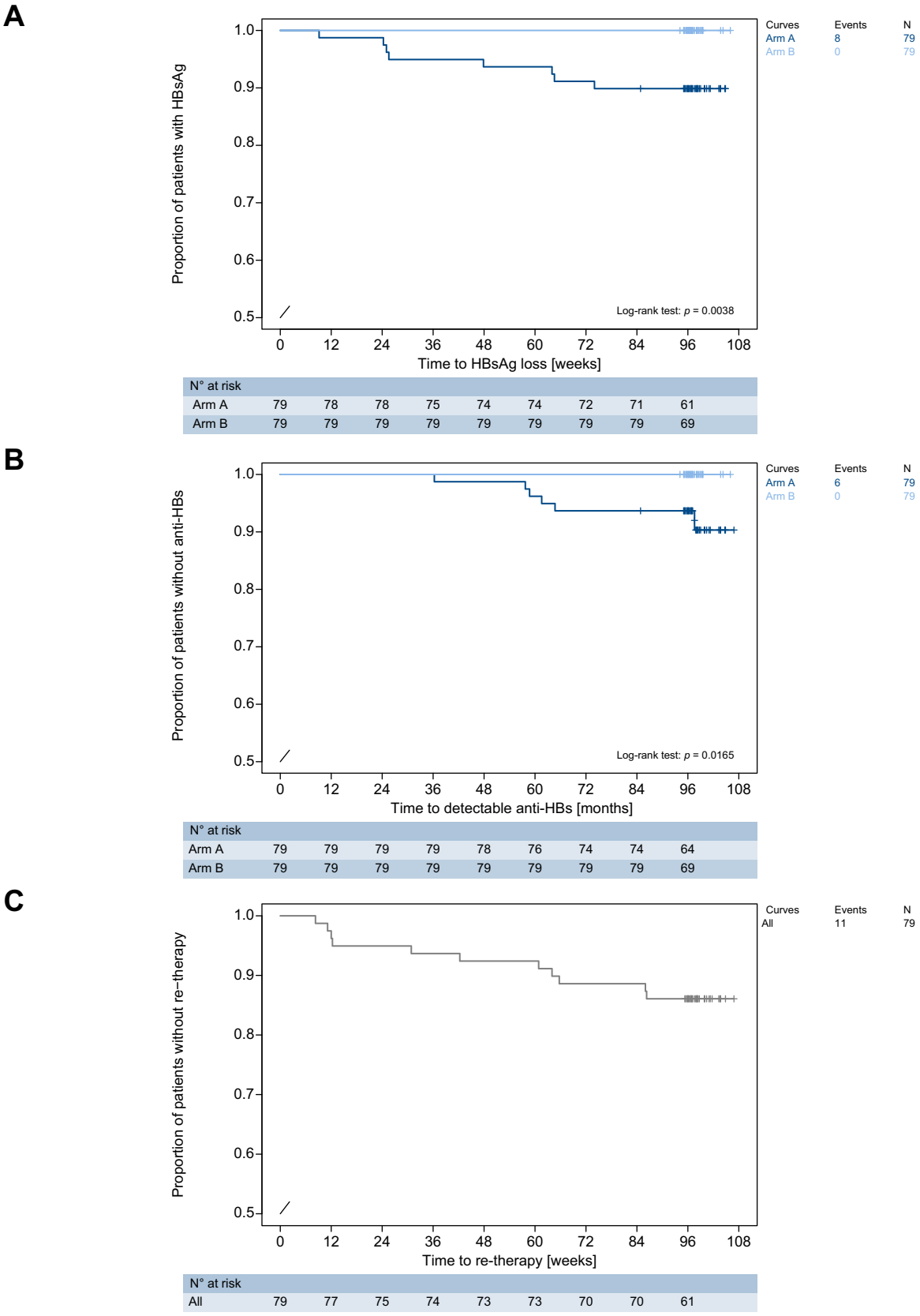

Fig. 2. HBsAg loss and HBsAg seroconversion after NUC treatment discontinuation. (A) Time to HBsAg loss. Levels of significance $p = 0.0038$ (Log-rank test, Kaplan-Meier estimates). (B) Time to HBsAg seroconversion. Levels of significance $p = 0.0165$ (Log-rank test, Kaplan-Meier estimates). (C) Time to re-treatment in trial Arm A (Kaplan-Meier-estimate). Please note that for better visibility of the time course, the proportions (y-axes) range from 0.5 to 1. NUC, nucleos(t)ide analogue.

Table 2. Outcomes.

	Arm A (n = 79)	Arm B (n = 79)	*p* value[6]
Primary endpoint			
HBsAg loss up to week 96, n (%)	8 (10.1)	0 (0)	0.006
Comparative secondary endpoints			
HBsAg seroconversion up to week 96, n (%)	6 (7.6)	0 (0)	0.028
Changes in liver stiffness (week 96 to baseline), kPA[1]	-0.3±1.8	-0.5±1.9	0.385
HBsAg reduction ≥1 log IU/ml up to week 96[2]	16 (20.3)	1 (1.3)	<0.001
Descriptive secondary endpoints for Arm A only			
Sustained remission up to week 96[3,4], n (%)	32/78 (41.0)	n.a.	
Virologic response at week 96[4], n (%)			
HBV DNA >20 IU/ml	53/78 (67.9)	0 (0)	
HBV DNA ≤20 IU/ml	14/78 (17.9)	79 (100)	
HBV DNA value not informative because of prior re-treatment	11/78 (14.1)		
Biochemical response at week 96, n (%)			
ALT >ULN	7 (8.9)	2 (2.5)	
ALT ≤ULN	61 (77.2)	77 (97.5)	
ALT value not informative because of prior re-treatment	11 (13.9)		
Patients with at least one ALT flare up to week 96[5], n (%)	28 (35.4)	0 (0)	
Patients with re-treatment up to week 96, n (%)	11 (13.9)	n.a.	

ALT, alanine aminotransferase; ULN, upper limit of normal.
[1]Mean ± SD, *p* value from ANCOVA, available for n = 64 in Arm A, n = 56 in Arm B.
[2]*Post hoc* exploratory endpoint.
[3]HBV DNA <2,000 IU/ml and normal ALT levels in all subsequent assessments after the first occurrence of remission.
[4]Please note that for one patient, central lab result at the final visit (week 96) is missing, thus reducing the denominator for sustained remission and virologic response status by one.
[5]ALT >3x ULN.
[6]Fisher's exact test for HBsAg loss, HBsAg seroconversion, HBsAg reduction ≥1 log IU/ml and *p* value from ANCOVA for liver stiffness.

group (*p* <0.001) (Fig. 5A). In patients with at least one event of ALT >ULN, median maximum ALT levels were 4.5x ULN (range, 2–43.4) in the TDF group compared to 4.4x ULN (range, 2.5-38.2) in the ETV group (*p* = 0.048); the ALT maximum occurred after a median of 10.8 (95% CI 9.9–12.1) weeks in the TDF group compared to 26.0 (95% CI 24.1–48.0) weeks in the ETV group (Fig. 5B). Time to re-treatment was similar in the TDF (n = 6) and the ETV (n = 4) groups (*p* = 0.937).

Re-treatment and safety

Re-treatment with NUCs was initiated in 11 (13.9%) patients in Arm A. Re-treatment decisions were based on trial protocol criteria in eight patients (ALT >10x ULN for at least 7 days in five, ALT >5 and ≤10x ULN for at least 28 days in one, and ALT >2 and ≤5x ULN for at least 112 days together with viral relapse with HBV DNA >20,000 IU/ml in two patients), and on an individual decision by the treating physician in three patients due to elevated HBV DNA levels. The duration to fulfil re-treatment criteria in Arm A ranged from 8-86 weeks, with a median of 42 weeks (Fig. 2C). A decrease in HBV DNA to undetectable levels and normalization of elevated ALT levels was achieved in all patients (Fig. S2).

Adverse events

In Arm A, no serious adverse events related to NUC treatment interruption occurred (Table S2).

Discussion

In this prospective, randomized, controlled multicenter trial, we observed an HBsAg loss rate of 10.1% at week 96 after NUC treatment discontinuation in HBeAg-negative patients without cirrhosis compared to 0% in those who continued NUC therapy. HBsAg loss rates were up to 28% (7/25) in patients who stopped NUC treatment with HBsAg levels <1,000 IU/ml at baseline (Fig. 3A), whereas only one patient with HBsAg levels <1,000 IU/ml showed a decrease in HBsAg levels >1 log IU/ml in the control arm (n = 26) (Fig. 3B). Apart from HBsAg loss, a considerable proportion of patients achieved HBV DNA <2,000 IU/ml and normal ALT values at the end of observation (Fig. 4), and many patients were transferred into the HBeAg-negative chronic infection phase (inactive carrier state) (40.5%) defined in our protocol as HBV DNA <2,000 IU/ml and normal ALT in all subsequent assessments after first occurrence. Importantly, only a minority of patients needed re-treatment (13.9%) (Table 2). These findings confirm, in a large randomized trial, that NUC treatment termination is currently the only available approach that succeeds in achieving functional cure in a relevant proportion of HBeAg-negative patients, which is defined as the optimal endpoint in this patient population.[4–6] Stopping NUC treatment was associated with increased bilirubin in two patients, of whom one experienced transient jaundice. No other serious adverse events were related to stopping NUCs. Hepatitis B reactivations were reliably controlled for those patients who required re-treatment with NUCs. This trial offers clear evidence that the discontinuation of NUC therapy in HBeAg-negative patients without cirrhosis is effective, safe and exceeds the rates of functional cure that have previously been documented during long-term NUC treatment.[4–7]

The mechanism by which HBsAg loss occurs after cessation of NUC treatment is not yet clear. It has been hypothesized that under NUC treatment some patients experience a reinvigoration of the exhausted HBV-specific immune phenotype. The reappearance of HBV replication after NUC discontinuation is then considered as the essential trigger which may ultimately lead to complete immune control of HBV infection, also described as autovaccination.[23–27] Long-term observations are required to study the involvement of this newly achieved immune control. Indeed, in our trial, HBsAg losses occurred continuously over the entire observation period of 96 weeks (Fig. 2A). Interestingly, in addition to the eight patients with

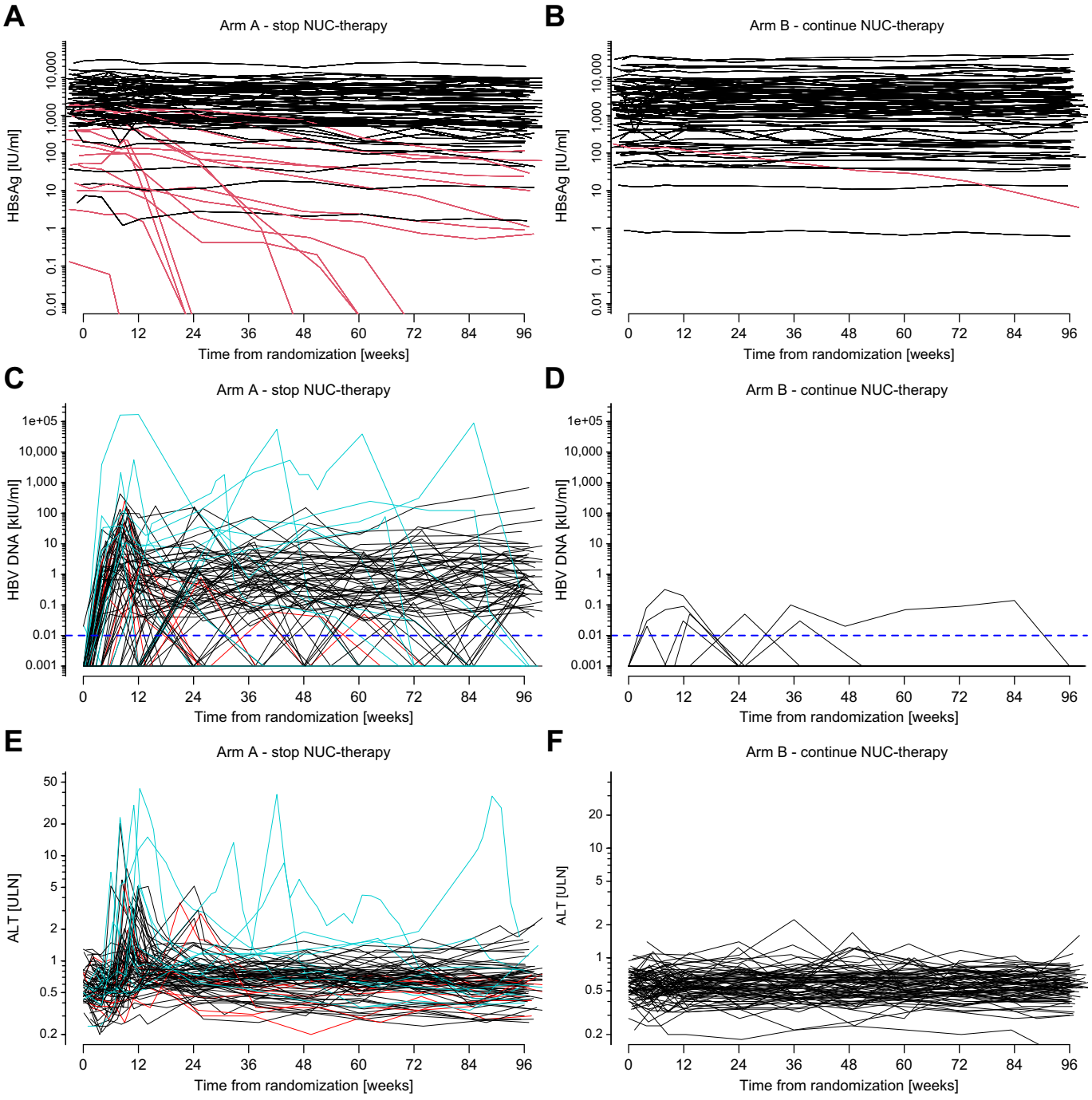

Fig. 3. Individual courses of HBsAg, HBV DNA and ALT levels in patients who stopped NUC treatment, or controls. (A) Individual time course of HBsAg in patients in the NUC discontinuation Arm A (patients with decreases >1 log IU/ml are displayed in red). (B) Individual time course of HBsAg in patients in the NUC continuation Arm B (patients with decreases >1 log IU/ml are displayed in red). (C) HBV DNA levels in Arm A (patients with HBsAg loss are displayed in red, patients with re-treament in turquoise). (D) HBV DNA levels in Arm B. (E) ALT levels in Arm A (patients with HBsAg loss are displayed in red, patients with re-treament in turquoise). (F) ALT levels in Arm B. Please note for (C) and (D): In order to make visible cases of undetectable HBV DNA (≤10 IU/ml (0.01 kIU/ml)) in the plot, these cases have been arbitrarily set to 0.001 kIU/ml, which is below the detection threshold (indicated by the dashed blue line). ALT, alanine aminotransferase; NUC, nucleos(t)ide analogue.

HBsAg loss, a continuous decline in HBsAg >1 log IU/ml was observed in another eight patients in Arm A (Fig. 3A), whereas this occurred in only one patient in Arm B, giving evidence that the drop in HBsAg in Arm A is associated with NUC treatment cessation (Fig. 3B). To address the potential increase in the HBsAg loss rate over time, we have now obtained further

funding for the STOP-NUC trial from the German Ministry of Research (BMBF), allowing us to perform a long-term follow-up of the trial cohort.

The current basis for NUC treatment discontinuation is inconsistent across many studies, and some studies show few or even an absence of HBsAg losses.[12,17] The reasons for this

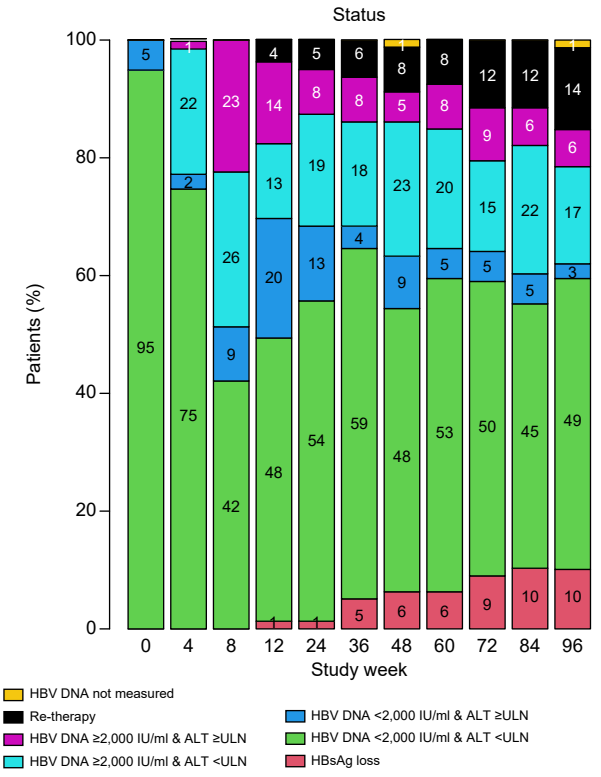

Fig. 4. HBsAg loss, HBV DNA and ALT levels, and re-treatment during 96 weeks after NUC treatment cessation. HBV DNA results were missing for one patient each at week 48 and week 96. ALT, alanine aminotransferase; NUC, nucleos(t)ide analogue; ULN, upper limit of normal.

heterogeneity are unclear, but may include factors such as HBsAg levels at the time of NUC discontinuation, or type and duration of NUC treatment. In particular, the duration of NUC treatment might be important in this regard as in many other trials the NUC treatment durations were as short as 1 year.[20,21] In contrast, in our trial, suppression of HBV DNA by NUC treatment over a period of at least 4 years was an inclusion criterion, similar to the initial trial by Hadziyannis and colleagues.[9] We considered a period of 4 years of viral replication suppressed below detection to be a rational prerequisite for achieving immune reinvigoration under therapy, which is ultimately necessary for the induction of a functional cure after treatment cessation. Another variable factor across studies is the timing of re-treatment in patients developing flares. Indeed, it was demonstrated in a previous study that patients with clinical relapses after NUC discontinuation who remained untreated had a significantly higher incidence of HBsAg loss than those who were immediately re-treated.[28] To safeguard against re-treating patients too early, we designed our re-treatment criteria to ensure patient safety by interrupting severe ALT flares early on but tolerating milder flares for defined periods at the same time. These criteria proved effective in preventing serious adverse events in patients with flares. Interestingly, in our trial, ALT flares seemed not to be associated with the probability of achieving HBsAg loss in Arm A, and none of the patients who required re-treatment due to very high ALT flares achieved HBsAg loss (Fig. 5E). Accordingly, HBV DNA relapses were highest in patients requiring re-treatment in Arm A, and similar in patients without re-treatment and with or without HBsAg loss (Fig. 5C). It therefore seems questionable whether the extent of such virologic and biochemical flares is causal for the clearance of HBsAg or merely a phenomenon reflecting the trigger of the immune response. Although we want to ensure maximal safety, we feel that early re-treatment of all ALT flares would lead to overtreatment, especially in those patients who had spontaneous development of an inactive carrier stage after the flare.

However, perhaps it is not the HBV DNA relapse *per se*, but the magnitude of the increase in HBV DNA after termination of NUC treatment that should be considered as a precursor to a severe ALT flare. Accordingly, a case of a patient who stopped NUC treatment as part of a trial and developed fulminant liver failure after a rapid increase in HBV DNA was recently published.[29] The fact that high HBV DNA relapses after NUC termination were not associated with HBsAg loss in our study (Fig. 3C) suggests that earlier treatment of HBV DNA relapses does not affect the chances of response to NUC termination.

In any case, a better understanding of the nature of virologic and biochemical flares after NUC cessation is necessary to optimize flare management. In addition, regional factors influencing HBsAg loss rates such as HBV genotype, the duration of HBV infection and host genetic factors, which could be responsible for the consistently lower HBsAg loss rates in Asian studies, must be considered.[15,17,18,20,21,28]

In our trial, seven out of eight patients in Arm A who achieved functional cure had HBsAg levels <1,000 IU/ml at the time point of NUC discontinuation (Fig. 3A). This observation is in line with previous studies reporting that the most consistent predictor of functional cure is low HBsAg level at the time of NUC treatment discontinuation.[30] Apart from HBsAg, experimental HBV serum biomarkers such as HBV RNA or hepatitis B core-related antigen, which are both markers of intrahepatic covalently closed circular DNA activity, have been associated with response to NUC treatment discontinuation.[21,31–34] These markers have not been validated and are not currently available for treating physicians. However, a possible improvement in the prediction of HBsAg loss represents a major clinical need; thus, the use of HBV RNA or hepatitis B core-related antigen must be investigated in future studies.

The STOP-NUC trial achieved its enrolment plan and had a very low drop-out rate of 4.8% (Fig. 1). A limitation of our trial is the focus on European patients. We therefore believe that similar trials should be performed in Asian and African regions with high HBV incidences and different HBV genotypes. Future studies should also investigate the role of intra- and extrahepatic immune cells that are involved in the establishment of functional cure of HBV infection defined by HBsAg loss.

In summary, we demonstrate the effectiveness of controlled discontinuation of NUC treatment in HBeAg-negative patients without advanced liver disease who previously received effective long-term NUC treatment over 96 weeks. Cessation of NUC therapy in HBeAg-negative patients was effective in reaching treatment endpoints that cannot be achieved with long-term NUC treatment, and it therefore holds the potential to revolutionize the treatment for these patients. The effectiveness of NUC treatment discontinuation is demonstrated by an increase in the functional cure rate, especially in European patients with HBsAg levels <1,000 IU/ml, but also by the absence of a therapeutic indication in many patients with higher HBsAg levels. This is shown in Table 2, with sustained remission up to

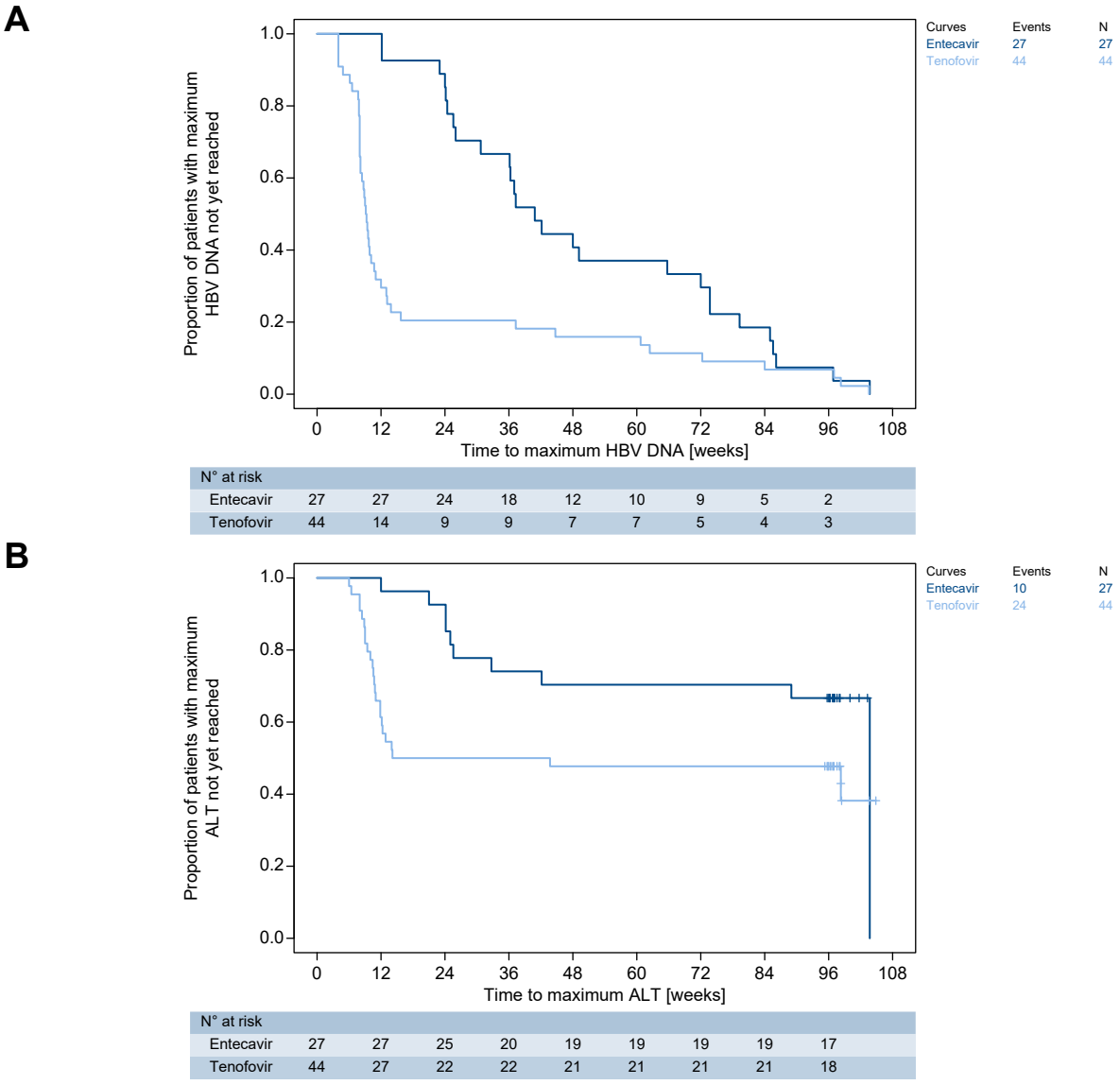

Fig. 5. Time to maximum flares in HBV DNA levels and ALT levels after discontinuation of tenofovir or entecavir treatment. (A) Time to maximum HBV-DNA in patients in Arm A, by type of prior NUC therapy (Kaplan-Meier estimates). (B) Time to maximum ALT (in case of ALT >ULN) in patients in Arm A, by type of prior NUC therapy. Patients with continuous ALT ≤ULN are censored (Kaplan-Meier estimates). ALT, alanine aminotransferase; NUC, nucleos(t)ide analogue; ULN, upper limit of normal.

week 96 in 41% and normal ALT at week 96 without re-therapy in 77% of patients. The powerful effect of NUC treatment cessation will need to be explored over longer time periods, but it may represent a step towards functional cure for many patients, especially those with low HBsAg levels, and it needs to be considered for novel treatment approaches (*e.g.,* via RNA interference mechanisms or immune stimulation) given in combination with NUCs for finite treatment durations.

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Abbreviations

ALT, alanine aminotransferase; ANCOVA, analysis of covariance; CI, coincidence interval; ETV, entecavir; HBeAG, hepatitis B e antigen; HBsAG, hepatitis B surface antigen; HBV, hepatitis B virus; NUCs, nucleos(t)ide analogues; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

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Conflict of interest

All authors disclose any conflicts of interest related to the present work.

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

Authors' contributions

FvB, OB and TB designed the study. Statistical analyses were performed by OB. The manuscript was prepared and written by FvB and OB. FvB, KS, RH, JP, PB, CB, SZ, AS, MS, ES, AP, UvA, VK, JL, KGS, CT, AT, DH, MC, FL, PI, RZ, HH, AZ, HK, JSzW and TB have collected data. AS was responsible for trial management and supervision. All authors have reviewed and approved the final submitted manuscript. FvB is deputy of the academic sponsor of the study, University of Leipzig.

Data availability statement

Individual participant data pertinent to the results reported in this publication will be shared after de-identification to researchers who provide a methodologically sound and ethically approved research proposal. To gain access, data requestors will need to sign a data-access agreement.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.12.018.

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