Effect on HBs antigen clearance of addition of pegylated interferon alfa-2a to nucleos(t)ide analogue therapy versus nucleos(t)ide analogue therapy alone in patients with HBe antigen-negative chronic hepatitis B and sustained undetectable plasma hepatitis B virus DNA: a randomised, controlled, open-label trial

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Summary

Background: Uncontrolled studies suggest that addition of Pegylated-Interferon (PEGIFN) in patients with HBeAg negative chronic hepatitis B (CHB) receiving nucleos(t)ide analogs (NUCs) with undetectable plasma HBV DNA may increase HBsAg clearance. We aimed to evaluate this strategy.

Method: In this multicentre, open-label, parallel group, 1:1 ratio randomized controlled trial, patients with HBeAg negative CHB and documented negative HBV DNA while on stable NUC regimens for at least one year were enrolled in 30 Hepatology wards in France. Patients with PEGIFN contra-indications were not eligible. A centralized randomization used computer-generated tables with stratification on HBsAg titers (< or $\geq$ 2·25 $\log_{10}$ IU/mL) to allocate patients to receive a 48 weeks course of 180 μg/week of PEGIFN alfa-2a in addition to the NUC regimen (PEGIFN arm) or no additional therapy (control arm). The primary end point was HBsAg loss at week 96 by intent-to-treat analysis. This trial is closed and registered with ClinicalTrials.gov, number NCT01172392.

Findings: Between Jan 20, 2011 and Jul 18, 2012, 401 patients had study proposal: 208 were screened and 185 were randomized (PEGIFN n=92; control n=93). Two patients from the PEGIFN arm were excluded from analyses because of withdrawal of consent or violation of inclusion criteria. At week 96, loss of HBsAg was reported in 7/90 (7·8%) in the PEGIFN *vs* 3/93 (3·2%) in the control arms, difference 4·6% (95% CI -2.6%; 12.5%), P=0·1521. Eighty-five patients started PEGIFN; 3 had a PEGIFN dose reduction and 17 had an early PEGIFN discontinuation (16 for serious adverse events). Grade 3 and 4 adverse events were more frequent in the PEGIFN arm.

Interpretation: Addition of a 48 weeks course of PEGIFN to NUCs therapy in HBeAg negative CHB patients with undetectable HBV DNA for a least one year did not result in a significant increase of HBsAg clearance.

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INTRODUCTION:

The goal of antiviral therapy in chronic hepatitis B (CHB) is to stop the progression of liver disease initially by means of immunological and virological control over Hepatitis B virus (HBV) replication and ultimately through eradication of the virus [1-3]. Suppression of HBV DNA is the primary endpoint of HBV therapy but durable loss of serum HBsAg and with or without sustained HBs seroconversion is the definition of functional cure and currently appears to be the ultimate goal of antiviral therapy in HBeAg negative CHB [3] [2,4]. Currently there are only two types of approved therapies for HBV: interferon alpha or its pegylated form (PEGIFN) and nucleosides (lamivudine, telbivudine and entecavir) or nucleotides (adefovir dipivoxyl (ADV) and tenofovir disoproxil fumarate (TDF)) analogs (NUCs). Interferon and PEGIFN are used as finite-duration treatment between 48 to 96 weeks whereas NUCs must be taken frequently life-long to prevent rebound.

In HBe antigen (HBeAg) negative CHB patients HBs seroconversion occurred in 3% of patients treated with PEGIFN for 48 weeks and 5.8% for those treated for 96 weeks[5,6]. The rate of HBsAg clearance increased during follow up reaching 9%[7] at three years and 12% at five years[8]. Patients who have no decline in HBsAg titers and <2log decline in HBV DNA at week 12 of treatment with PEGIFN have a very low chance of achieving a sustained virological response and discontinuing therapy is warranted for such patients[9] [10] NUCs are very effective to suppress HBV DNA levels within the first year of treatment in those patients but loss of HBsAg and anti-HBs seroconversion are seldom achieved (<1% at one, three, five and seven years) [11,12] [13] [14] [8,15-17].

NUCs therapies has been shown to partially restore the adaptive immunity whereas PEGIFN boost innate immunity, trigger T-cell mediated immune responses, prevents the formation of HBV proteins and deplete the intrahepatic cccDNA pool, which leads to more HBsAg loss when

compared to analogues [18] [19-21]. Initial treatment with combination of NUCs and PEGIFN for a 48 weeks duration did not demonstrated any benefit in term of HBV DNA suppression or HBsAg loss in HBeAg positive CHB population[5]. However combination of TDF and PEGIFN for 48 weeks followed by TDF therapy demonstrated a higher rate of viral control and HBsAg loss (9% versus 1%) compared to PEGIFN alone or analogs alone (0%) in HBeAg negative CHB population [22].

For these reasons, in patients who have HBV DNA suppression for a long period of time, a current concept is to try to enhance HBsAg loss by adding PEGIFN to NUCs. Among HBeAg positive patients a early add-on strategy have been proven to be superior to combination therapy in term of sustained HBsAg reduction[23], although the primary end-point (HBeAg loss with HBV DNA < 200 IU/ml) was not reached [24].

Among HBeAg negative CHB patients, this add-on PEGIFN strategy has been already reported in case reports[25,26] and in two uncontrolled pilot studies [27,28]. They, all show a deep decline in HBsAg titers on add-on treatment and a high rate of HBsAg loss and HBs seroconversion.

Therefore we designed a randomized controlled trial in order to investigate efficacy, safety, patient's reported outcomes and predictors of response of adding-on PEGIFN for 48 weeks during analogs therapy in HBeAg negative patients with CHB.

METHODS

Study design

The ANRS HB06 PEGAN study was a multicentre randomized, open-label, parallel group, 1:1 allocation ratio trial, conducted in 30 Hepatology tertiary care wards in France, with

patients enrolled between January 20, 2011 and July 18, 2012. The study was sponsored and funded by the French National Institute for Health and Medical Research – French National Agency for Research on AIDS and Viral Hepatitis (Inserm-ANRS) and was approved by the « Sud-Méditerranée I » Ethics Committee (Marseille, France). The protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research. The protocol was registered at http://www.clinicaltrials.gov (NCT01172392). Written informed consent was obtained from each patient before enrollment.

Participants

Patients were eligible to participate if they were aged between 18 years and 75 years, had a long-standing controlled chronic viral hepatitis B infection defined by positive serum HBsAg, negative HBeAg and undetectable HBV DNA in plasma for at least 12 months under NUCs therapy. HBV DNA was measured by local laboratories with the COBAS® TaqMan® Roche V2.0 (limit of detection 20 IU/mL) or by another method with equivalent sensitivity (e.g. Abbott RealTime® HBV with a limit of detection of 10 IU/mL). Undetectable HBV DNA was defined as HBV DNA below the limit of detection and had to be confirmed on at least two measurements including one measurement during the pre-inclusion visit. Antiviral treatment had to be unchanged over the last three months and did not include telbivudine. Other eligibility criteria were alanine aminotransferase (ALT) below 5 times the upper normal range (due to the risk of hepatic flare with interferon-based therapy), no evidence of hepatocellular carcinoma at ultrasound examination or serum alpha fetoprotein < 50 ng/mL, normal dilated fundus oculi examination, and a negative pregnancy test in women.

Patients were not eligible if: they had neutropenia (<1·5x10^9/L neutrophils); thrombocytopenia (platelets <70x10^3/µL); Human Immunodeficiency Virus (HIV) or Hepatitis

C Virus (HCV) or Hepatitis D Virus (HDV) co-infection; decompensated cirrhosis (defined as a Child-Pugh score ≥ 7 or a episodes of ascites, edemas, hepatic encephalopathy, gastrointestinal bleeding in the last 6 weeks); other chronic liver diseases (such as hemochromatosis, auto-immune hepatitis, Wilson's disease and alpha-1 antitrypsin deficiency, alcoholic or toxic liver disease); allergy to interferon alpha or to a component of the tested product ; psychiatric disorders (history of major depression or other uncontrolled psychiatric disorders); a history of seizures; cardiovascular disease; a history of cancer in the last 5 years (except basocellular skin cancer or in situ cancer) ; uncontrolled thyroid disorders and/or autoimmune disorders; renal insufficiency ; had been treated with immunosuppressive or immunomodulatory drugs with last intake less than 1 year before pre-inclusion visit (including interferon) or had received more than 4 consecutive weeks of systemic corticosteroid therapy; or if they were active intravenous drug-users or reported daily alcohol intake greater than 30 g (women) or 40 g (men). Women were not eligible if they were unwilling to use effective contraception.

Randomization and masking

Randomization was stratified according to the HBsAg titer (<2·25 $\log_{10}$ IU/mL vs ≥ 2·25 $\log_{10}$ IU/mL) at the pre-inclusion visit (week -6). The cut-off value of 2·25 $\log_{10}$ IU/mL was based on a preliminary report in CHB patients receiving PEGIFN and adefovir showing that loss of HBsAg differed according to this threshold [29]. Randomization was managed by the central data centre (Inserm U1136, Paris, France). The randomization list used random permuted blocks of size 4. It was concealed to the investigators who assigned participants to the treatment groups through a dedicated website after validating eligibility and stratification criteria at inclusion visit (week 0).

Intervention

All potentially eligible patients were proposed to enter the trial. Eligible patients who agreed to participate and who fulfilled satisfied inclusion criteria were centrally and randomly assigned (1:1) to receive subcutaneous injections of 180 μg PEGIFN alfa-2a (Pegasys®, Roche) once weekly for 48 weeks in addition to the NUCs regimen (PEGIFN arm) or to continue the NUCs regimen alone (control arm). Pegasys® was kindly provided by Roche France for all patients. Patients assigned in the PEGIFN arm had monthly follow-up visit during the 48 weeks on therapy then were followed every 3 months up to week 144. Patients assigned in the control arm were followed every 3 months up to week 144.

Biochemical, hematologic, anti-HBs antibodies and HBV DNA tests were analyzed in local laboratories at each study visit. Blood samples for HBsAg quantifications were collected at screening and day 0 then every 3 months in all patients up to week 144 (13 points) and were analyzed in a centralized laboratory. Non-invasive markers of liver fibrosis (Fibrotest® and Fibrometer®) at day 0, week 96 and week 144 were analyzed in a centralized laboratory.

Adverse events were recorded at each study visit. Adverse events were graded 1 (mild) to 4 (life-threatening), using the ANRS grading system (supplementary text 2). Reduction in the PEGIFN alfa-2a dosage to manage adverse events or laboratory abnormalities was left to the physician in charge of the patient. Patients who discontinued therapy prematurely because of adverse effects were encouraged to remain in the study. Instructions were given to the investigators to pay attention to any increase of HBV DNA or ALT or AST during the study and the follow-up, and modify antiviral treatment in case of emerging antiviral resistance. Antiviral treatment discontinuation was allowed in non-cirrhotic patients with sustainable HBsAg loss (> 24 weeks confirmed on 3 different measurements).

An independent data monitoring committee consisting of a statistician, a gastroenterologist and a hepatologist, reviewed safety and efficacy data during the trial. The committee had no role in the decision to submit the manuscript.

Outcomes

The primary outcome was the proportion of HBsAg loss at week 96. Other efficacy outcomes were kinetics of HBsAg titers, proportions of HBsAg loss and anti-HBs seroconversion up to week 144 and assessment of predictive factors associated with loss of HBsAg at week 96 including the treatment arm, age, sex, HBsAg titer at week 0 (in $\log_{10}$ scale), duration of undetectable HBV DNA, previous experience of interferon therapy (defined as any treatment with interferon in the past with last intake more than 1 year before entry in the trial), HBeAg status at the time of chronic HBV diagnosis, IL28B profile.

The detection and quantification of HBsAg titers on serum samples was performed in a central laboratory (Service de virologie, bactériologie-hygiène, mycologie-parasitologie, secteur hépatites-VIH, Hôpital Henri Mondor, Créteil, France). Technical staff was blinded to the arm allocation tested each sample. Levels of HBsAg in serum were quantified by a standardized electrochemiluminescent CMIA assay (Architect HBsAg, Abbott), having a lower detection limit of 0·05 IU/mL. Loss of HBsAg was defined when HBsAg titer was less than the detection limit. Samples with titers of more than the upper linearity limit of the assay (250 UI/ml) were retested after being diluted as recommended by the manufacturer. Anti-HBs seroconversion was defined as an anti-HBs antibodies titer above 10 mIU/mL.

Other secondary outcomes included the proportion and intensity of adverse events, the proportion of patients with HBV DNA suppression including HBV-DNA breakthrough

defined as a confirmed increase in HBV-DNA level of more than $1\log_{10}$ IU/mL [3] , the kinetics of ALT and AST, liver fibrosis progression using non-invasive markers (Fibrotest® and Fibrometer®), patients' reported outcomes (PROs), the acceptability of PEGIFN therapy (defined as the proportion of patients who agreed to enter the study) and any change in antiviral therapy.

PROs were obtained using self-administered questionnaires completed at week 0, 4, 48, 96 and 144 by all patients, and every 3-months for those in the PEGIFN arm. These questionnaires included an assessment of adherence to HBV treatment (ANRS scale), health-related quality of life (MOS SF-12 scale), functional impact of fatigue (MFIS scale), self-reported symptoms (ANRS scale) and depressive symptoms (CES-D scale)[30].

Statistical analysis

Our trial was designed as a superiority trial with 80% power to detect a difference of 9·5% (10% vs 0·5%) of loss of HBsAg between the PEGIFN and the control groups; these rates were based on observational data indicating that 8·7% of all patients receiving PEGIFN cleared HBsAg compared with none (0%) of the patients treated with NUC alone [31] they took into account early study discontinuation (which was expected to be 10%) and the calculation was done using the two-sided Fisher's exact test (PASS 11. NCSS, LLC. Kaysville, Utah, USA). The 1:1 ratio was chosen as it provides the lowest total sample size with respect to our hypotheses. No interim analysis was planned. Ninety-one patients had to be enrolled in each group.

Intention-to-treat (ITT) analysis was used as the primary analysis for all measures of efficacy or safety. As pre-specified in the protocol, ITT analysis included all randomized patients except patients who withdrew consent or patients violating major eligibility criteria.

Patients who missed the week 96 examination were considered to have detectable HBsAg (i.e. treatment failure) unless a loss of HBsAg could be observed in the last preceding 3 months. Missing measurements of HBsAg or anti-HBs antibodies at other visits were considered as detectable or lack of seroconversion, respectively. No quantitative imputation was made for these missing values in kinetic analyses as recommended [32] .

We performed secondary post-hoc analyses of efficacy outcomes selecting patients from the PEGIFN arm who initiated PEGFIN and who received full dose and duration of PEGIFN (full dose analysis set).

The Cochran-Mantel Haenszel test was used with stratification on pre-inclusion HBsAg titer to compare the proportions of patients with HBsAg loss between groups. Other proportions were compared using the Fisher exact test and continuous outcomes were compared using the Mann-Whitney test. The McNemar's chi square test for proportions and the Wilcoxon signed-rank test for continuous variables were used for matched data comparisons. Confidence limits for proportions or difference of proportions were calculated using exact methods [33] [34]. All statistical tests were 2-sided at 5% level of significance.

A linear mixed model for repeated measurements was used to compare the dynamics of HBsAg (in log10 scale), with group as a fixed effect, patient as a random effect and using a first-order autoregressive covariance matrix to handle within patient correlation between successive measurements. Exact logistic regression analysis was used to identify predictive factors of HBsAg loss. Covariates with p <0·05 in bivariable analyses were included in a multivariable model. The choice of a P-value < 0·05 prevented false positive findings due to the limited number of expected HBsAg loss. Of note, another threshold (e.g. P< 0.2) would have lead to strictly the same conclusions. Linear mixed models were used to compare

dynamics of ALT and AST (Week 0-Week 144) and to compare PRO's in the two arms over time (Week 0-Week 48). Statistical analyses were performed using SAS® v9.4 (Sas Institute Inc, Cary, NC) or STATA v12.1 (STATACORP, Texas, USA).

Role of the funding source

The funder of the study oversaw trial management, data collection, data analyses and writing of the report. The funder had no role in study design or data interpretation. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Between January 20, 2011 and July 18, 2012, 401 patients with HBeAg negative CHB who were HBV DNA negative on NUCs therapy had study proposal among whom 208 (acceptability rate 52%, 95% Confidence Interval (CI) 47%-57%) agreed to participate (Figure 1). Main reasons for declining participation were fear of adverse events and constraints linked with PEGIFN treatment. One hundred and eighty five patients were randomized. Two patients, both in the PEGIFN arm, were secondary excluded from all analyses for violation of major inclusion criteria (HBeAg positive) or consent withdrawal. Therefore our primary intent-to-treat analysis included 90 patients in the PEGIFN arm (14 in the HBsAg titer <2·25 log_{10} IU/mL strata, 76 in the HBsAg titer ≥ 2·25 log_{10} IU/mL strata) and 93 patients in the control arm (15 in the HBsAg titer <2·25 log_{10} IU/mL strata, 78 in the HBsAg titer ≥ 2·25 log_{10} IU/mL strata). Baseline characteristics were similar between groups, except for more alcohol use in the PEGIFN arm (table 1). Nine patients (4 in the PEGIFN arm, 5 in the control arm) had HBV DNA undetectability less than 1 year before entry. All these patients had a long period of undetectability in their history and the last detectable event was classified as a blip by the investigator. Five patients from the PEGIFN arm did not start therapy (patient's decision). Among 85 patients who started PEGIFN, 65 received full dose and duration of PEGIFN (full dose analysis set). Eleven patients from the PEGIFN arm (five patients who did not start PEGIFN, five patients with premature treatment discontinuation and 1 patient with full dose and duration of PEGIFN) and four from the control arm missed the week 96 visits and were considered as treatment failure. All these patients were HBsAg positive at their last evaluation before week 96.

In the primary intent-to-treat analysis, a loss of HBsAg at week 96 was reported in 7/90 (7·8%) in the PEGIFN *vs* 3/93 (3·2%) in the control arms, difference 4·6% (95% CI -2·6%; 12·5%),

P=0·1521 (table 2). Proportions of HBsAg loss were however significantly higher in the PEGIFN arm compared to the control arm at week 48 (7/90: 7·8% vs 0/93: 0·0%, difference 7·8% (95% CI 2·8%; 15·8%), P= 0·0057) or at any time point when considering the full dose analysis set.

At week 48, patients in the PEGIFN arm experienced a significant decline from baseline values in HBsAg titers compared to the control arm (-0·86 log_{10} IU/mL *vs* -0·22 log_{10} IU/mL, P=0·0006, Figure 2) and the difference remained stable thereafter: it was -0·85 log_{10} IU/mL *vs* -0·34 log_{10} IU/mL, P=0·0163 at week 96 and -0·95 log_{10} IU/mL *vs* -0·36 log_{10} IU/mL, P=0·0002 at week 144, respectively. The average decline from baseline in natural units were respectively (-2400 IU/mL vs -590 IU/mL, P=0·0224 at week 48; -2425 IU/mL vs -991 IU/mL, P=0·1117 at week 96 and -2306 IU/mL vs -856 IU/mL, P=0·0289 at week 144). In the PEGIFN arm, patients who achieved HBsAg loss at week 96 had a significant decline in HBsAg titers as early as week 12 compared to those who did not achieve HBsAg loss (-1·39 log_{10} IU/mL *vs* -0·21 log_{10} IU/mL, P<0·0001, appendix p1). However, the decline was not significantly different if HBsAg was treated in natural units (-1371 IU/mL vs -682 IU/mL, P=0·5152).

The proportions of patients with anti-HBs seroconversion were significantly higher in the PEGIFN arm compared to the control arm at weeks 48 and 96 (table 2). Among seven patients with HBsAg loss at week 96 in the PEGIFN arm, six had anti-HBs seroconversion at week 96 and one had anti-HBs seroconversion at week 108; all seven remained anti-HBs antibodies positive at week 144 and discontinued their NUCs treatment. In addition, two patients from this arm lost HBsAg and one had an anti-HBs seroconversion between week 132 and 144. In the control arm, among three patients with HBsAg loss at week 96, one patient had an anti-HBs seroconversion at week 96 and two had an anti-HBs seroconversion at week 144. In addition, one patient had HBsAg loss and anti-HBs seroconversion between week 132 and 144.

In intent-to-treat analysis set, HBsAg titers at week 0 was the unique factor associated with HBsAg loss at week 96: odds-ratio of HBsAg loss (OR) per 1log_{10} increase of HBsAg titer at week 0 = 0·36 (95% CI 0·17-0·76), P=0·0058 (Figure 3 and appendix p2). Of note, we found no association between NUCs regimen at entry and loss of HBsAg (appendix p3). In the full dose analysis set, HBsAg titer at week 0 (OR per 1log_{10} increase = 0·29 (95% CI 0·12-0·66), P=0·0024) and PEGIFN treatment (OR = 5·55 (1·02-43·8), P=0·0463) were independently associated with HBsAg loss at week 96. The benefit in HBsAg loss appeared more marked in patients with baseline HBsAg titers between 2 and 3 log_{10} IU/mL (figure 3).

Paired samples for centralized fibrosis staging using non-invasive markers at week 0 and week 144 were available in 70 patients in the PEGIFN arm and 76 patients in the control arm for fibrotest® and in 70 and 70 patients for fibrometer®, respectively. Using fibrotest®, 12 of 50 patients (24%) classified as F0-F2 at week 0 were classified F3-F4 at week 144 in the PEGIFN arm *vs* 6 of 51 patients (12%) in the control arm, P=0·1256. Conversely, 2 of 20 (10%) patients classified as F3-F4 at week 0 were classified as F0-F2 at week 144 *vs* 7 of 25 (28%) in the control arm, P=0·2604. Similar findings were found using fibrometer® (not shown).

Among the 85 patients who started PEGIFN, three had a PEGIFN dose reduction and 17 had an early PEGIFN discontinuation after a median duration of 19 weeks (Interquartile range: 13-26 weeks). Among them, 7 had a PEGIFN discontinuation due to a serious adverse event (thrombopenia (n=1), rash (n=1), anaemia (n=1), hepatic cytolysis (n=2), discovery of an hepatocellular carcinoma (n=1), cholestasis and jaundice (n=1)), 8 had a discontinuation for a combination of signs and symptoms not reported as serious adverse events (mostly fever or flu-like illness, vertigo, asthenia, myalgia, depression), one patient discontinued for non-adherence and one patient discontinued due to lack of HBsAg loss and adverse events. Over the first 48 weeks, five patients had a NUCs substitution or simplification (a combination of

Lamivudine and Adefovir was switched to Tenofovir for simplification in 2 patients; Tenofovir was switched to Entecavir for renal impairment in 1 patient; Lamivudine was stopped in 1 patient also treated with Tenofovir; Adefovir was stopped in 1 patient also treated with Entecavir) and one had a Tenovofir dose modification in the PEGIFN arm because of side effects, three patients had a NUCs substitution in the control arm. Nine patients (10%) in the PEGIFN arm experienced an episode of detectable HBV DNA (>20 IU/mL) versus six patients (6·5%) in the control arm (P=0·4288). No patient experienced HBV DNA breakthrough during the study.

Severe (Grade 3) and Life threatening (Grade 4) adverse events were more frequent in PEGIFN arm and were mainly laboratory abnormalities related with the use of PEGIFN (Table 3). A significant increase of ALT and AST levels was observed in the PEGIFN arm during the treatment period, with return to the baseline values at week 60 (appendix p4).

Physical and mental health-related quality of life (HRQL), the fatigue impact scale and self-reported symptoms) showed a significant change (impairment) during PEGIFN treatment and return to baseline values at weeks 96 compared to the control group. By contrast no significant difference was observed for depressive symptoms in the two arms.

After multiple adjustment for gender (women were generally at higher risk of impairment of PROs) or follow-up time and baseline values of the outcomes, the effect of the arm on the physical and the mental dimension of HRQL was no longer significant but individuals in the PEGIFN arm continue to experience both a higher functional impact of fatigue (P=0·0020) and a significant increase (P=0·0011) in the number of self-reported symptoms. At week 96, no significant difference for all PROs was found between the two arms.

After adjustment for age, the number of self-reported symptoms was a major predictor of PEGIFN discontinuation (OR= 1·3 (95%CI 1·1-1·5)). Independently, individuals with flu-like

symptoms had a 16-fold risk (P=0·0095) and those with sleep disturbances a 8-fold risk (P=0·0208) of PEGIFN discontinuation.

Discussion

In this randomized controlled trial in HBeAg negative CHB patients with undetectable HBV DNA for at least one year, we did not demonstrate that the addition of forty-eight weeks of PEGIFN alpha-2a to NUCs therapy results in a higher rates of HBs Ag loss and HBs seroconversion in our primary intent-to-treat analysis. However, HBSAg loss rates were significantly higher in patients who achieved a full 48 weeks course of PEGIFN. Moreover anti-HBs seroconversion was significantly higher in the PEGIFN arm at weeks 48 and 96. All 7 patients from the PEGIFN arm who lost HBsAg at week 96 achieved anti-HBs seroconversion at week 144 whereas it was observed in two of three patients in the NUCs group control arm. These results are in line with those reported in uncontrolled studies [27 28]. Another important finding was the strong link between baseline HBsAg titers and HBsAg loss. Even if this link have been already demonstrated with interferon therapy or combination therapy [10 35], this have been already suggested but never demonstrated by the previous add-on studies. Neither fibrosis stage, duration of HBV DNA undetectability under NUCs therapy, previous IFN treatment or IL28B status were related to HBsAg loss but our comparisons may have been underpowered due to the limited number of HBsAg loss. Regarding the fluctuation of fibrosis stage according to fibrotest or fibrometer, nearly 60% of our population had no significant change whatever treatment arms. Forty percent of our population had either an improvement or a deterioration of their fibrosis stage between baseline and week 144, not significally different between the two arms and may be due to treatment-unrelated causes. According to our findings the usefulness of this add-on strategy in HBeAg negative CHB patients appears to

be meaningful only among patients with HBsAg titer at baseline below 3 log_{10} IU/ml. In this selected population who represent 42% of our HBeAg negative population under NUCs, HBsAg loss is achieved in 23% of patients who received full-dose of PEGIFN.

The lack of HBV genotype information is a potential gap of our RCT. Obviously HBV genotype was not available in these patients with undetectable HBV DNA at entry in the trial. HBV genotyping is not part of the baseline assessment of HBeAg negative CHB patients in France and is not recommended by EASL guidelines [3]. Therefore very few patients had HBV genotype in their data. In France genotypes D, E and A are the most prevalent in CHB patients representing 86% of genotyped virus in the last decade [36] [37]. Several studies have demonstrated that the HBsAg titers are different according to HBV genotype and that HBsAg kinetics on interferon treatment are influenced by HBV genotype [38] [39] [40]. However the difference of HBsAg kinetics on PEGIFN treatment between genotype D and A , the two major potential genotypes in our population was not significantly different in one study [39].

One of the limitations of our study is the fixed 48 week duration of the PEGIFN treatment. In a pilot study it was suggested that the duration of PEGIFN treatment should be tailored according to the kinetic of HBsAg titers decline [28]. However in that study, four patients loss HBsAg after 48 weeks of treatment and two patients required 96 weeks of treatment to achieve HBsAg loss. In our study, the individual HBsAg titer kinetic did not suggest any beneficial effect of extending treatment duration up to 96 weeks as HBsAg titer decline occurs rapidly at week 12 and 24 after PEGIFN initiation in patients who achieve HBsAg loss or anti-HBs seroconversion. Moreover in our long term follow-up only two patients had a late HBsAg loss between week 132 and 144 and one of them achieve HBs seroconversion. Whether PEGIFN had a role for this late response remain questionable.

The second main limitation of our trial was lack of power. A continuous outcome such as a decline of HBsAg titer would have provided more power. However, loss of HBsAg is considered as a first step towards a functional cure of HBV infection, and a potential robust surrogate for clinical outcomes related to chronic HBV infection [41]. The proportion of HBsAg loss was higher than hypothesized in the control arm (3·2% instead of 0·5%) and a higher number of missing values, imputed as detectable HBsAg, was observed at week 96 in the PEGIFN (11) than the control arm (4). In addition, lack of blinding affected compliance to treatment, which in turn also affected the statistical power of our trial as only 72% in the PEGIFN arm received the full dose and duration of PEGIFN. However, maintaining blinding would have decreased the external validity of our trial. Indeed, because participants were not blinded, our findings are closed to real-life situations in which patients will be proposed a PEGIFN treatment. To this respect, our study is the first to provide a comprehensive evaluation of efficacy, safety and patients' reported outcomes of adding PEGIFN in HBV chronically infected patients. Finally, no restriction was made in relation with baseline HBsAg titer and it is likely that a selection of patients with low titers would have also increase the power of the trial. However, in HBeAg negative patient with stable NUC regimen, there was no published HBsAg threshold to indicate who may benefit from this add-on strategy of PEGFIN. Therefore we decided to include all patients irrespective of their HBsAg level and to stratify randomization on HBsAg titer to ensure proper balance on this criteria between arms. Our trial is the first to confirm the predictive value of HBsAg level at baseline in this setting.

This study clearly shows that the major barriers to initiation of and persistence with this regimen, in a population who is already HBV DNA undetectable on NUCs regimen, are related to the toxicity burden associated with PEGIFN. Half of eligible patients declined participation in the study, mainly due to the fear of adverse events. Moreover, the tolerance of the

treatment was poor and 17% of patients had an early treatment discontinuation that was associated with adverse events and patients' experience during treatment expressed by the number of self-reported symptoms. As flu-like symptoms and sleep disturbances are a major issue in this population, future assessment of efficacy in possible responders should assure an adequate management of such symptoms and better counsel older individuals.

Concluding, in this randomized controlled trial, we did not demonstrate an increase in HBsAg loss associated with the add-on strategy of PEGIFN for 48 weeks in HBeAg negative CHB population. PEGIFN appears poorly tolerated, and associated with severe adverse events and impairment in patients reported outcomes leading to frequent discontinuation. However, secondary post hoc analyses showed that patients who had a baseline level of HBsAg titers below 3 $\log_{10}$ IU/ml could benefit from this add-on strategy to achieve HBsAg loss and anti-HBs seroconversion.

Declaration of interest

Dr Bourliere is member of advisory board, had consulting and is speaker for Roche, BMS, Gilead, Janssen MSD and AbbVie.

Ms. RABIEGA has nothing to disclose.

Mr. BARTHE has nothing to disclose.

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Author Contribution

Conception or design of the work: MB, DT, LS, FZ, IB, FC

Acquisition of data: MB, NGC, PM, DT, DG, CH, MP, XC, VL, JPB, GR, IR, PA, JMM, YB, AT, JDG, FZ, HF

Analysis, or interpretation of data for the work: PR, YB, PC, IB, MBA, FC

Drafting the work: MB, FC

Revising the work for important intellectual content: all authors

Final approval of the version to be published: all authors

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Figure 1: Trial profile

Figure 2: Dynamics of HBsAg levels. Mean values of $\log_{10}$ HBsAg are estimated using linear mixed models (see text). Error bars represent 95%CI confidence intervals.

Figure 3: Loss of HBsAg at week 96 according to treatment arm stratified by HBsAg titer (in $\log_{10}$ IU/mL) at week 0.

Table 1: Demographic and clinical characteristics

Table 2: HBsAg loss and HBs seroconversion

Table 3: Adverse events and laboratory abnormalities from week 0 to week 48

Figure 1: Trial profile

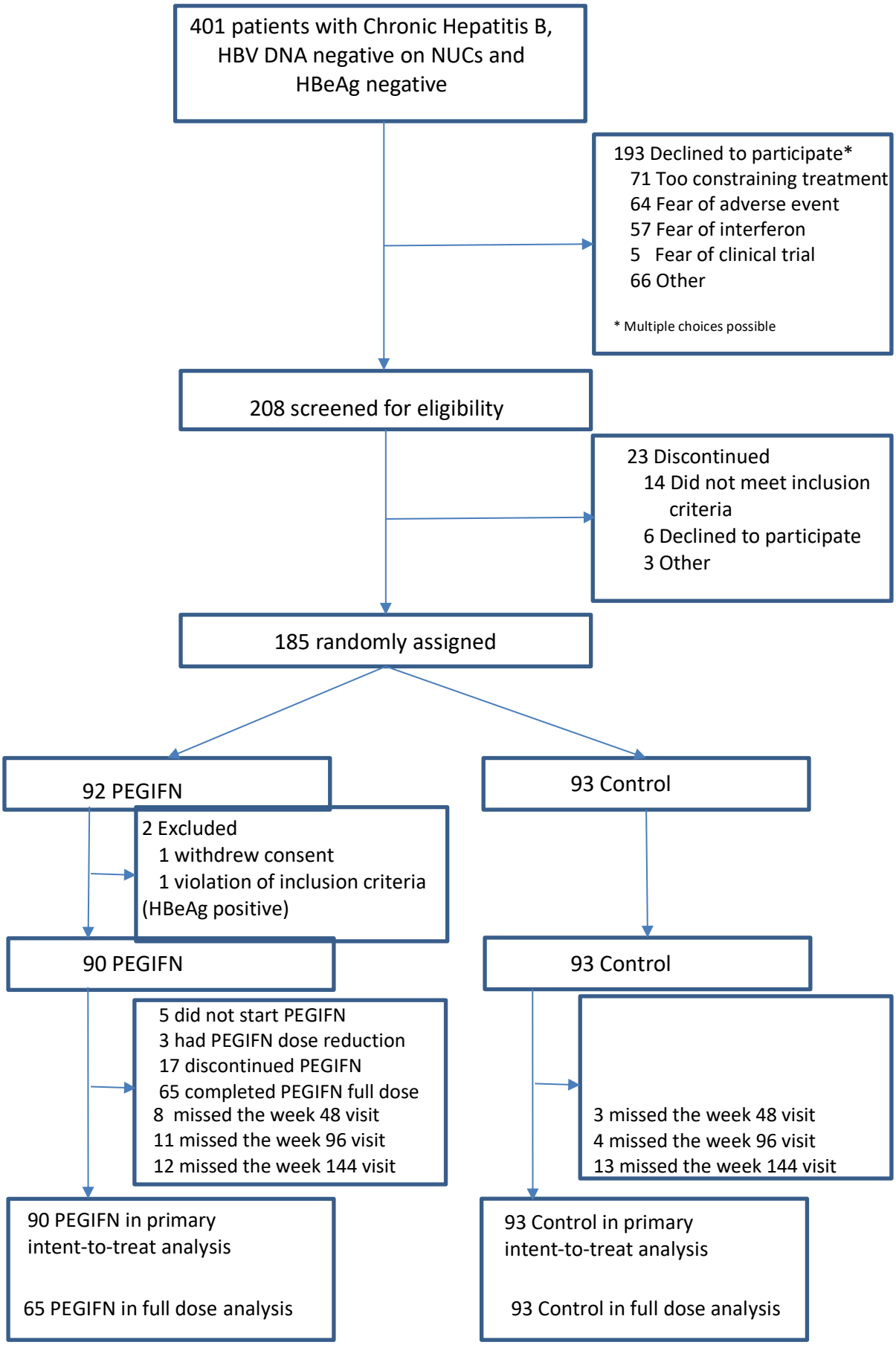

401 patients with Chronic Hepatitis B, HBV DNA negative on NUCs and HBeAg negative
193 Declined to participate*
71 Too constraining treatment
64 Fear of adverse event
57 Fear of interferon
5 Fear of clinical trial
66 Other
* Multiple choices possible
208 screened for eligibility
23 Discontinued
14 Did not meet inclusion criteria
6 Declined to participate
3 Other
185 randomly assigned
92 PEGIFN
93 Control
2 Excluded
1 withdrew consent
1 violation of inclusion criteria (HBeAg positive)
90 PEGIFN
93 Control
5 did not start PEGIFN
3 had PEGIFN dose reduction
17 discontinued PEGIFN
65 completed PEGIFN full dose
8 missed the week 48 visit
11 missed the week 96 visit
12 missed the week 144 visit
3 missed the week 48 visit
4 missed the week 96 visit
13 missed the week 144 visit
90 PEGIFN in primary intent-to-treat analysis
65 PEGIFN in full dose analysis
93 Control in primary intent-to-treat analysis
93 Control in full dose analysis

Figure 2: Dynamics of HBsAg levels. Mean values of log_{10} HBsAg are estimated using linear mixed models (see text). Error bars represent 95%CI confidence intervals.

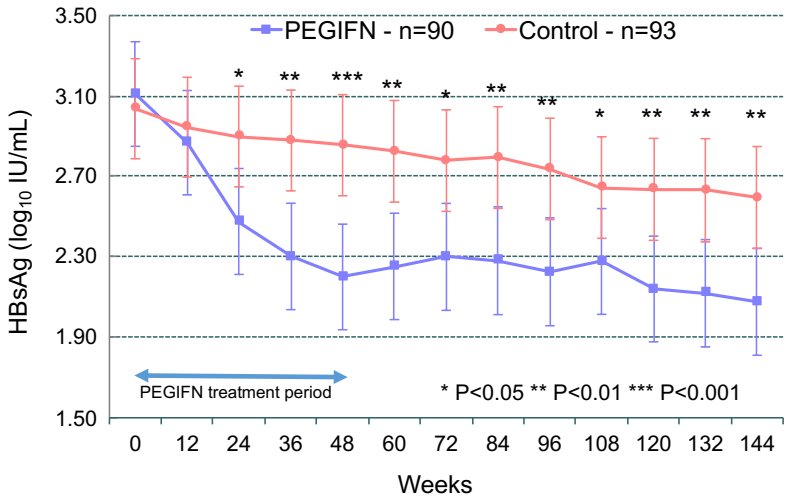


Figure 3. Loss of HBsAg at week 96 according to treatment arm stratified by HBsAg titer (in log_{10} IU/mL) at week 0.

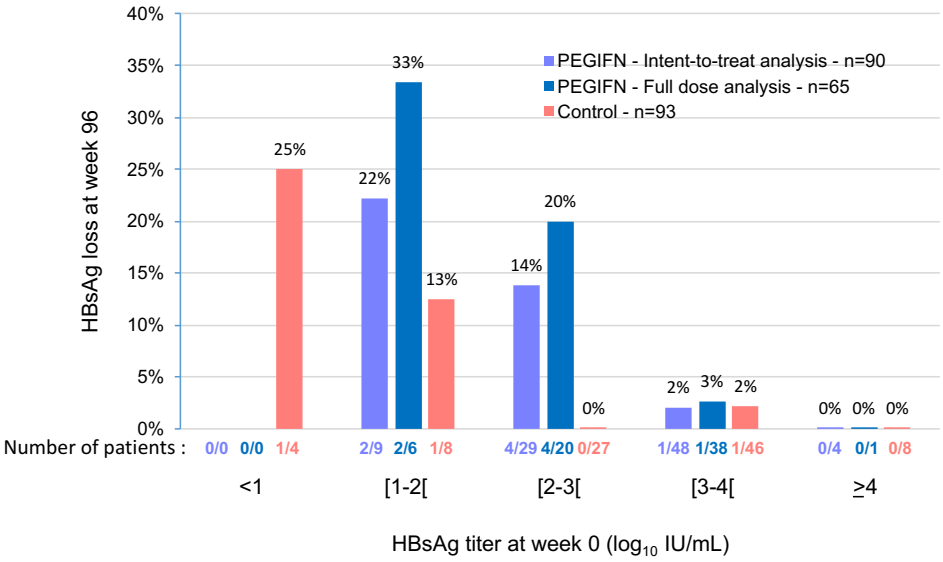


Table 1: Demographic and clinical characteristics (intent-to-treat analysis)

	PEGIFN n=90	**Control n=93**
Age, years, Mean (SD)	48·2 (10·1)	47·1 (10·2)
Gender male, N (%)	75 (83)	83 (89)
BMI, kg/m^2, Mean (SD)	25·7 (4·1)	25·0 (3·3)
Geographic origin, N (%)		
Africa	28 (31)	36 (39)
Asia	12 (13)	18 (19)
Caribbean island	3 (3)	5 (5)
Europe & North America	43 (48)	31 (33)
Middle East	4 (4)	3 (3)
Alcohol use, N (%)	21 (23)	11 (12)
Tobacco use (>5 cigarettes/day), N (%)	13 (14)	10 (11)
HBsAg titers at Week-6, Mean (SD)		
IU/mL	2901 (4534)	3172 (5622)
Log_{10} (IU/mL)	3·0 (0·7)	2·9 (0·9)
Duration of undetectable HBV DNA before inclusion, years, Median (IQR)	2·9 (1·7-4·6)	3·0 (1·6-4·6)
HBeAg serology status at the time of first HBV diagnosis, N (%)		
Negative	64 (71)	56 (60)
Positive	19 (21)	33 (35)
Not Available	7 (8)	4 (4)
NUCs treatment at entry, N (%)		
Entecavir	32 (36)	26 (28)
Tenofovir	54 (60)	53 (57)
Adefovir	12 (13)	11 (12)
Lamivudine	15 (17)	24 (26)
1 NUC	67 (74)	72 (77)
2 NUCs	23 (26)	21 (23)
Duration of NUCs treatment, years, Median (IQR)	2·7 (2·0-4·9)	3·3 (2·1-5·0)
Previous Interferon treatment*, N (%)	34 (38)	40 (43)
Fibrosis score (Metavir, F), N (%)		
*Liver biopsy***	52 (58)	58 (62)
Fibroscan®	33 (37)	31 (33)
Not Available	5 (6)	4 (4)
F0-F2	54 (60)	66 (71)
F3	16 (18)	9 (10)
F4	15 (17)	14 (15)
ALT level, IU/L, Mean (SD)	35 (21)	33 (15)
AST level, IU/L, Mean (SD)	31 (14)	30 (10)

IL28B genotype, N (%)			
	CC	33 (37)	31 (33)
	CT	32 (36)	30 (32)
	TT	11 (12)	10 (11)
	Not available	14 (17)	22 (24)

* any treatment with interferon in the past with last intake less than 1 year before entry in the trial

** was considered liver biopsy less than 1 year if METAVIR score was F0-F3 or liver biopsy irrespective of the date if METAVIR score was F4

Table 2: HBsAg loss and HBs seroconversion

HBsAg titers at Week-6 (Log10 IU/ml)		PEGIFN			Control			P-Value
		< 2·25	**>=2·25**	**Total**	**< 2·25**	**>=2·25**	**Total**	
Intent-to-treat analysis		***(n=14)***	***(n=76)***	***(n=90)***	***(n=15)***	***(n=78)***	***(n=93)***	
Week 48	HBsAg loss	3 (22%)	4 (5·2%)	7 (7·8%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0·0057
	Anti-HBs seroconversion	2 (14%)	2 (2·6%)	4 (4·4%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0·0384
Week 96	**HBsAg loss (primary outcome)**	4 (29%)	3 (3·9%)	7 (7·8%)	2 (13%)	1 (1·3%)	3 (3·2%)	0·1521
	Anti-HBs seroconversion	3 (21%)	3 (3·9%)	6 (6·7%)	0 (0%)	1 (1·3%)	1 (1·1%)	0·0465
Week 144	HBsAg loss	4 (29%)	5 (6·6%)	9 (10%)	3 (20%)	1 (1·3%)	4 (4·3%)	0·1135
	Anti-HBs seroconversion	4 (29%)	4 (5·2%)	8 (8·9%)	2 (13%)	1 (1·3%)	3 (3·2%)	0·0920
Full-dose analysis		***(n=10)***	***(n=55)***	***(n=65)***	***(n=15)***	***(n=78)***	***(n=93)***	
Week 48	HBsAg loss	3 (30%)	4 (7·3%)	7 (11%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0·0011
	Anti-HBs seroconversion	2 (20%)	2 (3·6%)	4 (6·2%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0·0145
Week 96	HBsAg loss	4 (40%)	3 (5·5%)	7 (11%)	2 (13%)	1 (1·3%)	3 (3·2%)	0·0415
	Anti-HBs seroconversion	3 (30%)	3 (5·5%)	6 (9·2%)	0 (0%)	1 (1·3%)	1 (1·1%)	0·0131
Week 144	HBsAg loss	4 (40%)	5 (9·1%)	9 (14%)	3 (20%)	1 (1·3%)	4 (4·3%)	0·0226
	Anti-HBs seroconversion	4 (40%)	4 (7·3%)	8 (12%)	2 (13%)	1 (1·3%)	3 (3·2%)	0·0203

Table 3: Adverse events and laboratory abnormalities from week 0 to week 48

		PEGIFN N= 90		Control N= 93		p-value Fisher's test
		AEs	N (%)	AEs	N (%)	
All Adverse events		1018	85 (94)	195	74 (80)	0·0038
	grade 1	613	4 (4)	130	35 (38)	
	grade 2	319	36 (40)	54	30 (32)	<0·0001
	grade 3	61	26 (29)	5	3 (3)	
	grade 4	25	19 (21)	6	6 (6)	
Events imputed to PEGIFN		562	76 (84)			
	grade 1	352	15 (17)			
	grade 2	163	31 (34)			
	grade 3	37	22 (24)			
	grade 4	10	8 (9)			
Most frequent grade 1 or 2* and all grade 3,4, or 5 events						
Asthenia	*grade 1&2*	48	46 (51)			
	grade 3	3	3 (3)			
Decreased appetite	*grade 1&2*	13	13 (14)			
Insomnia	*grade 1&2*	10	10 (11)			
Headache	*grade 1&2*	11	11 (12)			
Influenza-like illness	*grade 1&2*	29	29 (32)			
Myalgia	*grade 1&2*	19	15 (16)			
GGT** increased	*grade 1&2*	18	18 (20)			
	grade 3	2	2 (2)			
	grade 4	1	1 (1)			
Hepatocellular injury	*grade 1&2*	48	28 (31)			
	grade 3	3	1 (1)			
	grade 4	2	2 (2)			
Cholestasis	*grade 3*	1	1 (1)			
Leukopenia	*grade 1&2*	42	31 (34)			
	grade 3	12	12 (13)			
Lymphopenia	*grade 1&2*	16	15 (16)			
Neutropenia	*grade 1&2*	33	27 (30)			
	grade 3	8	8 (9)			
	grade 4	5	4 (4)			
Thrombocytopenia	*grade 1&2*	19	16 (18)			
	grade 3	2	2 (2)			
Anaemia	*grade 3*	1	1 (1)			
Irritability	*grade 1&2*	11	11 (12)			

Depression	*grade 3*	1	1 (1)
Hallucination	*grade 3*	1	1 (1)
Rash	*grade 4*	1	1 (1)
Arthralgia	*grade 3*	1	1 (1)
Sciatica	*grade 3*	1	1 (1)

*events occurring in more than 10%; **GGT: gamma-glutamyltransferase

Research in context:

Evidence before this study

Sustained HBs seroconversion after treatment cessation is the goal of HBV "functional cure" in patients with HBeAg negative chronic hepatitis B. This goal is rarely achieved either by pegylated interferon alpha (PEGIFN) finite duration treatment or by nucleos(t)ides (NUCs) life long duration treatment. NUCs therapies has been shown to partially restore the adaptive immunity whereas PEGIFN boost innate immunity, trigger T-cell mediated immune responses, prevents the formation of HBV proteins and deplete the intrahepatic cccDNA pool, which leads to more HBsAg loss when compared to analogues. For these reasons, in patients who have HBV DNA suppression for a long period of time, a current concept is to try to enhance HBsAg loss by adding PEGIFN to NUCs. We searched PubMed using the terms HBV and NUCs and PEGIFN and add-on. Up to day this add-on PEGIFN strategy has been only reported in a case report[25] and in two uncontrolled pilot study in HBeAg negative CHB patients [27,28]. They, all show a deep decline in HBsAg titers on add-on treatment and a high rate of HBsAg loss and HBs seroconversion.

Added value of this study

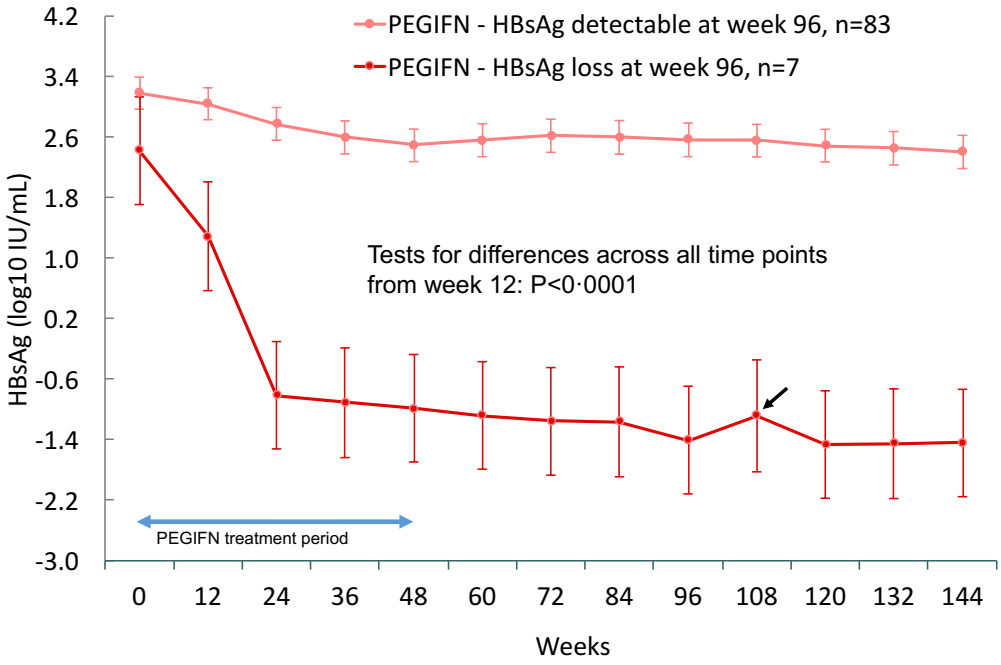
Our study is the first randomized controlled study to investigate efficacy, safety, patient's reported outcomes and predictors of response of adding-on PEGIFN for 48 weeks during analogs therapy in HBeAg negative CHB patients. More over this study provide long-term follow-up results up to week 144.

Implication of all available evidence

Our results do not support this add-on strategy in all HBeAg negative CHB. However, HBs seroconversion was significantly higher in patients who achieved a full 48-week course of PEGIFN. We found that the only predictive factor of HBsAg loss was the baseline low HBsAg

titer. Secondary post hoc analysis showed that patients who had a baseline level of HBsAg titers below 3 log_{10} IU/ml could benefit from this add-on strategy to achieve HBsAg loss and anti-HBs seroconversion allowing NUCs discontinuation. However, our study clearly shows the limitation of such treatment with the fear of PEGIFN in our population before treatment and on-treatment the poor tolerance with more severe adverse events and patient's reported outcomes.

Dynamics of HBsAg levels in the PEGIFN arm according to loss of HBsAg at week 96. Mean values of $\log_{10}$ HBsAg are estimated using linear mixed models (see text). Error bars represent 95%CI confidence intervals. The small increase at week 108 in patients with HBsAg loss at week 96 (as indicated by the black arrow) was due to one missing value in one patient.



Predictors of HBsAg loss at week 96 (intent-to-treat analysis set).
Exact logistic regression results.

Factors	N of HBsAg loss / N patients (%)	Bivariable OR (95% CI)	P-value
Arm			
Control	3/93 (3·2%)	1 [reference]	
PEGIFN (ITT)	7/90 (7·8%)	2·52 (0·55-15·6)	0·3037
Age, per 10 y increase		0·90 (0·47-1·70)	0·7602
Gender			
Male	9/158 (5·7%)	1 [reference]	
Female	1/25 (4·0%)	0·69 (0·02-5·40)	0·9999
HBsAg titer at week 0, per $1\log_{10}$ increase		0·36 (0·17-0·76)	0·0058
HBV DNA undetectable period before inclusion, per year increase		0·99 (0·73-1·27)	0·9534
HBeAg serology at the time of first HBV diagnosis*			
Positive	3/52 (5·8%)	1 [reference]	
Negative	7/120 (5·8%)	0·99 (0·16-4·56)	0·9999
Previous IFN treatment			
No	4/109 (3·7%)	1 [reference]	
Yes	6/74 (8·1%)	2·31 (0·53-11·5)	0·3341
IL28B genotype**			
CC	5/64 (7·8%)	1 [reference]	
CT	2/62 (3·2%)	0·40 (0·04-2·54)	0·4664
TT	2/21 (9·5%)	1·24 (0·11-8·36)	0·9999

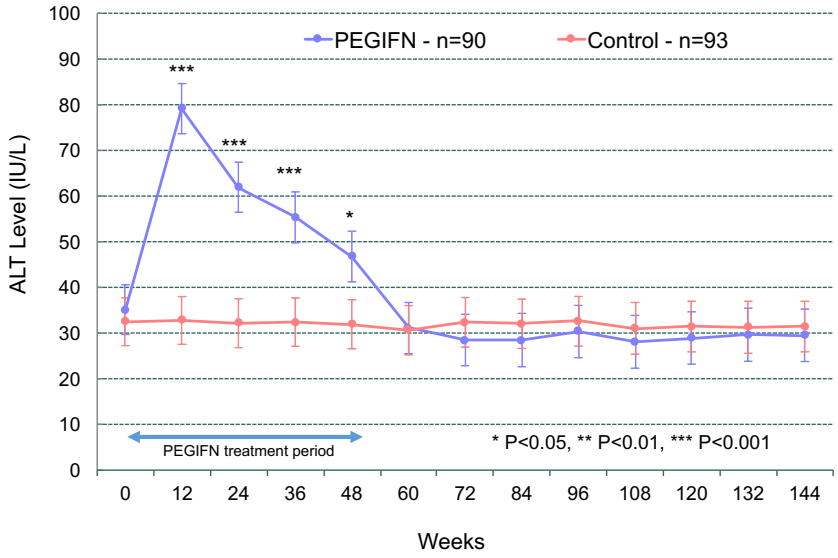
*11 missing, **36 missing

HBsAg loss according to NUCs regimen (intent-to-treat analysis set)
bivariable analysis**.**

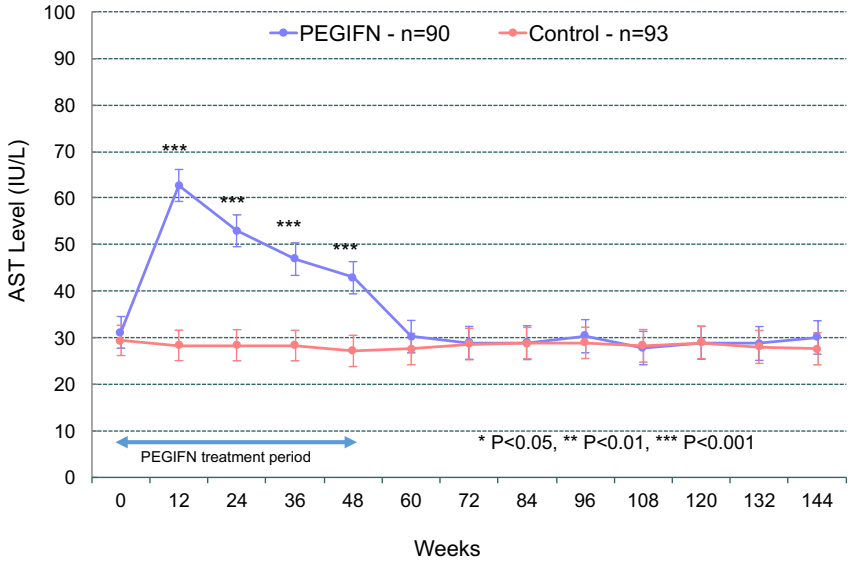
NUCs	N of HBsAg loss / N patients (%)	Bivariable OR (95% CI)	P-value
Entecavir			
No	7/125 (5·6%)	1 [reference]	
Yes	3/58 (5·2%)	0·92 (0·15-4·22)	0·9999
Tenofovir			
No	5/76 (6·6%)	1 [reference]	
Yes	5/107 (4·7%)	0·70 (0·15-3·15)	0·8076
Adefovir			
No	7/160 (4·4%)	1 [reference]	
Yes	3/23 (13·0%)	3·25 (0·50-15·7)	0·2310
Lamivudine			
No	7/144 (4·9%)	1 [reference]	
Yes	3/39 (7·7%)	1·63 (0·26-7·57)	0·7226

Dynamics of ALT (a) and AST (b). Mean values are estimated using linear mixed models (see text). Error bars represent 95%CI confidence intervals. P-values are for comparisons between the PEGIFN and the control arm at various time points.

a.



b.



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327	Pr. Christophe Hezode Dr. Ariane Mallat	Hôpital Henri Mondor Creteil	8
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71	Pr. Fabien Zoulim	Hôpital Hotel Dieu Lyon	4
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387	Pr. Manh Thông Dao	Centre Hospitalier Universitaire Côte de Nacre Caen	1