Earlier Alanine Aminotransferase Normalization During Antiviral Treatment Is Independently Associated With Lower Risk of Hepatocellular Carcinoma in Chronic Hepatitis B

Jonggi Choi, MD, PhD[1], Gi-Ae Kim, MD, PhD[2], Seungbong Han, PhD[3] and Young-Suk Lim, MD, PhD[1]

OBJECTIVES: **It was suggested that normalization of serum alanine aminotransferase (ALT) levels at 1 year of antiviral treatment is associated with a lower risk of hepatic events in patients with chronic hepatitis B (CHB). However, it remains unclear whether earlier ALT normalization is associated with lower hepatocellular carcinoma (HCC) risk, independent of fatty liver or cirrhosis and on-treatment virological response (VR), in patients with CHB.**

METHODS: **We analyzed 4,639 patients with CHB who initiated treatment with entecavir or tenofovir using landmark analysis and time-dependent Cox analysis. We defined normal ALT as ≤35 U/L (men) and ≤25 U/L (women) and VR as serum hepatitis B virus DNA <15 IU/mL.**

RESULTS: **During a median 5.6 years of treatment, 509 (11.0%) patients developed HCC. ALT normalization occurred in 65.6% at 1 year and 81.9% at 2 years and was associated with a significantly lower HCC risk in landmark ($P < 0.001$) and time-dependent Cox analyses (adjusted hazard ratio [AHR] 0.57; $P < 0.001$). Compared with ALT normalization within 6 months, delayed ALT normalization at 6–12, 12–24, and >24 months was associated with incrementally increasing HCC risk (AHR 1.40, 1.74, and 2.45, respectively; $P < 0.001$), regardless of fatty liver or cirrhosis at baseline and VR during treatment. By contrast, neither earlier VR (AHR 0.93; $P = 0.53$) nor earlier hepatitis B e antigen seroclearance (AHR 0.91; $P = 0.31$) was associated with a significantly lower HCC risk.**

DISCUSSION: **In patients with CHB treated with entecavir or tenofovir, earlier ALT normalization was independently associated with proportionally lower HCC risk, regardless of fatty liver or cirrhosis at baseline and on-treatment VR.**

SUPPLEMENTARY MATERIAL accompanies this paper at https://links.lww.com/AJG/B339

Am J Gastroenterol 2019;00:1–9. https://doi.org/10.14309/ajg.0000000000000490

INTRODUCTION

Hepatitis B virus (HBV) infection is the most common cause of primary liver cancer, which is the second leading cause of cancer-related mortality worldwide (1). Global deaths from liver cancer due to HBV are projected to double by 2040 (1). Hepatocellular carcinoma (HCC) represents >90% of primary liver cancer cases (2).

The goal of treatment for patients with chronic hepatitis B (CHB) is to improve survival by preventing disease progression and HCC (3,4). Ideally, for hepatitis B therapies to be approved, they should demonstrate efficacy in preventing HCC and liver-related deaths. However, these clinical endpoints evolve over years or decades. Therefore, intermediate surrogate endpoints that are easy to assess, occur frequently, and are considered to correlate with clinical outcomes have been used for the evaluation of treatment efficacy. Those surrogate endpoints include virological, biochemical, and serological biomarkers.

In natural-course studies on CHB, normalization of serum alanine aminotransferase (ALT) levels, marked decrease of HBV DNA levels, and hepatitis B e antigen (HBeAg) seroclearance have been associated with a reduced incidence of HCC and mortality (5,6). However, few studies have investigated whether these variables correlate with clinical outcomes during long-term treatment with potent nucleos(t)ide analogues (NUCs).

[1]Department of Gastroenterology, Liver Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; [2]Department of Internal Medicine, School of Medicine, Kyung Hee University, Seoul, Republic of Korea; [3]Department of Applied Statistics, Gachon University, Seongnam-si, Gyeonggi-do, Republic of Korea. **Correspondence:** Young-Suk Lim, MD, PhD. E-mail: limys@amc.seoul.kr.
Received July 9, 2019; accepted November 8, 2019; published online January 2, 2020

A recent study suggested that ALT normalization at 1 year of NUC treatment was associated with a lower risk of hepatic events in patients with CHB (7). However, the data for virological response (VR) during treatment and fatty liver disease at baseline, the important potential confounders, were lacking and could not be adjusted for in the study. Furthermore, it is still unclear whether earlier achievement of ALT normalization is associated with a lower risk of HCC.

Therefore, the aim of this large-scale historical cohort study was to comprehensively explore the impact of on-treatment surrogate endpoints on the risk of HCC in patients with CHB treated with entecavir (ETV) or tenofovir disoproxil fumarate (TDF).

METHODS

Study population

We obtained data from adult treatment-naive CHB patients who initiated treatment with ETV (0.5 mg/d) or TDF (300 mg/d) at Asan Medical Center, a 2,700-bed academic tertiary referral hospital in Seoul, Korea, between January 2007 and December 2016 (Figure 1). All patients had been positive for hepatitis B surface antigen (HBsAg) for at least 6 months and did not have a history of HCC or other malignancies. We excluded patients who met any of the following criteria: (i) age <20 or >80 years; (ii) serum HBV DNA at baseline <2,000 IU/mL (or undetectable); (iii) >2 weeks of previous treatment with other antiviral agents; (iv) insufficient medical records (no baseline ALT or HBV DNA data); and (v) coinfection with hepatitis C virus, hepatitis D virus, human immunodeficiency virus, or other hepatotropic viruses.

After further excluding patients treated for <1 year or who developed clinical outcomes (i.e., HCC, death, or transplantation) within 1 year of treatment, 4,639 patients were included in the 1-year landmark analysis. The 2-year landmark analysis included 4,152 patients after exclusion of those with follow-up of <2 years or occurrence of clinical outcomes in 2 years of treatment.

The Institutional Review Board of Asan Medical Center approved the study.

Clinical and laboratory variables

Data on clinical information and outcomes were extracted from electronic medical records. All patients had undergone standard clinical examinations; liver function tests; and assays for HBeAg, hepatitis B e antibody, and HBV DNA levels, at baseline and every 3–6 months during follow-up. Serum HBV DNA levels were measured using a real-time polymerase chain reaction assay (linear dynamic detection range, 15–10^9 IU/mL; Abbott Laboratories, Chicago, IL). HBV genotypes were not determined because >98% of Korean patients with CHB have HBV genotype C.

Cirrhosis was defined as the presence of any of the following findings: coarse liver echotexture and nodular liver surface on ultrasonography, clinical features of portal hypertension (e.g., ascites, splenomegaly, or varices), or thrombocytopenia (<150,000/mm^3). The presence of fatty liver was determined using ultrasonography.

Surrogate endpoints

VR was defined as undetectable serum HBV DNA levels (<15 IU/mL). Normal ALT was defined as ≤35 U/L for men and ≤25 U/L for women, following the recommendation of the American Association for the Study of Liver Diseases (4).

Clinical outcomes

The primary study outcome of interest was the development of HCC, and secondary outcomes included all-cause mortality and liver transplantation.

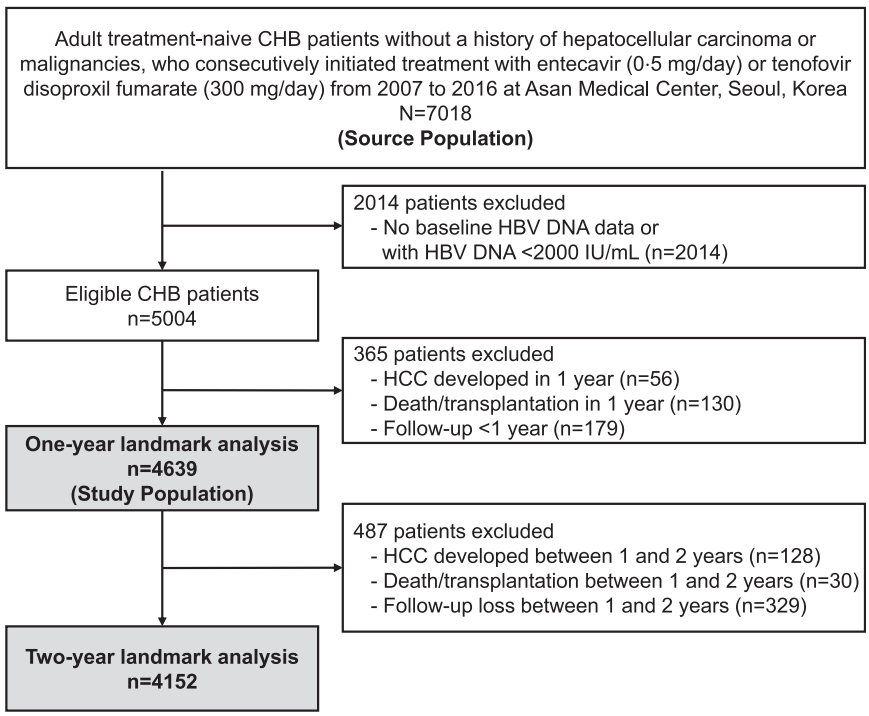

Figure 1. Study flow. CHB, chronic hepatitis B; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

The patients underwent regular surveillance for HCC with liver ultrasonography and serum alpha-fetoprotein measurements every 6 months. HCC was diagnosed histologically or by using noninvasive diagnostic criteria based on corresponding international guidelines of HCC at the time of HCC diagnosis (8,9). All patients were advised to continue the treatment with ETV or TDF even after HBeAg seroclearance until achieving HBsAg seroclearance.

The patients were followed up until the earliest of the following events: diagnosis of HCC, death of any cause, liver transplantation, last follow-up date, or December 31, 2018. Information on vital status and HCC diagnosis for all patients was validated using the Korean National Health Insurance Service database, which covers >99% of the Korean population.

Statistical analysis

The cumulative incidence rates of HCC and death/transplantation were estimated using the Kaplan-Meier method and compared using the log-rank test.

The timing of ALT normalization, VR, or HBeAg seroclearance varies among patients under antiviral treatment. The rate of clinical outcomes may be underestimated in patients achieving the surrogate endpoints after the baseline and overestimated in those who do not achieve the surrogate endpoints, leading to immortal time bias or guarantee time bias (10,11). Therefore, 2 statistical methods were applied to avoid immortal time bias (11). First, landmark analysis was used by redefining time zero as a specific landmark time (at 1 year and 2 years after treatment initiation), in which patients being treated at the landmark time were separated into categories described by the classifying event and followed forward in time. For example, if a patient achieved VR after the "landmark point," then the patient would be placed in the no-VR group because this patient already had a time at risk of HCC while not achieving VR. Second, a time-dependent Cox regression analysis was used considering the time variation of surrogate endpoints. Hence, the regression coefficients could be estimated more accurately (12).

All statistical analyses were performed using the R program (http://cran.r-project.org/). All reported P values are 2-sided, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of the study population

A total of 4,639 patients with CHB who were treated with ETV or TDF without the occurrence of clinical outcomes during the initial 1 year were included in the 1-year landmark analysis (Figure 1). The mean patient age was 47.0 years, and 64.5% of the patients were men (Table 1). Cirrhosis was present in 43.7% of the patients, and 64.2% were HBeAg-positive. The median HBV DNA level was 6.7 $\log_{10}$ IU/mL, and the median ALT level was 93 U/L. Fatty liver was present in 24.2% of the patients.

The 2-year landmark analysis included 4,152 patients who continued the treatment without the occurrence of clinical outcomes during the initial 2 years (Figure 1). Most of the baseline characteristics were similar between the patients included in the 1-year and 2-year landmark analyses (Table 1).

Table 1. Baseline characteristics of patients with CHB treated with ETV or TDF

Characteristics	Patients included in 1-yr landmark analysis	Patients included in 2-yr landmark analysis
No. of patients	4,639	4,152
Age, mean ± SD, yr	47.0 ± 10.9	46.8 ± 10.8
Men, n (%)	2,990 (64.5)	2,682 (64.6)
Cirrhosis, n (%)	2,027 (43.7)	1,811 (43.6)
HBeAg positivity[a], n (%)	2,732 (64.2)	2,475 (64.9)
HBV DNA, median (IQR), $\log_{10}$ IU/mL	6.7 (5.5–7.9)	6.7 (5.5–7.9)
AST, median (IQR), U/mL	74 (46–131)	74 (47–132)
ALT, median (IQR), U/mL	93 (47–184)	95 (47–186)
Albumin, median (IQR), g/dL	4.0 (3.6–4.2])	4.0 (3.6–4.2)
Total bilirubin, median (IQR), mg/dL	1.0 (0.8–1.4)	1.0 (0.8–1.4)
Prothrombin time, median (IQR), INR	1.1 (1.0–1.1)	1.1 (1.0–1.1)
Platelets, median (IQR), 1,000/mm^3	159 (116–199)	160 (117–199)
Creatinine, mean ± SD, mg/dL	0.8 (0.7–0.9)	0.8 (0.7–0.9)
BMI, mean ± SD	24.1 ± 3.4	24.0 ± 3.4
Diabetes mellitus, n (%)	281 (6.1)	234 (5.6)
Fatty liver[b], n (%)	1,124 (24.2)	1,047 (25.2)
Hypertension, n (%)	333 (7.2)	280 (6.7)
ETV/tenofovir, n (%)	3,065/1,574 (66.1/33.9)	2,803/1,349 (67.5/32.5)
CU-HCC score, mean ± SD	15.8 ± 12.9	15.6 ± 12.8
GAG-HCC score, mean ± SD	95.6 ± 22.4	95.5 ± 22.3
PAGE-B score, mean ± SD	13.4 ± 5.0	13.3 ± 4.9
REACH-B score, mean ± SD	11.2 ± 2.4	11.1 ± 2.4

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHB, chronic hepatitis B; CU, Chinese University; ETV, entecavir; GAG, guide with age, gender; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HBV DNA, core promoter mutations and cirrhosis; HCC, hepatocellular carcinoma; INR, international normalized ratio; IQR, interquartile range; PAGE-B, risk score based on age, gender, and platelets; REACH-B, risk estimation for hepatocellular carcinoma in chronic hepatitis B; TDF, tenofovir disoproxil fumarate.
[a]Information was unavailable in 382 patients.
[b]Information was unavailable in 59 patients.

Clinical outcomes

Among the total 4,639 patients, 509 developed HCC with an annual incidence rate of 1.99 per 100 person-years during the median treatment period of 5.6 years (range 1.0–10.9 years). The 3-, 5-, and 10-year cumulative HCC risk in Kaplan-Meier analysis was 5.1%, 9.2%, and 18.8%, respectively. The annual HCC incidence was significantly higher in patients with cirrhosis than in those without cirrhosis (3.7% vs 0.6%; $P < 0.001$).

Death or transplantation occurred in 220 patients with an annual incidence rate of 0.81 per 100 person-years. The cumulative incidence rates of death or transplantation were 1.9%, 3.9%, and 8.0% at 3, 5, and 10 years, respectively.

ALT normalization and HCC risk

ALT normalization occurred in 4,135 (89.1%) patients during the overall treatment period with cumulative rates of 65.6%, 81.9%, and 90.9% at 1, 2, and 5 years of treatment, respectively, in Kaplan-Meier analysis (see Figure 1a, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

ALT normalization at 1 and 2 years of treatment was significantly associated with a reduced HCC risk during the overall follow-up period in the 1-year landmark analysis ($P < 0.001$; Figure 2a) and the 2-year landmark analysis ($P < 0.001$; Figure 2b).

In the 2-year landmark analysis, earlier normalization of ALT levels in the first 2 years of treatment was associated with an incrementally decreasing risk of HCC in a dose-dependent manner ($P < 0.001$; Figure 3a). The HCC risk was the lowest in patients with ALT normalization at 6 months, followed by those with ALT normalization between 6 and 12 months, ALT normalization between 12 and 24 months, and no ALT normalization by 24 months.

At baseline, 3,066 (73.8%) patients showed no evidence of fatty liver disease on ultrasonography. In these patients, delayed ALT normalization was associated with an incrementally increasing risk of HCC during the follow-up period ($P < 0.001$; Figure 3b).

Of the 3,054 patients who achieved VR in the first 2 years of treatment, 2,641 (86.5%) also achieved ALT normalization in the same period. In this subgroup of patients, earlier ALT normalization was again associated with an incrementally decreasing risk of HCC during the follow-up period ($P < 0.001$; Figure 3c).

Regardless of presence of cirrhosis, earlier ALT normalization in the first 2 years of treatment was significantly associated with decreasing risk of HCC ($P < 0.001$ for both of cirrhosis and noncirrhosis subcohorts; see Table 1 and Figure 2a, b, Supplementary Digital Content 1, http://links.lww.com/AJG/B339). The similar results were consistently observed by separate analyses according to the type of antiviral drug (see Table 2 and Figure 3a, b, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

Virological response and HCC risk

A total of 4,040 (87.1%) patients achieved VR during the overall treatment period. The cumulative VR rates in Kaplan-Meier analysis were 45.4%, 72.6%, and 86.8% at 1, 2, and 5 years of treatment, respectively (see Figure 1b, Supplementary Digital Content 1, http://links.lww.com/AJG/B339). In patients without VR at 2 years of treatment, the median HBV DNA level was 300 IU/mL (interquartile range 54–9,900 IU/mL), and 69.1% had HBV DNA levels <2,000 IU/mL.

In the 2-year landmark analysis, the HCC risk was not significantly different between patients who achieved VR at 2 years of treatment and those who did not achieve VR in the same period ($P = 0.32$; Figure 4a).

HBeAg seroclearance and HCC risk

Among 2,732 HBeAg-positive patients at baseline, 1,119 (41.0%) achieved HBeAg seroclearance during the overall treatment period. The cumulative HBeAg seroclearance rates were 14.5%, 24.6%, and 40.8% at 1, 2, and 5 years of treatment, respectively (see Figure 1c, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

In the 2-year landmark analysis, HBeAg seroclearance at 2 years of treatment was not associated with a significantly reduced HCC risk during the overall follow-up period ($P = 0.22$; Figure 4b).

Time-dependent cox analyses

In multivariable time-dependent Cox regression analysis, earlier ALT normalization during the overall treatment period was an independent factor that was significantly associated with a lower HCC risk (adjusted hazard ratio [AHR] 0.57; 95% confidence interval [CI] 0.45–0.71; $P < 0.001$; Table 2).

Compared with ALT normalization within 6 months of treatment, delayed ALT normalization at 6–12, 12–24, and >24 months was associated with an incremental risk of HCC with AHR of 1.40 (95% CI 1.05–1.87; $P = 0.02$), 1.74 (95% CI 1.29–2.35; $P < 0.001$), and 2.45 (95% CI 1.89–3.17; $P < 0.001$), respectively (Table 3).

This incremental HCC risk associated with delayed ALT normalization was consistently observed in various subgroups,

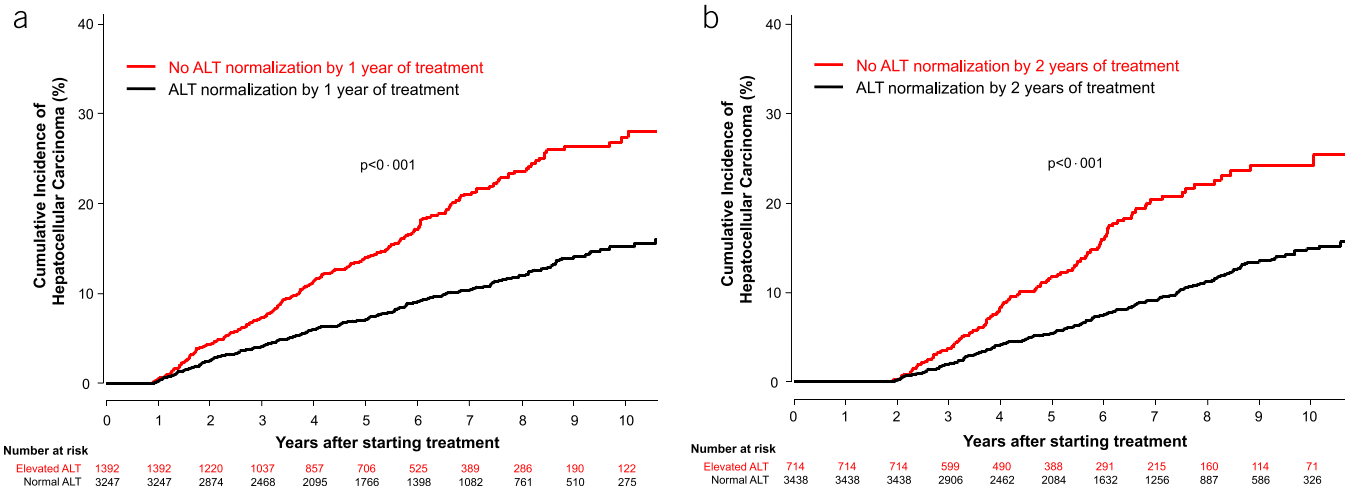

Figure 2. Risk of HCC according to ALT normalization during treatment in chronic hepatitis B patients. (**a**) One-year landmark analysis. (**b**) Two-year landmark analysis. ALT, alanine aminotransferase; HCC, hepatocellular carcinoma.

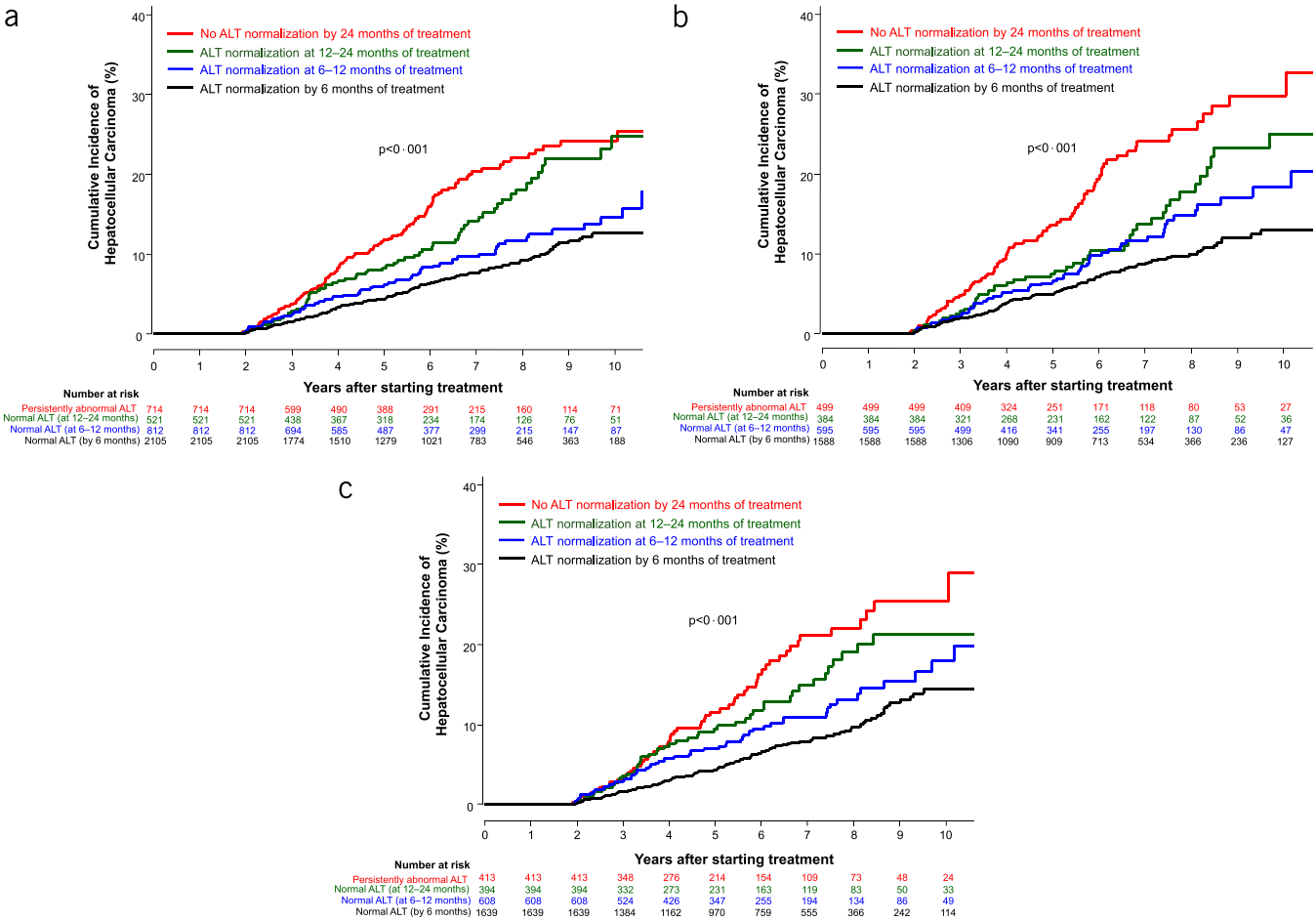

Figure 3. Risk of HCC according to the timing of ALT normalization by 2-year landmark analysis. (**a**) Entire cohort (n = 4,152). (**b**) Patients without fatty liver disease at baseline (n = 3,066). (**c**) Patients who achieved virological response by 2 years of treatment (n = 3,054). ALT, alanine aminotransferase; HCC, hepatocellular carcinoma.

including patients without fatty liver disease, those with VR, and those with cirrhosis ($P < 0.05$ for all; Table 3). This dose-response relationship persisted in patients with fatty liver (see Table 3, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

By contrast, neither earlier VR (AHR 0.93; 95% CI 0.73–1.17; $P = 0.53$) nor earlier HBeAg seroclearance (AHR, 0.91; 95% CI 0.75–1.10; $P = 0.31$) was independently associated with a significantly lower HCC risk in the multivariable time-dependent Cox regression analysis (Table 2).

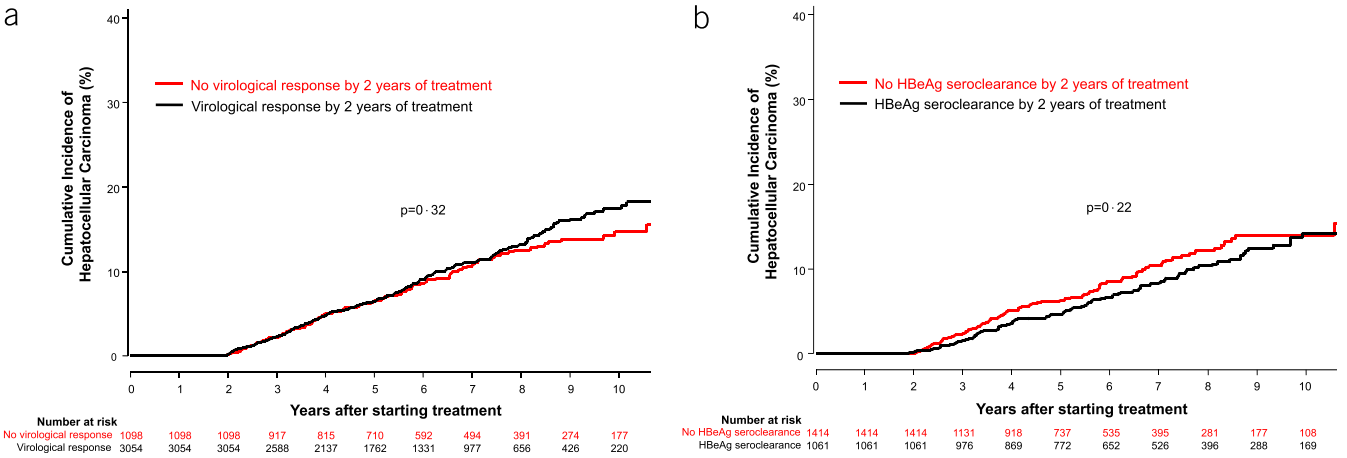

Figure 4. Risk of HCC according to virologic response and HBeAg seroclearance by 2-year landmark analysis. (**a**) According to virological response. (**b**) According to HBeAg seroclearance. HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma.

Table 2. Time-dependent Cox regression analysis for factors predictive of HCC in patients with CHB treated with entecavir or tenofovir

Variables	Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P* value	AHR (95% CI)	*P* value
On-treatment variables[a]				
ALT normalization during treatment[b]	0.54 (0.43–0.67)	<0.001	0.57 (0.45–0.71)	<0.001
VR during treatment[c]	0.99 (0.79–1.23)	0.90	0.93 (0.73–1.17)	0.53
HBeAg Positivity during treatment	0.68 (0.57–0.81)	<0.001	0.91 (0.75–1.10)	0.31
Baseline variables				
Age, yr	1.07 (1.06–1.08)	<0.001	1.06 (1.05–1.07)	<0.001
Sex, male	1.67 (1.36–2.05)	<0.001	2.45 (1.98–3.03)	<0.001
Cirrhosis	5.84 (4.64–7.34)	<0.001	2.30 (1.77–2.99)	<0.001
HBV DNA, $\log_{10}$ IU/mL	0.85 (0.80–0.90)	<0.001	0.91 (0.86–0.98)	0.01
Albumin, g/dL	0.46 (0.41–0.53)	<0.001	0.67 (0.57–0.80)	<0.001
Total bilirubin, mg/dL	1.04 (1.01–1.06)	0.01	0.97 (0.93–1.01)	0.09
Prothrombin time, INR	2.88 (2.31–3.58)	<0.001	0.80 (0.49–1.30)	0.37
Platelets, ×1,000/mm^3	0.98 (0.98–0.99)	<0.001	0.99 (0.99–0.99)	<0.001
Diabetes	1.92 (1.44–2.56)	<0.001	1.11 (0.83–1.49)	0.49
Fatty liver	0.74 (0.60–0.91)	0.005	1.13 (0.92–1.39)	0.24

Total number of patients, 4,639; number of events (HCC), 509.
AHR, adjusted hazard ratio; ALT, alanine aminotransferase; CHB, chronic hepatitis B; CI, confidence interval; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; HR, hazard ratio; INR, international normalized ratio; VR, virological response.
[a]Analyzed as time-varying variables.
[b]Normal ALT was defined as ≤35 U/L for men and ≤25 U/L for women.
[c]VR was defined as serum HBV DNA levels <15 IU/mL.

Death or liver transplantation

ALT normalization at 1 and 2 years of treatment was significantly associated with a reduced risk of overall death or transplantation during the follow-up period in the 1-year landmark analysis ($P < 0.001$; see Figure 4a, Supplementary Digital Content 1, http://links.lww.com/AJG/B339) and the 2-year landmark analysis ($P < 0.001$; see Figure 4b, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

Factors associated with ALT normalization

Of 3,438 patients who achieved ALT normalization at 2 years of treatment, 2,105 (61.2%) achieved early (<6 months) ALT normalization, whereas 1,333 (38.8%) achieved late (7–24 months) ALT normalization. By multivariable analysis, factors associated with early ALT normalization were male, lower HBV DNA at baseline, absence of fatty liver, and treatment with TDF (see Table 4, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

Table 3. Summary of time-dependent cox regression analysis for the risk of HCC according to the timing of ALT normalization in various patient subgroups

Timing of ALT normalization during treatment	Entire population (n = 4,152)		Patients without fatty liver (n = 3,066)		Patients with VR (n = 3,054)		Patients with cirrhosis (n = 1,811)	
	AHR[a] (95% CI)	*P* value	AHR[a] (95% CI)	*P* value	AHR[b] (95% CI)	*P* value	AHR[c] (95% CI)	*P* value
At ≤6 mo	1.00		1.00		1.00		1.00	
At 6–12 mo	1.40 (1.05–1.87)	0.02	1.48 (1.08–2.04)	0.02	1.59 (1.16–2.20)	0.004	1.44 (1.06–1.96)	0.02
At 12–24 mo	1.74 (1.29–2.35)	<0.001	1.58 (1.11–2.24)	0.01	1.68 (1.19–2.37)	0.003	1.49 (1.07–2.07)	0.02
At >24 mo	2.45 (1.89–3.17)	<0.001	2.58 (1.93–3.45)	<0.001	2.50 (1.80–3.47)	<0.001	2.22 (1.68–2.94)	<0.001

AHR, adjusted hazard ratio; ALT, alanine aminotransferase; CI, confidence interval, HBV, hepatitis B virus; HCC, hepatocellular carcinoma; VR, virological response.
[a]Adjusted for age, sex, HBV DNA levels, albumin levels, platelet counts, cirrhosis, and diabetes at baseline.
[b]Adjusted for age, sex, HBV DNA levels, albumin levels, platelet counts, cirrhosis, diabetes, and fatty liver at baseline.
[c]Adjusted for age, sex, HBV DNA levels, albumin levels, platelet counts, diabetes, and fatty liver at baseline.

Among 3,054 patients who achieved VR at 2 years of treatment, 413 (13.5%) failed to achieve ALT normalization. Old age, female, cirrhosis, hyperbilirubinemia, and presence of fatty liver at baseline were independently associated with no normalization of ALT at 2 years of treatment by multivariable analysis (see Table 5, Supplementary Digital Content 1, http://links.lww.com/AJG/B339).

DISCUSSION

This study demonstrated that earlier on-treatment ALT normalization was a surrogate endpoint that was independently associated with proportionally lower HCC risk in patients with CHB who initiated treatment with ETV or TDF, regardless of fatty liver or cirrhosis at baseline and achievement of VR during treatment. This result was consistently observed in the 1- and 2-year landmark analyses and in time-dependent Cox analysis. Compared with ALT normalization within 6 months of treatment, delayed ALT normalization at 6–12, 12–24, and >24 months was associated with an incrementally increasing risk of HCC during the overall follow-up period.

Similar to our results, a recent large-scale historical cohort study from Hong Kong showed that normal on-treatment ALT in the first 12 months of antiviral treatment was associated with a significantly reduced risk of hepatic events including HCC (7). Unfortunately, the confounding effects of fatty liver, VR, and HBeAg seroclearance on the association between on-treatment normal ALT and clinical outcomes were not assessed in this study. In this regard, the results of this study provide novel information proving the association between on-treatment ALT normalization and the risk of HCC and death/transplantation, which is independent of fatty liver disease, VR, and HBeAg seroclearance. However, on-treatment ALT normalization should not be interpreted as a criterion for discontinuation of NUCs.

Our previous study showed that the achievement of HBsAg seroclearance during NUC treatment is significantly associated with improved clinical outcomes and can be used as a criterion for a safe discontinuation of the therapy (13). However, HBsAg seroclearance is rare, if ever achievable, and needs long-term (almost indefinite) NUC therapy in most patients with CHB. Without HBsAg seroclearance, HCC can occur even during long-term continuous treatment with highly potent NUCs, as repeatedly demonstrated by our study and other studies (14–17). Therefore, in this study, we focused on the validation of surrogate endpoints during continuous treatment with highly potent NUCs.

The clinical benefit of on-treatment VR during continuous NUC therapy has been demonstrated in a randomized trial comparing lamivudine and placebo (18). Therefore, VR has been considered a key indicator of a good treatment response (3,4). However, with the current preferred NUCs (ETV, TDF, or tenofovir alafenamide [TAF]), most patients achieve VR with negligible risk of drug resistance during long-term therapy (19–22). The lack of an association between VR and HCC risk in this study may be explained by the fact that most patients achieved VR, and even those without VR maintained a very low level of viremia (HBV DNA level < 2,000 IU/mL).

VR is often associated with normalization of ALT levels. Failure of ALT normalization despite VR with potent antiviral treatment could reflect the presence of nonviral causes of elevated ALT, such as fatty liver disease. Indeed, patients who had no risk factors for metabolic syndrome, which is highly related to fatty liver disease, were more likely to achieve ALT normalization during antiviral treatment (20,21). This suggests that the presence of metabolic syndrome may have a negative impact on ALT normalization during antiviral treatment. Nonetheless, in this study, the association of early ALT normalization with a lower HCC risk was observed regardless of the presence of fatty liver disease. This implies that there could be a still unrevealed mechanism. Other viral or host factors that are not readily susceptible to the profound suppression of viral replication may be associated with a prolonged elevation of ALT and continued risk of HCC. These factors may include pre-existing integration of HBV DNA into the host genome and clonal expansion of transformed hepatocytes (23–26), which may be present before the treatment onset and cause persistent instability of hepatocytes during treatment. Further studies are needed to clarify these hypotheses.

Considerable attention has been given to on-treatment ALT normalization because several recent studies have shown that the rate of ALT normalization would be different among patients using different NUCs, and that early on-treatment ALT normalization is associated with clinical outcomes. In 2 phase 3 trials comparing TAF with TDF (20,21,27), the rate of ALT normalization was significantly higher in the TAF group than in the TDF group at all time points. Even after excluding the risk factors for metabolic syndrome in the analysis, the TAF group still had a significantly higher ALT normalization rate than the TDF group (57% vs 42%, respectively) (20,21). Interestingly, in our recent historical cohort study, TDF treatment was associated with a significantly higher rate of ALT normalization at 1 year of treatment and a significantly lower risk of HCC compared with ETV treatment (17). These results suggest that a NUC for HBV is not like the other in terms of ALT normalization rate and also possibly HCC risk.

This study results also raise the question regarding the optimal on-treatment monitoring strategies for patients with CHB, especially in resource-limited settings in low- and middle-income countries. To widen the treatment coverage in low- and middle-income countries, it is essential to develop simple and validated on-treatment monitoring strategies that are feasible and affordable (28,29). This is especially important because current NUC treatment should be continued lifelong for most patients, and the cost of NUC treatment should no longer be the main obstacle (<US$ 50 per year) (30). Our results suggest that on-treatment ALT levels could be used as a single validated surrogate biomarker to assess the long-term clinical benefit in situations in which HBV DNA and HBeAg tests are not feasible, provided that highly potent antiviral agents can be used with high level of medication adherence.

This study has several limitations. First, owing to the nature of a historical cohort study, some unavoidable biases may exist. Because surrogate endpoints occurring at different time points were evaluated, care was taken not to overestimate the impact of surrogate endpoints owing to immortal time bias. Therefore, multiple rigorous statistical methods were used, including landmark analysis at 2 different time points and time-dependent Cox regression analysis. Second, the population selected for this study included only patients of Korean ethnicity, which is mostly infected by HBV genotype C. Thus, the findings of this study should be validated in patients with CHB of other ethnicities and HBV genotypes. Third, this real-world study used

ultrasonographic criteria for the diagnosis of fatty liver and cirrhosis in part. This may underestimate the prevalence of cirrhosis or fatty liver in the study population. However, the prevalence of fatty liver in this study (24.5%) was similar to the prevalence of histologically proven fatty liver (21.8%) among patients with CHB in South Korea (31). Fourth, there would be a possibility that intermittent elevation of ALT levels during treatment was not captured in our study. Finally, recent advances in imaging techniques for HCC diagnosis and assessment of hepatic fibrosis and fatty liver were not applicable to our study, and liver biopsy was not conducted in most of our patients.

In conclusion, our comprehensive analysis including a large number of patients with CHB treated with ETV or TDF demonstrated that earlier ALT normalization was independently associated with significantly lower risks of HCC and death/transplantation, after adjustment of fatty liver at baseline and achievement of VR during treatment. Although the biological mechanism that explains this association should be the subject of future studies, our results suggest that efforts should be made to achieve ALT normalization as early as possible during NUC treatment to minimize the risk of HCC and mortality.

ACKNOWLEDGEMENTS

We thank Drs. Gwang Hyeon Choi, Danbi Lee, Ju Hyun Shim, Kang Mo Kim, Han Chu Lee, Young-Hwa Chung, Yung Sang Lee, and the Information Technology Service Management team at Asan Medical Center for their help in data collection. We also thank the Scientific Publications Team at Asan Medical Center for the editorial assistance in preparing this manuscript. None of them received compensation for their work.

CONFLICTS OF INTEREST

Guarantor of the article: Young-Suk Lim, MD, PhD.

Specific author contributions: All authors have full access to all data used in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. J.C. and Y.-S.L. were responsible for the conception and design of the study; acquisition, analysis, and interpretation of data; and drafting of the manuscript. J.C. and S.H. performed statistical analyses. All authors approved the final version of the manuscript.

Financial support: This study was supported by grants from the Korean National Health Clinical Research (NHCR) project, Ministry of Health & Welfare, Republic of Korea (HC15C3380); the Korean Health Technology R&D Project, Ministry of Health & Welfare (HI19C0790); National Evidence-based Healthcare Collaborating Agency (NECA, no. NH-16-008); National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, no. 2017R1A2B4011233); and the Technology Innovation Program (10079271) funded by the Ministry of Trade, Industry & Energy (MOTIE) of Republic of Korea.

Potential competing interests: No industry funded or supported this study. The interpretation and reporting of the data were the sole responsibility of the authors. Y.-S.L. is an advisory board member of Bayer Healthcare and Gilead Sciences and receives investigator-sponsored research funding from Bayer Healthcare and Gilead Sciences. No other disclosures are declared.

Writing assistance: Dr Joon Seo Lim from the Scientific Publications Team at Asan Medical Center provided editorial assistance in preparing this manuscript without financial compensation.

Study Highlights

WHAT IS KNOWN

- ✓ It was suggested that normal on-treatment serum ALT in the first 12 months of antiviral treatment with nucleos(t)ide analogues is associated with a lower risk of hepatic events in patients with CHB.
- ✓ It is unclear whether earlier ALT normalization is associated with lower HCC risk, independent of fatty liver or cirrhosis and on-treatment virologic response (VR), in patients with CHB.

WHAT IS NEW HERE

- ✓ Compared with ALT normalization within 6 months after initiation of treatment with ETV or tenofovir, delayed ALT normalization at 6–12, 12–24, and >24 months was associated with incrementally increasing risk of HCC, in patients with CHB.
- ✓ This dose-response relationship persisted after exclusion of patients with fatty liver disease and in patients with VR during treatment.
- ✓ By contrast, neither VR nor HBeAg seroclearance during treatment was associated with a significantly lower HCC risk.
- ✓ Efforts may have to be made to achieve ALT normalization as early as possible during treatment in patients with CHB to minimize HCC risk.

REFERENCES

1. Foreman KJ, Marquez N, Dolgert A, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: Reference and alternative scenarios for 2016-40 for 195 countries and territories. Lancet 2018;392:2052–90.
2. European Association for the Study of the Liver. EASL clinical practice guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69:182–236.
3. European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–98.
4. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560–99.
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. Yang HI, Lu SN, Liaw YF, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347:168–74.
7. Wong GL, Chan HL, Tse YK, et al. Normal on-treatment ALT during antiviral treatment is associated with a lower risk of hepatic events in patients with chronic hepatitis B. J Hepatol 2018;69:793–802.
8. Song DS, Bae SH. Changes of guidelines diagnosing hepatocellular carcinoma during the last ten-year period. Clin Mol Hepatol 2012;18:258–67.
9. Song P, Tobe RG, Inagaki Y, et al. The management of hepatocellular carcinoma around the world: A comparison of guidelines from 2001 to 2011. Liver Int 2012;32:1053–63.
10. Suissa S, Azoulay L. Metformin and the risk of cancer: Time-related biases in observational studies. Diabetes Care 2012;35:2665–73.
11. Giobbie-Hurder A, Gelber RD, Regan MM. Challenges of guarantee-time bias. J Clin Oncol 2013;31:2963–9.
12. Zhou Z, Rahme E, Abrahamowicz M, et al. Survival bias associated with time-to-treatment initiation in drug effectiveness evaluation: A comparison of methods. Am J Epidemiol 2005;162:1016–23.
13. Kim GA, Lim YS, An J, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: Clinical outcomes and durability. Gut 2014;63:1325–32.
14. Choi J, Han S, Kim N, et al. Increasing burden of liver cancer despite extensive use of antiviral agents in a hepatitis B virus-endemic population. Hepatology 2017;66:1454–63.
15. Lim YS, Han S, Heo NY, et al. Mortality, liver transplantation, and hepatocellular carcinoma among patients with chronic hepatitis B treated with entecavir vs lamivudine. Gastroenterology 2014;147:152–61.

16. Kim GA, Han S, Kim HD, et al. Higher risk of hepatocellular carcinoma in chronic hepatitis B vs chronic hepatitis C after achievement of virologic response. J Viral Hepat 2017;24:990–7.
17. Choi J, Kim HJ, Lee J, et al. Risk of hepatocellular carcinoma in patients treated with entecavir vs tenofovir for chronic hepatitis B: A Korean Nationwide Cohort Study. JAMA Oncol 2019;5:30–6.
18. Liaw YF, Sung JJ, Chow WC, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351: 1521–31.
19. Buti M, Tsai N, Petersen J, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci 2015;60:1457–64.
20. Chan HL, Fung S, Seto WK, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of HBeAg-positive chronic hepatitis B virus infection: A randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol 2016;1: 185–95.
21. Buti M, Gane E, Seto WK, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of patients with HBeAg-negative chronic hepatitis B virus infection: A randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol 2016;1:196–206.
22. Chang TT, Lai CL, Yoon SK, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2010;51:422–30.
23. Zoulim F, Mason WS. Reasons to consider earlier treatment of chronic HBV infections. Gut 2012;61:333–6.
24. Kennedy PT, Sandalova E, Jo J, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143:637–45.
25. Mason WS, Gill US, Litwin S, et al. HBV DNA integration and clonal hepatocyte expansion in chronic hepatitis B patients considered immune tolerant. Gastroenterology 2016;151:986–98.
26. Svicher V, Salpini R, Battisti A, et al. The integration of Hepatitis B virus into human genome is a common event in the setting of HBeAg negative disease: Implications for the treatment and management of CHB. J Hepatol 2019;70(Suppl 1):e83.
27. Agarwal K, Brunetto M, Seto WK, et al. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol 2018;68:672–81.
28. The World Health Organization. Global health Sector Strategy on Viral Hepatitis 2016–2021. WHO Press: Switzerland, Geneva, 2016.
29. The World Health Organization. Global Hepatitis Report, 2017. WHO Press: Switzerland, Geneva, 2017.
30. Shimakawa Y, Njie R, Ndow G, et al. Development of a simple score based on HBeAg and ALT for selecting patients for HBV treatment in Africa. J Hepatol 2018;69:776–84.
31. Lee YB, Ha Y, Chon YE, et al. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. Clin Mol Hepatol 2019;25:52–64.