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ABSTRACT

Aim: The optimal combination of the two currently available agents with different mechanisms of action, a nucleos(t)ide analog and pegylated interferon-α (PegIFNα), must be determined to improve treatment of chronic hepatitis B.

Methods: In this study, 24 patients with chronic hepatitis B (14 hepatitis B e-antigen [HBeAg]-positive and 10 HBeAg-negative) received entecavir for 36–52 weeks, followed by entecavir plus PegIFNα-2a for 4 weeks and finally by PegIFNα-2a alone for 44 weeks.

Results: A sustained biochemical, virological, and serological response was obtained in 7/24 (29%) patients at 48 weeks post-treatment (2/14 [14%] in HBeAg-positive vs. 5/10 [50%] in HBeAg-negative patients, $P = 0.085$). At baseline, patients with a sustained response had a significantly lower γ-glutamyl transferase level ($P = 0.0023$), a lower aspartate aminotransferase-to-platelet ratio index ($P = 0.049$) and a lower α-fetoprotein level ($P = 0.042$) than those without a sustained response. The decline in hepatitis B surface antigen (HBsAg) levels during the first 24 weeks of PegIFNα-2a treatment in patients with a sustained response was greater than that in patients without ($P = 0.017$). HBsAg seroclearance was achieved in two patients (8.3%): one HBeAg-positive and one HBeAg-negative patient.

Conclusion: The outcomes of sequential therapy involving an early switch from entecavir to PegIFNα-2a were unsatisfactory in Japanese patients with chronic hepatitis B. In addition to viral factors, host metabolic characteristics and liver fibrosis/tumor markers can be used for prediction of a sustained response to therapy, but accurate prediction of the therapeutic response is difficult.

Key words: Combination; genotype C; HBV; nucleoside analog; IFN

INTRODUCTION

Hepatitis B virus (HBV) infection affects nearly 250 million people worldwide and is the major cause of end-stage liver disease, accounting for 780,000 deaths annually [1]. At present, available antiviral treatments for chronic hepatitis B, nucleos(t)ide analogs and interferon-α (IFNα), can rarely eradicate HBV from infected hepatocytes [2-5]. Nucleos(t)ide analogs have little or no effect on the decrease in the intrahepatic HBV replicative intermediate, a covalently closed circular DNA. Although the immunomodulatory activity of IFNα can induce cytotoxic T-cell activity for clearance of infected cells, an adequate immune response against HBV is induced in only a minority of patients. More than 30 agents are currently under investigation in clinical trials [6], but at least several years will be required for approval of next-generation antiviral agents. Therefore, effective regimens comprising either simultaneous, add-on or sequential combination of the two currently available agents with different mechanisms of action are urgently needed in clinical practice [7-9].

Sequential therapy initiated with a nucleos(t)ide analog followed by IFNα has been evaluated. One objective of sequential therapy starting with a nucleos(t)ide analog is to lower the viral load, thereby restoring sensitivity to IFNα treatment. Another objective of sequential therapy is to prevent relapse of hepatitis after terminating the nucleos(t)ide analog through the use of IFNα. The protocols varied among the studies; those switching to IFNα after the use of a nucleos(t)ide analog for about 1 year (referred to as early switch) might be aiming for the former goal [10-19], and those using a nucleos(t)ide analog for several years (referred to as late switch) the latter [20,21]. However, even among studies only of an early switch, the results were conflicting. This might be caused by differences in the included HBV genotypes, since HBV genotypes have specific geographic distributions and can affect the response to IFNα therapy [22,23]. The outcomes of sequential therapy have been unsatisfactory in Japanese

studies (including ours) [24-28], at least in part because genotype C is the most prevalent type of HBV, and is associated with a low likelihood of a favorable response to IFNα treatment.

In our previous studies [27,28], the rate of a sustained response to sequential therapy initiated with lamivudine or entecavir followed by IFN in Japanese, hepatitis B e-antigen (HBeAg)-positive patients was 21–29%. Pegylation of IFNα improves its pharmacokinetic activity and prolongs its half-life. An international phase II study showed that once-weekly pegylated IFNα-2a (PegIFNα-2a) resulted in a higher rate of sustained HBeAg seroconversion, HBV DNA suppression, and alanine aminotransferase (ALT) normalization than thrice-weekly non-pegylated IFNα-2a (nonPegIFNα-2a) for 24 weeks in HBeAg-positive patients (24% vs. 12%; $P = 0.036$) [29]. In Japanese phase II and III registration studies, 90 μg or 180 μg PegIFNα-2a for 48 weeks produced a higher rate of a triple response than did nonPegIFNα for 24 weeks in HBeAg-positive and -negative patients with chronic hepatitis B (17.1–19.5% vs. 7.0%) [30]. Use of PegIFNα may therefore improve the outcome of sequential therapy starting with entecavir compared to use of nonPegIFNα. Although PegIFNα-2a use for 48 weeks was approved for both HBeAg-positive and -negative patients in Japan in 2011, the efficacy of sequential therapy with entecavir and PegIFNα-2a has not yet been reported.

In this study, we evaluated the efficacy of sequential therapy involving an early switch from entecavir to PegIFNα-2a in HBeAg-positive and -negative chronic hepatitis B patients in Japan. In addition, the clinical characteristics of patients with a sustained response to the sequential therapy were compared with those of patients without a sustained response.

METHODS

Patients

This study included 24 Japanese patients with chronic hepatitis B (16 males and 8 females; mean age, 35 ± 7 years; 14 HBeAg-positive and 10 HBeAg-negative) who had received sequential therapy with entecavir followed by PegIFNα-2a between October 2010 and October 2013. The inclusion criteria were as follows: 1) persistent or fluctuating elevations of serum ALT levels for at least 6 months before the start of therapy; 2) presence of hepatitis B surface antigen (HBsAg) in serum; 3) presence of HBV DNA > 10^4 copies/mL (equivalent to 2,000 IU/mL); 4) no use of corticosteroids or immunomodulatory drugs, including IFN, within 1 year before the start of therapy; 5) no use of nucleos(t)ide analogs, such as lamivudine, within 1 year before the start of therapy; 6) absence of resistance to nucleos(t)ide analogs; 7) absence of antibodies to hepatitis C virus and other likely causes of chronic liver disease; and 8) no clinical signs of decompensated cirrhosis or hepatocellular carcinoma. The procedures performed in this study were in accordance with the Helsinki Declaration of 1964 (2013 revision), and were approved by the ethics committee of each center. Written informed consent was obtained from each patient. This study was registered in the UMIN Clinical Trials Registry (registration ID number, UMIN000006943).

Treatment

Patients were treated with entecavir alone for 36 to 52 weeks, followed immediately by both entecavir and PegIFNα-2a for 4 weeks and finally by PegIFNα-2a alone for 44 weeks. Entecavir (Baraclude; Bristol-Myers K.K., Tokyo, Japan) was given orally at a dose of 0.5 mg, once daily. PegIFNα-2a (Pegasys, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was given by subcutaneous injection at a dosage of 180 μg

once per week for 48 weeks. The dose of PegIFNα-2a was modified because of adverse events in accordance with the manufacturers' recommendations. All patients were followed up for at least 48 weeks after completion of treatment, and responses to therapy were assessed as follows: a *biochemical response* was defined as a decrease in serum ALT levels to within the normal range; a *virological response* was defined as a decrease in serum HBV DNA to $< 10^4$ copies/mL; and a *serological response* was defined as loss of serum HBeAg. A sustained response was defined as fulfillment of the criteria for biochemical, virological, and serological responses at 48 weeks after the end of therapy.

Laboratory assays

The following variables were determined for all enrolled patients: complete blood counts, serum aspartate aminotransferase (AST), ALT and γ-glutamyl transferase (GGT) activities, the Fibrosis-4 (FIB-4) index, AST-to-platelet ratio index (APRI), type IV collagen 7S, α-fetoprotein, HBsAg, HBeAg, anti-HBe, hepatitis B core-related antigen (HBcrAg), HBV DNA levels, HBV genotype, and proportion of mutants in the precore and basal core promoter regions of HBV DNA.

Complete blood counts and serum AST, ALT and GGT activities were determined by standard procedures. The FIB-4 index was calculated using Sterling's formula: age (years) × AST (IU/L)/platelet count ($\times 10^9$/L) × √ALT (IU/L)) [31]. The APRI score was calculated using Wai's formula (AST/upper limit of normal)/platelet count (expressed as platelets × 10^9/L) × 100 [32]. Serum concentrations of type IV collagen 7S were measured by radioimmunoassay (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). Serum α-fetoprotein levels were determined by chemiluminescence enzyme immunoassay. HBsAg was measured by chemiluminescent microparticle immunoassay (Architect HBsAg QT, Abbott Japan Corp., Tokyo, Japan) as described elsewhere [33]. HBeAg

and anti-HBe were detected by chemiluminescence enzyme immunoassay. For quantitative evaluation of serum HBeAg levels, serial dilutions of the reference standard of PE HBeAg (Paul-Ehrlich Institute, Langen, Germany) were used to define the linear range of the assay and create a reference curve for linear regression, as described previously [34]. A standard curve was produced, and linear regression was used to convert assay results into appropriate units (PEIU/mL). HBcrAg was also detected by chemiluminescence enzyme immunoassay (Fuji-Rebio, Tokyo) [35]. HBV DNA was measured by real-time polymerase chain reaction assay (COBAS TaqMan HBV Test, ver. 2.0; Roche Diagnostics K.K., Tokyo, Japan) [36]. Genotypes of HBV were identified by enzyme-linked immunosorbent assay with monoclonal antibodies to type-specific epitopes in the preS2-region (Institute of Immunology, Tokyo, Japan) [37]. Mutations at nucleotide (*nt*) 1896 in the precore region and at *nt* 1762 and *nt* 1764 in the basal core promoter region of HBV DNA were identified by means of an enzyme-linked minisequence assay (Genome Science Laboratory, Tokyo, Japan).

Histopathological evaluations

When informed consent had been obtained, liver biopsy was performed before starting therapy. Histopathological findings were assessed by grading inflammatory activity and staging fibrosis according to the METAVIR scoring system [38]. An experienced pathologist blinded to the clinical data performed these evaluations.

Statistical analysis

Statistical analysis was conducted using JMP software (ver. 12.0; SAS Institute, Cary, NC, USA). Distributions of continuous variables were analyzed by the Mann-Whitney *U* test. Differences in proportions were tested by Fisher's exact test. A two-tailed *P*-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Rate of response to therapy

The proportions of patients with biochemical, virological, and serological responses at the start of PegIFNα-2a therapy, at the end of PegIFNα-2a therapy and at 48 weeks post-treatment, are shown in **Figure 1**. Overall, drug-resistant mutant variants did not emerge in any patient during entecavir treatment. At the start of PegIFNα-2a treatment (about 1 year after the start of entecavir treatment), most patients had normal ALT levels and serum HBV DNA levels of $< 10^4$ copies/mL (92% and 100%, respectively). However, serum ALT and HBV DNA levels increased in some patients during PegIFNα-2a treatment. Finally, at 24 and 48 weeks after completion of sequential therapy, a biochemical, virological, and serological response was obtained in 9 (38%) and 7 (29%) of the 24 patients, respectively.

At baseline, only 21% of 14 HBeAg-positive patients achieved HBeAg loss during entecavir treatment (**Fig. 1B**). During PegIFNα-2a treatment, HBeAg levels sometimes fell and the proportion of HBeAg-negative patients rose to 64%, while HBV DNA sometimes rose and the proportion of patients with HBV DNA $< 10^4$ copies/mL fell from 100% to 57%. Viral relapse also occurred after the end of treatment, and a combined response was achieved in 3 (21%) and 2 (14%) patients at 24 and 48 weeks after completion of sequential therapy, respectively. Hepatitis flare (defined as an increase in ALT level to 10-fold the upper limit of normal) occurred in one patient during PegIFNα-2a treatment, and in another patient after the end of treatment. Peak ALT levels in these patients were 813 and 308 IU/L, respectively, but none had jaundice or decompensation.

A minority of 10 HBeAg-negative patients at baseline (**Fig. 1C**) exhibited reappearance of HBeAg and breakthrough in HBV DNA to $\geq 10^4$ copies/mL during and after PegIFNα-2a treatment. A combined response was achieved in 6 (60%) and 5 (50%)

of the patients at 24 and 48 weeks after completion of sequential therapy, respectively. The rate of sustained response in HBeAg-negative patients was marginally higher than that in HBeAg-positive patients (2/14 [14%] vs. 5/10 [50%], $P = 0.085$). Hepatitis flare did not occur in any HBeAg-negative patient.

Baseline characteristics of patients according to response to therapy

The baseline demographic, hematological, biochemical, virological, and histological characteristics of patients at the start of entecavir treatment are classified according to the response to sequential therapy in **Table 1**. At baseline, patients with a sustained response had significantly lower GGT ($P = 0.0023$), APRI ($P = 0.049$), and α-fetoprotein ($P = 0.042$) levels than those without a sustained response. Marginal significance was found in the patients with a sustained response, with respect to lower ALT ($P = 0.053$) and type IV collagen 7S ($P = 0.062$) levels, a lower proportion of HBeAg-*positive* patients ($P = 0.085$) and a lower HBcrAg level ($P = 0.086$) than in those without a sustained response.

On-treatment characteristics of patients according to response to therapy

The on-treatment characteristics of the patients are shown according to the response to sequential therapy in **Table 2**. The change in HBcrAg levels during entecavir therapy ($P = 0.024$), and change in HBsAg levels during the first 24 weeks of PegIFNα-2a therapy ($P = 0.017$), were significantly different between patients with and without a sustained response.

Positive and negative predictive values of baseline factors for a therapeutic response

We evaluated the positive and negative predictive values (PPV and NPV, respectively) of baseline factors for a sustained response to sequential therapy (**Table 3**). When the cut-off value was set at 27 IU/L GGT, the PPV and NPV for a sustained response were 76% and 86%; when set at 2.8 ng/mL of α-fetoprotein, the PPV and NPV were 58% and 89%; when set at 3.4 ng/mL type IV collagen 7S, the PPV and NPV were 55% and 87%; when set at 0.57 of APRI, the PPV and NPV were 81% and 78%, respectively.

Presentation of cases with HBsAg seroclearance

As shown in **Figure 2**, seroclearance of HBsAg was achieved in two patients (8.3%). The first was a 28-year-old, male, IFN-naïve, HBeAg-positive patient with genotype C infection. At baseline, his ALT activity was 43 IU/L, GGT 27 IU/L, APRI 0.35, type IV collagen 7S 2.7 ng/mL, α-fetoprotein 5.1 ng/mL, HBsAg 19,118 IU/mL, HBeAg 794 PEIU/mL, HBcrAg 8.0 $\log_{10}$ U/mL and HBV DNA ≥ 9.1 $\log_{10}$ copies/mL. A liver biopsy specimen showed mild inflammation and mild fibrosis. The HBsAg level did not change significantly during entecavir and the first half of PegIFNα-2a treatment, but decreased immediately after week 24 of PegIFNα-2a treatment and then became undetectable (**Fig. 2A**). The second was a 37-year-old, male, HBeAg-negative patient with genotype C infection who did not respond to a previous nonPegIFNα treatment. The baseline ALT activity was 63 IU/L, GGT 18 IU/L, APRI 0.45, type IV collagen 7S 4.5 ng/mL, α-fetoprotein 2.8 ng/mL, HBsAg 902 IU/mL, HBcrAg 2.9 $\log_{10}$ U/mL and HBV DNA 5.0 $\log_{10}$ copies/mL. A liver biopsy specimen showed mild inflammation and mild fibrosis. The HBsAg level did not change significantly during entecavir treatment, but decreased immediately after switching to PegIFNα-2a and then became undetectable (**Fig. 2B**).

DISCUSSION

In this study, a sustained biochemical, virological, and serological response was achieved in 7 (29%) of 24 Japanese patients with chronic hepatitis B at 48 weeks after completion of sequential therapy involving an early switch from entecavir to PegIFNα-2a (**Fig. 1**). When analyzed separately in 14 HBeAg-positive and 10 HBeAg-negative patients, the sustained response rate was 14% and 50%, respectively. The response rate in HBeAg-positive patients was lower than the rate in our previous study of sequential therapy with entecavir and nonPegIFNα, which included only HBeAg-positive patients [28]; use of PegIFNα did not increase the rate of response to sequential therapy. The response rate in HBeAg-negative patients was higher than that in HBeAg-positive patients; therefore, HBeAg-negative patients might be good candidates for sequential therapy. Although the difference was not statistically significant (14% vs. 50%, $P = 0.085$), HBeAg status is an important factor in determining a therapeutic response.

Next, we identified the baseline and on-treatment characteristics of patients showing a favorable response to sequential therapy (**Table 1**). The patients with a sustained response had a lower GGT level. In the case of hepatitis C, such metabolic factors could accelerate the progression of disease and impair the response to IFNα treatment [39], but its association in chronic hepatitis B has been investigated less extensively [40]. A large cohort study of Taiwanese men with chronic HBV infection aged 40-65 years associated a high burden of metabolic risk factors with increased risk of hepatocellular carcinoma [41]. *In vitro* studies showed that oxidative stress can impair the cellular response to IFNα via interference with Janus kinase/signal transducers and activators of the transcription pathway [42]. These metabolic factors could reduce the efficacy of PegIFNα therapy by the same mechanisms in patients with HCV. The APRI were lower in patients with than without a sustained response. In

contrast, there were no significant differences between the two groups with respect to liver histopathology. The discrepancy may be caused by the majority of the patients having only mild inflammation and mild fibrosis in liver biopsy. Quantitative, noninvasive indices and markers can predict liver fibrosis more sensitively than semi-quantitative liver histology scoring.

Among viral markers, baseline HBeAg and HBcrAg levels in patients with a sustained response were lower than those in patients without a sustained response, albeit not significantly. Previous studies showed that low viral load was associated with an increased likelihood of a sustained response to IFNα [43,44]. The HBcrAg assay measures serum levels of all antigens transcribed from the pre-core/core gene—including a 22 kDa precore protein and the hepatitis B core and e-antigens—by using monoclonal antibodies that recognize epitopes common to the denatured antigens [35,45]. By scoring the HBsAg and HBcrAg levels, as surrogate markers of covalently closed circular DNA in the liver, Matsumoto *et al.* [46] proposed a model for predicting relapse of hepatitis after discontinuation of nucleos(t)ide analog therapy.

Regarding on-treatment factors (**Table 2**), the decline in HBcrAg levels during entecavir therapy in patients with a sustained response was smaller than that in patients without a sustained response. This unexpected finding may be due to patients with a sustained response having a lower baseline HBcrAg level, and to the subsequent decrease being smaller than in patients without a sustained response. The decline in HBsAg levels during the first 24 weeks of PegIFNα-2a treatment in patients with a sustained response was greater than that in patients without a sustained response. This result is consistent with previous reports, showing that serum HBsAg drop in the first 24 weeks is useful for predicting the response to PegIFNα-2a treatment [47,48]. The treatment outcome could be predicted at an earlier stage using the HBsAg level at week 12 of PegIFNα-2a treatment [49,50]. Unfortunately, however, neither HBsAg level at week 12, nor HBsAg decline during the first 12 weeks of PegIFNα-2a treatment, was

significantly predictive of a response to therapy in this study. Two patients achieved HBsAg seroclearance, which is the ultimate goal in treatment of chronic hepatitis B. In one patient, the HBsAg level was unchanged during entecavir therapy and then declined steeply in the first quarter of PegIFNα-2a treatment, and in the second half in the other patient (**Fig. 2**).

For prediction of a sustained response to sequential therapy by baseline factors (**Table 3**), serum HBcrAg and HBeAg levels could identify patients who are unlikely to respond to treatment (NPVs of 89% and 78%, respectively). In addition to viral factors, host factors, including GGT, α-fetoprotein and type IV collagen 7S levels, as well as APRI, in patients with a sustained response were different from those in patients without. However, of the two patients with HBsAg seroclearance, one HBeAg-positive patient had HBcrAg ≥5.0 $\log_{10}$ U/mL and α-fetoprotein level >2.8 ng/mL in serum, and the other HBeAg-negative patient had a type IV collagen 7S level of ≥ 3.5 ng/mL. Therefore, accurate prediction of the outcome of sequential therapy is difficult.

The JSH Guidelines [5] suggest that Peg-IFN monotherapy should be considered the first choice treatment for chronic hepatitis, irrespective of HBeAg status or HBV genotype. Retreatment using Peg-IFN should be considered in patients with chronic hepatitis when recurrence of hepatitis occurs following treatment with conventional IFN or Peg-IFN. However, at the time of a hepatitis flare, IFN is generally contraindicated because of concerns regarding decompensation. The use of entecavir before induction of PegIFNα in sequential therapy involving an early switch can be recommended in patients following a hepatitis flare. Sequential therapy involving an early switch can also be considered for treatment-experienced patients, to enhance the therapeutic efficacy by lowering the viral load, thereby restoring treatment sensitivity. However, evidence is still lacking.

This study had several limitations. First, this study was not a controlled trial. In Japanese phase II and III studies of 90 or 180 μg PegIFNα-2a monotherapy, the rate of a

sustained triple response (17.1–19.5% in 48-week treatment arms) was lower than that in this study [30]. However, we cannot conclude that short-term use of entecavir enhanced the effectiveness of PegIFNα-2a treatment, because of differences in patient backgrounds and treatment response definitions between the two studies. Second, we defined a sustained response as a triple response at 48 weeks post-treatment. However, relapse beyond 48 weeks post-treatment was not rare in our previous study in the long-term [51].

In conclusion, the outcomes of sequential therapy involving an early switch from entecavir to PegIFNα-2a were unsatisfactory in Japanese patients with chronic hepatitis B. In general, HBeAg-negative patients are good candidates for sequential therapy, but HBeAg-positive patients can also achieve HBsAg seroclearance. In addition to viral factors, host metabolic factors and liver fibrosis/tumor markers can be used for prediction of a sustained response to therapy, but accurate prediction of the outcome of sequential therapy using baseline factors is difficult.

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Table 1 Baseline characteristics of patients according to response to therapy

Variable	Sustained responders (n = 7)	Non-responders (n = 17)	*P* value
Age (years) [b]	34 ± 11	36 ± 6	0.14
Female gender [a]	4 (57%)	4 (24%)	0.17
Body mass index [b]	21.5 ± 2.6	23.9 ± 3.6	0.13
Previous interferon history [a]	4 (57%)	10 (59%)	0.99
ALT (IU/L) [c]	43 (15, 63)	119 (35, 783)	0.053
GGT (IU/L) [c]	18 (14, 27)	58 (28, 131)	0.0023
Platelet ($x10^3/\mu L$) [b]	193 ± 16	199 ± 63	0.85
FIB-4 index[c]	0.82 (0.63, 1.03)	1.12 (0.75, 2.05)	0.11
APRI [c]	0.45 (0.37, 0.57)	0.89 (0.46, 3.45)	0.049
Type IV collagen 7S (ng/mL) [c]	3.3 (2.7, 4.6)	4.3 (3.6, 7.6)	0.062
α-fetoprotein (ng/mL) [c]	2.2 (1.9, 4.6)	3.6 (3.0, 14.1)	0.042
HBsAg ($\log_{10}$ IU/mL) [b]	3.80 ± 0.76	3.72 ± 0.69	0.78
Qualitative HBeAg-*positive* [a]	2 (29%)	12 (71%)	0.085
Quantitative HBeAg (PEIU/mL) [c]	< 0.15 (< 0.15, 794)	120 (< 0.15, 891)	0.31
HBcrAg ($\log_{10}$ U/mL) [b]	5.1 ± 2.4	6.9 ± 1.9	0.086
HBV DNA ($\log_{10}$ copies/mL) [b]	6.0 ± 2.6	7.9 ± 1.6	0.14
HBV genotype C [a]	7 (100%)	16 (94%)	0.99
Precore G1896A [a]	5 (71%)	6 (35%)	0.22
Basic core promoter A1762T/G1764A[a]	2 (29%)	5 (29%)	0.95
Grade of inflammation (A1/A2/A3)[a]	6/0/0	12/3/1	0.40
Stage of fibrosis (F1/F2/F3)[a]	6/0/0	12/3/1	0.40

[a]Numbers of patients; [b]Mean ± SD; [c]Median (interquartile range).

ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; FIB-4, Fibrosis-4; GGT, γ-glutamyl transferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus

Table 2 On-treatment characteristics of patients according to response to therapy

Variable	Sustained responders (n = 7)	Non-responders (n = 17)	*P* value
At the start of PegIFNα-2a			
ALT (IU/L) [c]	22 (9, 28)	20 (13, 27)	0.61
Platelet (x10^3/μL) [b]	202 ± 13	217 ± 67	0.24
HBsAg (log_{10} IU/mL) [b]	3.53 ± 0.59	3.52 ± 0.65	0.95
Qualitative HBeAg-*positive* [a]	2 (29%)	10 (59%)	0.37
Quantitative HBeAg (PEIU/mL) [c]	< 0.15 (< 0.15, 38.0)	0.23 (< 0.15, 20.4)	0.45
HBcrAg (log_{10} U/mL) [b]	4.7 ± 1.9	5.2 ± 1.6	0.39
HBV DNA (log_{10} copies/mL) [c]	< 2.1 (< 2.1, < 2.1)	< 2.1 (< 2.1, 2.3)	0.31
At week 12 of PegIFNα-2a			
HBsAg (log_{10} IU/mL) [b]	3.09 ± 1.19	3.45 ± 0.62	0.63
Qualitative HBeAg-*positive* [a]	2 (29%)	6 (35%)	0.99
Quantitative HBeAg (PEIU/mL) [c]	< 0.15 (< 0.15, 6.03)	< 0.15 (< 0.15, 5.37)	0.90
HBcrAg (log_{10} U/mL) [b]	4.6 ± 1.8	5.1 ± 1.5	0.36
At week 24 of PegIFNα-2a			
HBsAg (log_{10} IU/mL) [b]	2.61 ± 1.87	3.38 ± 0.81	0.43
During entecavir therapy			
Changes in HBsAg (log_{10} IU/mL) [c]	–0.06 (–0.35, 0.01)	–0.03 (–0.43, 0.09)	0.78
Changes in HBcrAg (log_{10} U/mL) [c]	–0.1 (–0.6, 0.0)	–1.6 (–2.1, –0.5)	0.024
During the first 12 weeks of PegIFNα-2a therapy			
Changes in HBsAg (log_{10} IU/mL) [c]	–0.31 (–0.39, –0.02)	–0.08 (–0.17, 0.09)	0.081
Changes in HBcrAg (log_{10} U/mL) [c]	0.0 (–0.1, 0.0)	–0.1 (–0.3, 0.0)	0.27
During the first 24 weeks of PegIFNα-2a therapy			
Changes in HBsAg (log_{10} IU/mL) [c]	–0.49 (–0.65, –0.22)	–0.08 (–0.31, 0.10)	0.017
During 48 weeks of PegIFNα-2a therapy			
Peak ALT (IU/L) [c]	36 (30, 84)	53 (37, 80)	0.73

[a]Number of patients; [b]Mean ± SD; [c]Median (interquartile range).

ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus

Table 3 Positive and negative predictive values of baseline factors for a therapeutic response

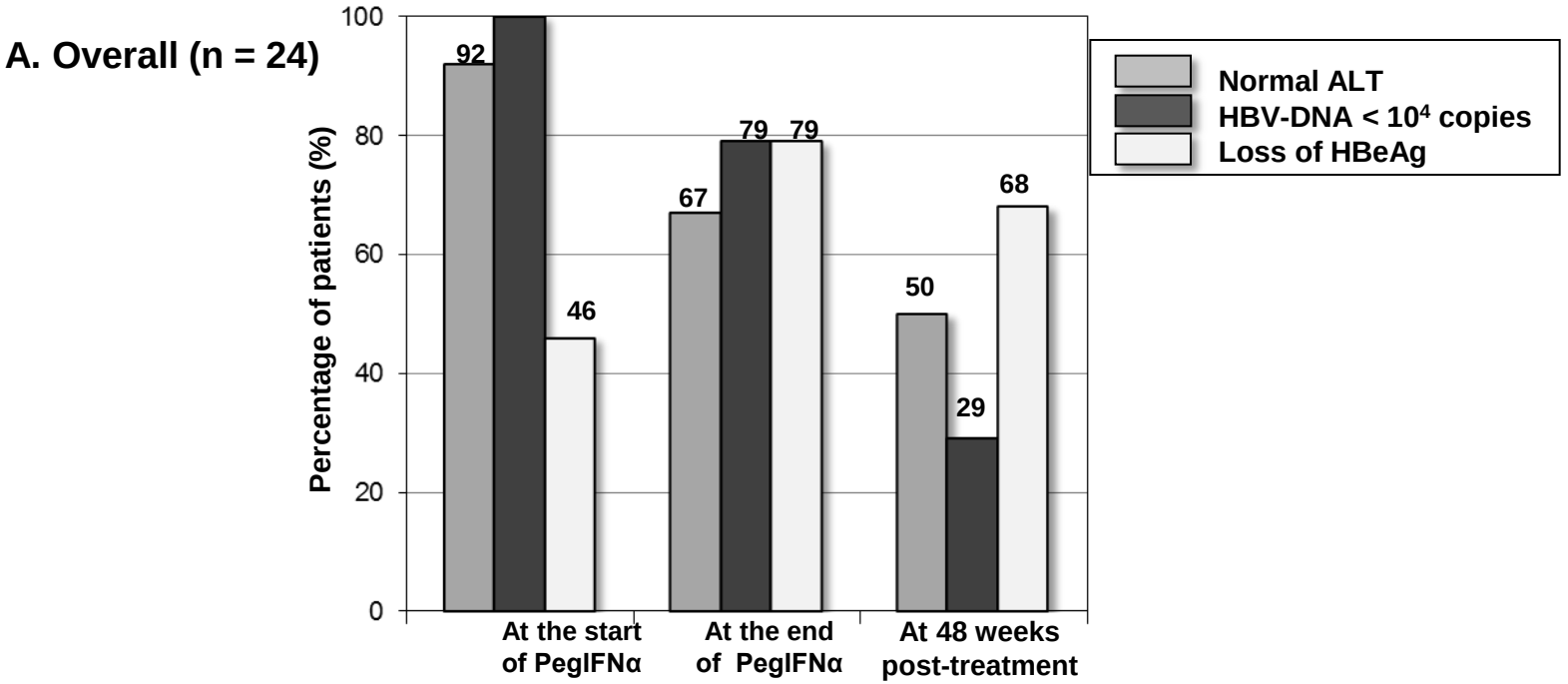
	Cut-off	Sensitivity (%)	Sensitivity (%)	PPV (%)	NPV (%)	AUROC
GGT	27 IU/L	76	76	76	86	0.90
α-fetoprotein	2.8 ng/mL	82	82	58	89	0.77
Type IV collagen 7S	3.4 ng/mL	80	80	55	87	0.77
APRI	0.57	71	71	81	78	0.76
ALT	50 IU/L	71	71	64	77	0.76
Baseline HBcrAg	4.9 $\log_{10}$ U/mL	82	82	59	89	0.73
Baseline HBeAg	positive	72	72	69	78	0.71

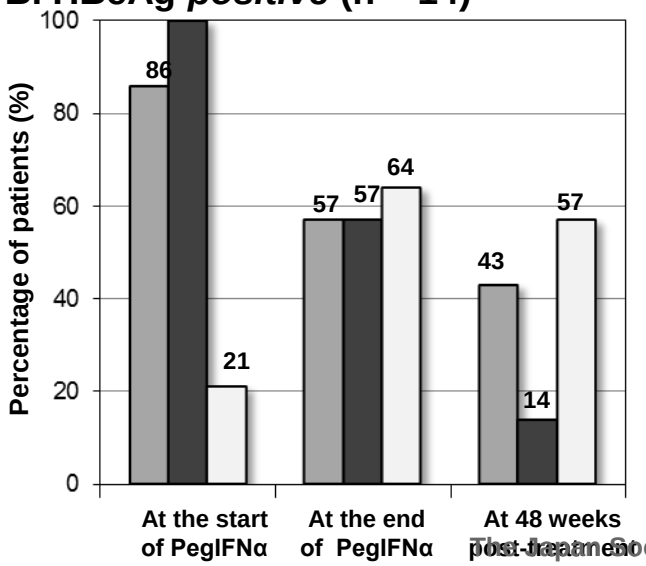
ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under the receiver operating characteristic curve; GGT, γ-glutamyl transferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; NPV, negative predictive value; PPV, positive predictive value

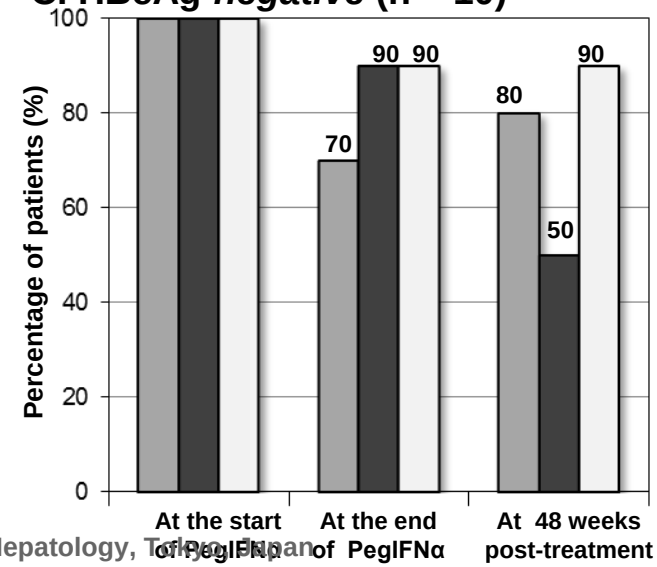
Figure Legends

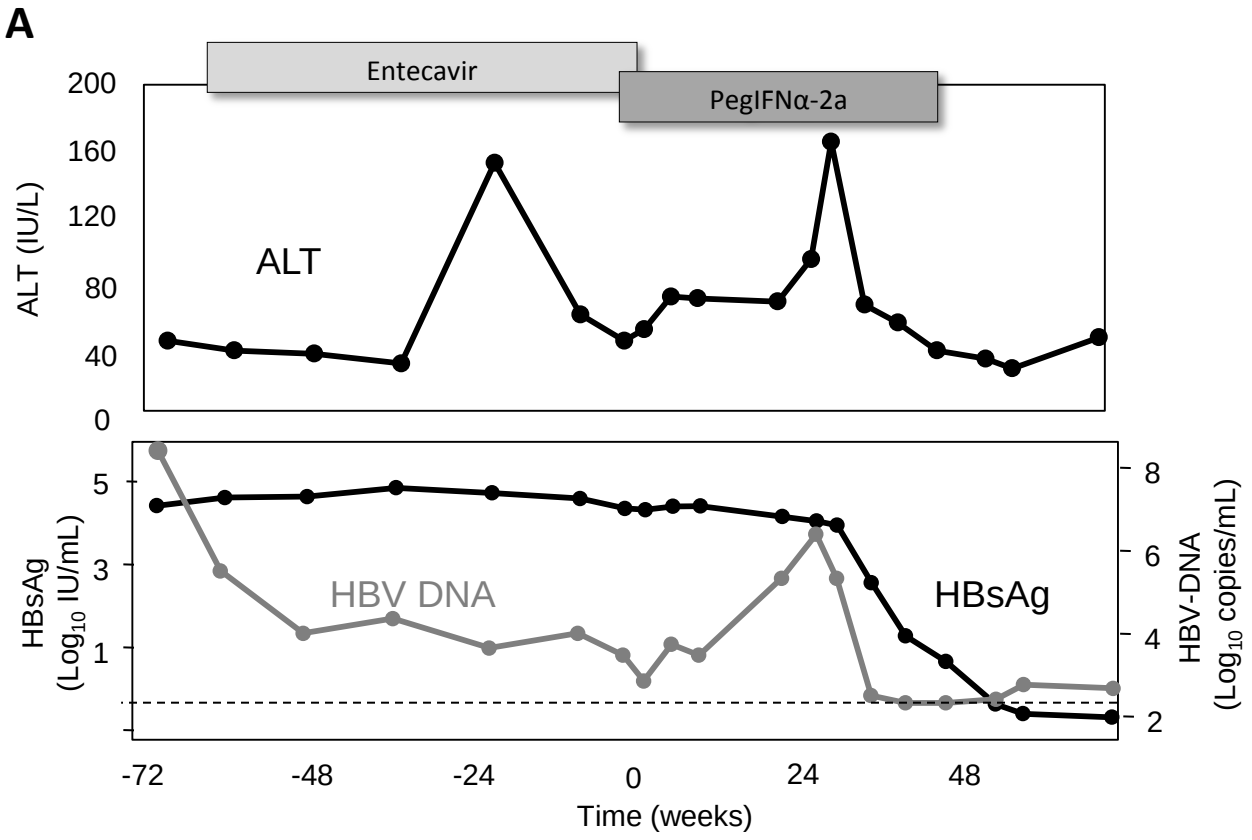
Fig. 1 Rates of biochemical, virological and serological responses during and after sequential therapy starting with entecavir followed by pegylated interferon-α2a (PegIFNα-2a) **(A)** in 24 patients with chronic hepatitis B comprising **(B)** 14 hepatitis B e-antigen (HBeAg)-positive patients and **(C)** 10 HBeAg-negative patients.

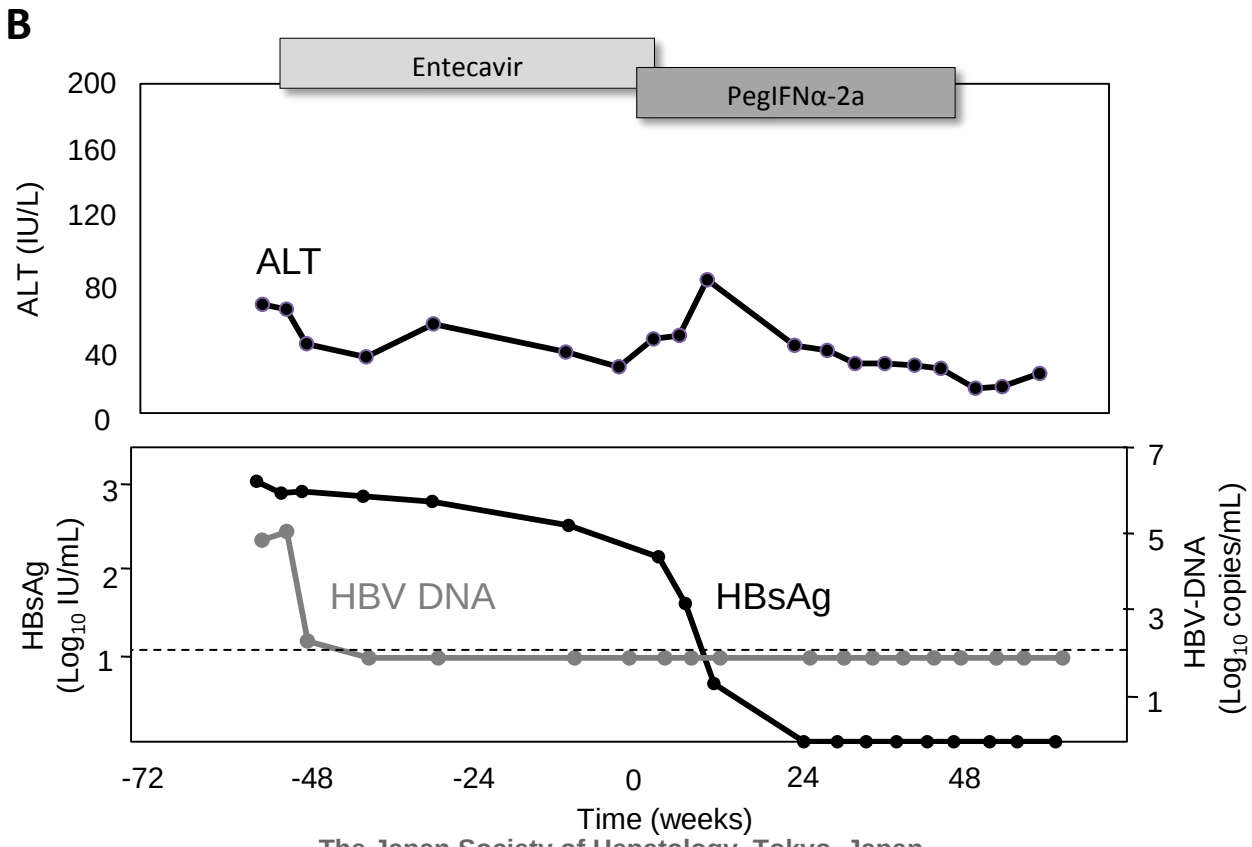
Fig. 2 Changes in alanine aminotransferase, hepatitis B surface antigen (HBsAg), and hepatitis B virus (HBV) DNA levels in two patients who achieved HBsAg seroclearance. **(A)** The first was a 28-year-old, male, treatment-naïve, hepatitis B e-antigen (HBeAg)-positive patient with genotype C infection. The HBsAg level did not change significantly during entecavir and the first half of pegylated interferon-α2a (PegIFNα-2a) treatment, but decreased immediately after week 24 of PegIFNα-2a treatment, and then became undetectable**. (B)** The second was a 37-year-old, male, HBeAg-negative patient with genotype C infection who did not respond to a previous nonPegIFNα treatment. The HBsAg level did not change significantly during entecavir treatment, but decreased immediately after switching to PegIFNα-2a, and then became undetectable. The broken line indicates the lower limit of quantification of HBV DNA (2.1 $\log_{10}$ copies/mL).


A. Overall (n = 24)
Normal ALT
HBV-DNA < 10^4 copies
Loss of HBeAg
Percentage of patients (%)
100
80
60
40
20
0
92
46
67
79
79
50
29
68
At the start of PegIFNα
At the end of PegIFNα
At 48 weeks post-treatment


B. HBeAg-*positive* (n = 14)
Percentage of patients (%)
100
80
60
40
20
0
86
21
57
57
64
43
14
57
At the start of PegIFNα
At the end of PegIFNα
At 48 weeks post-treatment


C. HBeAg-*negative* (n = 10)
Percentage of patients (%)
100
80
60
40
20
0
70
90
90
80
50
90
At the start of PegIFNα
At the end of PegIFNα
At 48 weeks post-treatment


A
Entecavir
PegIFNα-2a
ALT (IU/L)
200
160
120
80
40
0
ALT
HBsAg (Log10 IU/mL)
5
3
1
HBV DNA
HBsAg
HBV-DNA (Log10 copies/mL)
8
6
4
2
-72
-48
-24
0
24
48
Time (weeks)


B
Entecavir
PegIFNα-2a
ALT (IU/L)
200
160
120
80
40
0
ALT
HBsAg (Log10 IU/mL)
3
2
1
HBV DNA
HBsAg
HBV-DNA (Log10 copies/mL)
7
5
3
1
-72
-48
-24
0
24
48
Time (weeks)