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ORIGINAL RESEARCH

# Long-Term Telbivudine Treatment Results in Resolution of Liver Inflammation and Fibrosis in Patients with Chronic Hepatitis B

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## ABSTRACT

***Introduction***: The long-term goal of chronic hepatitis B (CHB) treatment is improvement of liver disease and prevention of cirrhosis. The aim of this study was to assess whether prolonged telbivudine treatment improves liver inflammation and fibrosis. The primary objective was to evaluate the proportion of patients with absence/minimal inflammation (Knodell necroinflammatory score ≤3) on liver biopsy at Year 5.

***Methods***: Fifty-seven patients aged 16–70 years with a clinical history of CHB and active viral replication (38 hepatitis B e antigen [HBeAg] positive and 19 HBeAg negative) were followed for 6 years: 33 received telbivudine 600 mg/day continuously for 5 years; 24 received lamivudine 100 mg/day for 2 years and then telbivudine for 3 years. Liver biopsies were taken pre-treatment and after 5 years of treatment.

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***Results***: At baseline, mean (standard deviation) serum hepatitis B virus (HBV) DNA load was 8.5 (1.7) $\log_{10}$ copies/mL, Knodell necroinflammatory score was 7.6 (2.9), and Ishak fibrosis score was 2.2 (1.1). After antiviral treatment (median duration: 261 weeks), liver histology improved with increased proportions of patients with absence/minimal liver inflammation (Knodell necroinflammatory score $\leq$3), from 16% (9/57) at baseline to 98% (56/57), and absence/minimal fibrosis (Ishak score $\leq$1), from 25% (14/57) at baseline to 84% (48/57). At Year 5, HBV DNA load was <300 copies/mL for all patients; cumulative HBeAg loss and seroconversion rates were 88% and 77%, respectively. At Year 6, 95% of patients with abnormal baseline glomerular filtration rate (60–90 mL/min/1.73 $m^2$) improved to normal GFR (>90 mL/min/1.73 $m^2$).

***Conclusion***: Long-term telbivudine treatment with profound and durable viral suppression significantly improved liver histology, thus achieving the long-term goals of CHB treatment. FibroScan® results after 5 and 6 years of treatment (in almost 20% of patients) were consistent with this information.

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**Keywords:** Chronic hepatitis B; Ishak fibrosis score; Knodell necroinflammatory score; Liver biopsy; Telbivudine

## INTRODUCTION

More than 240 million individuals are chronically infected with hepatitis B virus (HBV) worldwide. The prevalence of hepatitis B is high in sub-Saharan Africa and East Asia, where it exceeds >8%. About 80–90% of patients in the Asia–pacific region became infected with HBV during childhood, and 5–10% of the adult population is chronically infected. It is estimated that about 600,000 deaths occur each year due to acute or chronic hepatitis B (CHB) [1]. Between 15% and 20% of patients with chronic HBV replication will develop cirrhosis within 5 years [2, 3]. The REVEAL study demonstrated that progression to cirrhosis in hepatitis B-infected persons is strongly correlated with levels of HBV replication and serum HBV DNA level, with a DNA load $\geq$10,000 copies/mL a strong risk predictor of hepatocellular carcinoma [4, 5]. Moreover, increased serum HBV DNA levels also increased the mortality risk for all causes or causes related to chronic liver disease [6]. Several other studies have also correlated serum HBV DNA concentrations with the progression of chronic liver disease [7–10].

Viral replication is now recognized as the key driver of liver injury, and international guidelines recommend that permanent suppression of HBV replication is the primary aim of CHB treatment [1, 11, 12]. The ultimate goal of antiviral therapy is to improve survival by preventing liver disease progression to cirrhosis, liver failure, and hepatocellular carcinoma [12]. Liver fibrosis is a tissue-repair response that involves a range of cell types including mediators to encapsulate injury [13]. Although

previously considered irreversible, recent studies indicate that profound and durable viral suppression is associated with the regression of liver fibrosis, which improves clinical outcomes in patients with CHB or chronic hepatitis C [14–16].

Telbivudine provides effective treatment in CHB patients, as shown in randomized, double blind, multicenter phase 3 trials. The 2-year GLOBE study demonstrated the superiority of telbivudine 600 mg/day over lamivudine 100 mg/day with greater HBV DNA suppression and less genotypic resistance [17, 18]. Study 015, with a similar design to the GLOBE study, was conducted entirely in China and also showed greater antiviral and clinical efficacy responses for telbivudine, with less resistance, when compared with lamivudine after 2 years of treatment [19]. Telbivudine-treated hepatitis B e antigen (HBeAg)-positive patients achieved a higher cumulative seroconversion rate, suggesting long-lasting benefits of telbivudine treatment [20].

Long-term treatment with oral antiviral agents, such as lamivudine, adefovir, entecavir, and tenofovir slows the clinical and histologic progression of CHB in patients with advanced fibrosis and cirrhosis [14, 21–25]. However, no information is available on liver histology after long-term treatment with telbivudine. This study evaluated the impact of telbivudine treatment on liver inflammation and fibrosis in CHB patients after 5 years.

## METHODS

### Study Design

The study cohort consisted of patients who completed 2 years of telbivudine or lamivudine treatment in the phase 3 GLOBE or 015 studies, and subsequently continued with telbivudine for an additional 4 years (study CN04E1, ClinicalTrials.gov # NCT00877149). Liver biopsies were taken at baseline in the phase 3 studies and after 5 years of antiviral treatment (Fig. 1). The study was approved by each local independent ethics committee. Institutional Review Board and all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Written informed consent was obtained from each patient.

Studies were registered with the ClinicalTrials.gov identifiers NCT00057265 (GLOBE study), NCT00131742 (study 015), NCT00142298 (study 2303), and NCT00646503 (study CN04).

### Study Population

Detailed inclusion and exclusion criteria in GLOBE/015 studies have been described earlier [18, 19]. The key inclusion criteria were patients between 16 and 70 years of age with a clinical history of CHB and active viral replication. Patients who were willing to undergo a liver biopsy at Visit 2 (day 7 from baseline) and FibroScan® (Echosens, Paris, France) at Visits 2 and 4 (Week 52) were included in the study. Patients with the following abnormal laboratory values were excluded: hemoglobin <11 g/dL for men or <10 g/dL for women; absolute neutrophil count <1500/mm$^3$; platelet count <75,000/mm$^3$; serum creatinine ≥1.5 mg/dL; serum amylase or lipase ≥1.5 × upper limit of normal (ULN); prothrombin time prolonged by >3 s or prothrombin activity <70%; serum albumin <3.3 g/dL; or total bilirubin ≥2.0 mg/dL. At the screening visit, the following were

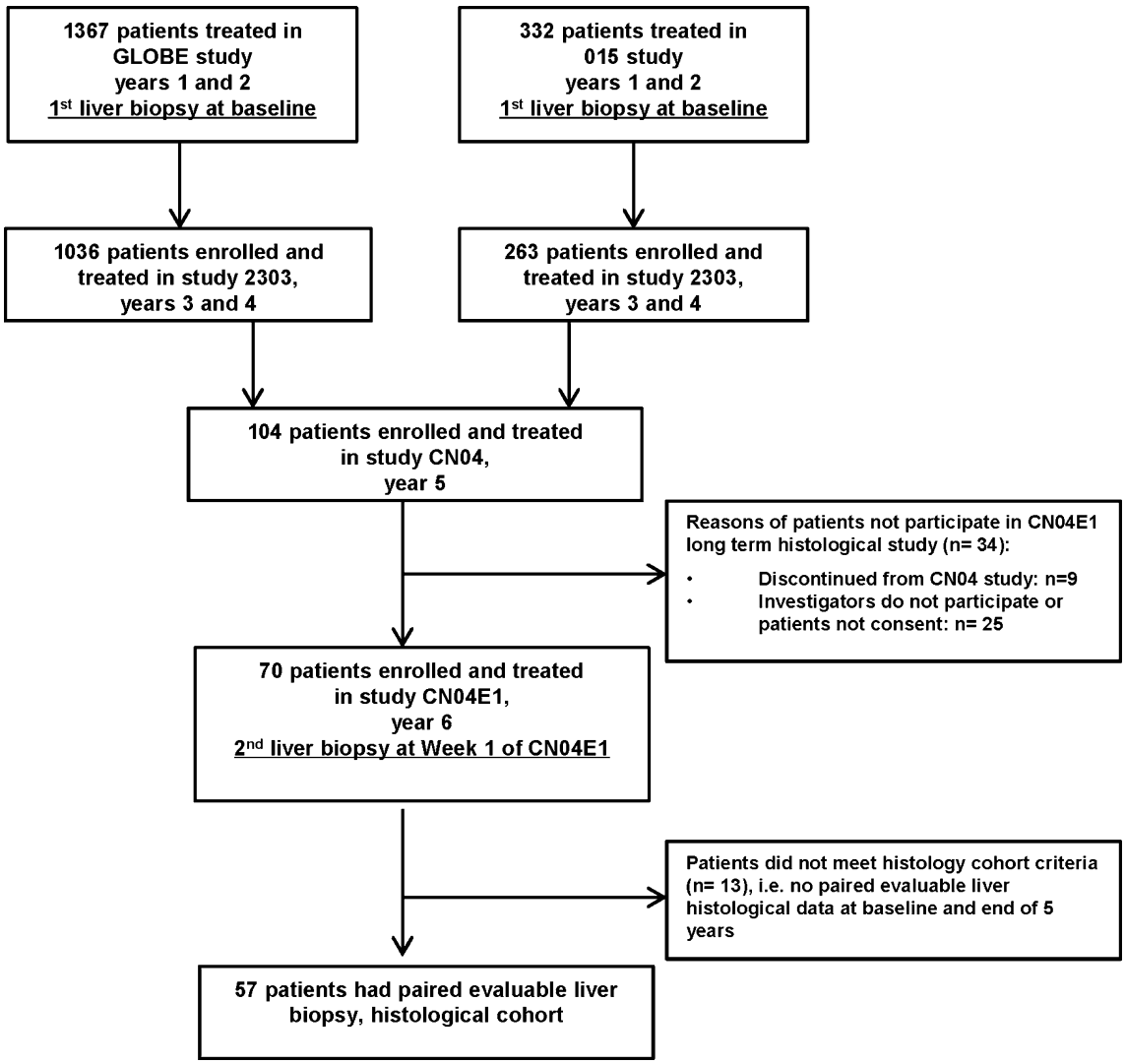


**Fig. 1** Patient flow diagram

documented: positive serum hepatitis B surface antigen (HBsAg), HBeAg positive or HBeAg negative, serum HBV DNA load $\geq 6$ $\log_{10}$ copies/mL, serum alanine aminotransferase (ALT) level $\geq 1.3 \times$ ULN but $< 10 \times$ ULN and a liver biopsy compatible with CHB obtained within 12 months prior to randomization.

## Histologic Evaluations

Histologic evaluation of the liver biopsies was performed centrally at the Armed Forces Institute of Pathology (Washington, DC) by a single pathologist who was blinded to the clinical and laboratory data of patients. Assessments were also made to calculate the proportion of patients with absence of inflammation on liver biopsy at study entry.

The Knodell histology activity index was used for the assessment of liver inflammation, with biopsy specimens graded in four categories: periportal necrosis (0–10), intralobular necrosis (0–4), portal inflammation (0–4), and fibrosis (0–4) [25]. The Knodell necroinflammatory score

(maximum of 18 points) is derived by adding the above scores, excluding the fibrosis score. A Knodell necroinflammatory score ≤3 represents absence/minimal inflammation of liver biopsy [24, 25]. To ensure a "reference standard", Prof. Zachary Goodman's hospital rules and guidelines on liver biopsy assessment (Center for Liver Diseases, Inova Fairfax Hospital, Falls Church, VA, USA) were followed. The protocol was established as the current practice in our hospital. Biopsies that did not match the criteria were excluded from the analysis.

Stages of fibrosis were assessed using the Ishak fibrosis score that ranges from 0 (no fibrosis) to 6 (cirrhosis; probable or definite) [26]. Changes in Ishak fibrosis score from baseline were categorized as: improvement (≥1-point decrease), worsening (≥1-point increase), or no change (post-baseline score = baseline score). Absence/minimal fibrosis of liver biopsy was defined as Ishak score ≤1; advanced fibrosis/cirrhosis was defined as Ishak score ≥4.

### Virologic, Serologic, and Biochemical Evaluations

Serum HBV DNA quantitation was performed at a central laboratory using the COBAS Amplicor™ HBV assay (Roche Diagnostics, Pleasanton, CA, USA) that utilizes polymerase chain reaction (PCR) methods and automated sample readout technologies (limit of detection: 300 copies/mL). Other HBV serologic markers (HBsAg/anti-HBs, HBeAg/anti-HBe) using standard commercially available assays were also assessed centrally.

### Serum Fibrosis Markers

Serum fibrosis markers were not assessed at baseline in the GLOBE/015 studies. These were centrally tested at baseline and Week 52 of CN04E1 study period, namely Year 5 or Year 6 of long-term antiviral treatment, using standard commercially available assays. Serum concentrations of procollagen III peptide and collagen IV were measured by radioimmunoassay (P III P RIA assay; Cis Bio International, Gif-sur-Yvette, France) and enzyme immunoassay (Type IV collagen EIA assay; Argutus Medical, Dublin, Ireland), respectively.

### Transient Elastography Measurements

Transient elastography (FibroScan) [27, 28] was used for diagnosis and overall assessment of hepatic fibrosis (by measuring liver stiffness) in a subgroup of 14 patients included in study CN04E1. No measurements at baseline or during the first 5 years of treatment were available.

### Safety

The assessment of safety was based on the analysis of adverse events (AEs), including serious AEs (SAEs) and AEs of special interest: muscle weakness, myopathy or myositis, and peripheral nephropathy, laboratory evaluations, vital signs, and physical examinations.

### Glomerular Filtration Rate Evaluations

Estimates of glomerular filtration rate eGFR) are the best indices of kidney function [29–31]. eGFR is estimated using prediction equations that take into account the serum creatinine concentration and some or all of the following variables: age, gender, race, and body size. In adults, Modification of Diet in Renal Disease (MDRD) equation [32] or the Cockcroft–Gault equation are often used. This study used the

MDRD equation because it is more accurate and precise for persons with GFR <90 mL/min per 1.73 $m^2$. The MDRD equation is as follows:

$$\text{eGFR} = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}).$$

### Statistical Analysis

The safety population consisted of all patients who received at least one dose of study drug in study CN04E1. All safety analyses were performed on the safety population throughout the whole telbivudine treatment period. The histology analysis was performed on the histology population of enrolled patients who had paired evaluable liver biopsy results at both feeder study baseline and study CN04E1 entry (or at the end of study 2303 if study CN04E1 entry biopsy was not available).

The primary endpoint was the proportion of patients with absence/minimal inflammation of liver biopsy at Year 5. The main secondary histologic endpoints were: mean change from baseline in the Knodell necroinflammatory score and Ishak fibrosis score; proportion of patients who achieved final Knodell score ≤3 points (in patients with baseline score ≥4); absence/minimal fibrosis of liver biopsy at Year 5; ≥1-point decrease of Ishak fibrosis; and change of Ishak score in patients with baseline advanced fibrosis/cirrhosis.

Changes of Ishak fibrosis score and Knodell necroinflammatory score from baseline were tested for statistical significance using Wilcoxon signed-rank test. Changes in HBV DNA level, ALT level, serum fibrosis markers, and eGFR (MDRD) were tested for significance using paired *t* test. All statistical tests were bilateral with a 0.05 alpha level of significance.

## RESULTS

The study population included 70 patients, of which 66 had at least one post-baseline assessment of serum HBV DNA (intent-to-treat population). Of these, 57 patients had paired evaluable liver biopsies at both baseline and after 5 years of antiviral treatment. The baseline characteristics of these 57 patients (Table 1) were similar in disease features to those in the GLOBE study and study 015. All patients were Chinese with HBV genotype either B or C. Mean baseline HBV DNA load was 8.5 $\log_{10}$ copies/mL, Knodell necroinflammatory score was 7.6, and the Ishak fibrosis score was 2.2. Six of the 57 patients (11%) had an Ishak fibrosis score ≥4, indicating advanced fibrosis or cirrhosis. Thirty-three patients received telbivudine 600 mg/day for 5 years and 24 patients received lamivudine 100 mg/day for 2 years followed by telbivudine for 3 years. Median duration of treatment was 261 weeks.

### Assessment of Liver Inflammation

Long-term treatment with telbivudine resulted in marked improvement of liver inflammation. Figure 2a shows microphotographs of biopsy samples from a representative patient whose Knodell score decreased from 10 (at baseline) to 1 (minimal inflammation) after long-term treatment with telbivudine. This observation was consistent among all 57 patients (Fig. 3a).

Long-term treatment resulted in effective control of HBV replication. The proportion of patients with no or minimal necroinflammation (defined as Knodell necroinflammatory score ≤3) increased from 16% (9/57) at baseline to 98% (56/57) after long-term treatment (Table 2). The mean change in baseline Knodell necroinflammatory

**Table 1** Baseline demographics and disease characteristics of CHB patients treated with telbivudine

| Characteristics | HBeAg positive ($n = 38$) | HBeAg negative ($n = 19$) | Total ($N = 57$) |
|---|---|---|---|
| Age, mean (SD), years | 26.9 (8.3) | 35.9 (9.7) | 29.9 (9.7) |
| Sex, male, *n* (%) | 33 (87) | 14 (74) | 47 (83) |
| Chinese patients, *n* (%) | 38 (100) | 19 (100) | 57 (100) |
| Genotype, *n* (%) | | | |
| B/C | 37 (100)[a] | 19 (100) | 56 (100) |
| A/D | 0 (0) | 0 (0) | 0 (0) |
| HBV DNA, mean (SD), $\log_{10}$ copies/mL | 9.1 (1.4) | 7.4 (1.7) | 8.5 (1.7) |
| $\geq 9$ $\log_{10}$ copies/mL, *n* (%) | 24 (63) | 5 (26) | 29 (51) |
| $<9$ $\log_{10}$ copies/mL, *n* (%) | 14 (37) | 14 (74) | 28 (49) |
| Serum ALT, mean (SD), IU/mL | 225 (151) | 143 (117) | 198 (145) |
| $<1 \times$ ULN, *n* (%) | 0 (0) | 3 (16) | 3 (5) |
| $\geq 1$ and $< 2 \times$ ULN, *n* (%) | 4 (11) | 8 (42) | 12 (21) |
| $\geq 2$ and $< 5 \times$ ULN, *n* (%) | 21 (55) | 3 (16) | 24 (42) |
| $\geq 5 \times$ ULN, *n* (%) | 13 (34) | 5 (26) | 18 (32) |
| Knodell necroinflammatory score, mean (SD) | 7.7 (2.6) | 7.6 (3.5) | 7.6 (2.9) |
| Ishak fibrosis score, mean (SD) | 2.1 (0.8) | 2.4 (1.6) | 2.2 (1.1) |

*ALT* alanine aminotransferase, *HBV* hepatitis B virus, *SD* standard deviation, *ULN* upper limit of normal, *HBeAg* hepatitis B e antigen

[a] Genotype was missing for one patient

score was 6.3-point reduction after long-term treatment with telbivudine ($p < 0.0001$). Among patients with a baseline Knodell necroinflammatory score $\geq 4$, almost all (98%; 47/48) achieved a Knodell score $\leq 3$ on long-term (Year 5) biopsy samples.

## Assessment of Liver Fibrosis

Long-term telbivudine treatment resulted in reversal of liver fibrosis (Fig. 2b). Figure 3b represents the evolution of Ishak fibrosis scores in all patients during long-term treatment. The proportion of patients with no or minimal fibrosis (defined as Ishak fibrosis score $\leq 1$) increased from 25% (14/57) at baseline to 84% (48/57) after long-term treatment (75%) (Table 2). The mean change from baseline in the Ishak fibrosis score was a 1.3-point reduction ($p < 0.0001$). The proportion of patients with $\geq 1$-point decrease of Ishak fibrosis score was 80% (44/55; baseline score $\geq 1$). Among patients with a baseline Ishak fibrosis score $\geq 2$, 84% (36/43) achieved an Ishak fibrosis score of 0 or 1 on the long-term biopsy samples.

Out of 57 patients, six (11%) had advanced fibrosis or cirrhosis at feeder study baseline: four males and two females; four with genotype C and one with genotype B (data missing for one patient); four were HBeAg negative and two were HBeAg positive. All six patients achieved undetectable HBV DNA at Week 24 (baseline range 5.18–9.81 $\log_{10}$ copies/mL). Of these, two

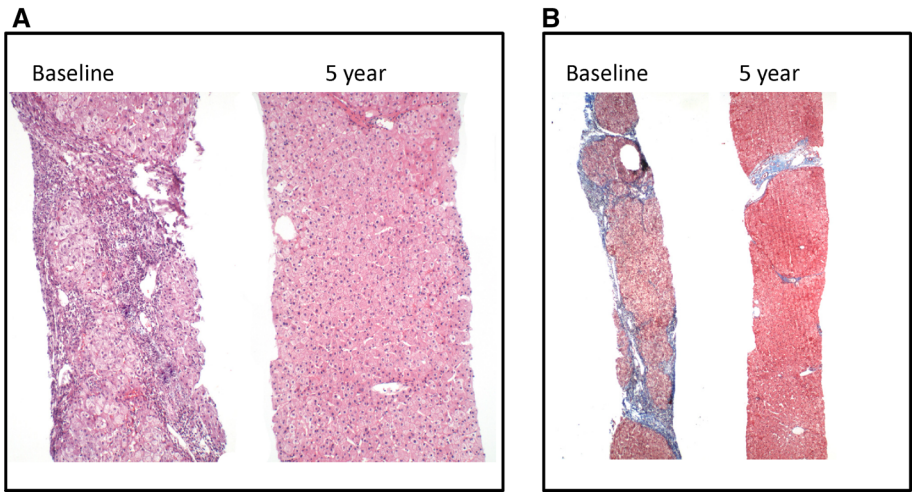


**Fig. 2** Liver biopsy samples: **a** Baseline liver biopsy (*left*) showing marked inflammation (Knodell inflammation score = 10); long-term follow up biopsy (*right*) with minimal inflammation (Knodell inflammation score = 1) (hemotoxylin and eosin stain; magnification ×100), and **b** baseline liver biopsy (*left*) showing incomplete cirrhosis (Ishak fibrosis score = 5); long-term follow up biopsy (*right*) with focal portal fibrosis (Ishak fibrosis score = 1) (Masson trichrome stain; magnification ×40)

patients stopped treatment at Year 6, and at 24 weeks post-treatment; undetectable HBV DNA was maintained in one patient with a DNA load of 2.88 $\log_{10}$ copies/mL for the other. At the end of 5 years' treatment, all patients had Knodell necroinflammatory scores ≤3 (baseline range 7–11) and an Ishak fibrosis score of 3 (baseline range 4–6). Median score reduction of Ishak fibrosis score was 3 (range 1–3; $p = 0.0313$). Procollagen III peptide and collagen IV normalized in all six patients.

## Serum Liver Fibrosis Markers

The mean (SD) levels of procollagen III peptide were 0.46 (0.18) U/mL at Year 5, and 0.45 (0.09) U/mL at Year 6 of prolonged treatment, without significant change ($p = 0.5031$). Both values were within the normal range of 0.3–0.8 U/mL. The mean (SD) levels of collagen IV were 90.0 (18.0) μg/L at Year 5 and increased to 111.1 (24.1) μg/L at the end of Year 6 ($p < 0.0001$), both values were within the normal range (range 53–145 μg/L).

## Liver Stiffness Measurements

In a subgroup of 14 patients with available data, liver stiffness measurement (LSM) from study CN04E1 entry to Week 52 (Year 5 to 6) decreased from 5.45 kPa (Year 5) to 4.75 kPa (Year 6) during treatment. At Week 24 post-treatment, the median LSM was 4.90 kPa.

## Virologic and Biochemical Response

At the time of long-term biopsy, 100% ($n = 66$) of patients had undetectable HBV DNA as determined by PCR. The median HBV DNA level decrease was −7.6 $\log_{10}$ copies/mL in HBeAg-positive patients ($p < 0.0001$) and −5.3 $\log_{10}$ copies/mL in HBeAg-negative patients ($p < 0.0001$). The median time to maintained PCR negativity (defined as HBV DNA <300 copies/mL for at least two consecutive visits) was 168 days in HBeAg-positive patients and 116 days in HBeAg-negative patients. After 6 years (2190 days) of treatment, median duration of maintained PCR negativity was

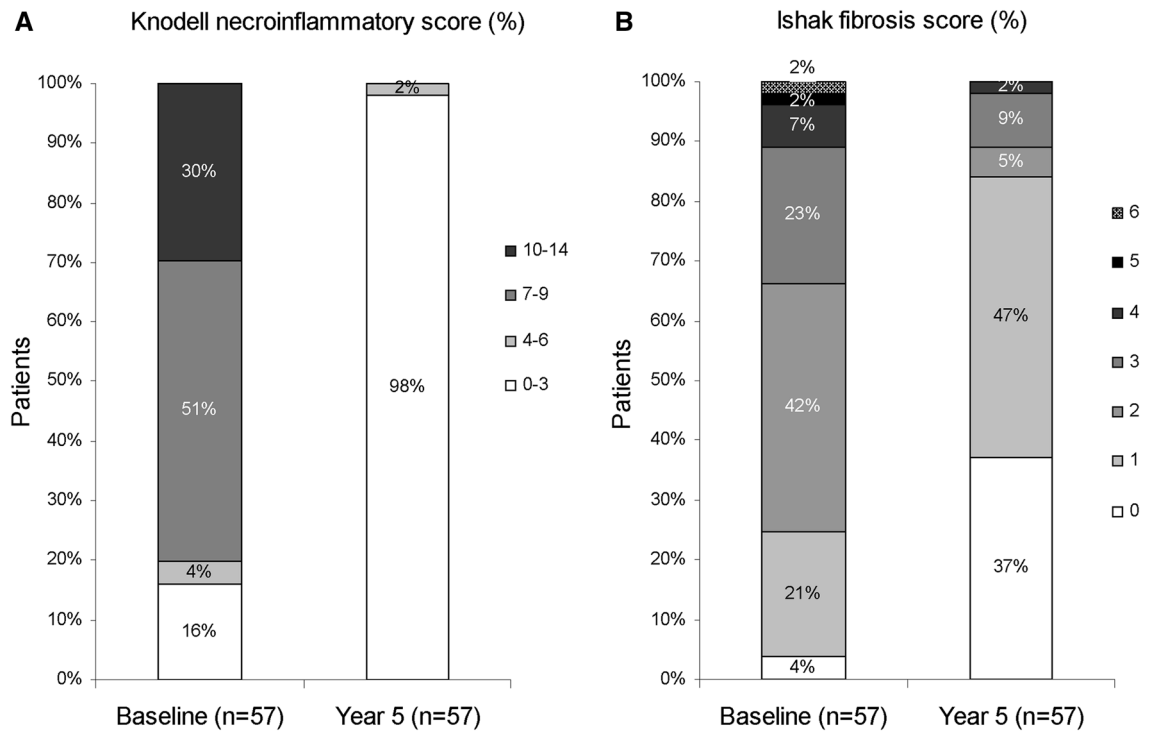


**Fig. 3** Distribution of **a** Knodell necroinflammatory score, and **b** Ishak score, at phase 3 baseline and after long-term treatment with telbivudine (5 years for 33 patients and 3 years for 24 patients after 2 years with lamivudine) for nucleoside-naïve CHB patients with histologically evaluable-paired biopsies

2019 days in HBeAg-positive patients and 2030 days in HBeAg-negative patients.

The proportion of patients who achieved serum ALT normalization at the time of long-term biopsy was 82% (50/61). Mean (SD) serum ALT levels decreased significantly from 201 (145) at feeder study baseline to 30 (18) IU/mL at the time of long-term biopsy ($p < 0.0001$).

## Changes in HBeAg and HBsAg

Among the 43 HBeAg-positive patients at baseline, 88% (38/43) achieved cumulative HBeAg loss and 77% (33/43) achieved HBeAg seroconversion during the long-term treatment period. The median time to maintained HBeAg loss (defined as HBeAg loss for at least two consecutive visits) was 703 days. Cumulative HBsAg loss was achieved in 6.1% of patients (4/66) (Table 2).

## Safety Evaluations

Among the safety population, telbivudine was well tolerated over the 6-year treatment period (95.7% of patients received telbivudine for ≥208 weeks). Eight patients (11.4%) reported SAEs (3 surgeries, 3 infections, 1 polymyositis, and 1 abortion); none of them were drug related. The patient with polymyositis recovered within 6 months while continuing telbivudine treatment. No death occurred during the study period (6 years). No AE caused permanent drug discontinuation.

**Table 2** Histologic, virologic, and biochemical responses of telbivudine-treated patients at the time of long-term biopsy

| | **Response rates, *n* (%)** |
|---|---|
| Liver inflammation response | $N = 57$ |
| Baseline Knodell necroinflammatory score ≥4/ ≤3 | 48/57 (84)/9/57 (16) |
| Absence/minimal inflammation in liver biopsy[a] | 56/57 (98) |
| Mean (SD) change from the baseline in the Knodell necroinflammatory score | −6.3 (2.8) |
| Knodell necroinflammatory score ≤3 in patients with a baseline score ≥4, *n* (%) | 47/48 (98) |
| Liver fibrosis response | $N = 57$ |
| Baseline Ishak fibrosis score ≥1 | 55/57 (96.5) |
| Baseline Ishak fibrosis score ≥2/ ≤1 | 43/57 (75)/14/57 (25) |
| Absence/minimal fibrosis in liver biopsy[b], *n* (%) | 48/57 (84) |
| Mean (SD) change from baseline in the Ishak fibrosis score | −1.3 (1.3) |
| ≥1-point decrease in patients with baseline score ≥1, *n* (%) | 44/55 (80) |
| ≥1-point decrease in patients with baseline score ≥2, *n* (%) | 36/43 (84) |
| Virologic, serologic, and biochemical responses | $N = 66$ |
| Serum HBV DNA <300 copies/mL, *n* (%) | 66/66 (100) |
| Cumulative HBeAg loss, *n* (%) | 38/43 (88) |
| Cumulative HBeAg seroconversion, *n* (%) | 33/43 (77) |
| Cumulative HBsAg loss, *n* (%) | 4/66 (6) |
| Cumulative HBsAg seroconversion, *n* (%) | 3/66 (5) |

*SD* standard deviation, *ULN* upper limit of normal, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus
[a] Defined as Knodell necroinflammatory score ≤3
[b] Defined as Ishak score ≤1

The most frequent AE of special interest was blood creatine kinase increase (11.4%; 8 patients). One patient (1.4%) experienced muscular weakness. According to the investigator's judgment, this moderately severe muscle-related AE was linked to telbivudine treatment. There were no severe AEs of special interest observed. There were no reports of myopathy, rhabdomyolysis, lactic acidosis, or pancreatitis as an AE in this study. On-treatment ALT flares (AASLD definition) [33] were reported in 17.1% (12/70) of patients, mainly during the first 6 months of treatment.

## Glomerular Filtration Rate Evaluations

GFR, assessed using the MDRD equation, increased steadily from baseline to the end of 6 years of treatment. Mean GFR increased by 60.5% at 6 years (Fig. 4). At Week 312 (year 6), 94.9% of patients with baseline GFR 60–90 mL/min/1.73 $m^2$ (mild renal insufficiency) shifted to GFR >90 mL/min/1.73 $m^2$. There was only

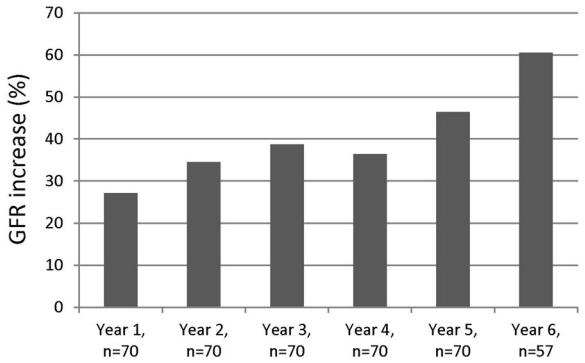


**Fig. 4** Glomerular filtration rate (GFR) improvement assessed by Modification of Diet Renal Disease (MDRD) change from baseline to year 6. The graph presents the percentage GFR increase for patients treated with telbivudine from Year 1 to Year 6

one HBeAg-negative patient who had presented with GFR <60 mL/min/1.73 $m^2$ at baseline and this patient shifted to GFR >90 mL/min/1.73 $m^2$ after 6 years of treatment. No patient with baseline GFR >90 mL/min/1.73 $m^2$ shifted to GFR 60–90 mL/min/1.73 $m^2$ at Year 6.

## DISCUSSION

The present study demonstrated that prolonged telbivudine treatment effectively controls HBV replication. This results in marked improvement of liver histology, resolution of inflammation, improvement (reversal) of liver fibrosis, and extends previous findings. In the GLOBE phase 3 study, histologic response (defined as a reduction of ≥2 points in the Knodell necroinflammatory score) was observed in 64.7% of patients as early as Week 48 [18]. Among patients who had marked fibrosis/cirrhosis, the Ishak fibrosis score improved (Ishak score ≤3) in 68% and 59% of HBeAg-negative and HBeAg-positive patients, respectively [18].

In the present analysis, the long-term improvement on fibrosis in nucleos(t)ide-naïve CHB patients was confirmed after a median exposure of 5 years. In patients with baseline Ishak score ≥1, 80% (44/55) had ≥1-point decrease after 5 years of treatment; in 43 patients with baseline Ishak score ≥2, 84% (36/43) presented ≥1-point decrease after 5 years of treatment. Six patients with advanced fibrosis at baseline showed marked reduction of Ishak score (median reduction of 3.0). This further highlights the potential benefit of successfully controlling HBV replication in the reversal of advanced liver fibrosis. Improvements in the liver biopsies correlated with normalized serum liver fibrosis markers, along with the normal FibroScan® values in a subgroup of patients after long-term treatment.

A high proportion of patients achieved HBeAg loss/seroconversion (88%/77%) [24]. Previous long-term histologic trials reported an improvement of necroinflammation and fibrosis, respectively, in 56% and 24% of patients treated with lamivudine (3 years); 67% and 60% of HBeAg-positive patients and 83% and 71–73% of HBeAg-negative patients treated with adefovir (5 years), respectively [14, 22, 23]. In a recent large-scale prospective study examining changes in liver histology during tenofovir treatment, paired biopsies from 348 patients showed that 176 (51%) patients demonstrated regression of fibrosis after 5 years of treatment [34]. The effects of telbivudine in regression of fibrosis (80–84% improvement in fibrosis) showed consistent efficacy reported with other antiviral trials.

Although these long-term histologic trials are not directly comparable, data from a 6-year entecavir trial suggested that telbivudine achieved numerically better histologic improvement, particularly in patients with high necroinflammation score at baseline: 98% and 75% of patients in telbivudine and entecavir treatment cohorts, respectively,

(baseline Knodell score ≥4) achieved necroinflammatory score ≤3. The proportion of patients with ≥1-point decrease in Ishak fibrosis score (baseline Ishak score ≥2) was 84% for telbivudine and 72% for entecavir. Better serologic results in the long-term cohorts were also in favor of telbivudine versus entecavir: HBeAg loss (88% vs. 55%, respectively), HBeAg seroconversion (77% vs. 33%), HBsAg loss (6% vs. 0%), and HBsAg seroconversion (5% vs. 0%). Moreover, the higher HBsAg loss is important since there were more patients with genotypes B/C in the telbivudine cohort (100% vs. 60% in entecavir cohort). Patients with genotype A/D are more likely to lose HBsAg compared with genotype B/C [35, 36]. As previously reported, the overall rate of HBsAg loss in telbivudine-treated patients with HBV genotype A/D (4/21, 19.0%) was significantly higher than in patients with genotype B/C (5/141, 3.5%; $p = 0.0174$) [37]. The significant improvement of renal function in CHB patients, especially with abnormal eGFR at baseline, shows potential benefit of telbivudine long-term treatment [34, 38]. The mechanism is not fully established and is under investigation.

The limited number of patients ($n = 57$) with available liver histology results at both baseline and post-treatment is mainly due to the lack of prospective design of investigating liver histologic change after long-term antiviral therapy in the telbivudine clinical development program. When the phase 3 (GLOBE and 015) studies were designed, only baseline liver biopsy or 1-year biopsy (for GLOBE study only) was required. During the implementation of these studies, after Novartis took over the ownership of this study from Idenix, Novartis made an amendment with the main objective of collecting post-treatment biopsy samples after 4 years of treatment in study CLDT600A2303. Many patients were lost because the effective date of the amendment was after the last patient's last visit. Meanwhile, as the Chinese study CN04 (5 year treatment) had enrolled the majority of Chinese patients after treatment in study 2303, an extension study was made to the CN04 study to collect post long-term treatment liver biopsy samples, which was the current CLDT600ACN04E1 study.

## CONCLUSION

In conclusion, long-term telbivudine treatment for nucleos(t)ide-naïve patients, including those with advanced fibrosis or cirrhosis at baseline, led to maintained suppression of HBV DNA, favorable cumulative HBeAg/HBsAg responses, and significant improvement of liver inflammation. Regression of fibrosis was also observed, which is supported by normalized levels of serum fibrosis marker and FibroScan® values. Histologic improvement observed at the time of the long-term biopsy was higher than the 1-year treatment values, thus substantiating the interest of long-term telbivudine treatment in CHB. Therefore, the present data support the conclusion that long-term treatment with telbivudine results in profound and durable virologic suppression and significant improvement in liver histology, thus achieving the ultimate antiviral treatment goals in CHB patients. The FibroScan® results at 5 and 6 years (in almost 20% of patients) were consistent with this information.

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***Conflict of interest.*** J-LH is on the Speakers' bureau for Roche, GSK, BMS, and Novartis, and has received contracts/grants from Roche, GSK, and Novartis. LW has received funding from BMS and Roche, and consulting fees from Abbvie, Gilead, JNJ, and BMS. DX, GS, MW, ZG, DT, QX, CC, JN, QW, HR, YW, and JJ have no conflict of interest. WB, YD, AT, and NVN are employees of Novartis.

***Compliance with ethics guidelines.*** The study was approved by each local independent ethics committee. Institutional Review Board and all procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008. Written informed consent was obtained from each patient.



## REFERENCES

1. World Health Organization (WHO). Hepatitis B-Fact sheet. 2013. http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed Jan 24, 2014.
2. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology. 1988;8:493–6.
3. Fattovich G, Brollo L, Giustina G, et al. Natural history and prognostic factors for chronic hepatitis type B. Gut. 1991;32:294–8.
4. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130:678–86.
5. Chen CJ, Yang HI, Su J, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.
6. Iloeje UH, Yang HI, Jen CL, et al. Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol. 2007;5:921–31.
7. Yu MW, Yeh SH, Chen PJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst. 2005;97:265–72.
8. Ohata K, Hamasaki K, Toriyama K, Ishikawa H, Nakao K, Eguchi K. High viral load is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Gastroenterol Hepatol. 2004;19:670–5.
9. Liu CJ, Chen BF, Chen PJ, et al. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis. 2006;193:1258–65.
10. Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic

liver disease in a prospective study. Am J Gastroenterol. 2006;101:1797–803.

11. Liaw YF, Leung N, Kao JH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263–83.

12. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol. 2009;50:227–42.

13. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2008;134:1655–69.

14. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology. 2006;131:1743–51.

15. Veldt BJ, Heathcote EJ, Wedemeyer H, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007;147:677–84.

16. Bruno S, Stroffolini T, Colombo M, et al. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. Hepatology. 2007;45:579–87.

17. Liaw YF, Gane E, Leung N, et al. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology. 2009;136:486–95.

18. Lai CL, Gane E, Liaw YF, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med. 2007;357:2576–88.

19. Hou J, Yin YK, Xu D, et al. Telbivudine versus lamivudine in Chinese patients with chronic hepatitis B: results at 1 year of a randomized, double-blind trial. Hepatology. 2008;47:447–54.

20. Hsu CW, Chen YC, Liaw YF, et al. Prolonged efficacy and safety of 3 years of continuous telbivudine treatment in pooled data from GLOBE and 015 studies in chronic hepatitis B patients. In: European Association for the Study of the Liver (EASL), 44th Annual Meeting, Copenhagen, Denmark; April 22–26, 2009.

21. Dienstag JL, Goldin RD, Heathcote EJ, et al. Histological outcome during long-term lamivudine therapy. Gastroenterology. 2003;124:105–17.

22. Marcellin P, Chang TT, Lim SG, et al. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology. 2008;48:750–8.

23. Yuen MF, Chow DH, Tsui K, et al. Liver histology of Asian patients with chronic hepatitis B on prolonged lamivudine therapy. Aliment Pharmacol Ther. 2005;21:841–9.

24. Chang TT, Liaw YF, Wu SS, et al. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology. 2010;52:886–93.

25. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology. 1981;1:431–5.

26. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–9.

27. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008;48:835–47.

28. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol. 2003;29:1705–13.

29. Amet S, Bronowicki JP, Thabut D, et al. Prevalence of renal abnormalities in chronic HBV infection: the HARPE study. Liver Int. 2015;35:148–55.

30. Qi X, Wang J, Chen L, et al. Impact of nucleos(t)ide analogue combination therapy on the estimated glomerular filtration rate in patients with chronic hepatitis B. Medicine (Baltimore). 2015;94:e646.

31. Ahn SH, Lee S, Park JY, et al. Comparison of the effects of telbivudine and entecavir treatment on estimated glomerular filtration rate in patients with chronic hepatitis B. Gut Liver. 2015. doi:10.5009/gnl14297 [Epub ahead of print].

32. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461–70.

33. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. Hepatology. 2009;50:661–2.

34. Marcellin P, Gane E, Buti M, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet. 2013;9(381):468–75.

35. Gane EJ, Marcellin P, Jacobson I, Heathcoate EJ, Dusheiko G, de Man R. HBsAg kinetics of decay and baseline characteristics of HBeAg-positive patients

with chronic hepatitis B following 3 years of tenofovir disoproxil fumarate (TDF) treatment [Abstract]. J Hepatol. 2010;52:S388.

36. Gish RG, Chang TT, Lai CL, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. J Viral Hepat. 2010;17:16–22.

37. Wursthorn K, Jung M, Riva A, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. Hepatology. 2010;52: 1611–20.

38. Gane E, Deray G, Piratvisuth T, et al. Renal function is improved for Chronic Hepatitis B (CHB) patients treated with telbivudine. In: 62nd Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA, USA; November 4–8, 2011. Poster #1439.