Long-term entecavir therapy results in reversal of fibrosis/cirrhosis and continued histologic improvement in chronic hepatitis B patients

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List of Abbreviations

CHB	chronic hepatitis B
HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HBeAg	hepatitis B e antigen
ALT	alanine aminotransferase
HAI	Histology Activity Index
ULN	upper limit of normal
HBsAg	hepatitis B s antigen
NC=M	Non-Completer=Missing
SAE	serious adverse event
NI	necroinflammatory

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Abstract [Word count: 219; limit: 275]

Background and rationale: One year of treatment with entecavir 0.5 mg daily in nucleoside-naïve patients with hepatitis B e antigen (HBeAg)-positive or HBeAg-negative chronic hepatitis B resulted in significantly improved liver histology and virologic and biochemical endpoints compared to lamivudine. *Methods:* Patients who received at least 3 years of cumulative entecavir therapy in phase III studies and a long-term rollover study and had a long-term liver biopsy were evaluated for improvement in histologic appearance. *Main results*: Sixty-nine patients [50 HBeAg-positive; 19 HBeAg-negative] receiving entecavir therapy underwent long-term liver biopsies (median time of biopsy was 6 years; range: 3–7 yrs). Histologic improvement was analyzed for 57 patients who had an adequate baseline biopsy, a baseline Knodell necroinflammatory score ≥2, and an adequate long-term biopsy. At the time of long-term biopsy, all patients in the cohort had HBV DNA <300 copies/mL and 86% had normalized ALT. Histologic improvement (≥2-point decrease in Knodell necroinflammatory score and no worsening of Knodell fibrosis score) was observed in 96% of patients and a ≥1 point improvement in Ishak fibrosis score was found in 88% of patients, including all ten patients with advanced fibrosis or cirrhosis at phase III baseline. ***Conclusion:*** The majority of nucleoside-naïve chronic hepatitis B patients treated with entecavir in this long-term cohort achieved substantial histologic improvement and regression of fibrosis or cirrhosis.

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Chronic hepatitis B (CHB) infection affects over 350 million people worldwide.(1) The REVEAL studies demonstrated that progression to liver cirrhosis, hepatocellular carcinoma (HCC) and liver-related mortality correlates strongly with the level of circulating hepatitis B virus (HBV) DNA.(2;3) The cumulative incidence of cirrhosis increased from 4.5% in patients with HBV DNA <300 copies/mL to 36.2% in patients with HBV DNA $\geq 10^6$ copies/mL ($p<0.001$).(3) Correspondingly, the cumulative incidence of HCC in the REVEAL study increased proportionately with serum HBV DNA, from 1.3% in patients with HBV DNA <300 copies/mL to approximately 15% when HBV DNA was $>10^6$ copies/mL.(2) Finally, all-cause and chronic liver disease mortality also increased with increasing HBV DNA levels.(4) This association between elevated serum HBV DNA and disease progression in CHB has been confirmed by several studies of similar design.(5-8)

The liver is a rapidly regenerating organ, and persistent liver injury leads to a process of healing and scar tissue formation resulting in fibrosis, and eventually cirrhosis. Liver injury leads to fibrosis through the transformation of hepatic stellate cells from vitamin A storage cells to activated hepatic stellate cells which secrete fibrillar collagens.(9-11) Although fibrosis was previously thought to be irreversible and relentlessly progressive, recent studies have challenged these ideas. Animal models of liver fibrosis have shown that removing the underlying source of liver injury results in clearance of the activated hepatic stellate cells, allowing resorption of the extracellular matrix and consequently, reversal of fibrosis.(12-14) Treatment of the underlying cause of inflammation has been

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shown clinically to result in reversal of fibrosis and cirrhosis in patients with liver disease from both viral and non-viral causes.(15-20)

Short-term antiviral therapy for CHB results in the suppression of viral replication,(21;22) and has been associated with improvement of liver histology in randomized clinical trials.(23) Treatment for 3 years with the oral antiviral agent lamivudine has also been shown to slow the clinical progression of liver disease in patients with advanced fibrosis and cirrhosis.(24) However, in this landmark study, disease progression was assessed clinically and not histologically, and serum HBV DNA results were not reported. Longer-term histologic data exist from studies in nucleoside-naïve CHB patients treated with lamivudine or adefovir.(25-27) Emergence of antiviral drug resistance negatively impacted the histologic benefits that were observed with lamivudine and the impact of resistance on histologic response was not reported in the adefovir studies.

Viral replication is now recognized as the key driver of liver injury and disease progression, and thus the primary aim of treatment for chronic HBV infection is long-term suppression of HBV replication to undetectable levels.(1;28;29) Entecavir is a potent HBV antiviral which demonstrated superior virologic, histologic and biochemical outcomes after 48 weeks of therapy compared to either lamivudine or adefovir in nucleoside-naïve patients.(21;22;30) In a study of nucleoside-naïve Japanese patients, 3 years of entecavir therapy resulted in potent virologic suppression and additional improvement in necroinflammatory and fibrosis scores compared to baseline and

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Week 48 values.(31) Virologic suppression increased through 5 years of entecavir treatment in long-term rollover studies, with minimal emergence of resistance.(32-34) The aim of the present evaluation was to determine if long-term treatment with entecavir is associated with continued histologic improvement and reversal of fibrosis or cirrhosis.

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Patients and Methods

Study design

The current analysis evaluated nucleoside-naïve patients from two Phase III entecavir studies (hepatitis B e antigen (HBeAg)-positive [ETV-022] and HBeAg-negative [ETV-027]) who subsequently entered an open-label rollover study (ETV-901) and received entecavir for a total duration of at least 3 years. During the Phase III program, patients received entecavir 0.5 mg daily and during the long-term rollover study, all patients received 1.0 mg of entecavir daily. Some patients received concurrent lamivudine 100 mg daily for a brief period of time early in the rollover study before continuing on entecavir monotherapy (1.0 mg daily) after the protocol was amended. Patients and investigators could discontinue entecavir therapy in the rollover study at any time and patients who discontinued therapy were to be followed for 24 weeks to assess safety.

The study protocol was approved by the ethics committees at all participating institutions and written informed consent was obtained from all patients. The study was carried out in accordance with the ethics principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines and local regulatory requirements.

Study population

Complete inclusion criteria for enrollment in studies ETV-022 (HBeAg-positive) and ETV-027 (HBeAg-negative) have been described previously.(21;22) Some key inclusion criteria were age ≥16 years; serologic diagnosis of CHB; compensated liver function;

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absence of co-infection with hepatitis C, hepatitis D, or HIV; no more than 12 weeks of prior lamivudine therapy; and no use of interferon-alfa, thymosin-α, or antiviral agents with anti-hepatitis B activity within 24 weeks of randomization.

A total of 293 nucleoside-naïve patients treated with entecavir in the two pivotal Phase III studies (ETV-022 and ETV-027) were enrolled into the ETV-901 long-term rollover study (Figure 1). Of these 293 patients, 69 (24%) consented to undergo a long-term liver biopsy (the Long-Term Histology Cohort). The primary reasons for not obtaining a long-term liver biopsy in the 224 patients not part of the Long-Term Histology Cohort were: 1) patient was off study (44%); 2) patient refused consent (33%); or 3) the investigator chose not to participate in the amended study (17%).

Evaluations

Liver biopsies were performed at baseline and again after 48 weeks of blinded entecavir therapy in the Phase III studies. In the long-term rollover study, optional liver biopsies were offered at two time points: after an additional 48 weeks of treatment in the rollover study and following a protocol amendment for patients who had received at least 3 years of cumulative entecavir therapy. All liver biopsies were evaluated by a single, central histopathologist. Necroinflammation and fibrosis were assessed using the original Knodell histology activity index (HAI) scoring system and the Ishak modification of this system.(35;36) The pathologist was blinded to treatment assignment, biopsy sequence and clinical outcome for the Phase III liver biopsies, and remained blinded concerning clinical outcomes when evaluating the long-term biopsies. Serum samples for virologic,

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biochemical and serologic end points were matched in time (± 12 weeks) with the corresponding long-term biopsy. Serum HBV DNA was assayed using the Roche Amplicor COBAS PCR assay (version 2.0; lower limit of quantification: 300 copies/mL [57 IU/mL], Pleasanton, CA) at 12-week intervals during the Phase III studies and the first year of the rollover study, and at 24-week intervals thereafter. HBV serologies were assessed every 12 weeks, centrally during the Phase III studies (Abbott AxSYM microparticle enzyme immunoassay [Abbott Laboratories, North Chicago, IL] and Diasorin enzyme immunoassay) and in local laboratories during study ETV-901. Alanine aminotransferase levels (ALT) were assessed in local laboratories.

Efficacy end points

The criteria for inclusion in the efficacy analyses were: 1) an adequate baseline liver biopsy; 2) a baseline Knodell necroinflammatory score of ≥2; and 3) an adequate long-term biopsy. The adequacy of the biopsy was determined by the histopathologist. The co-primary efficacy end points were histologic improvement (≥2-point decrease in the Knodell necroinflammatory score and no worsening in the Knodell fibrosis score) and improvement in the Ishak fibrosis score (≥1-point decrease). Secondary histologic end points included mean change from baseline in Knodell necroinflammatory score, mean change from baseline in Ishak fibrosis score, proportion of patients with baseline advanced fibrosis/cirrhosis (Ishak score ≥4) who demonstrated improvement, and proportion of patients with a baseline Knodell HAI score of ≥4 points who achieved a final score of ≤3 points. Non-histologic secondary end points included the proportions of patients achieving HBV DNA <300 copies/mL, ALT ≤1 x ULN, HBeAg loss, HBeAg

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seroconversion and HBsAg loss. All end points were assessed at Week 48 in the Phase III study, and at the time of the long-term biopsy.

Safety analyses

Safety analyses were performed for all patients who underwent long-term liver biopsy and were based on data collected during treatment in the long-term rollover study. Analyses included the incidence of adverse events, serious adverse events, laboratory abnormalities, and discontinuations due to adverse events.

Statistical analyses

Continuous variables were summarized using the mean, median, standard error, standard deviation, and minimum and maximum values. Binary variables were summarized by counts and percentages using the Non-Completer=Missing (NC=M) method of handling missing data.

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Results

Study population

Of the 69 patients who provided a long-term biopsy, 50 were HBeAg-positive and 19 were HBeAg-negative. Fifty-seven of the 69 patients met the criteria for inclusion in the efficacy analyses. Table 1 shows the baseline characteristics for these 57 patients compared with all entecavir-treated patients from the Phase III studies. Patients with long-term liver biopsies were comparable to all-treated patients, although a slightly higher proportion of patients with long-term biopsies were Asian (67% compared to 58% in ETV-022 and 38% in ETV-027). For these 57 patients, the mean baseline HBV DNA was 9.4 $\log_{10}$ copies/mL, with mean baseline Knodell necroinflammatory and Ishak fibrosis scores of 8.0 and 2.4, respectively; 10 of the 57 patients (18%) had an Ishak fibrosis score ≥4, indicating advanced fibrosis or cirrhosis.

The median time on entecavir treatment for these 57 patients at the time of the long-term biopsy was 280 weeks (approximately 6 years; range of 3–7 years), with a median gap of 3.3 weeks between end of dosing in the Phase III feeder study and the first date of dosing in the rollover study. The majority of patients (51/57) received lamivudine in combination with entecavir therapy for a median of 29 weeks early in the course of ETV-901, and received entecavir monotherapy for the remainder of the study.

Histologic response

All biopsies with at least three portal areas were evaluated with the understanding that small biopsies tend to be underscored for necroinflammation and fibrosis.(37) Baseline biopsies had a mean length of 12.1 mm (60%.≥10mm), Week 48 biopsies had a mean

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length of 11.6 mm (65% ≥10 mm), and the long-term biopsies had a mean length of 15.2 mm (79% ≥10 mm).

After long-term treatment with entecavir, 96% (55/57) of patients demonstrated histologic improvement, which was increased from 73% (41/56) of patients after 48 weeks of therapy (Table 2). The mean change from baseline in the Knodell necroinflammatory score was a 6.37-point reduction after long-term treatment, compared to a mean reduction of 3.39 points after 48 weeks of entecavir therapy. The proportion of patients in the cohort demonstrating at least a 1-point improvement in Ishak fibrosis score also increased, from 32% (18/56) after 48 weeks to 88% (50/57) after long-term treatment. The mean change from baseline in Ishak fibrosis score was a 1.53-point reduction after long-term treatment, which was increased from a 0.20-point reduction after 48 weeks of therapy.

Treatment for longer than 48 weeks resulted in an increasing proportion of patients with no or minimal necroinflammation by Knodell classification (Knodell HAI score ≤3), and no or minimal fibrosis by Ishak classification (Ishak score 0 or 1) (Table 2). Among patients with a baseline Knodell HAI score ≥4, the majority (75%, 41/55) achieved a Knodell HAI ≤3 on the long-term biopsy. Among patients with a baseline Ishak fibrosis score ≥2, the majority (31/43; 72%) achieved an Ishak fibrosis score of 0 or 1 on the long-term biopsy. Figures 2(a) and 1(b) show the change in distribution of Knodell necroinflammatory and Ishak fibrosis scores at the baseline, Week 48 and long term.

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One of the 57 patients had an increase in Ishak fibrosis score, rising from 1 at baseline to 2 at the long-term biopsy. This patient had undetectable HBV DNA and a normal serum ALT at the time of the long-term biopsy, as well as an improvement in the necroinflammatory score (from 3 at baseline to 1 at the long-term biopsy).

Advanced fibrosis and cirrhosis

Ten of the 57 patients had advanced fibrosis or cirrhosis (Ishak score ≥4) at baseline. With long-term entecavir therapy, all 10 patients demonstrated at least a 1-point reduction in Ishak fibrosis score, with a median reduction from baseline of 1.5 points. Four of the 10 patients had cirrhosis at baseline (Ishak fibrosis score ≥5), and all demonstrated an improvement in Ishak fibrosis score, with a median drop of 3 points (range: 1 to 4). Figure 3 shows photomicrographs of biopsies taken from a 60-year-old, HBeAg-negative Caucasian male patient. The baseline biopsy shows an Ishak fibrosis score of 6, indicating cirrhosis, which was unchanged at Week 48. Following long-term treatment with entecavir, the Week 268 biopsy shows an Ishak fibrosis score of 2, indicating minimal fibrosis.

Virologic and biochemical response

At the time of the long-term biopsy, 100% (57/57) of patients had HBV DNA <300 copies/mL (Table 2). This represents an increase from 70% (40/57) of patients with HBV DNA <300 copies/mL after 48 weeks of entecavir treatment. Similarly, the proportion of patients with ALT ≤1 x ULN increased from 67% (38/57) after 48 weeks of therapy to 86% (49/57) after long-term treatment. Genotypic testing for resistance was

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not performed because all the patients achieved a serum HBV DNA level <300 copies/mL.

Serologic response

According to the study design of ETV-022, patients who lost HBeAg (with or without seroconversion) during the first or second year of therapy and achieved undetectable serum HBV DNA by bDNA assay were to discontinue entecavir treatment and be followed off-treatment to determine sustained response. During the long-term rollover study, 55% (22/40) of the HBeAg-positive patients lost HBeAg and 33% (13/40) achieved HBe seroconversion. No patient in this cohort lost HBsAg.

Safety

The majority of patients (96%) experienced at least one adverse event at some time during entecavir treatment, and serious adverse events (SAE; the majority of which were grades 1 or 2) occurred in 25% of patients. However, no patient discontinued entecavir treatment due to an adverse event. Two patients experienced on-treatment ALT flares; both cases resolved with continued treatment. One patient died from myocardial ischemia during entecavir treatment; this death was not attributed to study medication.

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Discussion

In the original Phase III studies, histologic improvement was observed in the majority (73%) of patients as early as Week 48, but only a minority (32%) demonstrated an improvement in fibrosis. The current analyses of the Long-Term Histology Cohort summarize the effects of continued entecavir therapy on hepatic necroinflammation and fibrosis in nucleoside-naïve HBeAg-positive and -negative CHB patients. After a median exposure to entecavir therapy of approximately 6 years, histologic improvement and improvement of fibrosis increased to 96% and 88% of patients, respectively. Most patients (75%) in the cohort who had a baseline HAI score of ≥4 achieved a score of ≤3 by the long-term biopsy. These histologic analyses extend previous observations of the clinical efficacy of entecavir at 48 weeks in patients with advanced fibrosis or cirrhosis.(38) All patients who had evidence of advanced fibrosis or liver cirrhosis at Phase III baseline demonstrated improvement in fibrosis at the long-term assessment.

Suppression of viral replication to below the level of PCR assay detection (serum HBV DNA <300 copies/mL) occurred in all patients, and most patients (86%) also had a normal serum ALT level at the time of the long-term biopsy. Because of the sustained suppression of HBV DNA to <300 copies/mL, these patients were at minimal risk for antiviral drug resistance, and no evidence of virologic rebound or genotypic resistance to entecavir was observed in this study. A majority (55%) of patients lost HBeAg, and 33% experienced HBe seroconversion at the time of the long-term biopsy. Patients who did not demonstrate HBe seroconversion during long-term treatment also experienced improvements in liver histology and reversal of fibrosis, suggesting that these outcomes

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are more closely associated with HBV DNA suppression than with immunologic response to therapy.

The baseline demographics of the patients in the Long-Term Histology Cohort and the Phase III studies suggest that the two populations are comparable; however, the current data set has some limitations. For all patients who entered the rollover study, the dose of entecavir increased from 0.5 mg in the Phase III studies to 1.0 mg daily in the rollover study, and 51/57 patients (89%) in this cohort received a median of 29 weeks of concurrent lamivudine before continuing on entecavir monotherapy for the remainder of the observation period. Because amendments were made to the long-term rollover study as new data emerged, it is not possible to evaluate any potential contribution of the increased dose of entecavir or the brief period of concurrent lamivudine to the results. Although all 57 patients eventually went on to achieve HBV DNA <300 copies/mL by the time of their long-term biopsy, the additional increase in virologic suppression is likely related to the longer duration of entecavir therapy and absence of resistance, rather than the brief period of concurrent lamivudine therapy or the increased dose of entecavir. The histologic benefits observed in the Long-Term Histology Cohort are therefore more likely driven by the durable antiviral suppression and avoidance of antiviral drug resistance observed with entecavir therapy in these nucleoside-naïve patients. This assessment is supported by a separate long-term histology cohort of 19 Japanese patients who received continuous therapy with entecavir 0.5 mg once daily for 3 years, in whom histologic improvement and improvement in fibrosis was observed in 100% and 63% of patients respectively.(31)

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Clinical data on the degree of fibrosis or cirrhosis were not collected as part of the entecavir Phase III studies or the rollover study. Thus, is it not clear from this data set whether the macroscopic architectural abnormalities typically observed in patients with advanced fibrosis or cirrhosis remain among patients who have experienced histologic regression. However, the reductions in portal pressure observed among cirrhotic patients receiving treatment with entecavir or lamivudine would suggest that architectural remodeling does occur to some degree.(39;40)

The possibility that successful treatment of CHB could result in reversal of cirrhosis was first suggested in a case series of three patients who were treated with either interferon or lamivudine.(41) Three subsequent publications have reported the effects of nucleos(t)ide analogs on histologic outcome beyond 48 weeks. A cohort of previously nucleoside-naïve HBeAg-positive CHB patients were treated with lamivudine and followed for 3 years.(25) In this report, 35/65 patients (56%) experienced histologic improvement. Forty-one (63%) of these patients developed YMDD resistance, and the histologic improvement was lost in many of those patients. Two smaller cohorts of nucleoside-naïve HBeAg-negative CHB patients treated with adefovir were followed for 4 years (N=22) or 5 years (N=24).(26) In this report, 12/22 (55%) patients treated for 4 years and 17/24 (71%) patients treated for 5 years demonstrated improvements in Ishak fibrosis score. In a recently published cohort of 65 nucleoside-naïve HBeAg-positive CHB patients treated with adefovir for 5 years, 39% achieved a serum HBV DNA <1000 copies/mL, and resistance emerged to adefovir in 20% of patients. A subset of 15 patients had paired

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baseline and long-term biopsies, and improvement in necroinflammation and fibrosis was shown in 9/15 (60%) patients using the Knodell scoring system.(27)

These data support the conclusion that in most nucleoside-naïve patients, long-term entecavir therapy leads to potent suppression of HBV DNA, normalization of ALT and improvement in liver histology with accompanying regression of fibrosis, including those with advanced fibrosis or cirrhosis at baseline. Substantially more patients demonstrated histologic improvement at the time of the long-term biopsy compared to Week 48, confirming the value of long-term treatment for CHB. The safety profile, potent suppression of HBV replication, and low potential for antiviral drug resistance in nucleoside-naïve patients make long-term treatment of CHB with entecavir monotherapy possible.

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Figure Legends

Figure 1. Flowchart summarizing the composition of the Long-Term Histology Cohort.

Figure 2a. Distribution of Knodell necroinflammatory scores at Phase III baseline, after 48 weeks of entecavir treatment, and at the time of long-term biopsy (median: 6 years entecavir treatment [range 3–7 years]) among histologically evaluable patients in the Long-Term Histology Cohort (N=57)**.**

Figure 2b. Distribution of Ishak fibrosis scores at Phase III baseline, after 48 weeks of entecavir treatment, and at the time of the long-term biopsy (median: 6 years' entecavir treatment [range 3–7 years]) among histologically evaluable patients in the Long-Term Histology Cohort (N=57).

Figure 3: Liver biopsies stained with Masson's trichrome demonstrating a reduction in fibrosis following long-term entecavir therapy in a 60-year-old, HBeAg-negative, Caucasian male. The baseline biopsy shows an Ishak score of 6, indicating cirrhosis, which is unchanged at Week 48, whereas the Week 268 biopsy shows an Ishak score of 2, indicating minimal fibrosis.

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References

1. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
2. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
3. 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;130:678-686.
4. Iloeje UH, Yang HI, Jen CL, Su J, Wang LY, You SL et al. Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol 2007;5:921-931.
5. 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;101:1797-1803.
6. Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265-272.
7. Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH et al. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis 2006;193:1258-1265.
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;19:670-675.
9. Benyon RC, Iredale JP. Is liver fibrosis reversible? Gut 2000;46:443-446.
10. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655-1669.
11. Elsharkawy AM, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. Apoptosis 2005;10:927-939.
12. Iredale JP, Benyon RC, Pickering J, McCullen M, Northrop M, Pawley S et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J Clin Invest 1998;102:538-549.
13. Issa R, Williams E, Trim N, Kendall T, Arthur MJ, Reichen J et al. Apoptosis of hepatic stellate cells: involvement in resolution of biliary fibrosis and regulation by soluble growth factors. Gut 2001;48:548-557.

Accepted Preprint

14. Iredale JP. Models of liver fibrosis: exploring the dynamic nature of inflammation and repair in a solid organ. J Clin Invest 2007;117:539-548.

15. Dufour JF, DeLellis R, Kaplan MM. Regression of hepatic fibrosis in hepatitis C with long-term interferon treatment. Dig Dis Sci 1998;43:2573-2576.

16. Hammel P, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Flejou JF et al. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. N Engl J Med 2001;344:418-423.

17. Muretto P, Angelucci E, Lucarelli G. Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 2002;136:667-672.

18. Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z et al. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002;122:1303-1313.

19. Serejo F, Costa A, Oliveira AG, Ramalho F, Batista A, De Moura MC. Alpha-interferon improves liver fibrosis in chronic hepatitis C: clinical significance of the serum N-terminal propeptide of procollagen type III. Dig Dis Sci 2001;46:1684-1689.

20. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132:517-524.

21. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001-1010.

22. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011-1020.

23. Mommeja-Marin H, Mondou E, Blum MR, Rousseau F. Serum HBV DNA as a marker of efficacy during therapy for chronic HBV infection: analysis and review of the literature. Hepatology 2003;37:1309-1319.

24. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.

25. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL et al. Histological outcome during long-term lamivudine therapy. Gastroenterology 2003;124:105-117.

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26. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006;131:1743-1751.

27. Marcellin P, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S et al. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2008;48:750-758.

28. European Association For The Study Of The Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. J Hepatol 2009;50:227-242.

29. Liaw YF, Leung N, Kao J.H, Piratvisuth T, Gane E, Han KH et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008;2:538-549.

30. Leung N, Peng CY, Hann HW, Sollano J, Lao-Tan J, Hsu CW et al. Early hepatitis B virus DNA reduction in hepatitis B e antigen-positive patients with chronic hepatitis B: A randomized international study of entecavir versus adefovir. Hepatology 2009;49:72-79.

31. Takehara T, Hayashi N, Takaguchi K, Yokosuka O, Fujioka S, Shindo M et al. Efficacy and safety of 3 years treatment with entecavir in Japanese nucleoside-naive patients with chronic hepatitis B. Hepatology International 2008;2:A97-A98.

32. Tenney DJ, Pokornoski KA, Rose RE, Baldick CJ, Eggers BJ, Fang J et al. Entecavir at five years shows long-term maintenance of high genetic barrier to hepatitis B virus resistance . Hepatology International 2008;2:A88-A89.

33. Han SH, Chang TT, Chao YC, Yoon S, Gish RG, Cheinquer H et al. Five years of continuous entecavir for nucleoside-naive HBeAg(+) chronic hepatitis B: results from study ETV-901. Hepatology 2008;48(suppl. 1):705A-706A.

34. Shouval D, Lai CL, Chang TT, Gadano A, Wu SS, Halota W et al. Three years of entecavir re-treatment of HBeAg(-) entecavir patients who previously discontinued entecavir therapy: results from study ETV-901. Hepatology 2008;48(suppl. 1):722A-723A.

35. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431-435.

36. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.

37. Colloredo G, Guido M, Sonzogni A, Leandro G. Impact of liver biopsy size on histological evaluation of chronic viral hepatitis: the smaller the sample, the milder the disease. J Hepatol 2003;39:239-244.

Accepted Preprint

38. Schiff E, Simsek H, Lee WM, Chao YC, Sette H, Jr., Janssen HL et al. Efficacy and safety of entecavir in patients with chronic hepatitis B and advanced hepatic fibrosis or cirrhosis. Am J Gastroenterol 2008;103:2776-2783.

39. Manolakopoulos S, Triantos C, Theodoropoulos J, Vlachogiannakos J, Kougioumtzan A, Papatheodoridis G et al. Antiviral therapy reduces portal pressure in patients with cirrhosis due to HBeAg-negative chronic hepatitis B and significant portal hypertension. J Hepatol 2009;51:468-474.

40. Pozzi M, Pizzala DP, Maldini FF, Doretti A, Ratti L. Portal pressure reduction after entecavir treatment in compensated HBV cirrhosis. Hepatogastroenterology 2009;56:231-235.

41. Malekzadeh R, Mohamadnejad M, Rakhshani N, Nasseri-Moghaddam S, Merat S, Tavangar SM et al. Reversibility of cirrhosis in chronic hepatitis B. Clin Gastroenterol Hepatol 2004;2:344-347.

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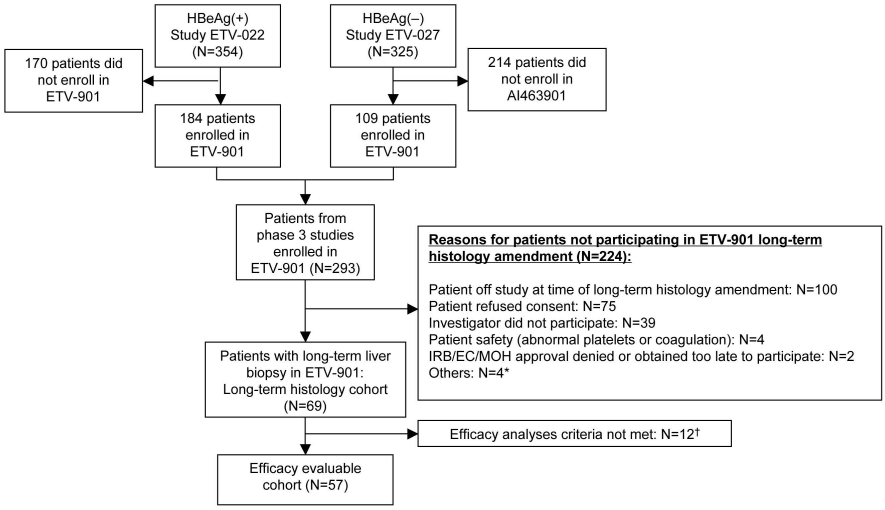

* 1 patient misclassified as ineligible; 2 subjects went off therapy shortly after amendment; 1 patient was relocating to a foreign job assignment and returned after conclusion of the amendment.
† For inclusion in the efficacy evaluable cohort, patients were required to have: a) an adequate baseline liver biopsy; b) a baseline Knodell necroinflammatory score of ≥2; and c) an adequate long-term biopsy.

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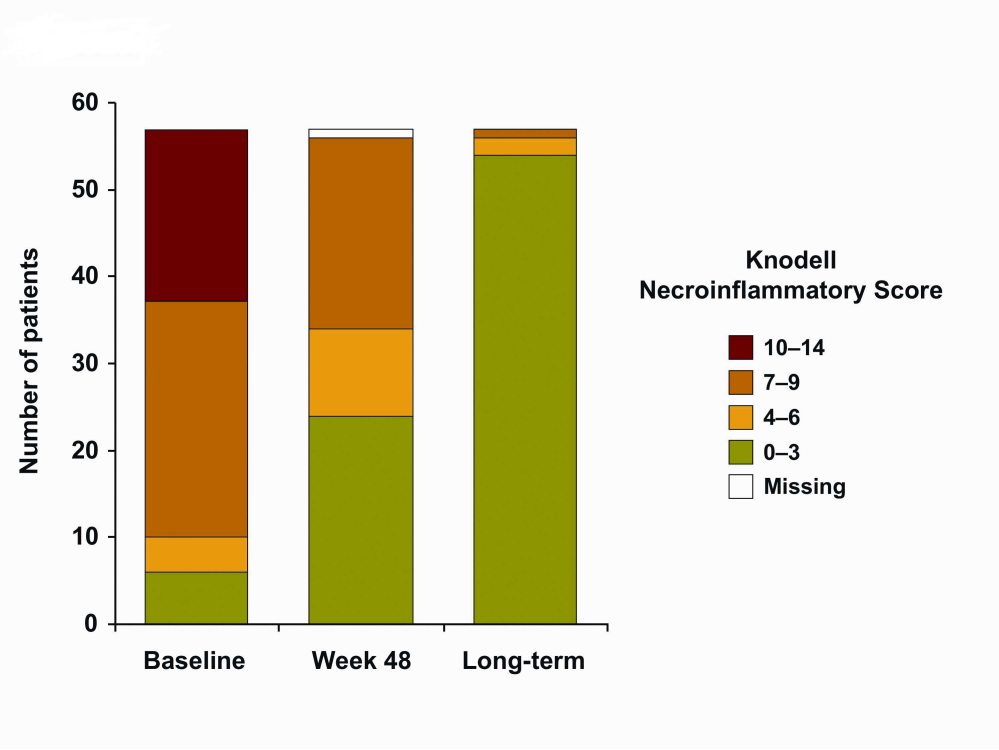

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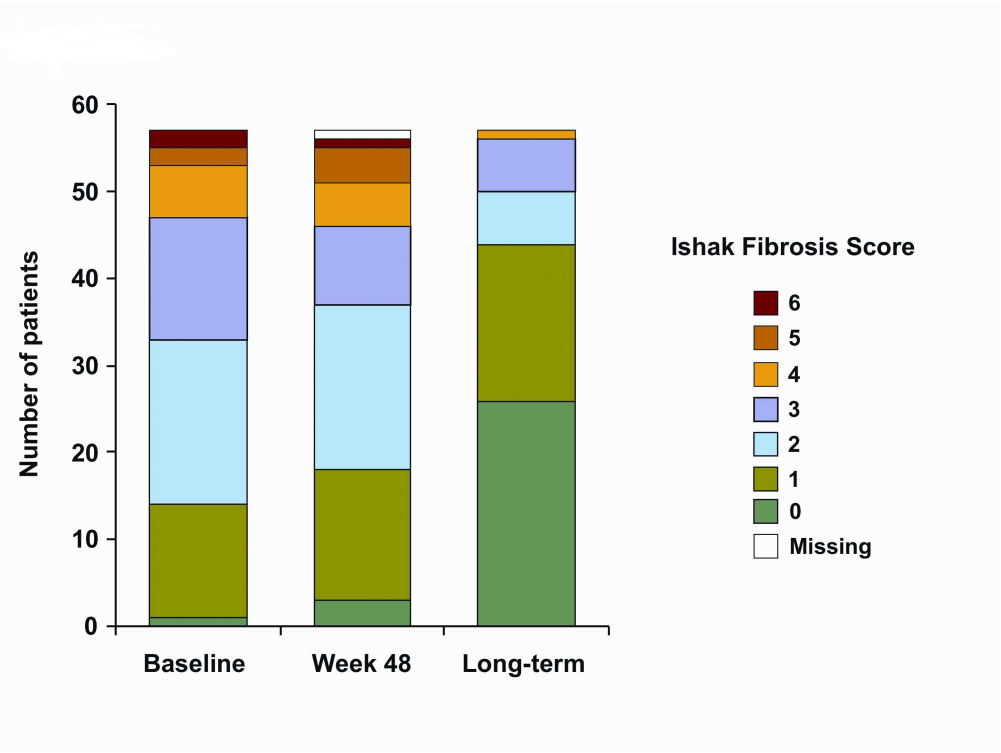

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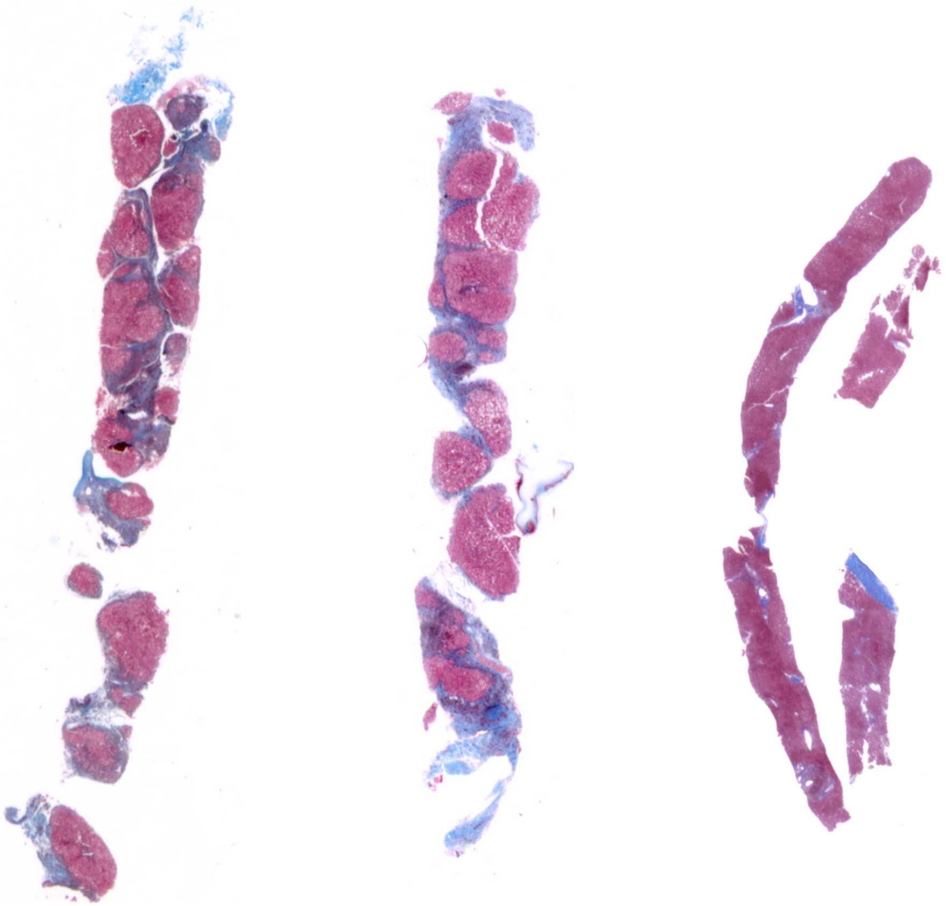

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Table 1. Demographic and baseline characteristics of the patients in the Long-Term Histology Cohort, compared to original Phase III studies

	ETV-022 Cohort (N=354) HBeAg-positive	ETV-027 Cohort (N=325) HBeAg-negative	Long-Term Histology Cohort (N=57)
Age, mean (years)	35	44	40
Male (%)	77	76	82
Race (%)			
Asian	58	38	67
Non-Asian	42	62	33
HBeAg-positive, %	98	1	72
HBV DNA by PCR, mean (log_{10} copies/mL)	9.6	7.6	9.4
Knodell NI score,[a] mean, (SD)	7.8 (3.0)	8.0 (2.7)	8.0 (2.6)
Ishak Fibrosis score,[a] mean (SD)	2.3 (1.3)	2.4 (1.2)	2.4 (1.3)
ALT, mean (U/L)	140	141	142
HBV genotype (%)			
A	27	10	18
B	19	14	27
C	31	18	33
D	10	48	13
Other	13	10	12

[a] For ETV-022, adequate biopsy specimens were available for 329 patients; for ETV-027, adequate biopsy specimens were available for 303 patients.
Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NI, necroinflammatory; PCR, polymerase chain reaction.

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Table 2. Histologic, virologic, and biochemical response following long-term treatment and at Week 48: Long-Term Histology Cohort.

	Week 48 (N=57)	**Long-Term Histology Cohort (N=57)[a]***
Primary end points		
Histologic improvement, n (%)	41/56 (73)[b]	55 (96)
Improvement in Ishak fibrosis score (≥1 point decrease), n (%)	18/56 (32)[b]	50 (88)
Secondary end points		
Mean change from baseline in Knodell NI score	-3.39	-6.37
Knodell HAI ≤3 in patients with baseline HAI ≥4, n (%)	12/54 (22)[b]	41/55 (75)
Mean change from baseline in Ishak fibrosis score	-0.20	-1.53
≥2-point decrease in Ishak fibrosis score, n (%)	3/42 (7)[b]	25/43 (58)
HBV DNA <300 copies/mL, n (%)	40/57 (70)	57/57 (100)
ALT ≤1 x ULN, n (%)	38/57 (67)	49/57 (86)
HBeAg loss, n (%)	1/41 (2)	22/40 (55)
HBeAg seroconversion, n (%)	1/41 (2)	13/40 (33)
HBsAg loss, n (%)	0/57 (0)	0/56 (0)

[a] Median time on entecavir therapy at the time of the long-term biopsy was 6 years (range: 3–7 years).
[b] One patient had an inadequate biopsy at Week 48.
Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HAI, histology activity index; NI, necroinflammatory;

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