Durability of Hepatitis B Surface Antigen Loss With Nucleotide Analogue and Peginterferon Therapy in Patients With Chronic Hepatitis B

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In patients with chronic hepatitis B (CHB), loss of hepatitis B surface antigen (HBsAg) is considered a functional cure. However, HBsAg loss is uncommon with existing therapies, and predictive factors associated with HBsAg seroreversion are unknown. Using pooled data from three phase 3 clinical trials of patients with CHB treated with nucleos(t)ide analogue (NUC) monotherapy or peginterferon (Peg-IFN) ± NUC combination therapy, we conducted a retrospective analysis to characterize patients who achieved sustained HBsAg loss, the predictors of HBsAg seroreversion, and the impact of hepatitis B surface antibody (anti-HBs) seroconversion on durability of HBsAg loss. In these three international trials, 1,381 adults with CHB received either NUC monotherapy for up to 10 years or Peg-IFN-containing regimens for up to 1 year. A total of 55 patients had confirmed HBsAg loss, defined as two or more consecutive negative-qualitative HBsAg results, with a minimum of one repeat result after the end of treatment. Throughout a median of 96 (quartile [Q]1, Q3, 46, 135) weeks follow-up after HBsAg loss, HBsAg loss was durable in 82% (n = 45) of patients, with 10 patients experiencing HBsAg seroreversion. Anti-HBs seroconversion was observed during follow-up in 78% of patients who lost HBsAg and in 60% of those who subsequently seroreverted. In analyzing predictors of HBsAg seroreversion, study treatment was significant, yet anti-HBs seroconversion and treatment duration after initial HBsAg loss were not. Risk of HBsAg seroreversion was observed to be lower if HBsAg loss was sustained through the off-treatment week 24 visit (8/10 seroreversions occurred by posttreatment week 24). *Conclusion:* HBsAg loss after NUC or Peg-IFN-containing regimens was durable in 82% of patients with CHB. Anti-HBs seroconversion and treatment duration after initial HBsAg loss were not significantly associated with durability of HBsAg loss. (*Hepatology Communications* 2020;4:8-20).

Worldwide, an estimated 257 million people are chronically infected with the hepatitis B virus (HBV), and more than 800,000 die annually due to HBV-related liver complications.[1] The goals of treatment for chronic HBV infection are to suppress viral replication and ultimately reduce or

Abbreviations: ALT, alanine aminotransferase; anti-HBs, hepatitis B surface antibody; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IU, international unit; KM, Kaplan-Meier; LLOD, lower limit of detection; NUC, nucleos(t)ide; Peg-IFN, peginterferon; Q, quartile; TDF, tenofovir disoproxil fumarate.

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prevent liver injury. Antiviral therapy has been shown to reduce the risks of cirrhosis, decompensated liver disease, and hepatocellular carcinoma (HCC) in patients with immune active HBV infection,[2] but few patients achieve seroclearance of hepatitis B surface antigen (HBsAg), which is widely accepted as a "functional cure."[3,4] However, HBsAg loss is uncommon with existing therapies, and durability of HBsAg loss and predictive factors associated with HBsAg seroreversion are unknown.

There is no standard or consistent definition of HBsAg loss when used as a treatment endpoint. Questions remain concerning the types of assays and sensitivity of assays used to detect HBsAg; whether HBsAg testing needs to be repeated and, if yes, after what interval to confirm sustained HBsAg loss; and whether seroconversion to hepatitis B surface antibody (anti-HBs) should be included in the definition of HBsAg loss. Clarification of these issues is important in designing clinical trials of new therapies aimed at an HBV functional cure. An important consideration in the choice of definition of HBsAg loss as an endpoint in clinical trials is its association with the durability of HBsAg loss after treatment is stopped.

We conducted a retrospective assessment of HBsAg loss using pooled data from three phase 3 clinical trials of patients with chronic hepatitis B (CHB) treated with nucleos(t)ide analogue (NUC) monotherapy or peginterferon (Peg-IFN)-containing combination therapy. The goals were to characterize patients with sustained HBsAg loss and to identify predictors of HBsAg seroreversion.

Patients and Methods

STUDY POPULATION

This analysis included patients who achieved HBsAg loss in three previously reported phase 3 studies.[5-8] In studies GS-US-174-0102 (patients who were hepatitis B e antigen [HBeAg] negative) and GS-US-174-0103 (patients who were HBeAg positive), patients received adefovir or tenofovir disoproxil fumarate (TDF) for 48 weeks then switched to TDF for up to 480 weeks. In GS-US-174-0149, patients who were HBeAg positive and patients who were HBeAg negative received Peg-IFN for 48 weeks, Peg-IFN plus TDF for 48 weeks, or Peg-IFN for 16 weeks plus TDF for 48 weeks. In all, 1,381 patients ≥18 years old with CHB received treatment across North America, Europe, and the Asia-Pacific region. All patients were HBsAg positive for at least 6 months before enrollment and were not taking any HBV antiviral treatment at the time of enrollment. Anti-HBs status was not evaluated at the time of enrollment. Key exclusion criteria were co-infection with human immunodeficiency virus 1 or hepatitis C or D virus, evidence of HCC or liver decompensation, or creatinine clearance <70 mL/minute.

ASSESSMENTS

Assessments were completed at planned study visits with visit windows ±1 week of the intended date. HBsAg loss was defined as a single-negative or nonreactive qualitative HBsAg result after the first dose

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of treatment. Confirmed HBsAg loss was defined as at least two consecutive negative HBsAg results and at least one negative HBsAg result ≥1 week after treatment discontinuation. The presence of HBsAg was assessed using the following qualitative Abbott Laboratories (Abbott Park, IL) assays: AUSZYME monoclonal enzyme immunoassay (lower limit of detection [LLOD], 0.04-0.13 international units [IU]/mL) in GS-US-174-0102 and -0103 and ARCHITECT Qualitative II assay (LLOD, 0.017-0.022 IU/mL; assay cutoff of 1.00 signal-to-cutoff) in GS-US-174-0149. Quantitative HBsAg was measured using the ARCHITECT assay (LLOD, 0.05 IU/mL) in all studies. Seroconversion to anti-HBs, defined as a single-positive anti-HBs test, was determined using AUSAB enzyme immunoassay (positive result >10 IU/L; Abbott Laboratories). Anti-HBs status was only assessed when an HBsAg result was negative. HBsAg seroreversion was defined as a positive HBsAg result after a negative HBsAg result. Loss of anti-HBs antibodies was defined as a negative anti-HBs result after a positive anti-HBs result. Serum HBV DNA was quantified using the Roche COBAS TaqMan assay (Roche Diagnostics, Branchburg, NJ; lower limit of quantification, 29 IU/mL). All specified laboratory tests were performed at a central laboratory.

STATISTICAL ANALYSES

Descriptive statistics were used to summarize baseline demographics and disease characteristics for patients with HBsAg loss. Differences in baseline demographics and disease characteristics between patients who experienced seroreversion and those who did not were tested using Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Durability of HBsAg loss was defined as the duration from the first HBsAg-negative result to the first redetection of HBsAg and estimated using the Kaplan-Meier (KM) method. On-treatment HBsAg loss was defined as HBsAg loss occurring during treatment, and off-treatment HBsAg loss was defined as HBsAg loss occurring after the last dose of treatment. Treatment duration from the time of initial HBsAg loss was defined as the duration of treatment from the first HBsAg-negative result to the last dose of treatment.

A list of key potential predictors and cutoffs were specified based on clinical relevance. Fisher's exact test was used to assess the association between potential predictors and HBsAg seroreversion. The analysis was then repeated within subgroups of each significant predictor to assess for potential interactions among predictors.

Results

Across the three phase 3 studies (Fig. 1), 74 patients with CHB had at least one negative HBsAg result (HBsAg <LLOD). Nineteen patients were excluded from the analysis either because they did not have a repeat visit after the initial HBsAg loss (n = 5), had only a single (unconfirmed) HBsAg-negative result (n = 5), or did not have an off-treatment repeat result at least 1 week after the initial HBsAg loss (n = 9). Among the 5 patients who only had a single HBsAg-negative result, all experienced HBsAg seroreversion: 3 patients during treatment and 2 patients during off-treatment follow-up; all remained HBsAg positive for the remainder of the study.

Durability of HBsAg loss off treatment was evaluated in 55 patients with confirmed HBsAg loss. Throughout a median follow-up of 96 (quartile [Q]1, Q3, 46, 135) weeks after the initial HBsAg loss, HBsAg loss was durable in 45 (82%) patients, while 10 (18%) patients experienced HBsAg seroreversion after treatment discontinuation.

BASELINE CHARACTERISTICS AND TREATMENT SUMMARY OF PATIENTS WITH AND WITHOUT HBsAg SEROREVERSION

Baseline characteristics and treatment summary of patients without HBsAg seroreversion (n = 45) compared to those with HBsAg seroreversion (n = 10) are summarized in Table 1. Overall, there were no significant differences between the two groups. The median (Q1, Q3) age was 39 (27, 48) years, and a majority were HBeAg positive (73%) before treatment or of non-Asian race (65%). HBV genotype A infection was most common. The median (Q1, Q3) baseline HBV DNA level was 8.0 (5.9, 8.0) $\log_{10}$ IU/mL, HBsAg level was 4.6 (3.4, 5.1) $\log_{10}$ IU/mL, and alanine aminotransferase (ALT) level was 110 (75, 176) U/L. The median (Q1, Q3) duration of

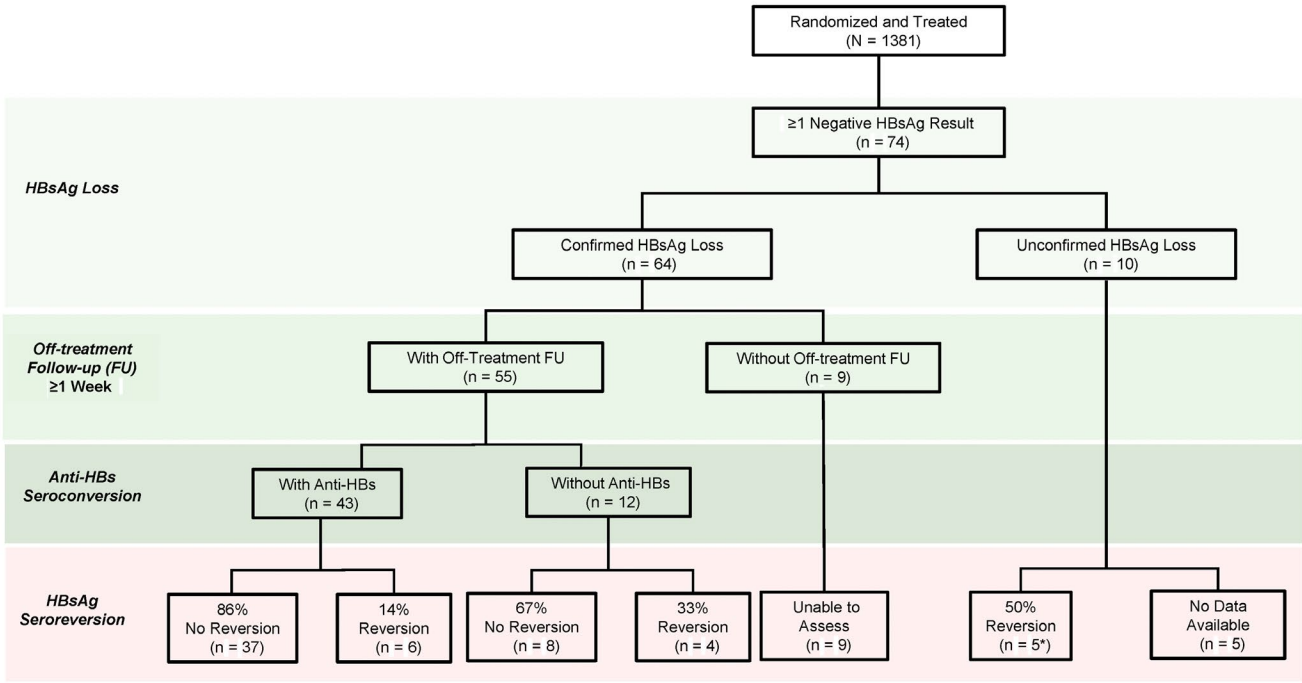


FIG. 1. Flow chart showing patient selection and outcomes. *HBsAg seroreversion occurred during treatment in 3 patients and off treatment in 2 patients; all remained HBsAg positive for the remainder of the study. Abbreviation: FU, follow-up.

treatment was 48 (48-120) weeks for patients with seroreversion, which was shorter compared with 109 (48-209) weeks in patients with no HBsAg seroreversion, but the median duration of off-treatment follow-up in the two groups was identical at 72 weeks. The median duration of treatment from the time of initial HBsAg loss in patients that experienced HBsAg seroreversion was similar to those with durable HBsAg loss (median, 24 weeks; Q1-Q3, 12-32 weeks versus median, 30 weeks; Q1-Q3, 24-48 weeks, respectively).

TIMING AND DURATION OF HBsAg LOSS

In the 55 patients with confirmed HBsAg loss, HBsAg loss occurred while on treatment in 47 (85%) patients (Table 2): 27 (93%) patients treated with NUC and 20 (77%) patients treated with Peg-IFN. The median (Q1, Q3) duration of treatment from the time of initial HBsAg loss was 40 (25, 84) weeks in patients treated with NUC and 24 (16, 32) weeks in patients treated with Peg-IFN. Eight (15%) patients achieved HBsAg loss after discontinuation of treatment: 2 patients treated with NUC and 6 patients treated with Peg-IFN.

Of the 55 patients, the median (Q1, Q3) duration of off-treatment follow-up after HBsAg loss was 57 (24, 73) weeks, with a range from 0.3 to 336 weeks. The median (Q1, Q3) number of off-treatment repeat HBsAg tests after HBsAg loss per patient was 11 (4, 14), with 91% and 67% of patients having at least one repeat HBsAg result at study visits ≥24 weeks and ≥48 weeks off treatment, respectively. In patients that maintained HBsAg loss at off-treatment study visits at weeks 24, 48, 96, and 144, the cumulative durability of HBsAg loss was 95% (38/40), 93% (27/29), 91% (10/11), and 90% (9/10), respectively.

OCCURRENCE OF ANTI-HBs SEROCONVERSION AND LOSS OF ANTI-HBs ANTIBODIES

Anti-HBs seroconversion was observed in 43 (78%) patients who achieved HBsAg loss (Table 2), with a similar rate in patients treated with NUC and Peg-IFN (79% vs. 77%, respectively). The majority

TABLE 1. BASELINE CHARACTERISTICS AND STUDY SUMMARY OF PATIENTS WHO LOST HBsAg WITH OR WITHOUT SEROREVERSION

	Total (n = 55)	No Seroreversion (n = 45)	Seroreversion (n = 10)	*P* value
Age, years	39 (27-48)	38 (28-48)	39 (27-45)	1.0
Sex, female, n (%)	10 (18)	9 (20)	1 (10)	0.67
Race, n (%)				0.07
Asian	19 (35)	15 (33)	4 (40)	
Black or African American	3 (6)	1 (2)	2 (20)	
White	33 (60)	29 (64)	4 (40)	
BMI, kg/m^2	25.4 (22.3-28.1)	25.4 (23.0-28.1)	25.5 (20.8-27.7)	0.87
Geographic region, n (%)				0.46
Europe	29 (53)	25 (56)	4 (40)	
North America	13 (24)	10 (22)	3 (30)	
Asia-Pacific	4 (7)	3 (7)	1 (10)	
India	5 (9)	3 (7)	2 (20)	
Australia/New Zealand	4 (7)	4 (9)	0 (0)	
HBV genotype, n (%)				0.79
A	24 (44)	19 (42)	5 (50)	
B	11 (20)	8 (18)	3 (30)	
C	6 (11)	6 (13)	0 (0)	
D	12 (22)	10 (22)	2 (20)	
F	2 (4)	2 (4)	0 (0)	
HBeAg positive, n (%)	40 (73)	32 (71)	8 (80)	0.71
HBV DNA level, $\log_{10}$ IU/mL	8.0 (5.9-8.0)	8.0 (5.9-8.0)	8.0 (6.6-8.3)	0.31
HBsAg level, $\log_{10}$ IU/mL	4.6 (3.4-5.1)	4.6 (3.5-5.1)	4.8 (3.3-5.1)	0.84
ALT level, U/L	110 (75-176)	110 (74-168)	117 (84-176)	0.74
Total study duration, week	123 (120-385)	123 (120-386)	120 (120-157)	0.20
Treatment duration	95 (48-179)	109 (48-209)	48 (48-120)	0.34
NUC (n = 29)	168 (120-327)	179 (137-348)	126 (109-160)	
Peg-IFN ± NUC (n = 26)	48 (48-48)	48 (48-48)	48 (48-48)	
Duration of treatment from time of initial HBsAg loss (n = 47)*	29 (23-48)	30 (24-48)	24 (12-32)	0.19
NUC (n = 27)	40 (25-84)	40 (25-96)	35 (16-67)	
Peg-IFN ± NUC (n = 20)	24 (16-32)	24 (16-32)	24 (0.1-32)	
Posttreatment follow-up duration	72 (40-75)	72 (44-75)	72 (25-72)	0.62
At time of HBsAg loss				
HBV DNA ≤29 IU/mL, n (%)	51 (93%)	41 (91%)	10 (100%)	1.0
HBeAg negative, n (%) (n = 45)	45 (100%)	36 (100%)	9 (100%)	1.0
Anti-HBe positive, n (%) (n = 37)	37 (100%)	30 (100%)	7 (100%)	1.0
ALT ≤ULN, n (%)	29 (53%)	24 (53%)	5 (50%)	1.0
HBsAg, IU/mL (n = 49)	0.1 (0.0-1.0)	0.1 (0.0-1.0)	0.1 (0.0-1.0)	0.57
APRI (n = 51)	0.4 (0.3-0.9)	0.4 (0.3-0.7)	0.7 (0.4-1.1)	0.21

Results are shown as median (Q1-Q3) in table.
*Excluded patients with off-treatment HBsAg loss.
Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; BMI, body mass index; ULN, upper limit of normal.

(77%) of anti-HBs seroconversion occurred during treatment, and 44% (n = 19) of anti-HBs seroconversion was detected on the same day as HBsAg loss. Anti-HBs seroconversion occurred in 60% of those who had HBsAg seroreversion and 82% of those who did not have HBsAg seroreversion. The median (Q1, Q3) time from HBsAg loss to anti-HBs seroconversion was 8 (0, 16) weeks: 12 (0, 24) weeks in patients treated with NUC and 8 (0, 16) weeks in patients treated with Peg-IFN. Loss of anti-HBs antibodies

TABLE 2. INCIDENCE AND TIMING OF HBsAg LOSS, ANTI-HBs SEROCONVERSION, AND HBsAg SEROREVERSION

	Total (n = 55)	NUC (n = 29)	Peg-IFN ± NUC (n = 26)
Overall HBsAg loss, n (%)	55 (100)	29 (100)	26 (100)
Time to first HBsAg loss, week	64 (32-120)	119 (80-240)	28 (21-48)
With anti-HBs seroconversion, n (%)	43 (78)	23 (79)	20 (77)
Time from HBsAg loss to anti-HBs seroconversion, week*	8 (0-16)	12 (0-24)	8 (0-16)
HBsAg seroreversion, n (%)	10 (18)	4 (14)	6 (23)
With anti-HBs seroconversion	6/43 (14)	3/23 (13)	3/20 (15)
Without anti-HBs seroconversion	4/12 (33)	1/6 (17)	3/6 (50)
Time from HBsAg loss to HBsAg seroreversion, week†	35 (12-96)	66 (32-162)	22 (5-48)
On-treatment HBsAg loss, n (%)	47 (85)	27 (93)	20 (77)
Time to first HBsAg loss, week	48 (24-120)	108 (64-168)	24 (16-32)
Duration of treatment from time of initial HBsAg loss, week	29 (23-48)	40 (25-84)	24 (16-32)
Duration of off-treatment follow-up, week	70 (26-75)	57 (25-207)	72 (45-73)
HBsAg seroreversion, n (%)	9 (19)	4 (15)	5 (25)
With anti-HBs seroconversion	5/39 (13)	3/23 (13)	2/16 (13)
Without anti-HBs seroconversion	4/8 (50)	1/4 (25)	3/4 (75)
Time from last dose to HBsAg seroreversion,† week	12 (6-25)	18 (9-102)	12 (5-24)
Off-treatment HBsAg loss, n (%)	8 (15)	2 (7)	6 (23)
Duration of treatment, week	48 (48-216)	433 (383-482)	48 (48-48)
Time from last dose to first HBsAg loss, week	17 (8-36)	12 (12-13)	29 (4-36)
HBsAg seroreversion, n (%)	1 (13)	0 (0)	1 (17)
With anti-HBs seroconversion	1/4 (25)	0/0 (0)	1/4 (25)
Without anti-HBs seroconversion	0/4 (0)	0/2 (0)	0/2 (0)
Time from HBsAg loss to HBsAg seroreversion,† week	4 (4-4)	-	4 (4-4)

Results are shown as median (Q1-Q3) in table.
*Excludes subjects who did not achieve anti-HBs.
†Only includes subjects who experienced seroreversion.

occurred in 12 (28%) patients with anti-HBs seroconversion; however, only 2 of these 12 patients also experienced HBsAg seroreversion.

HBsAg SEROREVERSION

Ten (18%) patients who lost HBsAg experienced seroreversion to HBsAg positivity during follow-up (Table 2). Patients with anti-HBs seroconversion had a higher rate of durable HBsAg loss than those without anti-HBs seroconversion (86% vs. 67%; Fig. 2). This difference was not statistically significant, possibly due to the relatively small sample size.

Of the 10 patients with HBsAg seroreversion, 4 had a single (unconfirmed) HBsAg-positive result after achieving confirmed HBsAg loss: in 2 patients HBsAg seroreversion occurred at the last study visit and 2 patients had transient HBsAg seroreversion that reverted to HBsAg negativity at the next study visit (marked with an asterisk in Fig. 3). Changes in

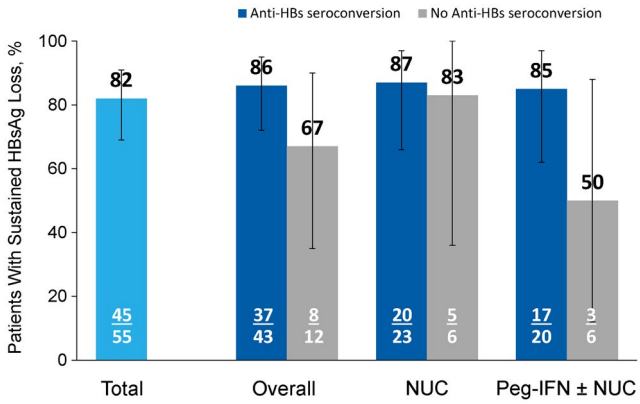


FIG. 2. Durability of HBsAg loss in patients with and without anti-HBs seroconversion. Bars represent 95% confidence intervals. White text within bars represent n/N.

HBV DNA, ALT, and HBsAg levels for each patient with HBsAg seroreversion are shown in Fig. 4. After the transient HBsAg seroreversion, these 2 patients,

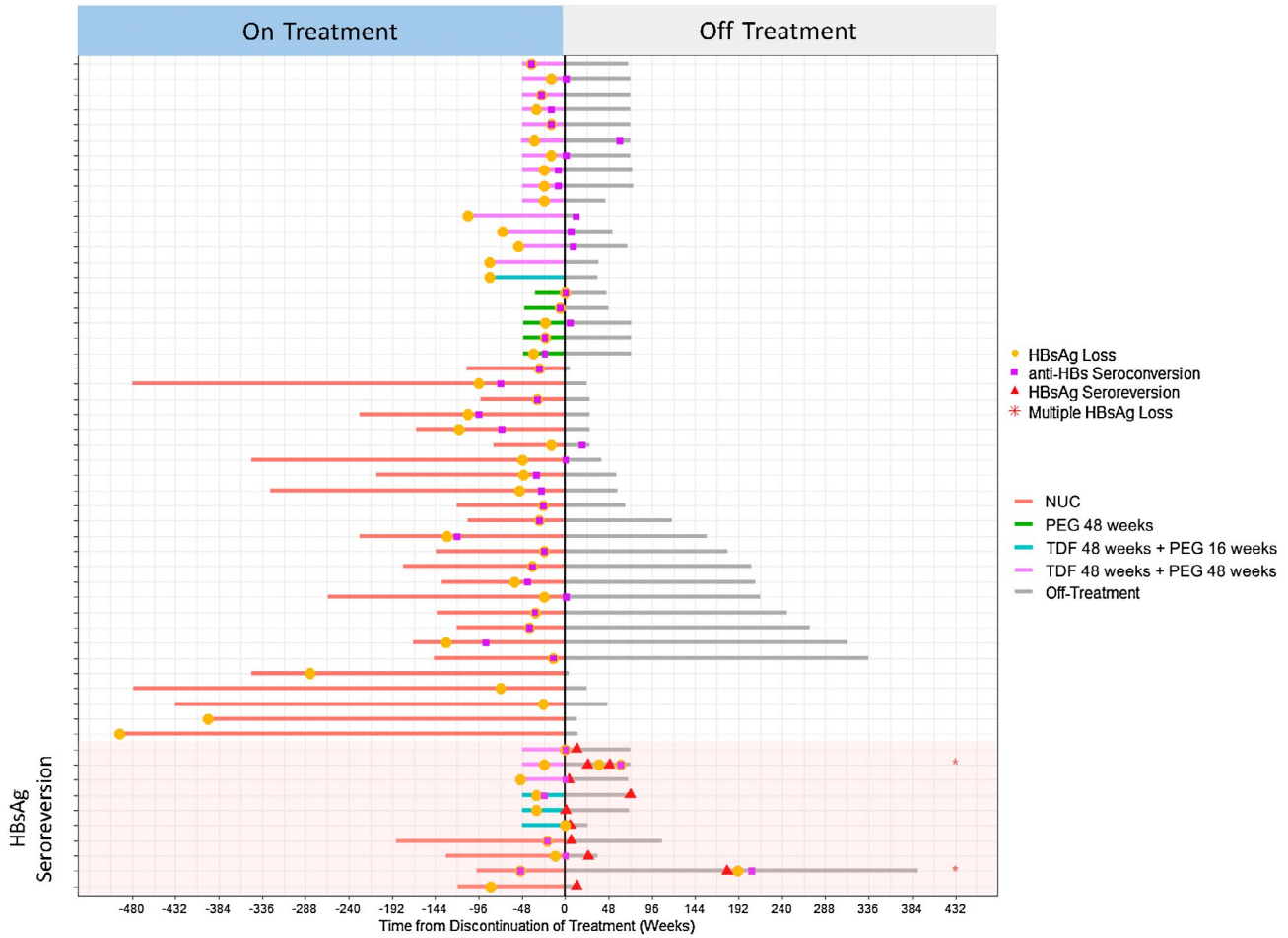


FIG. 3. Timing of HBsAg loss, anti-HBs seroconversion, and HBsAg seroreversion. Each line is an individual patient. The length of the line indicates treatment duration in the study, and the symbols indicate the key events. Patients are sorted from top to bottom in order by HBsAg seroreversion occurrence, treatment category, on- or off-treatment HBsAg loss, HBsAg seroconversion occurrence, and off-treatment HBsAg loss duration.

1 in each treatment group, maintained HBsAg loss through to the last follow-up visit 11 weeks (n = 1) and 199 weeks (n = 1) after HBsAg seroreversion. One of these 2 patients achieved anti-HBs seroconversion only after becoming HBsAg negative the second time. The other 6 patients remained HBsAg positive after the HBsAg seroreversion.

None of the 10 patients experienced seroreversion while on treatment. A majority (80%) of the patients that experienced seroreversion did so by the week 24 off-treatment study visit. Of the 2 patients that experienced seroreversion after the week 24 off-treatment study visit, 1 had an unconfirmed HBsAg-positive result at the last off-treatment follow-up visit (week 72) while the other had a single, transient, HBsAg-positive result at the off-treatment follow-up week 180 visit and remained HBsAg negative thereafter (Fig. 3). The median (Q1, Q3) interval from the last treatment dose to HBsAg seroreversion was 12 (5, 25) weeks: 19 (9, 102) weeks in patients treated with NUC and 9 (4, 24) weeks in patients treated with Peg-IFN.

The duration of follow-up time after HBsAg seroreversion varied among the 10 patients, with a median (Q1, Q3) of 54 (11, 71) weeks. The vast majority (80%) of these patients maintained HBV DNA and ALT levels below baseline levels after HBsAg seroreversion. Two patients initiated NUC treatment after HBsAg seroreversion (Table 3).

The KM estimated rate of durability of HBsAg loss up to the last available visit overall was 72% (Fig. 5A).

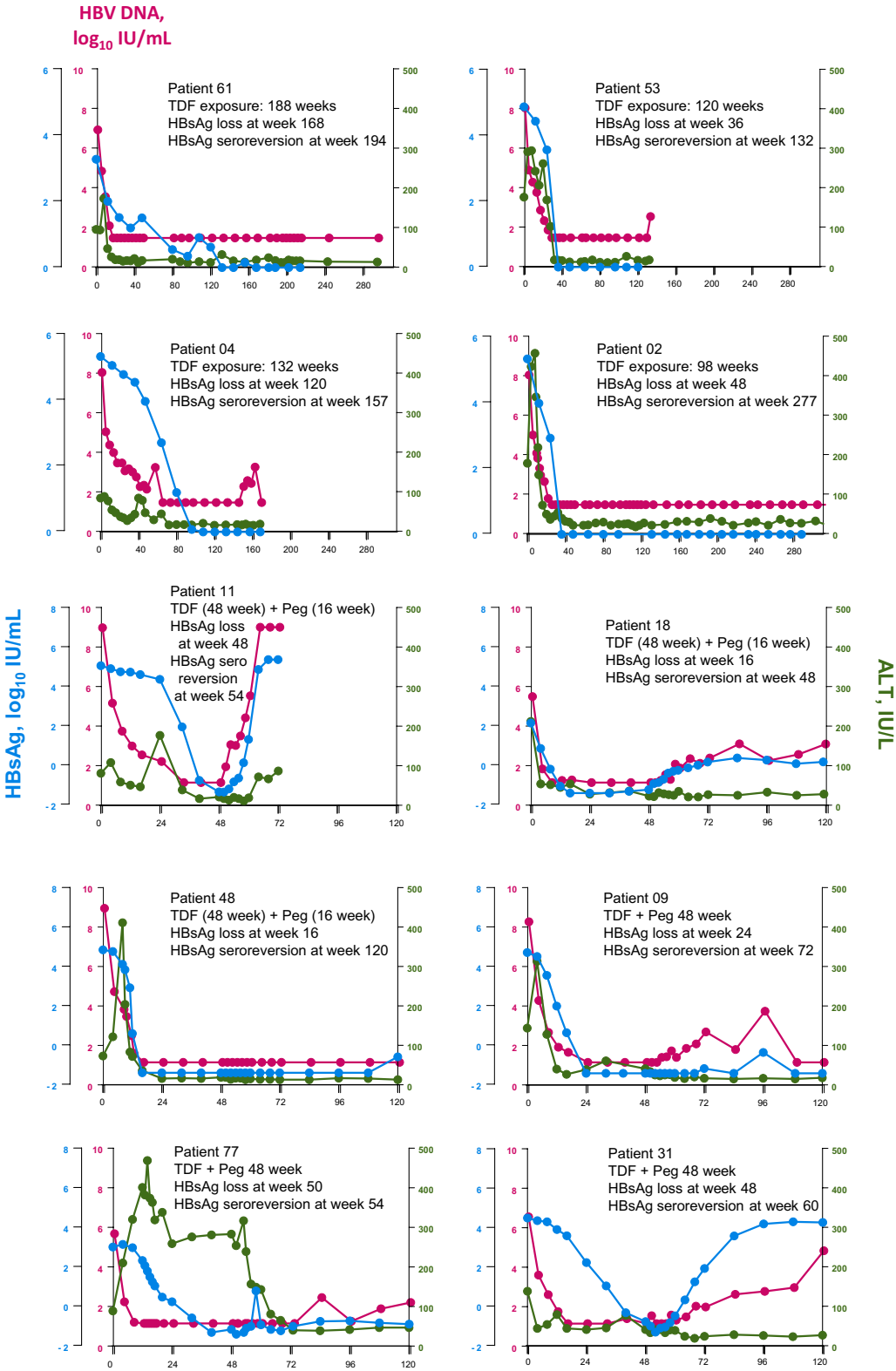


FIG. 4. Change in plasma HBV DNA (log_{10} IU/mL), serum ALT (log_2 U/L), and serum HBsAg levels in patients with HBsAg seroreversion. Quantitative changes in HBV DNA (red circles), ALT (green circles), and HBsAg (blue circles) levels over time in the 10 patients with HBsAg seroreversion.

TABLE 3. OUTCOMES OF PATIENTS AFTER HBsAg SEROREVERSION

Patient No.	Treatment	Confirmed HBsAg Seroreversion	Peak HBV DNA IU/mL	Peak ALT U/L	Resumed Treatment
04	TDF	Yes	1,810	18	Yes
11	TDF48PEG16	Yes	1,000,000,000	87	Yes
61	TDF	Yes	29	18	No
77	TDF48PEG48	Yes	285	239	No
18	TDF48PEG16	Yes	1,282	35	No
31	TDF48PEG48	Yes	69,329	39	No
02	TDF	No	29	43	No
09	TDF48PEG48	No	5,467	18	No
53	TDF	No further HBsAg test	342	17	No
48	TDF48PEG16	No further HBsAg test	14	13	No

Abbreviations: TDF48PEG16, TDF for 48 weeks and PEG for 16 weeks; TDF48PEG48, TDF for 48 weeks and PEG for 48 weeks.

The duration of off-treatment follow-up was shorter in patients treated with Peg-IFN (72 weeks vs. 336 weeks for patients treated with NUC). The estimated rate of durable HBsAg loss at week 72 overall was 81%: 74% for patients treated with Peg-IFN and 88% for patients treated with NUC. When the 2 patients with transient HBsAg seroreversion (1 treated with Peg-IFN and 1 with NUC) were excluded, the estimated overall durability of HBsAg loss was 83%. At week 72, the estimated rate of durable HBsAg loss was 78% in patients treated with Peg-IFN and 88% in patients treated with NUC (Fig. 5B). Among the patients treated with NUC, the estimated durability of HBsAg loss at week 336 was 88% when the patient with transient HBsAg seroreversion was excluded.

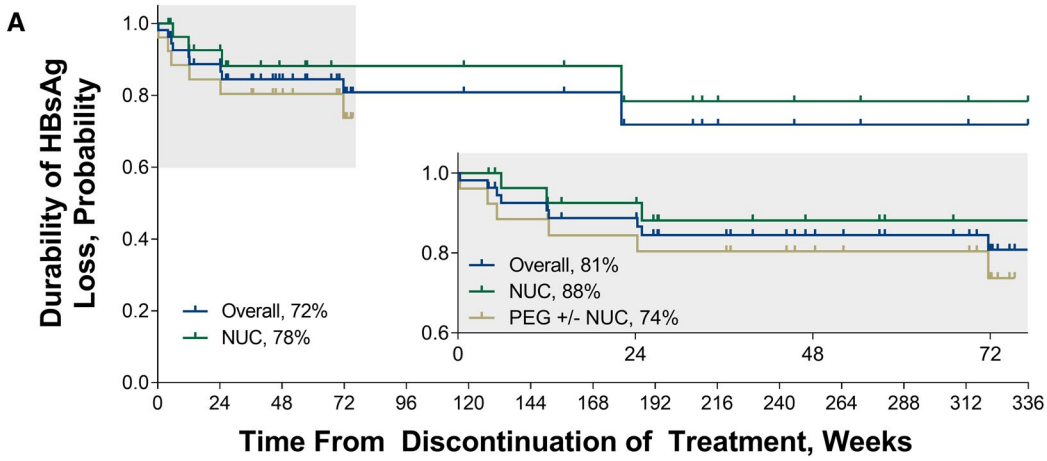
PREDICTORS OF HBsAg SEROREVERSION

In a univariate analysis (Fig. 6) of predictors of HBsAg seroreversion, significant associations were observed between study treatment arms (P = 0.02), duration of treatment ≤12 versus >12 weeks (P = 0.04) after HBsAg loss, and confirmation of HBsAg loss ≤12 versus >12 weeks apart (P = 0.04). The significant association between HBsAg seroreversion and study treatment arms showed that the association was specific to patients administered 16 weeks of Peg-IFN plus 48 weeks of NUC. These patients (n = 4) had a significantly higher rate of HBsAg seroreversion (75%) compared with the other treatment regimens (0% to 24%). Three of the 4 patients had on-treatment HBsAg loss (2 patients at week 16 and 1 patient at week 48), and of these, HBsAg seroreversion occurred 5, 0.3, and 72 weeks, respectively, after treatment discontinuation. The remaining patient had off-treatment HBsAg loss at week 84, which was 36 weeks after treatment discontinuation, and HBsAg loss was sustained until the last follow-up visit 36 weeks later.

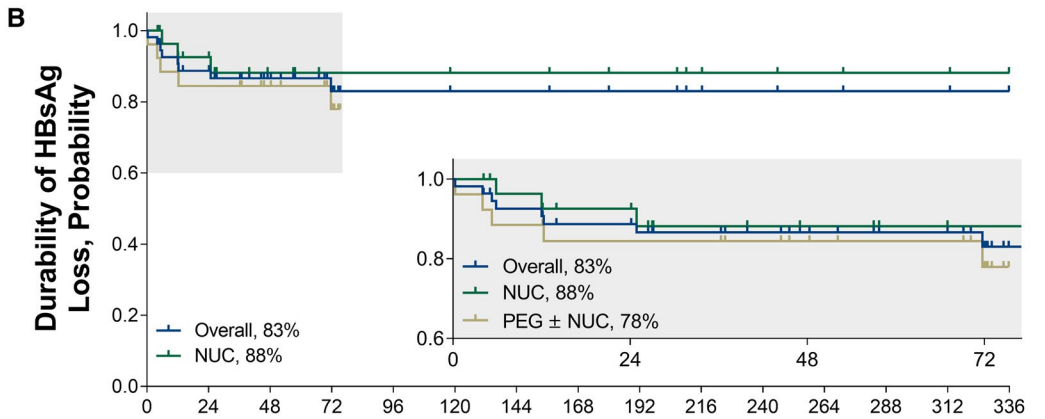
The absence of anti-HBs seroconversion was not significantly associated with HBsAg seroreversion. When the 4 patients who lost HBsAg after 16 weeks of Peg-IFN plus 48 weeks of NUC were excluded, no other predictors were significantly associated with HBsAg seroreversion.

Discussion

Improvement in our understanding of HBV biology and host immune response to HBV coupled with advances in the development of *in vitro* and animal models to study HBV replication have fueled the development of new antiviral and immune modulatory therapies.[9] While sterilizing or a complete cure may not be feasible,[10] a "functional cure" defined as HBsAg loss can be achieved in some patients with IFN, NUCs, or the combination.[3,11-15] Given that HBsAg loss will be used to assess the primary efficacy endpoint for phase 3 and possibly phase 2b clinical trials of newer targeted therapies, data to guide how HBsAg loss should be determined are urgently needed. Because HBsAg loss is rarely achieved with currently available therapies, we pooled data from patients with CHB who achieved HBsAg loss in three phase 3 clinical studies of NUC monotherapy or Peg-IFN-based



n at Risk (Events)															
Overall	55 (0)	44 (6)	30 (8)	21 (9)	11 (9)	10 (9)	10 (9)	9 (9)	7 (10)	5 (10)	4 (10)	3 (10)	2 (10)	2 (10)	0 (10)
NUC	29 (0)	23 (2)	14 (3)	11 (3)	11 (3)	10 (3)	10 (3)	9 (3)	7 (4)	5 (4)	4 (4)	3 (4)	2 (4)	2 (4)	0 (4)
PEG ± NUC	26 (0)	21 (4)	16 (5)	10 (6)	0 (6)										



n at Risk (Events)															
Overall	55 (0)	44 (6)	31 (7)	22 (8)	11 (8)	10 (8)	10 (8)	9 (8)	8 (8)	6 (8)	5 (8)	4 (8)	3 (8)	3 (8)	0 (8)
NUC	29 (0)	23 (2)	14 (3)	11 (3)	11 (3)	10 (3)	10 (3)	9 (3)	8 (3)	6 (3)	5 (3)	4 (3)	3 (3)	3 (3)	0 (3)
PEG ± NUC	26 (0)	21 (4)	17 (4)	11 (5)	0 (5)										

FIG. 5. KM plots of estimated probability of durable HBsAg loss to last study visit and to week 72. Patients treated with Peg-IFN had a shorter duration of follow-up, up to week 72 (insert) after HBsAg loss. (A) All 55 patients with confirmed HBsAg loss and all 10 patients with HBsAg seroreversion. (B) Modified KM estimate, including all 55 patients with confirmed HBsAg loss and 8 patients with confirmed HBsAg seroreversion.

therapies. Of a total of 1,381 patients, 74 had at least one negative HBsAg result. We focused on the 55 patients who became HBsAg negative and had at least one repeat off-treatment HBsAg test ≥1 week after HBsAg loss because the goal of HBV functional cure therapy is to achieve sustained HBsAg loss after completion of a finite course of treatment.

We found that HBsAg loss was durable in 82% (45/55) of patients, with similar results in patients who received NUC monotherapy (86%) or Peg-IFN ± NUC (77%) therapies. Eighteen percent of patients with confirmed HBsAg loss had HBsAg seroreversion; if, however, the 5 patients who could be assessed and who had unconfirmed HBsAg loss

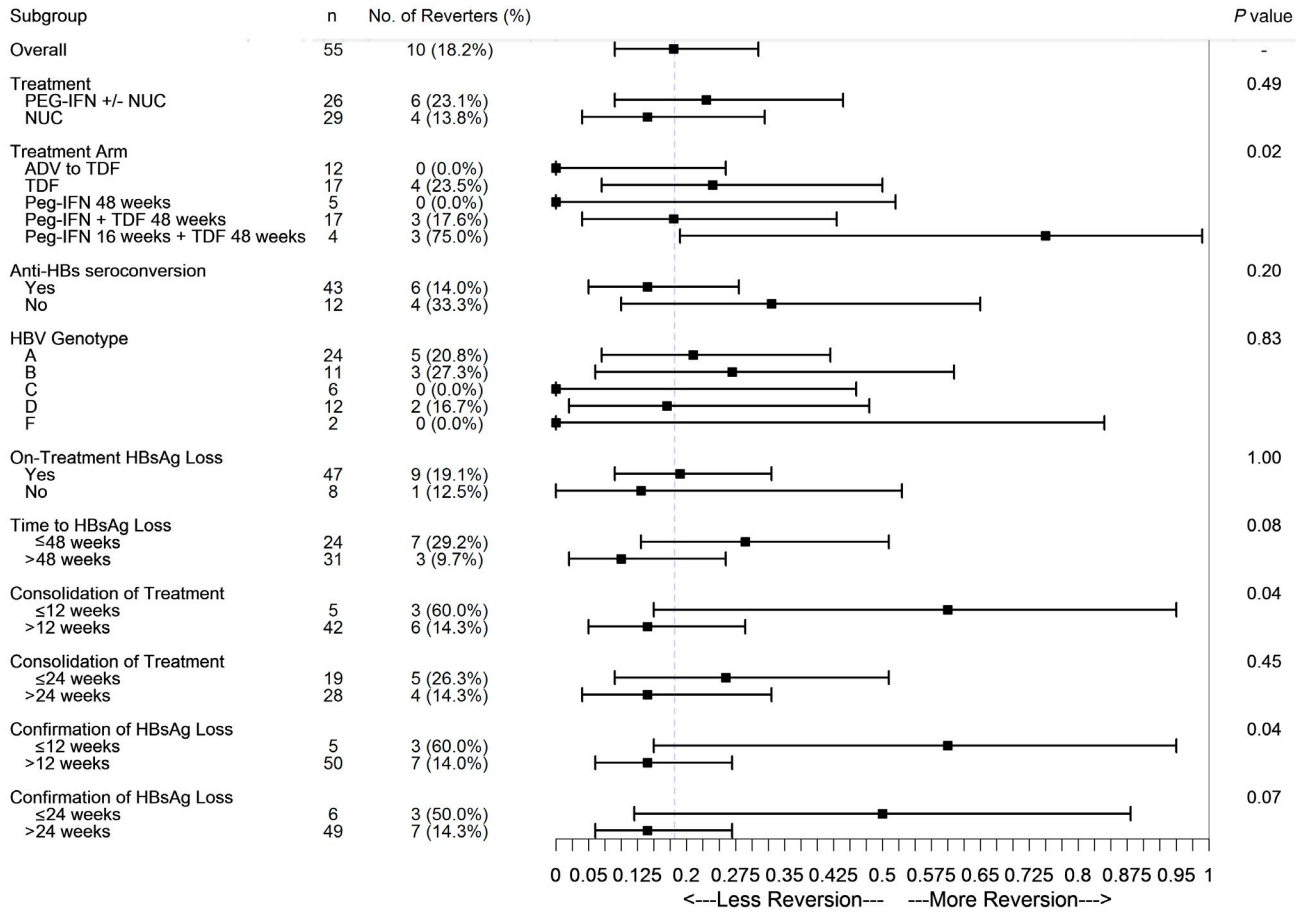


FIG. 6. Forest plot showing predictors of HBsAg seroreversion. Consolidation of treatment refers to the duration of treatment from time of initial HBsAg loss. Abbreviation: ADV, adefovir.

(i.e., reverted to HBsAg positive) after one negative result were included, 25% (15/60) would be considered to have HBsAg seroreversion. The overall KM estimated durability of HBsAg loss of 72% was lower than observed when accounting for censoring. When the KM analysis was repeated, excluding 2 patients with loss of HBsAg who experienced transient unconfirmed HBsAg seroreversion, the overall KM estimate of durable HBsAg loss was 83%. In the patients treated with Peg-IFN, the numerically lower rate of durable HBsAg loss was likely due to the higher rate of HBsAg seroreversion in patients that received suboptimal treatment with a 16-week Peg-IFN-containing regimen. Treatment with a 16-week Peg-IFN-containing regimen was the only significant predictor associated with HBsAg seroreversion.

All patients who lost HBsAg on treatment maintained HBsAg loss while on treatment. In the 10 patients that experienced HBsAg seroreversion, almost all (80%) experienced seroreversion by the week 24 off-treatment study visit. The 2 patients with late HBsAg seroreversion both had an unconfirmed HBsAg-positive result. In patients that maintained HBsAg loss at the off-treatment week 24 visit, the cumulative durability of HBsAg loss was 95% (38/40). Among the 19 patients who had one negative HBsAg result (unconfirmed) and were not included in this analysis, 5 experienced seroreversion to HBsAg positivity on the next repeat test and were not considered to have confirmed HBsAg loss. These data highlight the need to have confirmation of HBsAg loss at least once ≥24 weeks after the completion of treatment and to have confirmation of HBsAg seroreversion should it occur.

A major question regarding the nomenclature and definition of HBV functional cure (i.e., durable

HBsAg loss) is whether anti-HBs seroconversion should be required. Clinical studies demonstrating the benefit of HBsAg loss in reducing incidence of hepatic decompensation, HCC, and liver-related deaths have not analyzed the impact of anti-HBs seroconversion on clinical outcomes.[16-19] In our analysis, anti-HBs seroconversion occurred in 78% of patients with confirmed HBsAg loss. In nearly half of these patients, HBsAg loss and anti-HBs seroconversion were detected on the same day, and the median interval between the first negative HBsAg result and the first positive anti-HBs result was 8 weeks. Anti-HBs seroconversion was associated with a slightly higher rate of durable HBsAg loss (86% vs. 67%) compared with no anti-HBs seroconversion. However, the number of patients studied was small and the role of anti-HBs seroconversion in maintaining durable HBsAg loss remains unclear.

In this study (n = 55), the proportion with anti-HBs seroconversion increased from 35% on the day of HBsAg loss to 67%, 76%, and 76% within 24, 48, and 72 weeks of HBsAg loss, respectively. Given the variable and often long interval between HBsAg loss and anti-HBs seroconversion, requirement to demonstrate anti-HBs seroconversion as a therapeutic endpoint in clinical trials may be logistically difficult. Long intervals between HBsAg loss and anti-HBs seroconversion have been reported.[20-22] Roushan et al.[20] reported the cumulative probabilities of anti-HBs seroconversion after HBsAg loss at 1, 2, 3, 4, and 5 years were 8.7%, 24.3%, 37%, 49.1%, and 58%, respectively, in inactive CHB carriers with spontaneous HBsAg loss. Yip et al.[21] also reported an increasing proportion of patients with spontaneous or NUC-induced HBsAg loss over time; detectable anti-HBs seroconversion occurred in 37.6% of patients at the time of HBsAg loss, 42.6% within 1 year of HBsAg loss, and 53.1% at any time during follow-up. Anti-HBs seroconversion was not significantly associated with durable HBsAg loss in patients treated with NUC, leading the authors to conclude that anti-HBs seroconversion may not be needed before stopping NUC treatment.

Duration of consolidation treatment (duration of treatment from the time of initial HBsAg loss) has been shown to be important in maintaining durable HBeAg loss associated with NUC therapy[23] and may also affect durability of HBsAg loss.[21] In a cohort analysis, HBsAg seroreversion was more frequently observed in patients who received 6-12 months versus ≥12 months of consolidation NUC treatment.[21] In this analysis, we were not able to assess the impact of consolidation treatment (duration of treatment from the time of initial HBsAg loss) on durability of HBsAg loss because there were no consistent criteria for stopping treatment in patients who achieved HBsAg loss in the three phase 3 trials included. Based on the limited data available, duration of consolidation treatment did not appear to be associated with durability of HBsAg loss.

There are several limitations to our study, the most important being its retrospective design with heterogeneity of treatment regimens and lack of standardized criteria for stopping treatment after HBsAg loss. This study was also limited by the variations in the visit schedule as well as the differences in off-treatment follow-up durations. Two qualitative HBsAg assays were used to determine HBsAg loss; the limit of detection of the ARCHITECT Qualitative II assay (LLOD, 0.017-0.022 IU/mL), which was used to evaluate patients treated with Peg-IFN ± NUC, was slightly lower than the limit of detection for AUSZYME (LLOD, 0.04-0.13 IU/mL), which was used to evaluate patients treated with NUC. While this study included a larger number of patients with treatment-induced HBsAg loss than single-center studies or studies of patients in a single trial, the small sample size limits subgroup analyses. Our results may not apply to new therapies with different mechanisms of action or to studies that detect or measure HBsAg using assays with different sensitivities or different capabilities to detect HBsAg bound in immune complexes or HBsAg variants.[24] Finally, it should be noted that patients with a functional cure may have residual HBV genome, in the form of covalently closed circular DNA, that persists in the liver after HBsAg becomes undetectable, and HBV reactivation can occur in these patients.[25,26]

Defining how HBsAg loss should be determined is essential for designing clinical trials using HBsAg loss as an endpoint. Based on the results of our study, we propose that HBsAg loss should be confirmed on repeat tests ≥24 weeks after HBsAg first becomes negative and after completion of treatment. Additional testing at later time points to confirm long-term durability of HBsAg loss should be performed in post-treatment registry studies. Further studies are needed to delineate the role of anti-HBs seroconversion and the duration of treatment after HBsAg loss on durability of HBsAg loss.

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