




Low hepatitis B surface antigen and HBV DNA levels predict response to the addition of pegylated interferon to entecavir in hepatitis B e antigen positive chronic hepatitis B

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Summary

Background: Various treatment combinations of peginterferon (PEG-IFN) and nucleos(t)ide analogues have been evaluated for chronic hepatitis B (CHB), but the optimal regimen remains unclear.

Aims: To study whether PEG-IFN add-on increases response compared to entecavir (ETV) monotherapy, and whether the duration of ETV pretreatment influences response.

Methods: Response was evaluated in HBeAg positive patients previously treated in two randomized controlled trials. Patients received ETV pretreatment for at least 24 weeks and were then allocated to 24-48 weeks of ETV+PEG-IFN add-on, or continued ETV monotherapy. Response was defined as HBeAg loss combined with HBV DNA <200 IU/mL 48 weeks after discontinuing PEG-IFN.

Results: Of 234 patients, 118 were assigned PEG-IFN add-on and 116 continued ETV monotherapy. Response was observed in 38/118 (33%) patients treated with add-on therapy and in 23/116 (20%) with monotherapy ($P = 0.03$). The highest response to add-on therapy compared to monotherapy was observed in PEG-IFN naive patients with HBsAg levels below 4000 IU/mL and HBV DNA levels below 50 IU/mL at randomization (70% vs 34%; $P = 0.01$). Above the cut-off levels, response was low and not significantly different between treatment groups. Duration of ETV pretreatment was associated with HBsAg and HBV DNA levels (both $P < 0.005$), but not with response ($P = 0.82$).

Conclusions: PEG-IFN add-on to ETV therapy was associated with higher response compared to ETV monotherapy in patients with HBeAg positive CHB. Response doubled in PEG-IFN naive patients with HBsAg below 4000 IU/mL and HBV DNA below 50 IU/mL, and therefore identifies them as the best candidates for PEG-IFN add-on (Identifiers: NCT00877760, NCT01532843).

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1 | INTRODUCTION

The achievement of functional cure for chronic hepatitis B (CHB) infection remains difficult due to a persistent infection of hepatocytes with covalently closed circular DNA (cccDNA).^{1,2} CccDNA is a minichromosome that serves as a transcription template for hepatitis B virus (HBV) antigen and virion production. Nucleos(t)ide analogue (NA) therapy only marginally reduces levels of cccDNA such that cccDNA depletion would require years of NA treatment.^{3,4}

NA therapy effectively suppresses the HBV up to 8 years with few side-effects, but serological response rates remain low. The discontinuation of NA therapy leads to frequent virological relapse and patients therefore require long-term, if not indefinite NA therapy.^{5–10} In contrast, a finite course of pegylated interferon (PEG-IFN) achieves more sustained immune response than NA therapy.^{9,11,12} PEG-IFN is also able to directly target cccDNA and induce cccDNA decline in combination with NA therapy.^{13,14} PEG-IFN monotherapy, however, induces sustained response in only 30%–40% of patients and has limited tolerability.^{15,16}

These limitations of CHB therapy have led to the evaluation of various treatment combinations of NAs and PEG-IFN to maximise response rates, among which is the strategy of adding PEG-IFN to NA treatment (PEG-IFN add-on). One of the rationales for the PEG-IFN add-on strategy is that long-term NA treatment enables partial restoration of the liver-specific immunology of both the adaptive (T-cells) and innate immune system (natural killer cells).^{17–20} Viral load suppression could thus increase the immunomodulatory effect of PEG-IFN therapy resulting in increased HBsAg loss and HBeAg loss or accelerated HBsAg decline rates.¹¹

Several randomized controlled trials (RCT) employed a PEG-IFN add-on strategy in HBeAg positive and negative patients on long-term NA monotherapy.^{21–23} PEG-IFN add-on increased HBeAg seroconversion and viral antigen decline, but primary efficacy endpoints were not reached, possibly because of insufficient power or because the effect was limited to a subgroup of patients only. Clinical practice could benefit substantially if these responsive patients can be identified at the start of PEG-IFN therapy with readily available laboratory markers. Other remaining issues concern the optimal duration of PEG-IFN add-on and of NA pretreatment.

We therefore evaluated whether PEG-IFN add-on to ETV treatment increases serological response compared to ETV monotherapy in CHB, and whether the duration of ETV pretreatment or the length of PEG-IFN addition therapy influenced response. To this purpose, we performed an analysis in a large HBeAg positive CHB population that was previously treated in two global RCTs.

2 | MATERIALS AND METHODS

2.1 | Combined study design

We conducted a post hoc analysis of two international RCTs (ARES and PEGON; registered at ClinicalTrials.gov, Identifier: NCT00877760, NCT01532843).^{21,23} Detailed inclusion and exclusion criteria have

been previously described. In short, patients with CHB were eligible if they were HBeAg positive at randomization (baseline) and had a serum alanine aminotransferase (ALT) between 1.3 and five times the upper limit of normal (ULN). Patients had received pretreatment with ETV for at least 6 months. The main exclusion criteria were history of decompensated liver disease, coinfection with hepatitis C virus or HIV, other concomitant liver disease, and any contra-indication for interferon therapy.

After initial treatment with ETV (Baraclude, Bristol-Myers Squibb, New York, NY, 0.5 mg once-daily), patients were randomized to either 6–12 months of PEG-IFN addition or of continued ETV monotherapy (Figure 1). Patients treated within the ARES trial received PEG-IFN a2a (Pegasys, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 180 µg once-weekly) and patients in the PEGON study PEG-IFN a2b (PegIntron, Merck, Kenilworth, NJ, 1.5 µg/kg once-weekly). If patients achieved HBeAg seroclearance in combination with an HBV DNA level below 200 IU/mL at the end of PEG-IFN treatment (EOT) or at the corresponding time point for patients allocated to ETV monotherapy, ETV was discontinued after a minimum of 24 weeks consolidation therapy. Otherwise, ETV was continued until the end of follow-up (EOF), which was 48 weeks after EOT for all patients regardless of treatment response.

Several patients within the ARES study did not reach the designated primary endpoint at the end of treatment. These patients were allowed to enrol in the subsequent PEGON trial and were then randomized again to PEG-IFN add-on or ETV monotherapy. This study was approved by local ethics boards of all centres and performed in concordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written consent.

2.2 | Study endpoints

Response was defined as combined HBeAg loss with HBV DNA <200 IU/mL at EOF. We analysed the modified intention-to-treat population, which includes all patients who received at least one dose of the allocated treatment after baseline. Patients were considered non-responders in case of missing HBeAg status or HBV DNA at EOF. To assess the potential for functional cure, as studied with therapeutic compounds now in development, we also investigated specific other virological and serological outcomes (Table 2).

2.3 | Study follow-up and measurements

During PEG-IFN treatment, routine examination and laboratory testing were performed every 4 weeks. After PEG-IFN treatment was stopped, patients visited the out-patient clinic every 12 weeks until EOF. Patients on ETV monotherapy had study visits every 12 weeks throughout the entire study period. Routine biochemical and haematological tests were assessed locally at every visit. Serum ALT levels were standardised according to the ULN per centre and gender. Serum HBV DNA was measured with the Cobas TaqMan 48 polymerase chain reaction assay (lower limit of detection: 20 IU/mL; Roche Diagnostics, Basel, Switzerland). Serum HBeAg, anti-HBe and

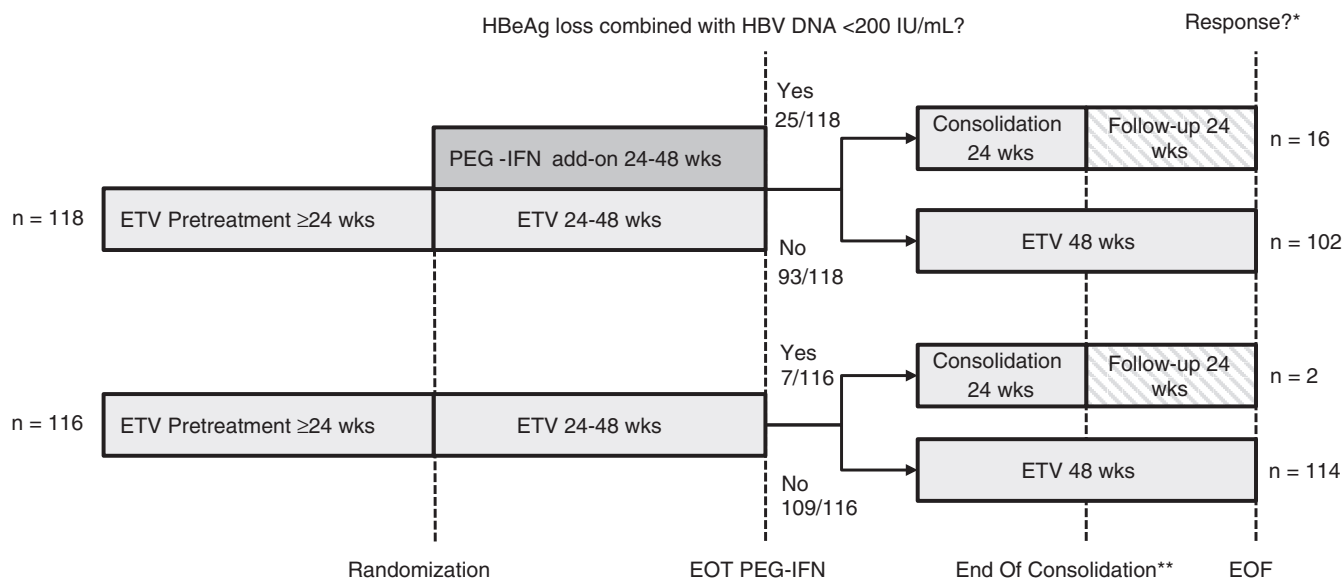


FIGURE 1 Combined study design. *Response: HBeAg loss in combination with HBV DNA <200 IU/mL at EOF for the intention-to-treat population. **Only for responders. Non-responders were treated with ETV until EOF. Of the 32 patients who reached response at EOT, 16/25 patients assigned PEG-IFN add-on and 2/7 patients assigned ETV monotherapy discontinued treatment after 24 weeks of consolidation therapy. Of these patients, 12/16 vs 2/2 patients allocated to PEG-IFN add-on vs ETV monotherapy sustained response at EOF. EOT, end of treatment; EOF, end of follow-up

HBsAg were evaluated with Architect (Abbott Laboratories, North Chicago, IL) or Cobas Elecsys 411 (lower limit of detection 0.30 and 0.05 IU/mL, respectively; Roche Diagnostics). HBV genotyping was performed with the INNO-LiPA HBV genotype assay (Fujirebio Europe, Ghent, Belgium). If HBV genotype could not be assessed due to undetectable HBV DNA levels at baseline, we reviewed HBV genotype data in medical charts where possible. The presence of cirrhosis was defined by Ishak stage 6 on liver biopsy, or an aspartate aminotransferase to platelet ratio index (APRI) score >1.0.²⁴

2.4 | Statistical analysis

Variables are summarised with mean \pm SD or frequency (%). Non-normally distributed variables were log-transformed. Differences in outcomes were evaluated by chi-squared test, Student's *t*-test or Mann-Whitney test, where appropriate. To study the influence of PEG-IFN addition on response and adjust for confounders, we performed logistic regression analysis. Predefined covariates included age, gender, HBV genotype, cirrhosis, previous use of PEG-IFN, duration of ETV pretreatment, ALT, HBV DNA, and HBsAg. The duration of ETV pretreatment and HBV DNA were categorised due to a skewed distribution. Predictors that were significantly associated with response in univariable regression ($P < 0.10$) were further evaluated in multivariable regression (backward stepwise selection). Interactions between response and baseline variables included in the final model were explored.

Cut-off values for HBV DNA and HBsAg at baseline were evaluated to find clinically useful starting rules for PEG-IFN add-on. HBsAg levels were dichotomised at thresholds between 2.7 and 5.0 log IU/mL in steps of 0.1. HBV DNA was categorised at 50, 100,

500, and 1000 IU/mL. The likelihood-ratio test and sum of log-likelihood ratios of the two treatment groups were calculated. We selected optimal cut-off values based on a minimum response difference of 15% between add-on and monotherapy; a significant likelihood ratio test of add-on vs monotherapy below the cut-offs, but not above; and the lowest sum of likelihood ratios. For each threshold receiver operating characteristic curves were constructed and AUCs were calculated and compared to each other. Furthermore, a sensitivity analysis was performed among non-responding patients within the ARES study who subsequently received retreatment in the PEGON study by modelling the correlated data in a generalised estimating equation.²⁵ Analyses were performed in *spss* (v. 22.0, Chicago, IL) and *SAS* v. 11.2 (SAS Institute Inc, Cary, NC). Two-sided $P < 0.05$ were considered significant.

3 | RESULTS

3.1 | Patient population

A total of 234 patients met the inclusion criteria. Excluded were five patients assigned PEG-IFN add-on and 10 assigned ETV monotherapy who had achieved HBeAg loss at baseline (during ETV pretreatment). At baseline, 118 patients were allocated to PEG-IFN add-on and 116 patients continued ETV monotherapy. Baseline characteristics were comparable between the two groups (Table 1). The mean age was 33 (SD 9) years, the majority of patients were male and of Asian ethnicity. HBV genotypes A/B/C/D/other were present in 4%, 17%, 41%, 24%, and 1% of patients, respectively. In total, 80/118 (68%) patients received PEG-IFN add-on for 24 weeks and 38/118

TABLE 1 Characteristics of the modified intention-to-treat population at randomization

	PEG-IFN add-on (n = 118)	ETV monotherapy (n = 116)
Age, y (SD)	33 (10)	33 (9)
Male gender (%)	87 (74)	83 (72)
Ethnicity (%)		
Asian	85 (72)	84 (72)
Caucasian	31 (26)	31 (27)
Other	2 (1.7)	1 (0.9)
HBV genotype (%)		
A	3 (2.5)	6 (5.2)
B	22 (19)	17 (15)
C	45 (38)	51 (44)
D	30 (25)	26 (22)
Other/unknown ^a	18 (14)	16 (14)
Cirrhosis ^b (%)	3 (2.5)	5 (4.3)
Previous (PEG-)IFN therapy (%)	16 (14)	20 (17)
ETV pretreatment (%)		
6-12 mo	80 (68)	79 (68)
1-2 y	12 (10.2)	9 (7.9)
2-3 y	16 (22)	28 (24)
Alanine aminotransferase, ULN (IQR)	0.5 (0.3-0.9)	0.5 (0.4-0.9)
HBV DNA (IU/mL) (%)		
Undetectable ^c	38 (32)	42 (36)
20-100	16 (14)	27 (23)
100-1000	27 (23)	18 (16)
>1000	37 (31)	29 (25)
Quantitative HBsAg, log IU/mL (SD)	3.7 (0.7)	3.6 (0.7)
Quantitative HBeAg, log IU/mL (IQR)	1.1 (0.5-2.0)	1.0 (0.4-1.9)
PEG-IFN duration (%)		
24 wk	80 (68)	—
48 wk	38 (32)	—

IQR, interquartile range; SD, standard deviation; ULN, upper limit of normal.

^aHBV genotyping was not possible for 32 patients (all Asian) due to undetectable HBV DNA at randomization.

^bCirrhosis was defined as Ishak stage 6 on liver biopsy; all 81 patients with unavailable biopsy data had an APRI. Score <1.0, which suggests absence of cirrhosis.

^c<20 IU/mL.

(32%) patients received PEG-IFN add-on for 48 weeks. Among patients included in the ARES study, 36 non-responders were reincluded in the subsequent PEGON trial. The baseline characteristics per trial are shown in Table S1.

3.2 | Response

Response was reached in 38/118 (33%) patients allocated to add-on therapy and in 23/116 (20%) patients with ETV monotherapy

($P = 0.03$; Figure 2 and Table 2). Other serological, virological, and biochemical outcomes are reported in Table 2. HBeAg seroconversion rates at EOF were also significantly higher in PEG-IFN add-on patients. The response group comprised significantly more males (84% vs 69%, $P = 0.03$), and had a higher frequency of genotype B (26% vs 13%) and fewer genotype D (12% vs 28%) compared to non-responders. Furthermore, responders had significantly lower ALT (0.4 vs $0.6 \times$ ULN, $P = 0.01$), HBsAg (3.3 vs 3.8 , $P < 0.005$) and HBeAg (0.5 vs 1.4 , $P < 0.005$) levels at baseline, and a higher frequency of undetectable HBV DNA at baseline (53% vs 28%, $P < 0.005$) than non-responders. Other baseline characteristics were comparable between patients with and without a response. Response occurred in 12/16 patients assigned to PEG-IFN add-on vs 2/2 assigned to ETV monotherapy ($P = 0.42$) in the subgroup that achieved HBeAg loss in combination with HBV DNA <200 IU/mL at EOT.

The two sensitivity analyses (cohort without 36 retreated non-responders and whole cohort with adjustment for correlated data) were consistent with our findings indicating that PEG-IFN add-on significantly increased response to ETV monotherapy (Table S2).

3.3 | HBsAg decline and loss

HBsAg decline >0.5 log IU/mL occurred more often in the PEG-IFN add-on group compared to the ETV monotherapy group at EOF (25 [23%] vs 11 [9.6%]; $P = 0.01$). HBsAg <1000 IU/mL was reached by 35/118 (30%) patients with PEG-IFN add-on and by 28/116 (24%) with ETV monotherapy ($P = 0.32$) at EOT, which increased to 27% at EOF in both groups ($P = 0.97$). The proportions of patients with HBsAg <100 IU/mL in PEG-IFN add-on vs ETV monotherapy were 1 (1%) vs 5 (4%) at baseline ($P = 0.09$), and 6 (5%) vs 5 (4%) at EOF ($P = 0.77$). The proportion of patients in the add-on group with HBsAg <100 IU/mL increased from baseline to EOF ($P = 0.06$). HBsAg loss was observed in one patient assigned to PEG-IFN add-on.

3.4 | Sustained response after ETV discontinuation

Among the EOT responders, 16 (64%) of 25 PEG-IFN add-on patients vs 2 (29%) of 7 ETV monotherapy patients discontinued ETV treatment after 24 weeks of ETV consolidation therapy. The remaining EOT responders continued ETV treatment despite response due to protocol violations. After ETV discontinuation, 12/16 vs 2/2 patients allocated to PEG-IFN add-on vs ETV monotherapy had a sustained response ($P = 0.42$). Within the total cohort, response was sustained 24 weeks after stopping ETV in 12/118 (10%) vs 2/116 (0.2%) patients assigned PEG-IFN add-on vs ETV monotherapy ($P = 0.01$). Similarly, disease remission (combined HBeAg loss, HBV DNA <200 IU/mL and ALT normalisation at EOF) in PEG-IFN add-on vs ETV monotherapy was achieved by 12/16 vs 2/2 patients ($P = 0.42$).

3.5 | Response prediction

By univariable analysis, response was associated with PEG-IFN add-on (odds ratio [OR] 1.9; 95% confidence interval [CI] 1.1-3.5;

TABLE 2 Outcome over time in 234 HBeAg positive patients

n (%)	Baseline Randomization			End of PEG-IFN Week 24-48			End of consolidation Week 72			End of follow-up Week 96		
	PEG-IFN add-on n = 118	ETV Mono n = 116	P	PEG-IFN add-on n = 118	ETV Mono n = 116	P	PEG-IFN add-on n = 118	ETV Mono n = 116	P	PEG-IFN add-on n = 118	ETV Mono n = 116	P
Response												
HBeAg loss + HBV DNA <200 IU/mL	—	—	—	25 (21)	7 (6.0)	<0.005 [†]	35 (30)	15 (13)	<0.005 [†]	38 (33)	23 (20)	0.03 [†]
Virological outcomes												
HBV DNA <2000 IU/mL	89 (75)	92 (79)	0.48	111 (95)	100 (86)	0.02 [†]	104 (89)	102 (88)	0.82	99 (85)	104 (90)	0.24
HBV DNA <200 IU/mL	64 (54)	75 (65)	0.11	102 (87)	88 (76)	0.03 [†]	93 (80)	92 (79)	0.97	95 (82)	97 (84)	0.62
HBV DNA undetectable ^a	38 (32)	41 (35)	0.61	37 (32)	41 (35)	0.55	37 (32)	41 (35)	0.55	36 (31)	40 (35)	0.54
Serological outcomes												
HBeAg loss	—	—	—	25 (22)	7 (6.0)	<0.005 [†]	36 (32)	15 (13)	<0.005 [†]	40 (36)	23 (20)	0.01 [†]
HBeAg seroconversion	—	—	—	19 (16)	2 (1.7)	<0.005 [†]	26 (22)	5 (4.3)	<0.005 [†]	28 (24)	11 (9.6)	<0.005 [†]
HBsAg loss	0 (0.0)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS	1 (0.8)	0 (0.0)	NS
HBsAg <1000 IU/mL	22 (19)	22 (19)	0.95	35 (30.0)	28 (24)	0.32	33 (28)	32 (28)	0.92	31 (27)	31 (27)	0.97
HBsAg <100 IU/mL	1 (0.8)	5 (4.3)	0.09	10 (8.5)	4 (3.4)	0.10	6 (5.1)	4 (3.4)	0.53	6 (5.2)	5 (4.3)	0.77
HBsAg decline >0.5 log IU/mL	—	—	—	30 (26)	2 (1.7)	<0.005 [†]	30 (26)	6 (5)	<0.005 [†]	25 (23)	11 (9.6)	0.01 [†]
Biochemical outcome												
ALT normalisation	96 (81)	90 (78)	0.56	73 (63)	94 (82)	<0.005 [†]	104 (91)	98 (86)	0.21	103 (92)	99 (86)	0.16

NS, not significant.

^a<20 IU/mL.**P* < 0.05.

P = 0.03), male gender (OR 2.3; 95% CI 1.1-4.9; *P* = 0.03), HBV genotype (*P* = 0.02), lower ALT (OR 0.3; 95% CI 0.1-0.7; *P* = 0.01), lower HBV DNA level (OR 0.5; 95% CI 0.3-0.7; *P* < 0.005), and lower HBsAg level at baseline (OR 0.4; 95% CI 0.2-0.6; *P* < 0.005; Table 3). The duration of ETV pretreatment was associated with HBsAg and HBV DNA at baseline (both *P* < 0.005), but not with response (1.3 years vs 0.1 year, OR 1.1; 95% CI 0.6-2.2; *P* = 0.76), nor was duration of the PEG-IFN add-on regimen (*P* = 0.92). In multivariable analysis, PEG-IFN add-on remained independently associated with response (OR 2.5; 95% CI 1.3-4.8; *P* = 0.01, when adjusted for HBV DNA and HBsAg level at baseline). Response rates to PEG-IFN add-on compared to ETV monotherapy increased especially in PEG-IFN naive patients with lower serum HBV DNA and HBsAg at baseline (Figure S1).

3.6 | Response-guided therapy using HBV DNA and HBsAg

To establish clinical starting rules for PEG-IFN add-on, the relationship between different cut-off values of HBsAg and HBV DNA at baseline and likelihood of response was evaluated (Figure S2 and Table S3). As previous use of PEG-IFN was strongly associated with a lack of response, we evaluated all PEG-IFN naive patients (198/234 [85%]). Based on this analysis, PEG-IFN naive patients with an

HBsAg level below 4000 IU/mL (3.6 log) and HBV DNA level below 50 IU/mL (1.7 log) at baseline achieved the largest gain in probability of response with PEG-IFN add-on compared to ETV monotherapy (70% vs 34%, *P* = 0.01; Figure 3). Patients who met one of the above criteria achieved a moderate gain in response from PEG-IFN add-on, compared to ETV monotherapy (44% vs 17%; *P* = 0.02). Above the proposed HBsAg and HBV DNA cut-off levels, response was very low and not significantly different between treatment groups (PEG-IFN add-on vs ETV monotherapy: 9.3% vs 5.9%; *P* = 0.58). The cut-off values combined had an AUC of 0.79 (95% CI 0.72-0.86) for probability of response.

4 | DISCUSSION

In this combined analysis of two global RCTs, PEG-IFN add-on to ETV increased response compared to ETV monotherapy in HBeAg positive patients with CHB. Response was 33% for add-on patients vs 20% for ETV monotherapy. HBeAg seroconversion rates at EOF were also significantly higher in add-on patients. The response to PEG-IFN add-on was especially high (up to 70%) among patients who were naive to PEG-IFN therapy and had low HBV DNA (<50 IU/mL) and HBsAg levels (<4000 IU/mL) at the start of PEG-IFN therapy.

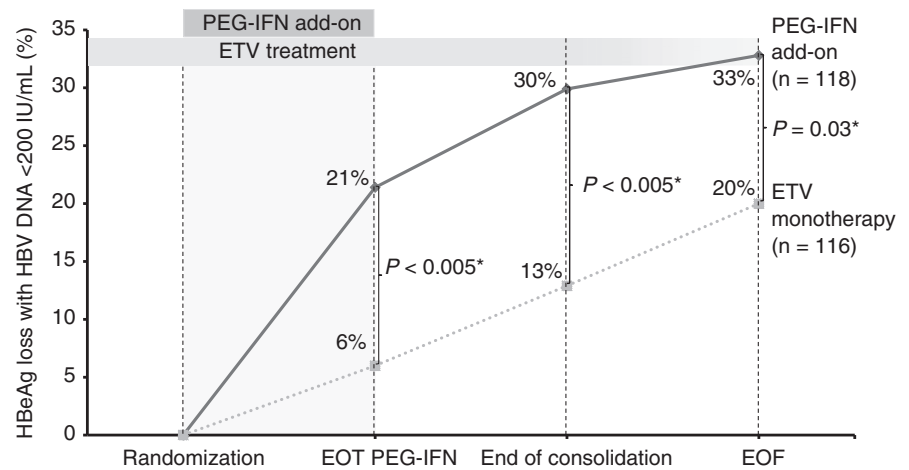


FIGURE 2 Response. * $P < 0.05$. Of 32 patients who reached combined HBeAg loss and HBV DNA <200 IU/mL at week 48, 18 discontinued treatment after ETV consolidation therapy. EOT, end of treatment; EOF, end of follow-up

TABLE 3 Logistic regression on response at end of follow-up

Variable	Univariable regression			Multivariable regression		
	OR	95% CI	P-value	OR	95% CI	P-value
Age, y	1.02	0.99-1.05	0.24			
Gender, male vs female	2.31	1.09-4.90	0.03*			NS
HBV genotype			0.02*			NS
C	Reference					
A vs C	1.50	0.35-6.47	0.59			
B vs C	2.09	0.95-4.59	0.07			
D vs C	0.43	0.17-1.07	0.07			
Other vs C	1.44	0.61-3.37	0.41			
Cirrhosis	1.76	0.41-7.59	0.45			
Duration of ETV, mo			0.79			
0-1 yr	Reference					
1-3 yr vs 0-1 yr	1.12	0.56-2.23	0.76			
>3 yr vs 0-1 yr	1.28	0.46-3.54	0.64			
PEG-IFN experienced vs naive	0.64	0.27-1.56	0.33			
PEG-IFN duration, 12 vs 6 mo	0.96	0.41-2.20	0.92			
PEG-IFN add-on, compared to ETV monotherapy	1.92	1.06-3.49	0.03*			
Within PEG-IFN naive				3.72	1.76-7.87	<0.005*
Within PEG-IFN experienced				0.24	0.04-1.66	0.15
ALT, × ULN	0.32	0.14-0.74	0.01*			NS
HBV DNA (IU/mL) ^a			<0.005*			0.02*
Undetectable	Reference			1.00		
20-100 vs undetectable	0.67	0.30-1.49	0.33	0.62	0.26-1.47	
100-1000 vs undetectable	0.53	0.24-1.17	0.12	0.47	0.19-1.16	
>1000 vs undetectable	0.10	0.03-0.29	<0.005*	0.12	0.04-0.42	
HBsAg, log IU/mL	0.38	0.24-0.60	<0.005*	0.51	0.29-0.89	0.02*

NS, not significant; ULN, upper limit of normal.

^aHBV DNA groups: <lower limit of detection (<20 IU/mL); 20-100 IU/mL; 100-1000 IU/mL; ≥1000 IU/mL.

* $P < 0.05$.

This is the first study demonstrating a higher response in patients allocated to PEG-IFN add-on compared to ETV monotherapy. The strengths of this study are inclusion of a large multiethnic

cohort of patients comprising treatment naive and experienced patients who after ETV treatment did not reach HBeAg seroconversion. These patients are representative of the majority of treatment

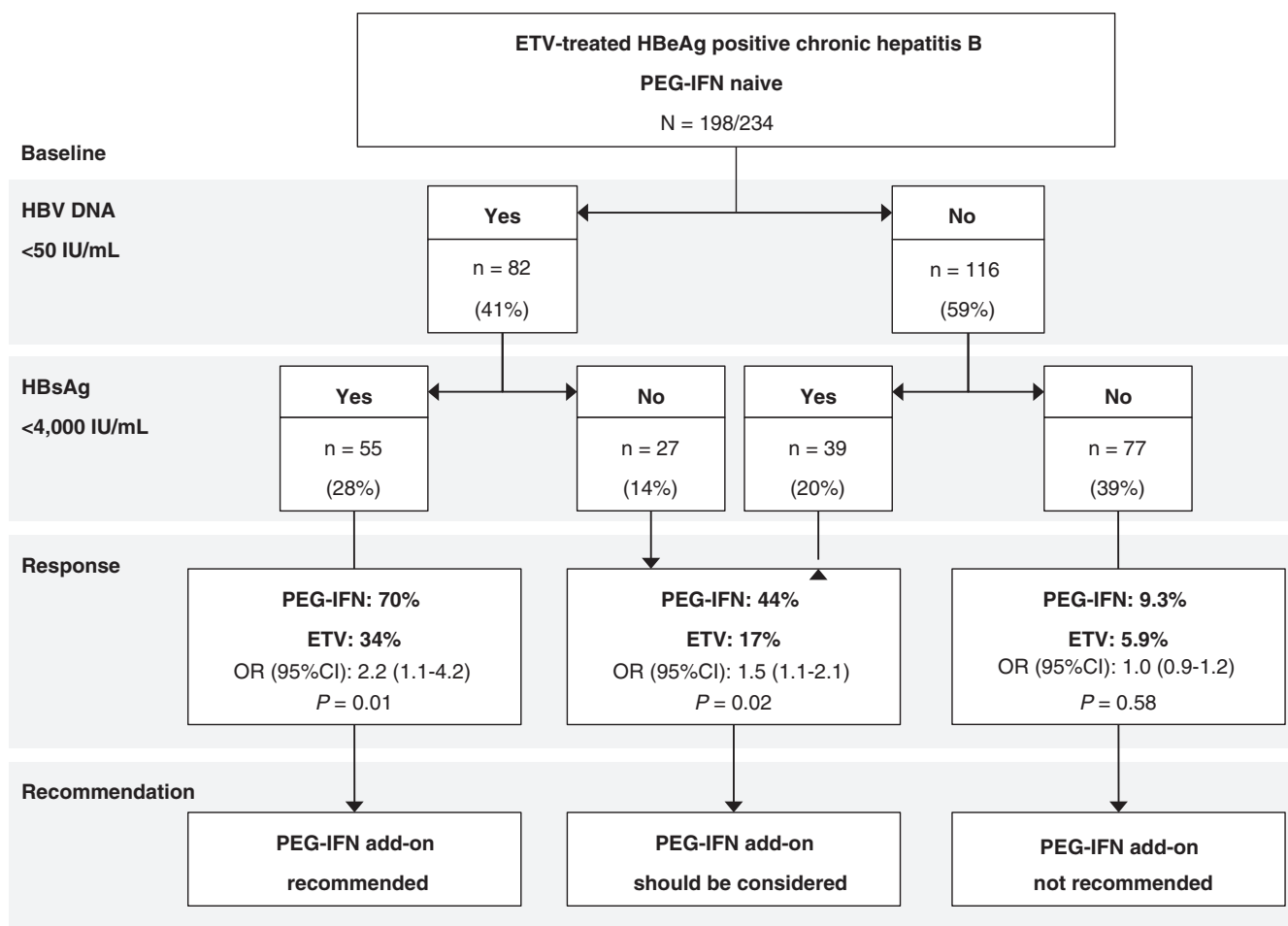


FIGURE 3 Algorithm for probability of response at end of follow-up based on HBV DNA and HBsAg at baseline

eligible patients seen in clinical practice who would otherwise continue NA therapy for longer duration. A finite PEG-IFN add-on regimen offers disease remission and discontinuation of treatment, thereby preventing additional costs and the potential of nonadherence and resistance associated with long-term or indefinite NA therapy.

To avoid unnecessary side-effects and costs of PEG-IFN it is essential to identify the optimal candidates for add-on therapy as only a subset will respond. The current HBV clinical practice guidelines only broadly mention the usefulness of quantifying HBV DNA and HBsAg to decide when and in whom to start PEG-IFN. Evidence to support one cut-off value over another is limited.^{26,27} We established clinical starting rules for PEG-IFN add-on based on widely available biomarkers. Based on results from this study, we recommend starting PEG-IFN add-on in PEG-IFN naive patients with an HBsAg level below 4000 IU/mL (3.6 logs) and HBV DNA below 50 IU/mL (1.7 log) at randomization. A sufficiently large subgroup (28% of PEG-IFN naive patients) had laboratory levels below these thresholds. PEG-IFN add-on response rates were nearly twice as high as the average PEG-IFN response in previous studies.^{15,16} In patients with values below either of the cut-off values, PEG-IFN add-on should be considered, as these patients have a moderately

high response to PEG-IFN. PEG-IFN add-on is not recommended in patients with both HBsAg and HBV DNA levels above the cut-off values, because of the low probability of response. Our HBsAg threshold is concordant with a threshold found in another study which showed that HBsAg <1500 IU/mL predicted response.²⁸ Moreover, the higher and thus more lenient HBsAg cut-off value established in this study would allow practitioners to identify even more candidates for PEG-IFN add-on at an earlier stage in their disease course. None of the previous add-on studies provided a comprehensive grid search to establish response-guided therapy. Apart from response, the side effects and cost-effectiveness should to be taken into consideration when deciding on a treatment strategy.

In recent RCTs that compared PEG-IFN add-on to continuing NA monotherapy, HBsAg decline rates were significantly higher in the add-on group, yet the primary endpoints (HBsAg loss at week 96; combined HBeAg loss with HBV DNA <200 IU/mL at week 96) were not reached, potentially due to a type II error.²¹⁻²³ In the ARES study response was achieved in 19% of patients in the add-on arm vs 10% in the monotherapy arm ($P = 0.095$); declines in HBsAg, HBeAg, and HBV DNA were also larger in the add-on group (all $P < 0.001$).²¹ Uncontrolled studies in HBeAg positive and negative patients reported similar findings.^{29,30}

The PEGAN study in HBeAg negative patients did not find a significant effect of PEG-IFN add-on on HBsAg loss at week 96, but was possibly underpowered and included older-generation NAs.²² This study showed that PEG-IFN add-on treatment resulted in significantly greater HBsAg declines and, within patients who received a full 48 week course, larger proportions of HBsAg loss, and seroconversion. Within patients with an HBsAg titre below 3 log IU/mL at baseline, 6/26 (23%) achieved HBsAg loss (full dose analysis). The PEGAN study suggested using add-on only in patients with baseline HBsAg levels of less than 3 log IU/mL. Other regimens of PEG-IFN and NA therapy, such as sequential or combination therapy have been evaluated in CHB, but the optimal strategy remains unclear.^{28,31}

The optimal duration of ETV pretreatment or PEG-IFN add-on therapy has not yet been established. Prolonged NA pretreatment partially restores immune function (NK and T cells).^{17–20} In our study the duration of ETV pretreatment correlated to baseline HBV DNA and HBsAg, but not to response. This suggests that levels of HBsAg and HBV DNA at the start of PEG-IFN therapy are more important in considering which patients to treat than the actual duration of ETV pretreatment. The duration of PEG-IFN add-on treatment did not correlate with response. A post hoc analysis in a previous study revealed larger HBsAg decline after 24 weeks of PEG-IFN add-on to ETV therapy compared to 52 weeks of combined PEG-IFN and LAM therapy.³² This suggests that a PEG-IFN course of 24 weeks is at least as effective as 52 weeks, while the shorter regimen would reduce the risk of IFN-related adverse events and treatment costs. Our analysis lacked a comparison to PEG-IFN monotherapy. However, the focus of this study was to investigate PEG-IFN add-on in the large population of patients currently on NAs, and not treatment naive patients. Furthermore, the relation between the type of PEG-IFN (a2a or a2b) and the respective PEG-IFN add-on trials could potentially have influenced response rates.

The endpoint of HBeAg seroclearance is clinically relevant because it is associated with a lower risk of HCC and improved survival.⁹ Since only a subset of patients stopped ETV therapy after receiving consolidation therapy the durability of sustained response after treatment discontinuation could not be studied in further detail. Long-term follow-up studies could focus on the effect on HBsAg loss or development of important clinical outcomes (decompensation, HCC and death), although such studies will be difficult to perform. Due to the fact that part of the patients had received long-term HBV suppressive therapy HBV genotype and cirrhosis status was not known for some patients. Nevertheless, the sensitivity analyses performed to adjust for these partially missing baseline characteristics also showed higher response and HBsAg decline achieved by PEG-IFN add-on compared to ETV monotherapy. It is important that our findings will be validated in new PEG-IFN add-on studies.

In conclusion, PEG-IFN add-on to ETV therapy was associated with a higher probability of response and HBeAg seroconversion compared to ETV monotherapy in HBeAg-positive CHB. Response was highest in patients who were naive to PEG-IFN therapy with levels of HBsAg below 4000 IU/mL and HBV DNA below 50 IU/mL. In particular these patients should be offered PEG-IFN add-on therapy.

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AUTHORSHIP

Guarantor of the article: HLA Janssen is acting as the submission's guarantor, taking responsibility for the integrity of the work as a whole, from inception to published article.

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REFERENCES

- Petersen J, Thompson AJ, Levrero M. Aiming for cure in HBV and HDV infection. *J Hepatol.* 2016;65:835-848.
- Lucifora J, Protzer U. Attacking hepatitis B virus cccDNA—the holy grail to hepatitis B cure. *J Hepatol.* 2016;64:S41-S48.
- Wong DK-H, Yuen M-F, Ngai VW-S, Fung J, Lai C-L. One-year entecavir or lamivudine therapy results in reduction of hepatitis B virus intrahepatic covalently closed circular DNA levels. *Antivir Ther.* 2006;11:909-916.
- Dandri M, Locarnini S. New insight in the pathobiology of hepatitis B virus infection. *Gut.* 2012;61:i6-17.
- Buti M, Tsai N, Petersen J, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. *Dig Dis Sci.* 2015;60:1457-1464.
- Marcellin P, Lau G, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. *N Engl J Med.* 2004;351:1206-1217.
- Chi H, Hansen BE, Yim C, et al. Reduced risk of relapse after long-term nucleos(t)ide analogue consolidation therapy for chronic hepatitis B. *Aliment Pharmacol Ther.* 2015;41:867-876.
- Jeng W-J, Sheen I-S, Chen Y-C, et al. Off-therapy durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis B patients. *Hepatology.* 2013;58:1888-1896.
- van Zonneveld M, Honkoop P, Hansen BE, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. *Hepatology.* 2004;39:804-810.
- Reijnders J, Perquin MJ, Zhang N, Hansen BE, Janssen HLA. Nucleos(t)ide analogues only induce temporary hepatitis B e antigen seroconversion in most patients with chronic hepatitis B. *Gastroenterology.* 2010;139:491-498.

11. Buster E, Flink HJ, Cakaloglu Y, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology*. 2008;135:459-467.
12. de Niet A, Stelma F, Jansen L, et al. Restoration of T cell function in chronic hepatitis B patients upon treatment with interferon based combination therapy. *J Hepatol*. 2016;64:539-546.
13. Lucifora J, Xia Y, Reisinger F, et al. Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA. *Science*. 2014;343:1221-1228.
14. Wursthorn K, Lutgehetmann M, Dandri M, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology*. 2006;44:675-684.
15. Janssen HLA, van Zonneveld M, Senturk H, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet*. 2005;365:123-129.
16. Lau G, Piratvisuth T, Luo KX, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med*. 2005;352:2682-2695.
17. Boni C, Laccabue D, Lampertico P, et al. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. *Gastroenterology*. 2012;143:e9.
18. Boni C, Lampertico P, Talamona L, et al. Natural killer cell phenotype modulation and natural killer/T-cell interplay in nucleos(t)ide analogue-treated hepatitis e antigen-negative patients with chronic hepatitis B. *Hepatology*. 2015;62:1697-1709.
19. Tjwa E, van Oord GW, Hegmans JP, Janssen HLA, Woltman AM. Viral load reduction improves activation and function of natural killer cells in patients with chronic hepatitis B. *J Hepatol*. 2011;54:209-218.
20. Sonneveld MJ, Zoutendijk R, Hansen BE, Janssen HLA. Peginterferon results in higher serological, but not virological, response rates when compared to continuous entecavir. *Antivir Ther*. 2012;17:1605-1608.
21. Brouwer WP, Xie Q, Sonneveld MJ, et al. Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: A multicenter randomized trial (ARES study). *Hepatology*. 2015;61:1512-1522.
22. Bourlière M, Rabiaga P, Ganne-Carrie N, et al. 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. *Lancet Gastroenterol Hepatol*. 2017;2:177-188.
23. Chi H, Hansen BE, Guo S, et al. Pegylated interferon alfa-2b add-on treatment in hepatitis B virus envelope antigen-positive chronic hepatitis B patients treated with nucleos(t)ide analogue: a randomized, controlled trial (PEGON). *J Infect Dis*. 2017;215:1085-1093.
24. Lin Z-H, Xin Y-N, Dong Q-J, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology*. 2011;53:726-736.
25. Generalized Estimating Equations 2015. <https://support.sas.com/rnd/app/stat/topics/gee/gee.pdf>. Accessed March 22, 2017.
26. Terrault NA, Bzowej NH, Chang K-M, et al. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology*. 2016;63:261-283.
27. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017;67:370-398.
28. Ning Q, Han M, Sun Y, et al. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). *J Hepatol*. 2014;61:777-784.
29. Kittner JM, Sprinzl MF, Grambihler A, et al. Adding pegylated interferon to a current nucleos(t)ide therapy leads to HBsAg seroconversion in a subgroup of patients with chronic hepatitis B. *J Clin Virol*. 2012;54:93-95.
30. Ouzan D, Pénaranda G, Joly H, Khiri H, Pironti A, Halfon P. Add-on peg-interferon leads to loss of HBsAg in patients with HBeAg-negative chronic hepatitis and HBV DNA fully suppressed by long-term nucleotide analogs. *J Clin Virol*. 2013;58:713-717.
31. Han M, Jiang J, Hou J, et al. Sustained immune control in HBeAg-positive patients who switched from entecavir therapy to pegylated interferon- α 2a: 1-year follow-up of the OSST study. *Antivir Ther*. 2016;21:337-344.
32. Brouwer WP, Sonneveld MJ, Xie Q, et al. Peginterferon add-on results in more HBsAg decline compared to monotherapy in HBeAg-positive chronic hepatitis B patients. *J Viral Hepat*. 2016;23:419-426.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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