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Original Article

Pegylated Interferon-based Treatment Improves Response Rates in Immune-tolerant Patients with Chronic Hepatitis B: A Prospective Trial

Min Liu[1,2], Lili Zuo[2], Yuting Zhang[2], Bing Bu[2], An Xiao[2], Ling Zhu[2], Xiuying Ma[2], Yilan Wang[2], Wei Yue[2], Jiawei Geng[2*] and Xueshan Xia[1,3*]

[1]Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan, China; [2]Department of Infectious Disease and Hepatic Disease, The First People's Hospital of Yunnan Province (The Affiliated Hospital of Kunming University of Science and Technology), Kunming, Yunnan, China; [3]School of Public Health, Kunming Medical University, Kunming, Yunnan, China

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Abstract

Background and Aims: The optimal management strategy for adults with immune-tolerant (IT) chronic hepatitis B infection remains undefined. This study aimed to investigate the efficacy and predictive factors of a pegylated interferon (Peg-IFN)-based treatment strategy in IT patients with chronic HBV infection. ***Methods:*** In this pilot, open-label, prospective study, 286 patients aged 18 to 60 years with IT characteristics were enrolled and allocated to one of three groups. The combination group received Peg-IFN for 48–96 weeks, with tenofovir disoproxil fumarate (TDF) initiated at week 12 and continued through week 96 (n = 103). The monotherapy group received TDF monotherapy alone (n = 125), and the control group was monitored without therapeutic intervention (n = 58). ***Results:*** No patients in the control group met any predefined efficacy endpoints. Intention-to-treat analysis showed that patients in the combination group achieved significantly higher virological response rates (71.8% vs. 53.6%, $p = 0.005$), hepatitis B e antigen seroconversion rates (15.5% vs. 1.6%, $p < 0.001$), and hepatitis B surface antigen (HBsAg) loss rates (10.7% vs. 0%, $p < 0.001$) compared with those in the monotherapy group at week 96. In the combination group, the cumulative rate of HBsAg loss was 5.4% at week 48 and increased to 11.8% by week 96. Independent predictors of achieving either hepatitis B e antigen seroconversion or HBsAg loss were baseline age under 30 years (odds ratio = 0.217, 95% confidence interval: 0.048–0.976, $p = 0.046$) and a decline in HBsAg level greater than 1 log10 IU/mL by week 24 (odds ratio = 13.976, 95% confidence interval: 2.506–77.932, $p = 0.003$).

Conclusions: A Peg-IFN-based treatment strategy significantly increases response rates compared with TDF monotherapy or observation in patients with IT characteristics.

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Introduction

The global burden of liver disease due to hepatitis B virus (HBV) infection is substantial, with approximately 257 million individuals chronically infected and 870,000 deaths annually attributable to HBV-related complications.[1] In response, the World Health Organization has set a goal of eliminating viral hepatitis as a major public health threat by 2030, aiming for a 90% reduction in new cases and a 65% reduction in mortality.[2] Achieving this goal depends on treating 80% of individuals eligible for treatment worldwide, which has prompted experts to simplify treatment criteria and expand antiviral therapy for chronic hepatitis B.[3,4] However, in this context, the management of the immune-tolerant (IT) phase of chronic HBV infection remains a contentious issue.[5]

Due to limited fibrosis and inflammation, limited treatment efficacy, indefinite treatment duration, increased risk of low-level viremia or drug resistance, and insufficient evidence supporting improved clinical outcomes with antiviral therapy, some experts do not recommend initiating antiviral treatment in IT phase patients.[6,7]

Our team previously conducted a systematic review and meta-analysis incorporating 23 studies and a total of 5,471 patients (321 children and 5,150 adults) in the IT phase to estimate the effects of antiviral therapy in this specific population. The stratified analysis showed that adults who received pegylated interferon (Peg-IFN) plus nucleos(t)ide analogue (NUC) during the IT phase achieved significantly higher rates of hepatitis B surface antigen (HBsAg) loss compared to those receiving NUC monotherapy (9% vs. 0%),

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***Correspondence to:** Jiawei Geng, Department of Infectious Disease and Hepatic Disease, The First People's Hospital of Yunnan Province (The Affiliated Hospital of Kunming University of Science and Technology), Kunming, Yunnan 650100, China. ORCID: https://orcid.org/0009-0002-5414-6112. Tel: +86-871-63638510, Fax: +86-871-63638049, E-mail: geng_edu2023@163.com; Xueshan Xia, Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan 650500, China. ORCID: https://orcid.org/0009-0001-1177-6387. Tel/Fax: +86-871-65920570, E-mail: oliverxia2000@aliyun.com.

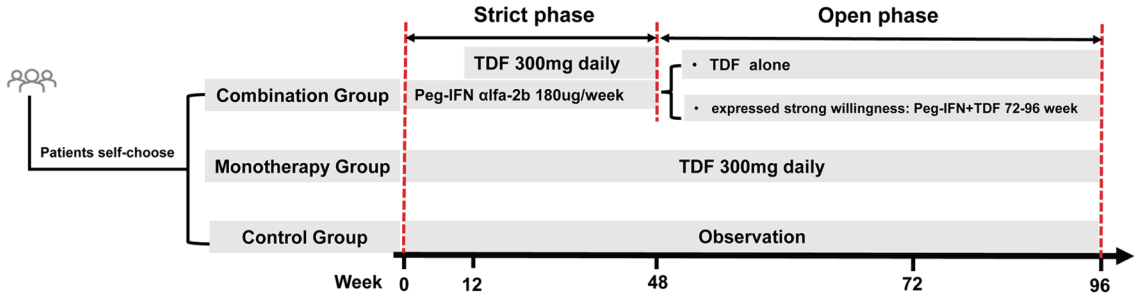

Fig. 1. Trial design. Peg-IFN, pegylated interferon; TDF, tenofovir disoproxil fumarate.

suggesting the potential benefits of Peg-IFN-based combination therapy.[8] However, this conclusion was associated with high heterogeneity due to the limited number of included studies and inconsistent findings across them.[9–13]

In China, Peg-IFN-based therapy is not routinely used in adults during the IT phase of chronic HBV infection. This study aimed to evaluate the effects of Peg-IFN-based therapy in this population, with the goal of providing evidence to support clinical management.

Methods

Study design

This pilot, open-label, prospective cohort trial was registered with the Chinese Clinical Trial Registry (www.chictr.org.cn, identifier: ChiCTR2100046860). After receiving comprehensive information regarding the potential benefits and risks of different treatment strategies, eligible participants voluntarily selected one of three groups: (a) The combination group initially received Peg-IFN alfa-2b monotherapy (180 μg/week) for 12 weeks, followed by the addition of tenofovir disoproxil fumarate (TDF) (300 mg/day) up to week 48, forming a structured initial treatment phase. From week 48 onward, participants entered a more flexible phase during which they could choose to continue TDF monotherapy until week 96 or extend combination therapy until week 72–96. (b) The monotherapy group received TDF for 96 weeks. (c) The control group underwent clinical follow-up without antiviral therapy until week 96. If a patient in the control group transitioned to the immune-active (IA) phase during follow-up, first-line antiviral therapy was initiated (Fig. 1). The IA phase is characterized by the presence of serum hepatitis B e antigen (HBeAg), high-level HBV DNA (10^4–10^7 IU/mL), and elevated alanine aminotransferase (ALT), as defined in the 2017 EASL Clinical Practice Guidelines on the management of hepatitis B virus infection.[14]

All patients were assessed at baseline and at weeks 12, 24, 48, 72, and 96. Beyond week 96, participants were followed up every three to six months. All participants provided written informed consent prior to study enrollment. The trial was approved by the research ethics committees of the First People's Hospital of Yunnan Province (ethics approval number: KHLL2021-KY051) and conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

Patients

Patients aged 18 to 60 years with IT characteristics who had not received antiviral therapy within the preceding six months were enrolled from the Department of Infectious Diseases and the Department of Hepatic Diseases at the First People's Hospital of Yunnan Province, China, between May 2021 and January 2023.

IT phase patients met the criteria defined in the 2017 EASL Guidelines[14]: (a) positive HBsAg for more than six months; (b) HBeAg positivity, HBV DNA > 10^7 IU/mL, and ALT < 40 U/L; and (c) liver biopsy or noninvasive test results showing absent fibrosis (FibroScan value < 7.3 kPa) and minimal inflammation.

Key exclusion criteria included a history of liver cirrhosis (past or current), malignancy, solid organ or bone marrow transplantation, baseline neutrophil count < 2.0 × 10^9/L, platelet count < 100 × 10^9/L, creatinine clearance < 50 mL/min, co-infection with hepatitis A, C, D, or E viruses or human immunodeficiency virus, severe alcohol use disorder, pregnancy or lactation, allergic predisposition, preexisting psychiatric or autoimmune disease, and any other medical condition that could be exacerbated by Peg-IFN alfa-2b or TDF therapy.

Endpoints

The primary efficacy endpoint was the virological response (VR) rate by week 96, defined as a serum HBV DNA level < 10 IU/mL. The co-primary endpoints were the rates of HBeAg seroconversion and HBsAg loss.

Secondary endpoints included the cumulative incidence of HBeAg seroconversion, cumulative incidence of HBsAg loss, and decline in HBsAg levels through week 96.

All adverse events were recorded and graded at each clinical visit.

Sample size

The primary efficacy endpoints of our trial were VR, HBeAg seroconversion, and HBsAg loss. Most prior clinical studies evaluating antiviral therapy in IT patients had used HBeAg loss or seroconversion as the primary efficacy endpoint. In contrast, data on HBsAg clearance remain extremely limited, and HBV DNA levels are highly susceptible to the timing of treatment cessation. Therefore, we selected the HBeAg loss rate as the basis for sample size calculation.

In IT patients who received observation or NUC monotherapy, the 96-week HBeAg loss rates were 0.1% and 2.0%, respectively.[15] Based on preclinical and clinical evidence suggesting potential synergistic antiviral effects of PEG-IFN and NUC,[10,16,17] the 96-week HBeAg loss rate for the combination therapy group was conservatively estimated at 10.0%.

Sample size calculation was performed using PASS software (Version 15.0, NCSS LLC, Kaysville, UT, USA), assuming 80% power and a two-sided α level of 0.05 for detecting differences among the three arms. Accounting for a projected 10% dropout rate and allocating participants in a 2:2:1 ratio (combination group: monotherapy group: control group), the required sample sizes are 90, 90, and 45

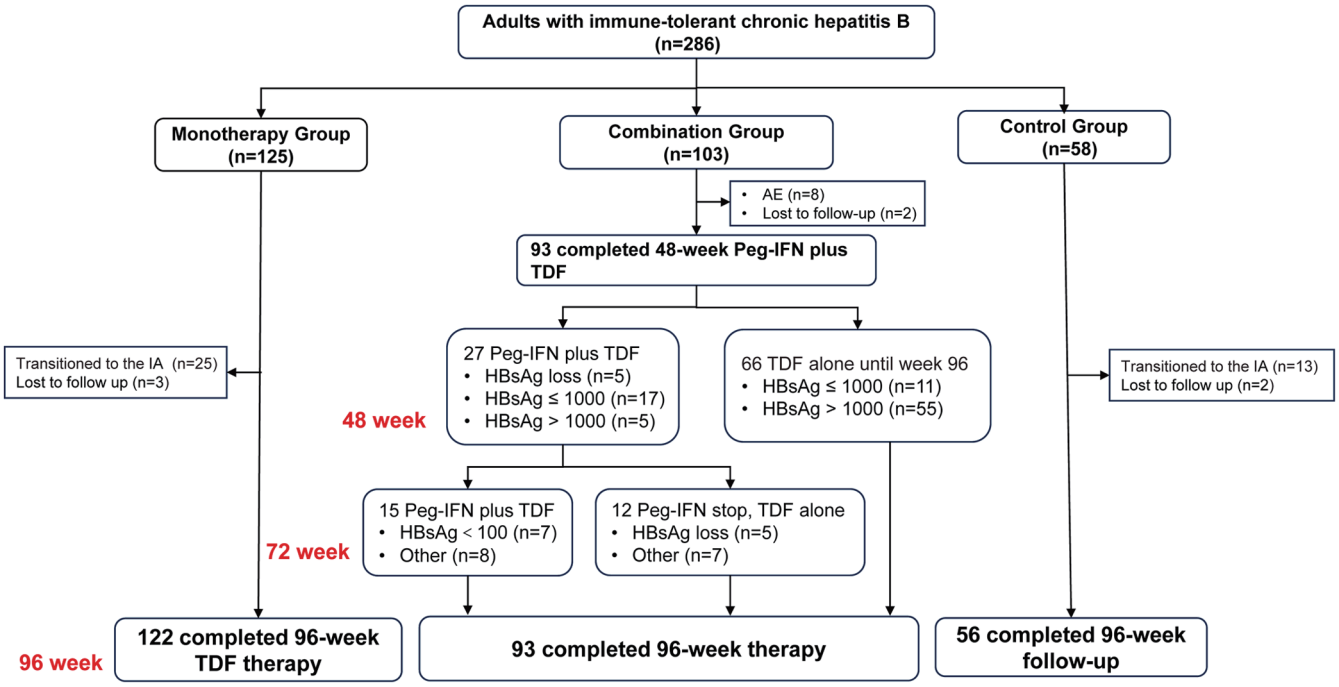

Fig. 2. Patient disposition. AE, adverse event; IA, immune-active; Peg-IFN, pegylated interferon; TDF, tenofovir disoproxil fumarate.

per group, respectively, yielding a total enrollment target of 225 participants.

Statistical analysis

Two populations were defined for post hoc analyses: the intention-to-treat (ITT) population and the per-protocol (PP) population. The ITT population included all eligible patients, while the PP population excluded those who discontinued treatment or had protocol deviations, including therapy switches. Primary analyses were conducted according to the ITT principle, with patients analyzed based on their originally assigned group regardless of the treatment actually received.

Continuous variables are presented as median (interquartile range), and categorical variables as frequencies and percentages. Group comparisons were performed using the Mann-Whitney U test for two groups or the Kruskal-Wallis test for three groups for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. Cumulative rates of HBeAg seroconversion and HBsAg loss were estimated using the Kaplan-Meier method and compared using the log-rank test.

Logistic regression analysis was performed to identify independent predictors of response, defined as HBeAg seroconversion and/or HBsAg loss, to antiviral therapy in the combination therapy group. All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$ and were conducted using SPSS 26.0 (SPSS, Chicago, IL, USA).

Results

Study cohort

A total of 286 patients who met the eligibility criteria were enrolled in the trial: 103 in the combination group, 125 in the monotherapy group, and 58 in the control group.

In the combination group, 2 patients were lost to follow-up, 8 discontinued treatment due to adverse events potentially associated with Peg-IFN alfa-2b, and 93 completed the scheduled 48-week initial therapy. After week 48, 66 patients continued with TDF monotherapy until week 96, while the remaining 27 chose to extend combination therapy based on personal preference: 15 continued Peg-IFN alfa-2b plus TDF until week 96; 5 who achieved HBsAg loss by week 48 received combination therapy until week 72 and completed treatment; and 7 continued combination therapy until week 72 before switching to TDF monotherapy until week 96.

In the monotherapy group, 25 patients transitioned to the IA phase during follow-up and elected to continue TDF treatment; additionally, 3 subjects were lost to follow-up before week 48, and 122 completed the scheduled 96-week initial therapy.

In the control group, 2 patients were lost to follow-up, and 13 transitioned to the IA phase and initiated antiviral therapy—11 chose TDF monotherapy and 2 selected Peg-IFN alfa-2b plus TDF (Fig. 2).

Patient characteristics

In the ITT population, the median age was 29 years (interquartile range: 24–33), and 54.9% (157/286) were female. Nearly all participants had HBV genotypes B or C. In addition, 35 patients were diagnosed with metabolic dysfunction-associated steatotic liver disease by abdominal ultrasound, and hepatosteatosis grade was assessed using the same modality in accordance with the EASL-EASD-EASO Clinical Practice Guidelines for the management of dysfunction-associated steatotic liver disease.[18] Baseline characteristics and demographic data are presented in Table 1, with no significant differences observed across the three groups.

Primary outcomes

ITT analysis revealed that patients in the combination group had significantly higher VR rates (71.8% vs. 53.6%, p =

Table 1. Baseline characteristics of enrolled patients in the ITT population

Characteristics	Combination group (n = 103)	Monotherapy group (n = 125)	Control group (n = 58)	*p*
Gender				0.769
Male, n (%)	44 (42.7%)	57 (45.6%)	28 (48.3%)	
Female, n (%)	59 (57.3%)	68 (54.4%)	30 (51.7%)	
Age (years)				0.069
Median (IQR)	27 (23, 33)	30 (25, 33)	27 (22, 32)	
<30	65 (63.1%)	62 (49.6%)	37 (63.8%)	
≥30	38 (36.9%)	63 (50.4%)	21 (36.2%)	
BMI (kg/m^2)	21.4 (19.7, 23.7)	22.0 (20.0, 24.1)	21.1 (19.6, 22.5)	0.125
Mode of transmission				0.200
Vertical	54 (52.4%)	56 (44.8%)	22 (37.9%)	
Others	49 (47.6%)	69 (55.2%)	36 (62.1%)	
Family history of cirrhosis or HCC, n (%)	24 (23.3%)	25 (20.0%)	11 (19.0%)	0.786
HBV genotype				0.779
B	47 (45.6%)	52 (41.6%)	28 (48.3%)	
C	55 (53.4%)	70 (56.0%)	30 (51.7%)	
Other	1 (1.0%)	3 (2.4%)	0 (0%)	
HBsAg (IU/mL)	50,554 (31,411, 71,481)	56,063 (39,856, 83,034)	60,938 (37,495, 78,892)	0.096
HBeAg (S/CO)	1,549.07 (1,159.40, 1,770.42)	1,597.53 (1,390.54, 1,800.89)	1,566.80 (1,267.62, 1,724.98)	0.445
HBV DNA (log_{10}IU/mL)	8.07 (7.83, 8.37)	8.19 (7.83, 8.45)	8.24 (7.84, 8.52)	0.151
ALT (U/L)	25 (20, 33)	25 (18, 34)	25 (20, 34)	0.996
FIB-4①	0.53 (0.41, 0.73)	0.56 (0.44, 0.70)	0.51 (0.40, 0.73)	0.584
FibroScan value (kPa)	5.9 (5.1, 6.9)	5.9 (5.1, 6.7)	5.9 (5.0, 6.4)	0.505
CAP (dB/m)	238	243	240	0.822
Hepatosteatosis grade②				0.125
Mild, n (%)	21 (20.4%)	37 (29.6%)	8 (13.7%)	
Moderate-severe, n (%)	14 (13.6%)	11 (8.8%)	6 (10.3%)	

①FIB-4 = age (years) × AST (U/L) / Platelet count (10^3/μL) × √ALT (U/L); ②Hepatosteatosis grade was assessed by abdominal ultrasound. ITT, intention to treat; IQR, interquartile range; BMI, body mass index; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; CAP: controlled attenuation parameter.

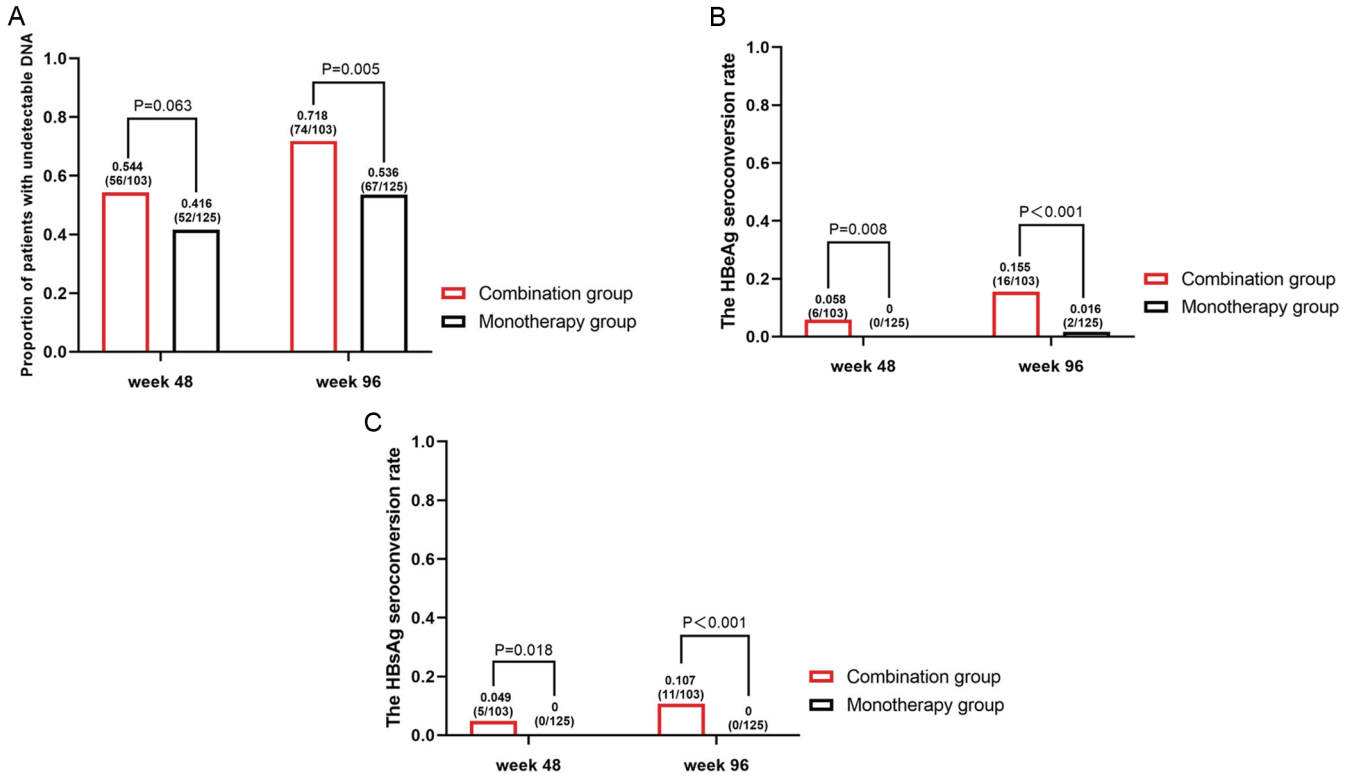

Fig. 3. Proportions of patients with responses. Comparison of virological response, HBeAg seroconversion, and HBsAg loss rates between the combination and monotherapy groups at week 48 and week 96 by ITT analysis. (A) Virological response rates. (B) HBeAg seroconversion rates. (C) HBsAg loss rates. ITT, intention to treat.

0.005), HBeAg seroconversion rates (15.5% vs. 1.6%, $p < 0.001$), and HBsAg loss rates (10.7% vs. 0%, $p < 0.001$) compared with those in the monotherapy group through week 96 (Fig. 3A–C). Among the combination group, 66 patients received TDF monotherapy from week 48 to week 96, of whom 48 (72.7%) achieved VR, 6 (9.1%) experienced HBeAg seroconversion, and none had HBsAg loss. Meanwhile, 27 patients chose to continue combination therapy, all of whom achieved VR; 7 achieved both HBeAg seroconversion and HBsAg loss, 3 achieved HBeAg seroconversion alone, and 4 achieved HBsAg loss while remaining HBeAg positive. Additionally, none of the 58 patients in the control group met any efficacy endpoints by the end of the observation period.

Secondary outcomes

Cumulative HBeAg seroconversion rates in the combination and monotherapy groups were 6.4% (95% confidence interval (CI): 6.2–22.7%) vs. 0% (95% CI: 0–0%) at week 48 and 17.2% (95% CI: 11.5–19.4%) vs. 1.6% (95% CI: 1.6–34.1%) at week 96, respectively, based on ITT analysis (all $p < 0.001$) (Fig. 4A). The cumulative HBsAg loss rate was 5.4% (95% CI: 5.3–24.2%) at week 48 and increased to 11.8% (95% CI: 9.7–18.8%) at week 96 in the combination group according to ITT analysis (Fig. 4C). Results from the PP analysis were consistent with those of the ITT analysis (Fig. 4B and D).

Additionally, the median levels of serum HBsAg quantification at each key visit for patients in the combination and monotherapy groups, as determined by PP analysis, are shown in Fig. 5. The results showed that patients in the combination group who did not achieve HBsAg loss experienced a rebound in HBsAg levels within 6 to 12 months after discontinuation of Peg-IFN alfa-2b. However, the combination group continued to demonstrate a greater reduction in HBsAg levels compared with the TDF monotherapy group through week 96 ($p < 0.001$).

Baseline and on-treatment predictors of response (HBeAg seroconversion and/or HBsAg loss) in the combination group based on PP analysis

In summary, by week 96, seven patients in the combination group achieved both HBeAg seroconversion and HBsAg loss, nine achieved HBeAg seroconversion alone, and four achieved HBsAg loss while remaining HBeAg positive.

Univariate and multivariate logistic regression analyses were performed to identify predictors of response (HBeAg seroconversion and/or HBsAg loss). Baseline variables included gender, age, HBsAg level, HBV DNA load, HBeAg status, and hepatosteatosis grade. On-treatment variables included ALT levels at weeks 12 and 24, as well as changes in HBsAg levels from baseline to weeks 12 and 24. The results revealed that age < 30 years (odds ratio = 0.217, 95% CI: 0.048–0.976, $p = 0.046$) and a decline in HBsAg level of more than 1 $\log_{10}$ IU/mL by week 24 (odds ratio = 13.976, 95% CI: 2.506–77.932, $p = 0.003$) were independent predictors of response (Table 2).

Safety

No serious adverse events, such as liver failure, hepatic decompensation, hepatocellular carcinoma (HCC), or death, occurred in any of the three groups during the follow-up period.

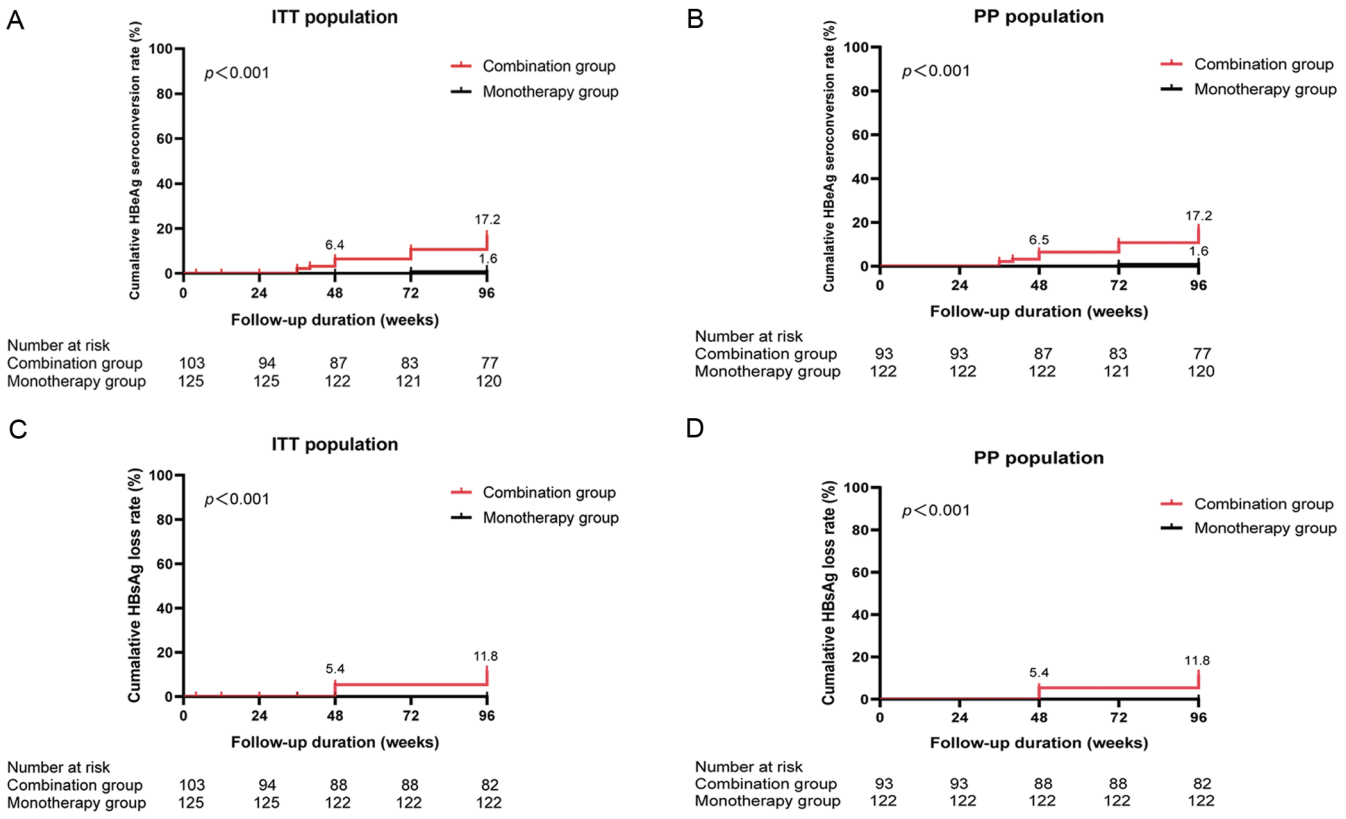

Fig. 4. Cumulative HBeAg seroconversion rate and cumulative HBsAg loss rate. Comparison of cumulative HBeAg seroconversion rate between the combination and monotherapy groups by ITT analysis (A) and PP analysis (B). Comparison of cumulative HBsAg loss rate between the combination and monotherapy groups by ITT analysis (C) and PP analysis (D). ITT, intention to treat; PP, per protocol.

In the combination group, eight patients discontinued Peg-IFN therapy due to adverse events considered possibly related to Peg-IFN alfa-2b: one patient at week 1 due to high fever, two at week 12 due to myalgia and insomnia, and five at week 24 due to anxiety, alopecia, and hyperthyroidism. Additionally, five patients experienced ALT elevations exceeding ten times the upper limit of normal, leading to a dose adjustment of Peg-IFN to 135 μg/week. The most com-

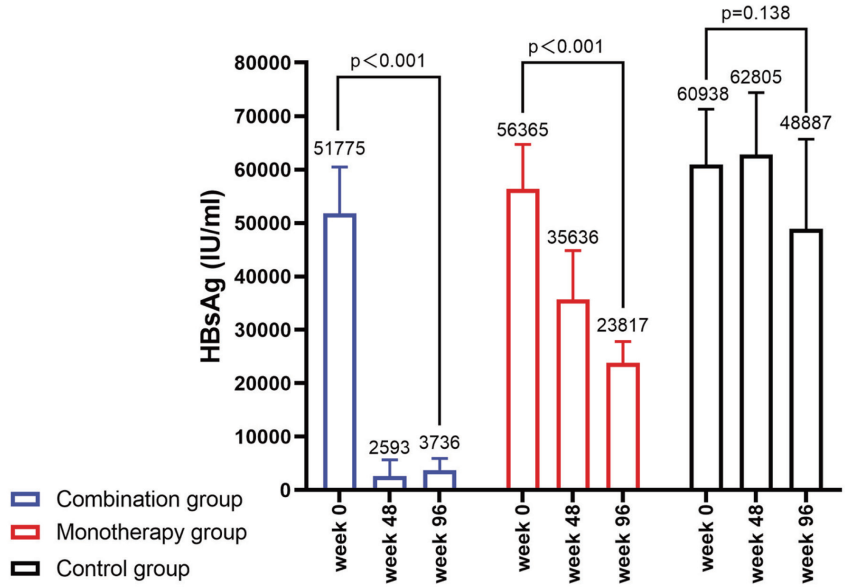

Fig. 5. Median HBsAg levels at each visit among the groups by PP analysis. PP, per protocol.

Table 2. Baseline and on-treatment variables predictive of responses (HBeAg seroconversion and/or HBsAg loss) in the combination group by PP analysis

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*	OR	95% CI	*p*
Baseline						
Gender	2.034	0.704–5.878	0.190	2.239	0.698–7.183	0.175
Age (years)	0.226	0.061–0.839	0.026	0.217	0.048–0.976	0.046
<30						
≥30						
HBsAg (IU/mL)	1.000	1.000–1.000	0.065	1.000	1.000–1.000	0.285
HBeAg (S/CO)	1.000	0.999–1.001	0.975	1.000	0.999–1.001	0.579
HBV DNA (log_{10} IU/mL)	0.294	0.094–0.915	0.035	0.420	0.124–1.425	0.164
Hepatosteatosis grade	0.898	0.420–1.920	0.781	1.523	0.602–3.855	0.374
None						
Mild						
Moderate-Severe						
On-treatment						
ALT week 12 (U/L)	1.909	0.683–5.331	0.217	0.898	0.263–3.070	0.864
< 2 ULN						
≥2 ULN						
ALT week 24 (U/L)	2.915	1.029–8.254	0.044	2.100	0.635–6.942	0.224
< 2 ULN						
≥2 ULN						
HBsAg week 12 change from baseline (IU/mL)	3.344	1.154–9.692	0.026	0.729	0.163–3.265	0.679
< 0.5 log 10						
≥ 0.5 log 10						
HBsAg week 24 change from baseline (IU/mL)	12.319	3.281–46.253	< 0.001	13.976	2.506–77.932	0.003
< 1.0 log 10						
≥ 1.0 log 10						

OR, odds ratio; CI, confidence interval. PP, per protocol; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase.

mon adverse effects were fever, occurring in 78.6% (81/103) of patients, and mild decreases in neutrophil and platelet counts, observed in 63.1% (65/103) and 42.7% (44/103) of patients, respectively.

In the monotherapy group, 25 patients (20%) experienced ALT elevations, 12 (9.6%) had reduced serum phosphorus levels, and 8 (6.4%) had decreased creatinine clearance. In the control group, 13 patients (22.4%) experienced ALT elevations, and 2 (3.4%) developed dyssomnia and anxiety. All adverse events are summarized in Table 3.

Discussion

Patients with the IT phase of chronic HBV infection are characterized by early-age infection, active viral replication, high HBsAg levels, HBeAg positivity, and absence of biochemical abnormalities.[19] In China, due to high contagiousness, psychological burden, career concerns, and social discrimination, among other factors, some individuals with the IT phase of chronic HBV infection, particularly younger patients, desire to achieve VR, HBeAg seroconversion, or even HBsAg loss through antiviral treatment. However, clinical experience in treating CHB in adults suggests that antiviral therapy yields the highest response rates in patients with elevated serum ALT levels and low HBsAg levels—features that are atypical of the IT phenotype.[20–23]

Recognition that high HBV DNA levels are associated with an increased risk of HCC has heightened interest in treating chronic HBV infection during the IT phase.[24] Trials of NUC monotherapy in patients in the IT phase of chronic HBV infection have demonstrated suppression of viral load, but the treatment duration is indefinite, and clearance of HBeAg and HBsAg is rare.[10–12,25] Data on the effect of Peg-IFN combination NUC treatment in adults with IT characteristics are limited. Feld *et al*. reported that among 27 adults in the IT phase, entecavir was administered for 48 weeks, with Peg-IFN alfa-2a added at week 8 and continued through week 48. Disappointingly, only one patient (4%) achieved HBeAg loss, and none achieved durable off-treatment suppression of HBV DNA to ≤1,000 IU/mL or HBsAg loss by week 96.[9]

Table 3. Adverse events across the three groups (ITT population)

Adverse events	N (%)
Combination group (n = 103)	
Discontinuation	
High fever	1 (0.9%)
Myalgia	1 (0.9%)
Insomnia	1 (0.9%)
Anxiety	1(0.9%)
Alopecia	2 (1.9%)
hyperthyroidism	2 (1.9%)
Dose modification	
ALT flare	5 (4.9%)
Others	
Fever	81 (78.6%)
Neutrophil count decrease	65 (63.1%)
Platelet count decrease	54 (52.4%)
Fatigue	45 (43.7%)
Alopecia	31 (30.1%)
Decreased appetite	23 (22.3%)
Muscle soreness	18 (17.5%)
Anti-nuclear antibody positive	16 (15.5%)
Rash	10 (9.7%)
Thyroid dysfunction	9 (8.7%)
Dyssomnia and anxiety	8 (7.8%)
Arthralgia	4 (3.9 %)
Monotherapy group (n = 125)	
ALT increased	25 (20%)
Serum phosphorus fell	12 (9.6%)
Decreased creatinine clearance	8 (6.4%)
Control group (n = 58)	
ALT increased Dyssomnia and anxiety	13 (22.4%) 2 (3.4%)

ALT, alanine aminotransferase; ITT, intention to treat.

To date, an optimized regimen of Peg-IFN-based therapy to improve response rates in adults with the IT phase has not been established.

The design of our trial was based on a randomized controlled trial conducted in pediatric patients in China. In that study, 46 children with IT characteristics received interferon therapy for the first 12 weeks, followed by either continued interferon monotherapy or combination therapy with lamivudine up to week 72. This treatment strategy yielded encouraging response rates, including 33% HBeAg loss and 22% HBsAg loss at week 96.[26] This randomized controlled trial provided valuable insights for our team: the key distinction between the IT phase and IA phase lies in the presence of an inflammatory milieu during the IA phase, which triggers nonspecific inflammation in response to T-cell recognition of HBV.[27] Studies in patients during the IA phase who experienced ALT flares have achieved sustained VR and even HBsAg clearance, suggesting the importance of immune stimulation.[20,28,29]

Peg-IFN exerts both direct antiviral effects and host immunomodulatory actions.[30,31] In patients with IT characteristics who are in a "noninflammatory" state, 12 weeks of interferon monotherapy can induce partial activation of HBV-specific immune responses and disrupt immune tolerance, followed by combination therapy with NUC and interferon to enhance the immune response. We hypothesized that using a more potent agent, such as TDF, which does not interfere with subsequent pregnancy planning in younger patients, may yield similar therapeutic outcomes in adults as those observed in pediatric populations.

Our results demonstrate that a lead-in strategy consisting of 12 weeks of Peg-IFN alfa-2b, followed by the addition of TDF, significantly increases the rates of VR, HBeAg seroconversion, and HBsAg loss compared to TDF monotherapy in adults with IT characteristics. The encouraging outcomes observed in the combination group—71.8% virological response, 15.5% HBeAg seroconversion, and 10.7% HBsAg loss—support our initial scientific hypothesis. Previ-

ous studies reported VR rates of 55–85.2%, HBeAg seroconversion rates of 3.3–5%, and no HBsAg loss following 48 to 192 weeks of NUC monotherapy in IT phase adults.[11,25] In our trial, the TDF monotherapy group achieved a VR rate of 53.6%, an HBeAg seroconversion rate of 1.6%, and no cases of HBsAg loss, which is consistent with findings from most prior studies.

Multivariate analysis showed that age less than 30 years was a favorable factor for HBeAg seroconversion or HBsAg loss in the combination group. Given the association between increased age and a higher risk of HCC, both national and international guidelines recommend antiviral therapy for patients with chronic HBV infection who are over 30 years of age.[14,32] In fact, young patients in the IT phase have been shown to exhibit a similar or greater frequency and reactivity of HBV-specific T cells in peripheral blood compared with older patients in the IA phase of CHB.[33] Our findings suggest that Peg-IFN-based therapy is likely to be beneficial and should be considered for patients in the IT phase who are under 30 years of age and have a strong willingness to undergo antiviral treatment, as younger patients demonstrated a more favorable response. A key point to emphasize is that the combination group was younger than the monotherapy group (p = 0.041) in our trial—a clinically relevant age difference that likely contributed to the higher treatment response rate observed in the combination group. In addition, consistent with previous studies showing that an early on-treatment decline in HBsAg levels predicts treatment response in patients with HBeAg-positive chronic hepatitis B at the end of therapy,[34,35] our study found that a decline in HBsAg of more than 1 $\log_{10}$ IU/mL by week 24 was associated with higher response rates among patients in the IT phase.

It has been reported that a reduction in serum HBsAg levels during Peg-IFN treatment is associated with a higher likelihood of sustained HBsAg loss over time, which may be linked to immune stimulation leading to decreased intrahepatic covalently closed circular DNA levels.[34–37] Hou *et al*. recently reported that among patients with chronic HBV infection who achieved virologic suppression with NUC therapy, treatment with xalnesiran (a small interfering RNA) plus Peg-IFN resulted in HBsAg loss and seroconversion exclusively in participants with baseline HBsAg levels below 1,000 IU/mL at screening.[38] In our study, 28 subjects (27.2%) in the combination group had HBsAg levels below 1,000 IU/mL at week 48. However, no subjects in the monotherapy group achieved HBsAg levels below 1,000 IU/mL until week 96.

Meanwhile, most researchers are concerned that discontinuing Peg-IFN may lead to a rebound in serum HBsAg levels among IT phase patients who have not achieved HBsAg loss, potentially resulting in a return to the pre-treatment "tolerant" state. In the present trial, 66 subjects in the combination group discontinued Peg-IFN after week 48 and received TDF monotherapy until week 96. Our data indicated a slight rebound in median HBsAg levels from 2,593 IU/mL at week 48 to 3,736 IU/mL at week 96 following 12 months of Peg-IFN cessation. Importantly, patients in the combination group still exhibited a greater reduction in HBsAg levels compared to those in the TDF monotherapy group at week 96. Additionally, 27 subjects opted to extend combination therapy from 48 to 72–96 weeks due to a significant decline in HBsAg levels relative to baseline, enabling an additional 22.2% (6/27) of patients to achieve HBsAg loss. The cumulative HBsAg loss rate was 5.4% at week 48 and increased to 11.8% at week 96 in the combination group based on ITT analysis.

The response rates may increase in IT patients who are under 30 years old and receive Peg-IFN-based therapy compared with NUC monotherapy. Additionally, those with a decline in HBsAg level of more than 1 $\log_{10}$ IU/mL at week 24 compared to baseline may consider extending the duration of Peg-IFN treatment to 72–96 weeks.

This study has several limitations. First, the study was an open-label, single-center trial, and the findings may be subject to selection bias and confounding. Although no significant differences were observed in baseline demographic or clinical characteristics among the three groups, the combination group was significantly younger than the monotherapy group (p = 0.041). Second, our criteria for identifying the IT phase in chronic HBV infection were primarily based on biochemical, serological, and virological indicators, as only a small number of participants (3%, 8/286, all had G0-1S1) underwent liver biopsy. Third, not all patients offered Peg-IFN therapy may choose to receive it, even if it could provide clinical benefit. Fourth, there was no Peg-IFN monotherapy group included for comparison. Fifth, data on antiviral therapy discontinuation at 24–48 weeks are lacking for patients who achieved HBsAg loss. These limitations should be addressed in future study designs.

Although the term may be a misnomer, adults with IT phase of chronic HBV infection remain difficult to treat. The "set goal, not set time" approach, featuring initial, intermittent, and retreatment antiviral strategies, may help improve the rate of HBsAg loss in this specific population.

Conclusions

In conclusion, response rates may be higher in IT phase patients under 30 years of age who receive Peg-IFN-based therapy compared to those receiving NUC monotherapy. Additionally, patients who achieve a decline in HBsAg level of more than 1 $\log_{10}$ IU/mL by week 24 relative to baseline may be considered for extension of Peg-IFN treatment duration to 72–96 weeks. In China, Peg-IFN-based therapy is not routinely used in adults during the IT phase of chronic HBV infection. This study provides evidence to inform and refine clinical decision-making in this understudied IT-phase patients.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (JG, ML, XX), data collection (ML, LZuo, YZ, BB), analysis and interpretation of data (ML, BB, LZhu), statistical analysis (ML, XM, WY, YW), drafting of the manuscript (ML, AX), and critical revision of the manuscript for important intellectual content (JG, XX). All authors have approved the final version and publication of the manuscript.

Ethical statement

The trial was approved by the research ethics committees of the First People's Hospital of Yunnan Province (ethics approval number: KHLL2021-KY051) and conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki (as revised in 2024). All participants provided written informed consent prior to study enrollment. Trial registration number: ChiCTR2100046860.

Data sharing statement

Individual participant data reported in this publication will be available, after de-identification, to researchers who provide an ethically approved research proposal from the corresponding authors upon reasonable request.

References

[1] Polaris Observatory Collaborators. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modelling study. Lancet Gastroenterol Hepatol 2023;8(10):879–907. doi:10.1016/S2468-1253(23)00197-8, PMID:37517414.

[2] WHO. Global health sector strategy on viral hepatitis 2016–2021: towards ending viral hepatitis 2016. Available from: https://www.who.int/publications/i/item/WHO-HIV-2016.06.

[3] Wong RJ, Kaufman HW, Niles JK, Kapoor H, Gish RG. Simplifying Treatment Criteria in Chronic Hepatitis B: Reducing Barriers to Elimination. Clin Infect Dis 2023;76(3):e791–e800. doi:10.1093/cid/ciac385, PMID:35594550.

[4] Zhang S, Wang C, Liu B, Lu QB, Shang J, Zhou Y, *et al*. Cost-effectiveness of expanded antiviral treatment for chronic hepatitis B virus infection in China: an economic evaluation. Lancet Reg Health West Pac 2023;35:100738. doi:10.1016/j.lanwpc.2023.100738, PMID:37424693.

[5] Martin P. Immune-Tolerant Hepatitis B: Maybe a Misnomer but Still Hard to Treat. Hepatology 2019;69(6):2315–2317. doi:10.1002/hep.30654, PMID:30964209.

[6] Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, *et al*. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67(4):1560–1599. doi:10.1002/hep.29800, PMID:29405329.

[7] Hui CK, Leung N, Yuen ST, Zhang HY, Leung KW, Lu L, *et al*. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. Hepatology 2007;46(2):395–401. doi:10.1002/hep.21724, PMID:17628874.

[8] Liu M, Zhao T, Zhang J, Bu B, Zhang R, Xia X, *et al*. Estimating the key outcomes and hepatocellular carcinoma risk in patients in immune-tolerant phase of chronic hepatitis B virus infection: A systematic review and meta-analysis. Rev Med Virol 2024;34(4):e2570. doi:10.1002/rmv.2570, PMID:38964866.

[9] Feld JJ, Terrault NA, Lin HS, Belle SH, Chung RT, Tsai N, *et al*. Entecavir and Peginterferon Alfa-2a in Adults With Hepatitis B e Antigen-Positive Immune-Tolerant Chronic Hepatitis B Virus Infection. Hepatology 2019;69(6):2338–2348. doi:10.1002/hep.30417, PMID:30549279.

[10] Liu N, Yang N, Ma W, Yang S, Hu C, Li J, *et al*. Efficacy of Antiviral Treatment in Liver Biopsy-Proven Immune-Tolerant Chronic Hepatitis B Patients: A Retrospective Cohort Study. Front Med (Lausanne) 2021;8:655530. doi:10.3389/fmed.2021.655530, PMID:33898489.

[11] Wu ZX, Chen FS, Zhou XL, Huang Q, Zhang SA, Wu HC, *et al*. Tenofovir and telbivudine combination therapy rapidly decreases viral loads in immune-tolerant chronic hepatitis B patients awaiting assisted reproduction: an open-label, randomized, controlled study. Eur J Gastroenterol Hepatol 2019;31(7):832–835. doi:10.1097/MEG.0000000000001345, PMID:30601336.

[12] Chang Y, Choe WH, Sinn DH, Lee JH, Ahn SH, Lee H, *et al*. Nucleos(t)ide Analogue Treatment for Patients With Hepatitis B Virus (HBV) e Antigen-Positive Chronic HBV Genotype C Infection: A Nationwide, Multicenter, Retrospective Study. J Infect Dis 2017;216(11):1407–1414. doi:10.1093/infdis/jix506, PMID:29029102.

[13] Lu J, Zhang S, Liu Y, Du X, Ren S, Zhang H, *et al*. Effect of Peg-interferon α-2a combined with Adefovir in HBV postpartum women with normal levels of ALT and high levels of HBV DNA. Liver Int 2015;35(6):1692–1699. doi:10.1111/liv.12753, PMID:25438657.

[14] 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(2):370–398. doi:10.1016/j.jhep.2017.03.021, PMID:28427875.

[15] Tseng TC, Kao JH. Treating Immune-tolerant Hepatitis B. J Viral Hepat 2015;22(2):77–84. doi:10.1111/jvh.12370, PMID:25424771.

[16] Hu X, Luo H, Tan G, Li Y, Qin B. The expression of interleukin-1β in patients with chronic hepatitis B treated with pegylated-interferon-alpha combined with tenofovir disoproxil fumarate and monotherapy. BMC Gastroenterol 2023;23(1):163. doi:10.1186/s12876-023-02812-5, PMID:37208599.

[17] Mieli-Vergani G, Bansal S, Daniel JF, Kansu A, Kelly D, Martin C, *et al*. Peginterferon Alfa-2a (40KD) Plus Lamivudine or Entecavir in Children With Immune-Tolerant Chronic Hepatitis B. J Pediatr Gastroenterol Nutr 2021;73(2):156–160. doi:10.1097/MPG.0000000000003118, PMID:33720089.

[18] European Association for the Study of the Liver (EASL). European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO), European Association for the Study of the Liver (EASL).. EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol 2024;81(3):492–542. doi:10.1016/j.jhep.2024.04.031, PMID:38851997.

[19] Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. Hepatology 2006;43(2 Suppl 1):S173–S181. doi:10.1002/hep.20956, PMID:16447285.

[20] Wong D, Littlejohn M, Edwards R, Jackson K, Revill P, Gaggar A, *et al*. ALT flares during nucleotide analogue therapy are associated with HBsAg loss in genotype A HBeAg-positive chronic hepatitis B. Liver Int 2018;38(10):1760–1769. doi:10.1111/liv.13716, PMID:29427368.

[21] Si-Ahmed SN, Pradat P, Zoutendijk R, Buti M, Mallet V, Cruiziat C, *et al*. Efficacy and tolerance of a combination of tenofovir disoproxil fumarate plus emtricitabine in patients with chronic hepatitis B: a European multicenter study. Antiviral Res 2011;92(1):90–95. doi:10.1016/j.antiviral.2011.07.003, PMID:21767570.

[22] Cao Z, Liu Y, Ma L, Lu J, Jin Y, Ren S, *et al*. A potent hepatitis B surface antigen response in subjects with inactive hepatitis B surface antigen carrier treated with pegylated-interferon alpha. Hepatology 2017;66(4):1058–1066. doi:10.1002/hep.29213, PMID:28407271.

[23] Ning Q, Wu D, Wang GQ, Ren H, Gao ZL, Hu P, *et al*. Roadmap to functional cure of chronic hepatitis B: An expert consensus. J Viral Hepat 2019;26(10):1146–1155. doi:10.1111/jvh.13126, PMID:31087479.

[24] Kim GA, Lim YS, Han S, Choi J, Shim JH, Kim KM, *et al*. High risk of hepatocellular carcinoma and death in patients with immune-tolerant-phase chronic hepatitis B. Gut 2018;67(5):945–952. doi:10.1136/gutjnl-2017-314904, PMID:29055908.

[25] Chan HL, Chan CK, Hui AJ, Chan S, Poordad F, Chang TT, *et al*. Effects of tenofovir disoproxil fumarate in hepatitis B e antigen-positive patients with normal levels of alanine aminotransferase and high levels of hepatitis B virus DNA. Gastroenterology 2014;146(5):1240–1248. doi:10.1053/j.gastro.2014.01.044, PMID:24462735.

[26] Zhu S, Zhang H, Dong Y, Wang L, Xu Z, Liu W, *et al*. Antiviral therapy in hepatitis B virus-infected children with immune-tolerant characteristics: A pilot open-label randomized study. J Hepatol 2018;68(6):1123–1128. doi:10.1016/j.jhep.2018.01.037, PMID:29452204.

[27] Bertoletti A, Kennedy PT. The immune tolerant phase of chronic HBV infection: new perspectives on an old concept. Cell Mol Immunol 2015;12(3):258–263. doi:10.1038/cmi.2014.79, PMID:25176526.

[28] ter Borg MJ, Hansen BE, Bigot G, Haagmans BL, Janssen HL. ALT and viral load decline during PEG-IFN alpha-2b treatment for HBeAg-positive chronic hepatitis B. J Clin Virol 2008;42(2):160–164. doi:10.1016/j.jcv.2008.02.007, PMID:18359663.

[29] Brakenhoff SM, de Knegt RJ, van Campenhout MJH, van der Eijk AA, Brouwer WP, van Bömmel F, *et al*. End-of-treatment HBsAg, HBcrAg and HBV RNA predict the risk of off-treatment ALT flares in chronic hepatitis B patients. J Microbiol Immunol Infect 2023;56(1):31–39. doi:10.1016/j.jmii.2022.06.002, PMID:35941076.

[30] Zhang M, Zhang Z, Imamura M, Osawa M, Teraoka Y, Piotrowski J, *et al*. Infection courses, virological features and IFN-α responses of HBV genotypes in cell culture and animal models. J Hepatol 2021;75(6):1335–1345. doi:10.1016/j.jhep.2021.07.030, PMID:34363922.

[31] Micco L, Peppa D, Loggi E, Schurich A, Jefferson L, Cursaro C, *et al*. Differential boosting of innate and adaptive antiviral responses during pegylated-interferon-alpha therapy of chronic hepatitis B. J Hepatol 2013;58(2):225–233. doi:10.1016/j.jhep.2012.09.029, PMID:23046671.

[32] Chinese Society of Hepatology, Chinese Medical Association, Chinese Society of Infectious Diseases, Chinese Medical Association. [Guidelines for the prevention and treatment of chronic hepatitis B (version 2022)]. Zhonghua Gan Zang Bing Za Zhi 2022;30(12):1309–1331. doi:10.3760/cma.j.cn501113-20221204-00607, PMID:36891718.

[33] Kennedy PTF, Sandalova E, Jo J, Gill U, Ushiro-Lumb I, Tan AT, *et al*. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143(3):637–645. doi:10.1053/j.gastro.2012.06.009, PMID:22710188.

[34] Ning Q, Han M, Sun Y, Jiang J, Tan D, Hou J, *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(4):777–784. doi:10.1016/j.jhep.2014.05.044, PMID:24915612.

[35] Hu P, Shang J, Zhang W, Gong G, Li Y, Chen X, *et al*. HBsAg Loss with Peg-interferon Alfa-2a in Hepatitis B Patients with Partial Response to Nucleos(t)ide Analog: New Switch Study. J Clin Transl Hepatol 2018;6(1):25–34. doi:10.14218/JCTH.2017.00072, PMID:29577029.

[36] Gan W, Gao N, Gu L, Mo Z, Pang X, Lei Z, *et al*. Reduction in Intrahepatic cccDNA and Integration of HBV in Chronic Hepatitis B Patients with a Functional Cure. J Clin Transl Hepatol 2023;11(2):314–322. doi:10.14218/JCTH.2022.00177, PMID:36643049.

[37] Song A, Lin X, Lu J, Ren S, Cao Z, Zheng S, *et al*. Pegylated Interferon Treatment for the Effective Clearance of Hepatitis B Surface Antigen in Inactive HBsAg Carriers: A Meta-Analysis. Front Immunol 2021;12:779347. doi:10.3389/fimmu.2021.779347, PMID:34804072.

[38] Hou J, Zhang W, Xie Q, Hua R, Tang H, Morano Amado LE, *et al*. Xalnesiran with or without an Immunomodulator in Chronic Hepatitis B. N Engl J Med 2024;391(22):2098–2109. doi:10.1056/NEJMoa2405485, PMID:39774313.