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ORIGINAL ARTICLE

Addition of PEG-interferon to long-term nucleos(t)ide analogue therapy enhances HBsAg decline and clearance in HBeAg-negative chronic hepatitis B

Multicentre Randomized Trial (PAS Study)

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Abstract

We studied whether 48 weeks of PEG-IFN alfa-2a add-on increases HBsAg-decline and clearance in HBeAg-negative patients on long-term nucleo(s)tide analogue (NA) therapy. In this investigator-initiated, randomized, controlled trial conducted in Europe and Canada, HBeAg-negative patients treated with NA > 12 months, with HBVDNA < 200 IU/mL, were enrolled. Patients were randomized 2:1 to 48 weeks of PEG-IFN alfa-2a add-on (180 µg per week) or continued NA-monotherapy with subsequent follow-up to Week 72. Endpoints were HBsAg decline (≥1 $\log_{10}$ IU/mL) and HBsAg clearance at Week 48. Of the 86 patients in the modified-intention-to-treat analysis, 58 patients received PEG-IFN add-on, and 28 continued NA monotherapy. At Week 48, 16(28%) patients achieved HBsAg decline ≥1 $\log_{10}$ in the add-on arm versus none on NA-monotherapy ($p < .001$), and HBsAg clearance was observed in 6 (10%) PEG-IFN add-on patients versus 0% NA-monotherapy ($p = .01$). HBVRNA was only detected in 2% after PEG-IFN treatment versus 19% in NA-monotherapy ($p = .002$) at Week 48. PEG-IFN add-on therapy was well tolerated in majority of patients. Low baseline HBsAg levels (<10 IU/mL) identified patients most likely to achieve HBsAg loss with PEG-IFN add-on, whereas an HBsAg level > 200 IU/mL at on-treatment Week 12 was highly predictive of non-response (NPV = 100%). Addition of PEG-IFN to long-term NA enhanced HBsAg decline and increased the chance of HBsAg clearance in HBeAg-negative patients on long-term NA. On-treatment HBsAg levels >200 IU/mL

Abbreviations: ALT, alanine aminotransferase; BCP, basal core promoter; ccc-DNA, covalently closed circular DNA; CHB, chronic hepatitis B; CR, combined response; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LoD, lower limit of detection; LoQ, lower limit of quantification; LTFU, long-term follow-up; NA(s), nucleos(t)ide analogue(s); PC, precore; PCR, polymerase chain reaction; PEG-IFN, peginterferon; qHBsAg, quantitative hepatitis B surface antigen; RACE, rapid amplification of cDNA ends; RBV, Ribavirin; SD, standard deviation; ULN, upper limit of normal.

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identify patients unlikely to benefit from PEG-IFN add-on and could be used as a potential stopping-rule for PEG-IFN therapy. Our findings support further exploration of immune modulation add-on to antiviral therapy, preferably using response-guided strategies, to increase functional cure rates in patients with CHB.

KEYWORDS
functional cure, HBsAg loss, HBVRNA, novel agents, short-term PEG-IFN

1 | INTRODUCTION

Chronic hepatitis-B (CHB) is one of the most serious public health issues affecting more than 250 million people worldwide.[1] Hepatitis-B e antigen (HBeAg)–negative CHB represents a late phase in the course of the infection, which is globally recognized with increasing prevalence.[2] Therapeutic intervention is often indicated for HBeAg-negative patients as spontaneous remission rarely occurs, and patients usually have more advanced liver disease in comparison with HBeAg-positive patients.[3,4]

The ultimate goal of antiviral therapy in CHB is to halt the progression of liver disease through viral eradication and immunologic control. Viral eradication is rarely achieved as the intrahepatic covalently closed circular DNA (cccDNA) often persists in the hepatocyte nuclei.[5,6] Serum hepatitis-B surface antigen (HBsAg)-loss is associated with a reduced risk of liver disease progression and development of hepatocellular carcinoma (HCC).[7] HBsAg-loss is therefore considered the optimal treatment endpoint of anvitiral therapy and is often referred to as functional cure.[8–10]

Nucleos(t)ide analogues (NA) and pegylated interferon (PEG-IFN) are considered the first-line treatment strategies in CHB.[8,9] Although NA therapy effectively inhibits viral replication, HBsAg-loss is rarely achieved. Moreover, treatment response may not be durable in a large proportion of patients after discontinuing therapy, which indicates the necessity of long-term and perhaps indefinite treatment.[11,12] In contrast, finite PEG-IFN treatment leads to higher rates of durable remission and serologic response by establishing sustained immune control.[13,14] Despite the side effects, PEG-IFN results in higher rates of HBsAg-loss compared with NA therapy in HBeAg-negative patients.

The adaptive immunity is partially restored after starting NA therapy, while PEG-IFN improves the innate immune response by preventing the formation of HBV proteins and promoting cccDNA pool depletion.[15–20] When combined, immune restoration through viral suppression and the concurrent effect of PEG-IFN through immune modulation and degradation of cccDNA[21] could enhance HBsAg decline and subsequent HBsAg-loss. In this regard, adding PEG-IFN to NA therapy may be necessary to induce a robust HBsAg response in HBeAg-negative patients. Thus, in this randomized, controlled trial, we aimed to investigate whether the addition of PEG-IFN alfa-2a for 48 weeks enhances HBsAg decline and induces HBsAg clearance in HBeAg-negative chronic hepatitis-B patients on long-term NA therapy.

2 | PATIENTS AND METHODS

2.1 | Trial design

This global, investigator-initiated, open-label, multicentre, randomized, controlled trial (PAS study; ClinicalTrials.gov registration number: NCT 01373684) was conducted at seven centres in the Netherlands and Canada. The recruitment was finalized in April 2017, follow-up ended in October 2019 and the database was closed in January 2020.

Patients were randomized 2:1 to either add-on treatment with 180 micrograms of PEG-IFN alfa-2a (F. Hoffmann-La Roche Ltd., Basel, Switzerland) once weekly for 48 weeks or to continue NA-monotherapy. After 48 weeks, patients from both arms continued NA treatment. All patients were followed for another 24 weeks until Week 72 (Figure S1).

Randomization was done centrally, stratified by study centre. A computer-generated randomization sequence prepared by the trial statistician (B.H.) was used. Randomization was done sequentially and communicated to the sites by e-mail, and treatment was assigned in random blocks of 3 or 6. The study was performed in accordance with the Declaration of Helsinki and good clinical practice guidelines and was approved by the ethics committee of each participating centre. All subjects gave written informed consent before the screening. All centres were monitored by the trial coordinating centre in Rotterdam.

2.2 | Study population

Adult patients with documented CHB (serum HBsAg positive >6 months) were eligible if they were as follows: (1) HBeAg-negative and hepatitis-B e antibody (anti-HBe) positive within 6 months prior to study initiation and (2) treated with nucleos(t)ide analogues for at least 1 year at screening and had HBV-DNA <200 IU/mL at least within 1 month before the study. Both PEG-IFN-naive and experienced patients were eligible for the study.

Patients were excluded for the following reasons: treatment with any investigational drug within 30 days before screening; current or previous treatment with telbivudine; ALT > 10× upper limit of normal (ULN); a history of decompensated cirrhosis defined as jaundice in the presence of cirrhosis, ascites, bleeding gastric or oesophageal varices, or encephalopathy; pre-existent

neutropenia (neutrophils < 1500 mm^3) or thrombocytopenia (platelets < 90,000/mm^3); evidence of a coinfection with hepatitis C, hepatitis D virus or human immunodeficiency virus; any other acquired or inherited liver disease: alcoholic liver disease, obesity induced liver disease, drug related liver disease, autoimmune hepatitis, hemochromatosis, Wilson's disease or alpha-1 antitrypsin deficiency; alpha-fetoprotein level of >50 ng/mL; hyper-or hypothyroidism (subjects requiring medication to maintain thyroid stimulating hormone levels in the normal range were eligible if all other inclusion/exclusion criteria were met); use of immunosuppressive medication within the preceding 6 months; contra-indications for IFN-α therapy such as suspected hypersensitivity to PEG-IFN or any known pre-existing medical condition that could interfere with the patient's participation in or completion of the study; pregnancy or lactation; other significant medical illness that might interfere with this study: significant pulmonary dysfunction in the previous 6 months, malignancy other than skin basal cell carcinoma in the past 5 years, immunodeficiency disorders (e.g. HIV positivity, auto-immune diseases, organ transplants other than cornea and hair transplant); any medical condition requiring or likely to require chronic systemic administration of corticosteroids during the study; substance abuse in the past 2 years (such as alcohol [<80 g/day]) or inhaled drugs in the past 2 years.

2.3 | Efficacy analysis

The efficacy analysis included all patients who fulfilled the inclusion criteria, were HBeAg-negative at Week 0, were randomized and received at least one dose of the assigned medication (modified intention-to-treat [mITT] population). The predefined primary endpoint was HBsAg-decline ≥ 1 $\log_{10}$ IU/mL from baseline at Week 48. Secondary endpoints included HBsAg-loss at Weeks 48 and 72, HBsAg-decline ≥ 1 $\log_{10}$ IU/mL at Weeks 24 and 72, and HBsAg-decline ≥ 0.5 $\log_{10}$ IU/mL at Weeks 24 and 48.

2.4 | Safety analysis

The safety analysis included all patients who were randomized and received at least one dose of study medication. Safety measures included adverse events (AEs; vital signs, and chemistry and haematology data, analysed according to the World Health Organization recommendations for toxicity grading, adapted for chronic liver disease); the causality of AEs was determined by the local investigator.

2.5 | Measurements

In the first 4 weeks of PEG-IFN treatment, study visits were conducted biweekly for routine examination and laboratory tests. Thereafter, patients were followed every 4 weeks until the end of the study. Patients who were assigned to NA-monotherapy were followed every 12 weeks.

Routine biochemical and haematological tests were performed locally. Serum ALT levels were standardized by using the centre- and sex-specific ULN values. Virological tests were performed at the central laboratory (Erasmus Medical Center, Rotterdam, The Netherlands). HBV-DNA was measured by Cobas TaqMan polymerase chain reaction assay (lower limit of quantification 20 IU/mL; Roche Diagnostics, Basel, Switzerland). Serum HBeAg, anti-HBe and HBsAg levels (lower limit of detection [LOD]:0.05 IU/mL) were measured by Architect (Abbott Laboratories, North Chicago, IL). Serum HBV-RNA was measured by Cobas 6800/8800 (linear range: 10-1e9; lower limit of quantification [<titre minimum]:10 cp/mL; lower limit of detection: 3.3. cp/mL; Roche Diagnostics, Basel, Switzerland).

2.6 | Statistical analysis

The power calculation was based on the primary endpoint. The assumed response rates (HBsAg-decline ≥ 1 $\log_{10}$ IU/mL) were 5% for the NA-monotherapy group and 35% for the PEG-IFN add-on group. The calculated number of patients per treatment arm needed to detect a statistically significant difference at a two-sided α-level of 0.05 with 80% power, anticipating a 20% drop-out rate, was 60 patients for the PEG-IFN add-on group, and 30 patients for the NA-monotherapy group. Hence, a total of 90 patients were needed for this study.

Patients were classified as non-responders in case of missing HBsAg levels at Week 48 or early discontinuation of PEG-IFN before Week 48. A statistical analysis plan was defined before the closure of the database. This prespecified statistical analysis plan included the adjustment of covariates possibly influencing the primary outcome using logistic regression analysis techniques and followed the European Medicines Agency guidelines for the selection of baseline variables for adjustment.[22] The most relevant factors included race, sex, previous use of IFN or NA therapy, duration of NA therapy, and serum HBV-DNA, HBsAg, and ALT values at baseline. Skewed laboratory values were log-transformed before analyses. Baseline characteristics were compared between treatment arms using the chi-square test, the Fisher exact test, the Student *t*-test or the Mann–Whitney test, where applicable. Dichotomous primary and secondary endpoints were analysed using the chi-square test or the Fisher exact test, whereas continuous endpoints were analysed using the Student *t*-test. Logistic regression analysis was performed to assess the association between baseline factors and the primary endpoint. We used a grid-search of cut-off points to identify an HBsAg level that could be used to identify patients unlikely to achieve HBsAg clearance, aiming for a negative predictive value (NPV) of >95%.

SPSS software (version 25.0; SPSS, Chicago, IL) and the SAS9.4 program (SAS Institute Inc., Cary, NC) were used to perform statistical analyses. Two-sided *p*-values < .05 were considered significant.

3 | RESULTS

3.1 | Baseline characteristics of the study population

Of the 91 patients screened, 90 met the eligibility criteria. Out of the eligible patients, 59 were randomized to receive PEG-IFN add-on, and 31 to continue NA monotherapy (Figure S2).

Group characteristics at baseline are shown in Table 1. In total, 86 patients were included in the mITT analysis: One patient from the PEG-IFN add-on group was withdrawn before receiving intervention at Week 0 due to travel reasons. Three patients were withdrawn from the NA-monotherapy group: one patient before receiving intervention at Week 0 because of refusal to undergo repetitive venipunctures, one appeared to be HBeAg-positive at Week 0, and one had HBV-DNA > 200 IU/mL at Week 0. The baseline characteristics were balanced between the treatment arms in the mITT analysis. All patients in the NA-monotherapy mITT group ($n = 28$) completed treatment and follow-up. During the PEG-IFN treatment period, six patients received a reduced dose of PEG-IFN (median [IQR]: 18 [12–44] weeks), and 12 patients discontinued PEG-IFN prematurely (2 lost to follow-up and 10 due to adverse events). Median time to PEG-IFN discontinuation was 16 [range 14–40] weeks.

3.2 | PEG-IFN add-on increases HBsAg decline and clearance

3.2.1 | HBsAg kinetics

Patients in the PEG-IFN add-on group had a significantly greater mean decline in HBsAg levels from Week 0 to Week 48 versus those who continued NA-monotherapy −0.79 (1.2) $\log_{10}$ IU/mL versus −0.08 (0.2) $\log_{10}$ IU/mL; $p < .0001$ (Figure 1A; Figure S3). At Week 72, a mean HBsAg decline of −0.65 (1.1) $\log_{10}$ IU/mL was observed in the PEG-IFN add-on group versus −0.11 (0.2) $\log_{10}$ IU/mL in the NA-monotherapy group ($p = .01$).

3.2.2 | HBsAg response

HBsAg-decline ≥1 $\log_{10}$ IU/mL at Week 48 (primary endpoint) was achieved in 16/58 (28%) PEG-IFN add-on patients versus 0/28(0%) NA-monotherapy patients ($p < .001$; Figure 1B). In the PEG-IFN add-on group, 21% and 19% achieved HBsAg-decline ≥1 $\log_{10}$ IU/mL from baseline to Weeks 24 and 72, respectively, whereas no patient in the NA-monotherapy group achieved a response at either

TABLE 1 Baseline characteristics.

Variable	PEG-IFN add-on $n = 58$	NA monotherapy $n = 28$
Demographics and clinical characteristics		
Mean age, year (SD)	49 (10)	46 (11)
Male, *n* (%)	51 (88)	23 (82)
Asian/Caucasian/Other (%)	64/29/7	71/21/7
Mean BMI, (SD)	25 (3.8)	25 (3.3)
Median NA therapy duration, year (IQR)	6.2 (6.0–6.53)	6.6 (4.3–7.7)
Previous PEG-IFN therapy, *n* (%)	9 (16)	8 (27)
TDF/ETV/LMV/ADV, %	55/33/7/3	64/29/7/0
Cirrhosis, *n* (%)	2 (3)	1 (3)
Laboratory results		
Mean ALT, ×ULN (SD)	0.7 (0.4)	0.7 (0.3)
Mean HBV DNA, $\log_{10}$ (IU/mL) (SD)	0.3 (0.2)	0.3 (0.4)
HBV DNA <20 IU/mL, *n* (%)	58 (100)	27 (96)[a]
Mean HBsAg, $\log_{10}$ (IU/mL) (SD)	2.6 (1.0)	2.6 (0.9)
HBV RNA > 10 cp/mL, *n* (%)	8 (14)	4 (15)
Mean total bilirubin, µmol/L	10.9 (4.2)	11.1 (4.0)
Mean albumin, g/L	43 (2)	44 (3)
Mean platelet, $\times 10^9$/L	200 (48)	188 (45)

[a]All Patients had HBV DNA below 200 IU/mL.

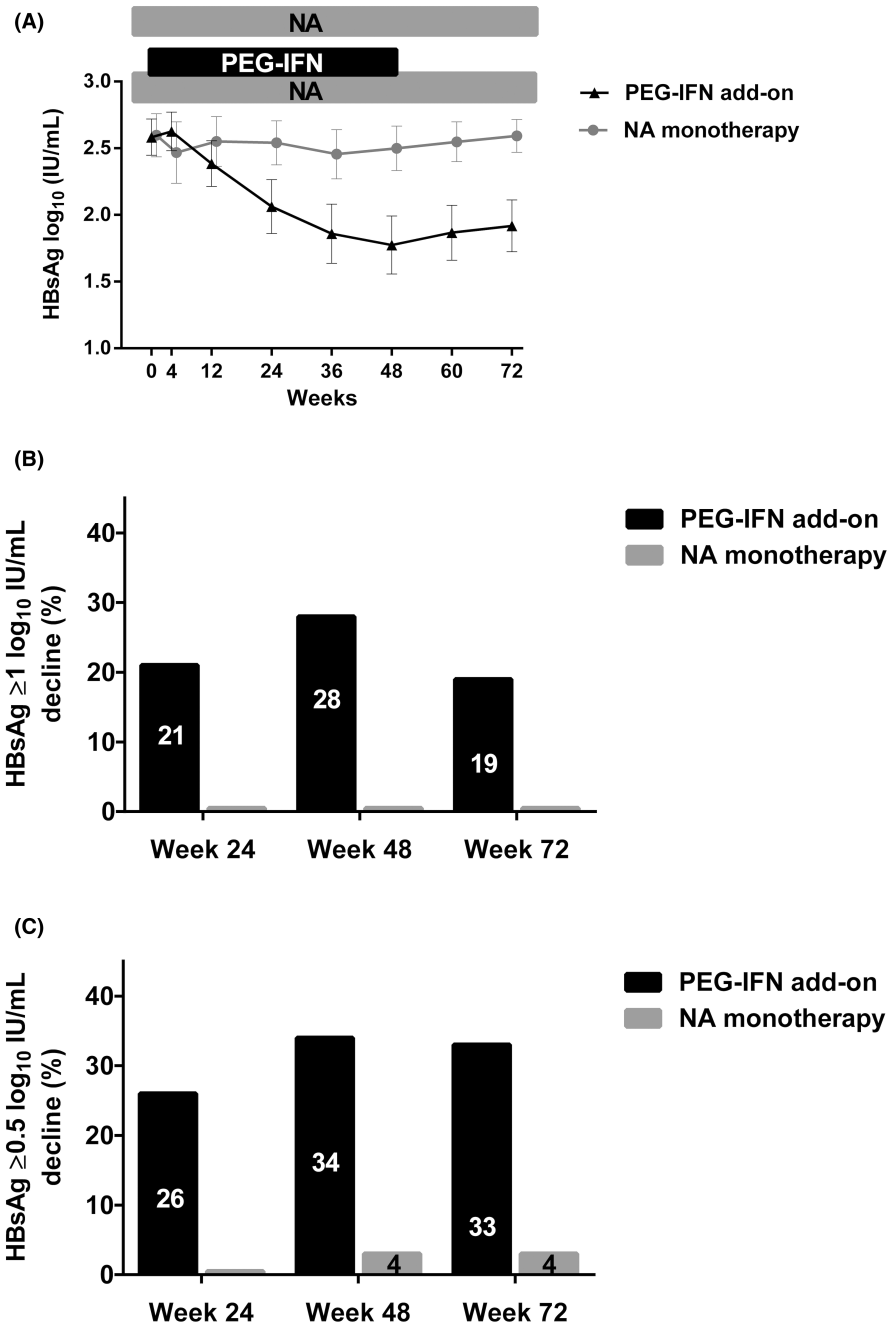

FIGURE 1 HBsAg changes during the study (A) HBsAg kinetics during treatment (Weeks 0–48) and follow-up (Weeks 49–72). (B) Primary endpoint response rates (HBsAg decline ≥ 1 $\log_{10}$) per treatment arm at Weeks 24, 48 and 72. (C) Rates of HBsAg decline ≥ 0.5 $\log_{10}$ per treatment arm at Weeks 24, 48 and 72.

time point ($p < .001$). HBsAg-decline ≥0.5 $\log_{10}$ IU/mL was observed in 34% in the PEG-IFN add-on group and in only one patient (4%) in the NA-monotherapy arm (Figure 1C).

3.2.3 | HBsAg loss

At Weeks 48 and 72, HBsAg-loss was observed in six (10%) PEG-IFN add-on patients versus none in the NA-monotherapy arm ($p = .01$; Figure 2A). At Week 72, HBsAg seroconversion had occurred in 2/6 (33%) patients who had achieved HBsAg-loss at Week 48. One patient with HBsAg-loss at Week 48 and subsequent seroconversion at Week 72 had a borderline HBsAg level of 0.1 IU/mL at Week 72. HBsAg-loss occurred at various timepoints during PEG-IFN treatment (Figure 2B).

3.2.4 | PEG-IFN add-on increases HBV RNA decline

At baseline, HBV-RNA was above LOD among 20/56(36%) of PEG-IFN add-on versus 12/27 (44%) of NA-monotherapy ($p = .6$). During PEG-IFN treatment period, four additional patients reached undetectable HBV-RNA levels as early as Week 4 compared with none in the NA-monotherapy group (Week 4 detectable HBV-RNA: 7% vs. 21%, $p = .05$). At Week 48, only 2% of the PEG-IFN group had detectable levels compared with 19% in the NA-monotherapy group ($p = .002$). Nine percent of patients in the PEG-IFN group had HBV-RNA detectable levels at Week 72 versus 19% in the NA-monotherapy group ($p = .06$) (Figure 3; Figure S4). Among patients with quantifiable levels at baseline, HBV-RNA declined profoundly as early as Week 4 in the PEG-IFN group

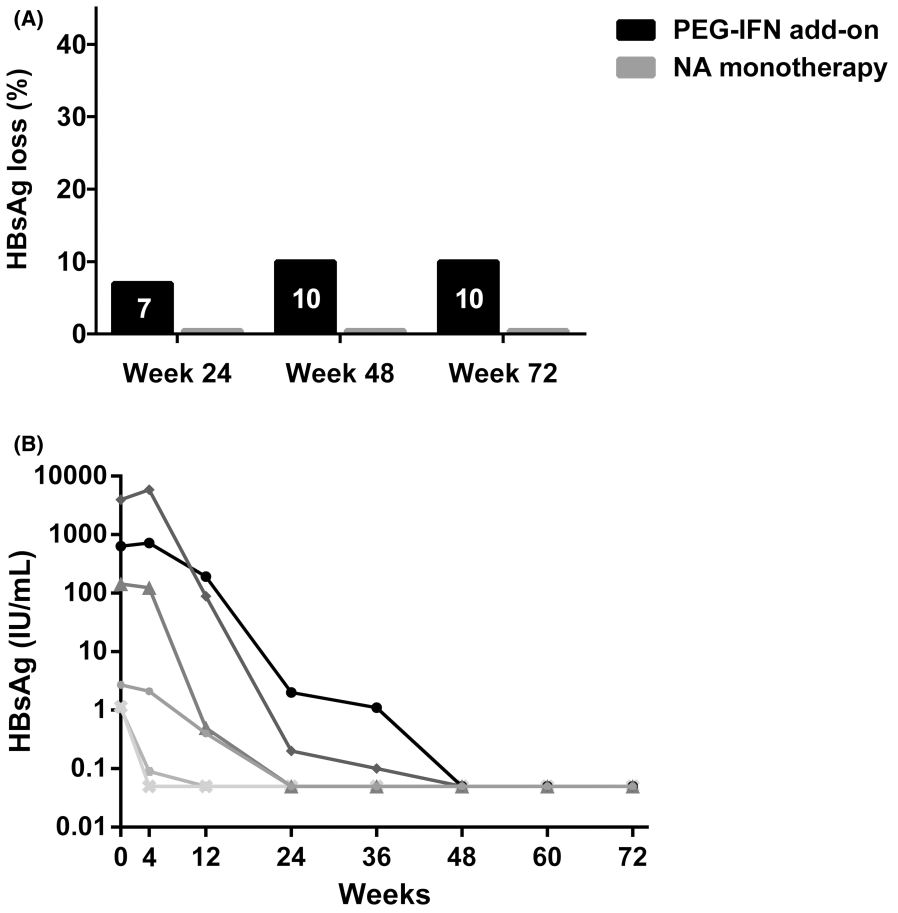

FIGURE 2 HBsAg loss achieved during the study (A) Rates of HBsAg loss per treatment arm at Weeks 24, 48 and 72. (B) Individual HBsAg changes Weeks 0–72 among patients with HBsAg loss by Week 72.

(Median [IQR] −112.0 [362.3 to −28.1] cp/mL) compared with NA-monotherapy (+7.2[−1.9 to 9.7] cp/mL; $p = .08$).

3.3 | Changes in ALT levels

ALT levels had increased significantly as early as Week 4 in the PEG-IFN add-on group versus the NA monotherapy group (+0.60 (0.6)×ULN vs. +0.01 (0.2)×ULN; $p < .0001$; Figure 4). At Week 12, four patients in the PEG-IFN add-on group had ALT >3×ULN, and three reached >5×ULN.

3.4 | Prediction of response

In univariable analysis, an HBsAg level < 10 IU/mL was associated with an increased chance of HBsAg loss at Week 48 ($p < .006$; Table S1). Additionally, HBsAg decline during PEG-IFN add-on treatment was more pronounced in patients achieving HBsAg loss at Week 48 (Week 12: −1.1(0.9) $\log_{10}$ IU/mL vs −0.08(0.5) $\log_{10}$ IU/mL; $p < .0001$), and HBsAg levels at Week 12 could discriminate patients with HBsAg loss from those without (AUROC 0.813, 95% CI: 0.52–1.0, $p = .01$). None of the patients with an HBsAg level above 200 IU/mL at Week 12 ($n = 20$, 35% of the cohort) achieved HBsAg loss (NPV = 100%).

3.5 | Safety and tolerability

Patients receiving PEG-IFN add-on therapy experienced AEs more frequently, especially during the 48 weeks of PEG-IFN treatment, than those receiving NA-monotherapy (Table 2). The most common AEs were those that were expected to appear during PEG-IFN therapy, such as fatigue, headache, myalgia, flu-like syndrome, neutropenia and thrombocytopenia.

In total, four (7%) serious adverse events (SAEs) occurred in the PEG-IFN add-on group, and none occurred in the NA-monotherapy group ($p < .001$). Two of the adverse events were reported to be related to PEG-IFN treatment (thyroiditis at Week 12 and appendicitis at Week 36), while the other two were not considered related to PEG-IFN treatment (transient ischemic attack at Week 30 and hepatocellular carcinoma (HCC) at Week 44). Of the four SAEs, three led to discontinuation of PEG-IFN treatment (thyroiditis, appendicitis and HCC).

4 | DISCUSSION

In this randomized, controlled trial of HBeAg-negative patients on long-term NAs, addition of PEG-IFN for 48 weeks increased HBsAg decline and HBsAg clearance. Early on-treatment HBsAg declines were predictive of subsequent HBsAg clearance, potentially paving

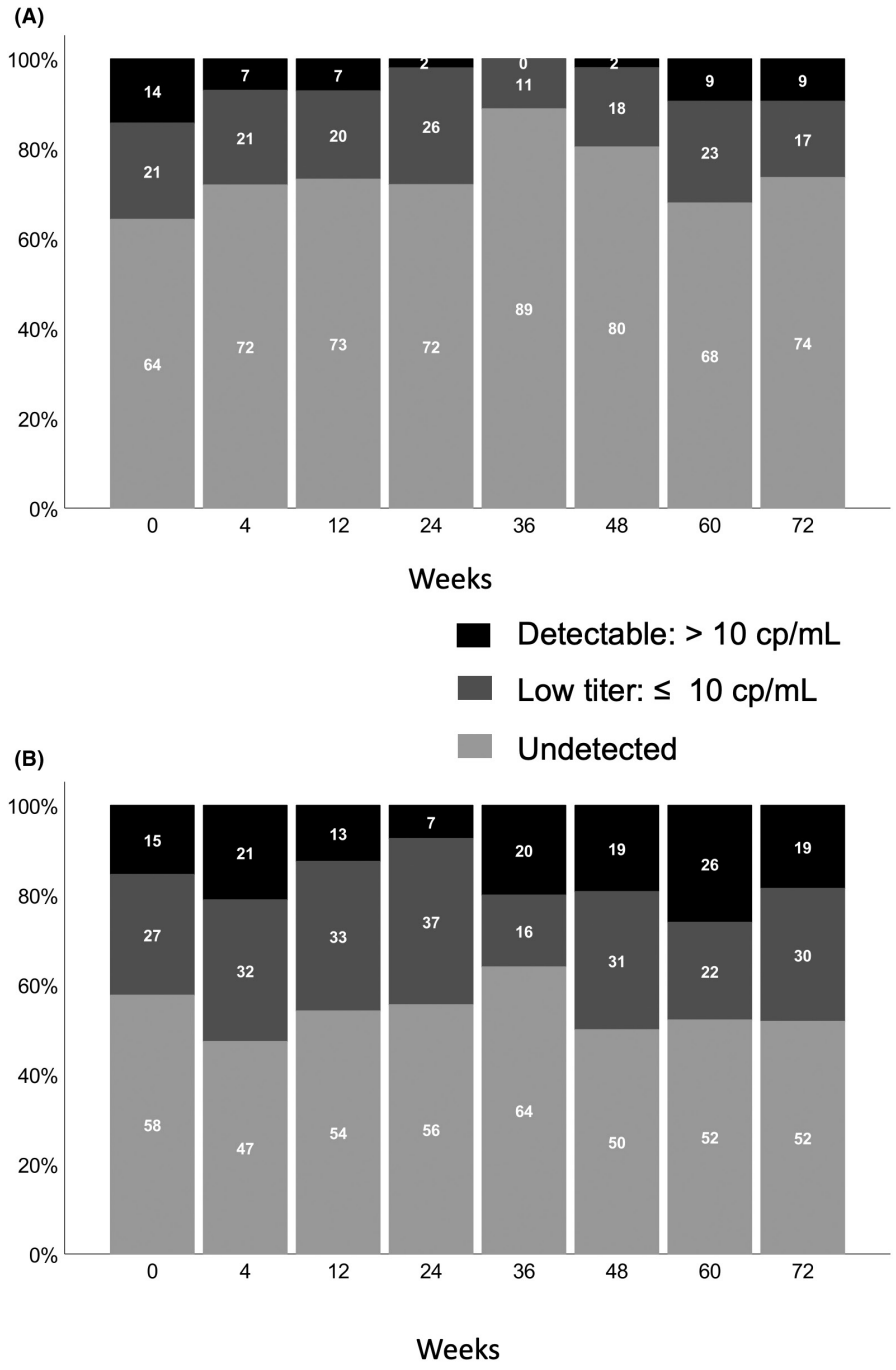

FIGURE 3 HBV-RNA changes during the study (A) HBV-RNA detectability in the PEG-IFN add-on group. (B) HBV-RNA detectability in the NA monotherapy group.

the way for response-guided addition of immunomodulatory treatment in NA treated CHB patients.

In CHB, the exhaustion of T cells are due to persistent exposure to viral antigens and increased expression of T-cell inhibitory responses.[23–25] For the restoration of robust HBV-specific immune responses, immune modulation is required along with viral inhibition to achieve immunological control. In this respect, suppressive therapy with NA would partially restore the adaptive immunity and reduce virally mediated T-cell exhaustion, allowing HBV-specific T cells to be more responsive upon PEG-IFN treatment. Addition of PEG-IFN may then enhance the innate immune response.[15–19] Thus, adding PEG-IFN to effective viral suppression with NA therapy may further promote the HBV-specific response, which is an important step to achieve HBsAg decline and functional cure.

Earlier trials combining lamivudine with PEG-IFN in HBeAg-negative CHB patients showed low response rates similar to PEG-IFN monotherapy.[26,27] However, combined treatment with more potent NAs such as tenofovir or entecavir has been associated with improved HBsAg decline and HBsAg-loss in uncontrolled trials.[28,29] A recent French study in which primarily Caucasian patients were pretreated with NAs for a median of 2.7 years showed no significant difference in the rate of HBsAg-loss between patients who received PEG-IFN add-on therapy for 48 weeks versus NA-monotherapy (8% vs. 3%; $p = .15$).[30] In our study, two-third was Asian and patients were

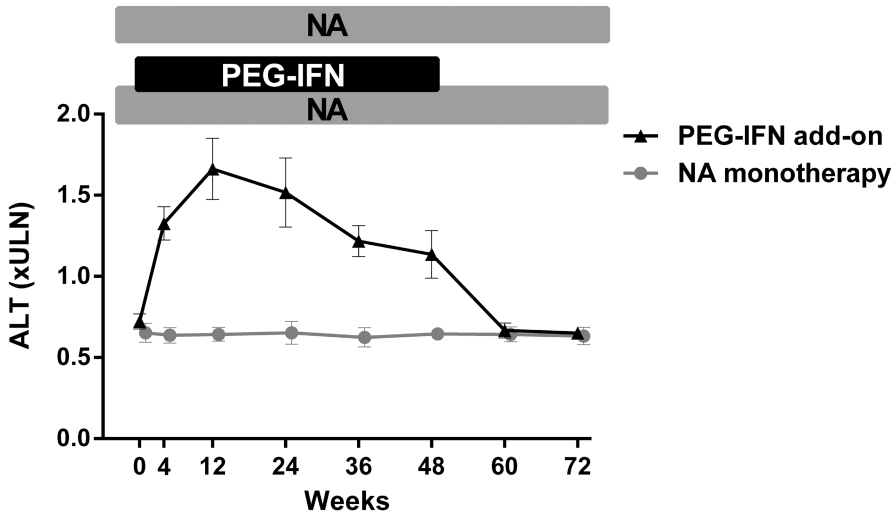

FIGURE 4 ALT changes during the study ALT kinetics during treatment (Weeks 0–48) and follow-up (Weeks 49–72).

pretreated with NAs for a median of 6.2 years. Although HBsAg loss rates were similar to our study, our HBsAg-loss was significantly higher in the PEG-IFN add-on versus NA-monotherapy group (10% vs. 0%; $p < .0001$). Our findings are similar to what is now being reported with Bepirovirsen, an antisense oligonucleotide that targets all HBV messenger RNA.[31] Our results could be due to differences in racial composition and longer duration of NA pretreatment, although these variables were not predictive of HBsAg-loss in the current study, likely due to lack of statistical power.

Achieving lower HBsAg levels is beneficial as high HBsAg levels are reported to be a risk factor for hepatocarcinogenesis in inactive HBeAg-negative CHB.[32] While this outcome is not yet reported among NA treated HBeAg-negative patients, it is important to note that HBsAg levels were significantly reduced in PEG-IFN add-on patients versus NA-monotherapy patients, in whom only negligible changes were observed. Although HBsAg levels decreased continuously throughout PEG-IFN treatment, the steepest decline occurred during the first 24 weeks of treatment (Week 24: −0.5 $\log_{10}$ IU/mL; Week 72: −0.7 $\log_{10}$ IU/mL). This early HBsAg decline supports the potential use of short-term PEG-IFN in combination trials with novel agents aiming at functional cure which is defined as sustained HBsAg-loss.

Although the increase in HBsAg loss rates with PEG-IFN add-on is encouraging, absolute response rates are limited. Selection of patients with the highest chance of success is therefore crucial. Previous studies have identified a wide range of factors associated with response to PEG-IFN monotherapy, but some of the most pivotal (such as serum HBV DNA and ALT levels) cannot be used in patients on NA therapy. Low pretreatment HBsAg levels have also been associated with HBsAg-loss in patients treated with PEG-IFN monotherapy, PEG-IFN and NA combination therapy, and HBeAg-positive patients treated with PEG-IFN add-on.[30,33,34] In our HBeAg-negative patients treated with PEG-IFN add-on, very low baseline HBsAg levels (<10 IU/mL) were shown to be strongly predictive of HBsAg-loss. Unfortunately, this cut-off identifies only very few patients. Future studies, preferably using larger datasets, should therefore focus on identifying additional factors associated with favourable response to immunomodulatory therapy, such as hepatitis B core-related antigen levels and/or serum levels of anti-HBc.

Another tool for optimizing the use of PEG-IFN in CHB is the application of response-guided stopping rules, which has become widely accepted for patients treated with de novo PEG-IFN.[8,35] The current study shows that early HBsAg declines at Week 12 of PEG-IFN add-on were associated with subsequent HBsAg loss, with an HBsAg level above 200 IU/mL at Week 12 of PEG-IFN therapy identifying patients with a negligible chance of achieving HBsAg loss. These findings pave the way for response-guided PEG-IFN add-on strategies in patients with HBeAg-negative CHB. This suggests a potential benefit in treating HBeAg-negative patients on long-term NA treatment with PEG-IFN, who would otherwise have minimal changes in HBsAg with continued NA-monotherapy.

In our HBeAg-negative population with long-term NA pretreatment, HBV-RNA levels were undetectable among majority of patients at baseline. This observation was previously reported in HBeAg-negative patients who were suppressed on long-term NA therapy.[35] For those with detectable HBV-RNA levels, we demonstrated that HBV-RNA significantly decreased early after introducing PEG-IFN compared with continued NA-monotherapy. The pronounced HBV-RNA decline may be explained by the substantial inhibitory effect of interferon on HBV-RNA production itself [36,37] as well as on the post-transcriptional steps of the HBV life cycle.[38]

Regarding safety and tolerability, PEG-IFN was generally tolerated. As we implemented targeted screening, all of the eligible and screened patients were enrolled in the study except one patient who was found to be HBeAg-positive. The observed AEs were expected with PEG-IFN treatment. PEG-IFN treatment was discontinued in 17% of the patients in the add-on group. The majority of those patients (63%) received at least 20 weeks of PEG-IFN treatment, which is considered an adequate treatment duration.

Despite the encouraging increase in the rates of HBsAg loss with PEG-IFN add-on observed in this study, the low overall response rates preclude widespread implementation of PEG-IFN add-on therapy. However, it is important to note that PEG-IFN plays a pivotal role in increasing the chances of functional cure with novel

TABLE 2 Adverse events (AEs).

Variable	PEG-IFN add-on *n* = 58	NA monotherapy *n* = 28	*p* Value
≥1 AE			
Weeks 0–48	56 (97)	10 (36)	<.001
Weeks 49–72	5 (9)	6 (33)	.02
Most common AEs			
Fatigue	29 (50)	0	<.001
Headache	18 (31)	2 (7)	.015
Myalgia	18 (31)	0	.001
Flu-like syndrome[a]	16 (28)	0	.002
Skin reaction[b]	11 (19)	0	.014
Cough	8 (14)	0	.05
Nasopharyngitis	7 (12)	0	.09
Arthralgia	6 (10)	0	.17
Dizziness	6 (10)	0	.17
Injection site reaction	6 (10)	0	.17
Abdominal discomfort	5 (9)	0	.17
Dry skin	5 (9)	0	.17
Dyspnoea	4 (7)	0	.30
Alopecia	4 (7)	0	.30
Depression	4 (7)	0	.30
Injection site erythema	4 (7)	0	.30
Seasonal allergy	4 (7)	0	.30
Nausea	4 (7)	2 (7)	1.0
Onychomycosis	4 (7)	0	.30
Lab abnormalities			
Neutropenia			
Grade 3	21 (36)	0	<.001
Grade 4	0	0	
Thrombocytopenia			
Grade 3	4 (7)	0	.40
Grade 4	1 (2)	0	
ALT Increase			
Grade 3	3 (5)	0	.55
Grade 4	0	0	

[a]Including Pyrexia.

[b]Rash, eczema, pruritus.

compounds.[39] The use of PEG-IFN in patients with CHB is therefore likely to increase significantly as these novel compounds enter advanced stages of development. Our findings suggest that pre-treatment selection and on-treatment stopping rules may allow for individualized use of PEG-IFN in these emerging treatment strategies aimed at HBV cure.

A key strength of our study is the inclusion of a racially-diverse, multicentre, international CHB cohort, which enabled us to study the efficacy of PEG-IFN add-on treatment in both Asian and Caucasian populations from Europe and North America. The present study encountered minimal drop-outs, and most of the patients remained compliant throughout the study. Limitations of the current study include lack of available HBV genotype information due to long-term viral suppression in 99% of the patients. Therefore, the role of race in HBsAg loss should be carefully interpreted. Additionally, there was no available data on the HBeAg status 6 months prior to inclusion. Thus, the potential effect of recent HBeAg loss on HBsAg kinetics cannot be assessed.

In conclusion, in this trial of HBeAg-negative CHB patients, the addition of PEG-IFN to long-term NA treatment was associated with substantial HBsAg decline and 10% HBsAg clearance. Low baseline HBsAg levels and early on-treatment HBsAg declines were predictive of subsequent HBsAg clearance. Our findings support further exploration of immunomodulator add-on to both current and emerging

antiviral therapies, preferably using response-guided strategies, as a means to increase functional cure rates in patients with CHB.

AUTHOR CONTRIBUTIONS

Study coordination and design, data collection, data analysis, writing of manuscript, approval of final version: MSF. Laboratory work, data analysis, critical review of the manuscript, approval of final version: Study coordination and design, data collection, critical review of the manuscript, approval of final version: HLAJ, Study coordination and design, data collection and analysis, manuscript draft: Data collection, critical review of the manuscript, approval of final version: MS. Study design, statistical analysis, critical review of the manuscript, approval of final version: BEH.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

Individual participant data will not be shared.

DISCLOSURES

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