의학 박사 학위논문

경구 항바이러스제를 사용한 만성 B 형간염 환자에서 ALT 정상화의 대리표지자로써의 간암 발생 예측

ALT Normalization during Treatment of Chronic Hepatitis B Is Independently Associated with Improved Clinical Outcomes

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최 종 기

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이 논문을 의학 박사 학위 논문으로 제출함

2018 년 12 월

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국문 요약

경구 항바이러스제를 사용한 만성 B 형간염 환자에서 ALT 정상화의 대리표지자로써의 간암 발생 예측

연구배경: 혈청 ALT 수치의 정상화, 바이러스 반응, HBe 항원 혈청소실은 HBe 항원 양성 만성 B 형간염 환자에서 대리 표지자로써 널리 사용되고 있다. 그러나 최근 사용되고 있는 강력한 경구항바이러스제를 사용하는 환자에서 이러한 대리 표지자가 장기 임상 예후에 어떤 영향을 미치는지에 대해서는 충분히 밝혀지지 않았다.

방법: 본 연구는 엔테카비르 혹은 테노포비어를 초치료로 선택한 HBe 항원 양성 만성 B 형간염환자들을 대상으로 2007 년부터 2016 년까지 국내 3 차병원에서 2,630 명의 환자가 포함되었다. 간암 발생, 사망 및 간이식 시행의 위험도를 연구 대상에서 분석하였으며, 정상 혈청 ALT 값은 남성의 경우 35 U/L, 여성의 경우 25 U/L 로 정의하였다. 또한 바이러스 반응은 혈청 B 형 간염 바이러스 수치가 15 IU/mL 로 정의하였다.

결과: 연구에 포함된 환자들의 평균 연령은 45.1 세 였으며, 대상의 65.1%는 남성이었다. 연구 포함 당시 38.7%의 환자가 간경화로 진단 되었으며, 추적 관찰 기간의 중위수는 5.1 년 이었다. 치료를 받는 기간 동안 216 명의 환자에서 간세포암이 발생 하였고, 107 명의 환자가 사망하거나 간이식을 시행 받았다. 대리 표지자와 장기 예후와의 관련성에서 ALT 정상화는 1 년 랜드마크 분석과 2 년 랜드마크 분석 모두에서 간세포암의 발생, 사망 및 간이식의 낮은 위험도와 연관이 있었다. (*P*<0.001) 바이러스 반응의 경우 1 년, 2 년 랜드마크 분석 모두에서 간세포암 발생의 위험도와는 연관이 없었다. 사망 및 간이식의 경우 1 년 랜드마크 분석에서는 유의한 연관성이 없었으나. 2 년 랜드마크 분석에서 바이러스 반응은 낮은 사망 및 간이식 위험도와 유의한 연관성이 있었다. (*P*=0.003) HBe 항원 혈청 소실의 경우 간세포암의 발생, 사망 및 간이식의 위험도와 유의한 연관성을 보여주지 않았다. 시간 의존형 콕스 모델로 분석 하였을때 혈청 ALT 의 정상화는 간세포암 발생의 유의한 예측 인자였으며 (위험도: 0.44, 95% 신뢰구간: 0.32-0.60, *P*<0.001), 또한 사망 및 간이식을 예측할 수 있는 유의한 인자 였다. (위험도: 0.47, 95% 신뢰구간: 0.30-0.72, *P*<0.001). 하지만 바이러스 반응의 경우 사망 및

간이식의 위험도와 연관된 인자 (위험도: 0.61, 95% 신뢰구간: 0.39-0.97, *P*=0.33)였지만, 간세포암 발생 위험 (위험도: 1.24, 95% 신뢰구간: 0.85-1.81, *P*=0.26)과는 연관이 없었다. HBe 항원 혈청 소실의 경우 간세포암의 발생 (위험도: 1.30, 95% 신뢰구간: 0.96-1.75, *P*=0.09), 사망 및 간이식 (위험도: 0.97, 95% 신뢰구간: 0.65-1.46, *P*=0.89) 위험과 유의하지 않았다.

결론: HBe 항원 양성 만성 B 형간염에서 초치료로 강력한 경구 항바이러스제 사용시 혈청 ALT 정상화는 장기 임상 예후, 즉 간세포암, 사망 및 간이식을 예측할 수 있는 유의한 인자였다. 바이러스 반응의 경우 간세포암의 발생과는 연관이 없었으나 사망 및 간의식의 위험과는 유의한 연관이 있었다. HBe 항원 혈청 소실은 간세포암, 사망 및 간이식의 위험과의 연관은 유의하지 않았다.

중심단어: ALT 정상화, HBe 항원 혈청 소실, 간세포암, 바이러스 반응, 대리 표지자

목 차

약어 목록: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; CHB, chronic hepatitis B; ETV, entecavir; HCC, hepatocellular carcinoma; HR, hazard ratio; IPW, inverse probability weighting; NUC, nucleos(t)ide analogues; PY, person-year; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; VR, virological response.

INTRODUCTION

The goal of treatment for patients with chronic hepatitis B (CHB) is to improve survival by preventing disease progression and hepatocellular carcinoma (HCC).[1,2] Ideally, hepatitis B therapies to be approved should demonstrate that they can prevent HCC and liver-related deaths. However, these clinical endpoints evolve over years or decades. Therefore, surrogate biomarkers that are easy to assess and correlate with clinical outcomes have been used to evaluate the treatment efficacy. Those intermediate surrogate endpoints include virological, biochemical, and serological responses.

Surrogate endpoints can substitute the clinical endpoints only when they are in the causal pathway to the clinical outcomes and when the effect of an intervention on the surrogate endpoint explains the effect on subsequent clinical outcomes.[3,4] However, in many circumstances, therapeutic interventions affect clinical endpoints in ways that are not entirely explained by the effects on the surrogate endpoints. This occurs in complex diseases in which a single biomarker may capture only a portion, or none, of the treatment effects.[3]

In the natural course studies for CHB, normalization of serum alanine aminotransferase (ALT) levels and 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 highly potent nucloes(t)ide analogues (NUC). Because recent

clinical trials have suggested that the rate of on-treatment ALT normalization is different among patients using different NUCs,[7,8] such investigations are needed not only to decide how to monitor treatment responses, but also to determine how to treat the patients with CHB.

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

METHODS

Study Population

We obtained data from 3,512 treatment-naïve adult HBeAg-positive CHB patients who consecutively initiated treatment with ETV (0.5 mg/day) or TDF (300 mg/day) 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 at baseline. We excluded the patients who had any of the following criteria: (i) age < 20 or > 80 years, (ii) serum HBV DNA at baseline < 2,000 IU/mL (or undetectable), (iii) more than two weeks of previous treatment with other antiviral agents, (iv) insufficient medical records (no baseline ALT, HBV DNA, or HBeAg status), (v) HCC, death, or liver transplantation within 6 months of treatment, and (vi) co-infection with HIV, hepatitis C virus, or other hepatotropic viruses. For the 1-year landmark analysis, data from patients who were treated for less than 1 year or who developed clinical outcomes (i.e., HCC, death or transplantation) within 1 year of treatment initiation were further excluded. Finally, 2,630 patients were included in the 1-year landmark analysis. In the 2-year landmark analysis, 2,249 patients were included after further excluding the patients who were treated for less than 2 years or who developed clinical outcomes within 2 years of treatment.

The Institutional Review Board of the Asan Medical Center approved the study, and waived the need for informed consents from the patients due to the historical nature of the cohort study.

Clinical and Laboratory Variables

We extracted clinical information including patients' demographics, laboratory parameters, history of antiviral treatments, and clinical outcomes systematically from the electronic medical records. All patients had received standard clinical examinations, liver function tests, and measurement of HBV-related serologic markers including HBeAg, anti-HBe, and HBV DNA levels every 6 months. Serum HBV DNA levels were measured with real-time PCR assay (linear dynamic detection range 15 IU/mL – 1.0×10^9 IU/mL, Abbott Laboratories, Chicago, IL). HBV genotypes were not determined because over 98% of Korean patients with CHB have the HBV genotype C.[9] Cirrhosis was defined if the patients had any of the following criteria: coarse liver echo texture and nodular liver surface on ultrasonography, clinical features of portal hypertension (e.g., ascites, splenomegaly, or varices), or thrombocytopenia (< 150,000/mm^3). The patients received ultrasonography with serum alpha-fetoprotein every 6 months for HCC surveillance. HCC was diagnosed radiologically or histologically based on current HCC guidelines.[10] After treatment initiation, all patients were advised to continue

the treatment even after HBeAg seroclearance, until achieving HBsAg seroclearance.

Surrogate Endpoints

We defined virological response (VR) as undetectable serum HBV DNA levels (< 15 IU/mL). Normal ALT was defined as ≤ 35 and ≤ 25 U/L for men and women, respectively, based on the recommendation of the American Association for the Study of Liver Diseases (AASLD).[2,11] We defined HBeAg seroclearance as the first detection of HBeAg negativity, regardless of the appearance of anti-HBe, once the HBeAg remained negative at subsequent tests throughout the study period.

Clinical Outcomes

The primary outcome of interest of this study was the development of HCC, and secondary outcomes included all-cause mortality and liver transplantation. The follow-up periods were calculated as person-years from the first date of ETV or TDF treatment initiation and until the earliest of the followings: diagnosis of HCC, death from any cause, liver transplantation, last follow-up time, or November 30, 2017. The vital status information and HCC diagnosis for all patients were

validated by using the Korean National Health Insurance Service database, which covers more than 99% of the Korean population.[12]

Statistical Analysis

The baseline characteristics of patients were analyzed by using Student's *t* test for continuous variables and Chi-square or Fisher's exact test for categorical variables as appropriate. The cumulative incidence rates of HCC and death/transplantation were estimated by using the Kaplan–Meier method and were compared by the log-rank test.

The timing of ALT normalization, VR, or HBeAg seroclearance varied among patients under antiviral treatment. Therefore, the rate of clinical outcomes may be underestimated in patients achieving the surrogate endpoints after the baseline, and overestimated in those who do not achieving the surrogate endpoints, leading to immortal time bias or guarantee time bias.[13] Thus, we applied three statistical methods to avoid the immortal time bias.[14] First, the landmark analysis was used, by redefining time zero at a specific landmark time at 1-year and 2-years of treatment, where the patients being treated at the landmark time are separated into categories described by the classifying event and followed forward in time.[15] For example, if a patient achieved VR after the "landmark point", then the patient would be placed in a no-VR group, because this patient already had a time at-risk of HCC while not achieving VR. Secondly, a

time-dependent Cox analysis was used considering the time variation of ALT normalization, VR, and HBeAg seroclearance. Hence, the regression coefficients were estimated more accurately.[16] Lastly, the inverse probability weighting (IPW) in a two-stage model was used.[14] The probability of achieving surrogate endpoints during follow-up time intervals was first modeled using patient baseline characteristics as predictors. Then, each patient was weighted by the inverse of the probabilities from the previous estimation. The weighted cumulative incidence of HCC and death or transplantation were assessed by using the Kaplan–Meier method. All statistical analyses were performed by using the R program (http://cran.r-project.org/). All reported *P* values are two sided, and *P* values of <0.05 were considered significant.

RESULTS

Data from a total of 2,630 HBeAg-positive patients with CHB who maintained treatment with ETV or TDF for at least 1 year without occurrence of clinical outcomes were analyzed. The mean patients' age was 45.1 years, 65.1 % were men while 34.9% were women (Table 1).

Clinical Outcomes

In total, 216 patients developed HCC with an annual incidence rate of 1.61 per 100 person-years (PYs) during the median follow-up period of 5.1 years (range: 1.0-10.9 years) with continued treatment. The 3-, 5-, and 10-year cumulative probabilities of HCC development were 4.1%, 7.6%, and 15.3%, respectively. The annual HCC incidence was significantly higher in patients with cirrhosis than in those without cirrhosis (3.33% vs. 0.46%, $P < 0.001$).

Death/transplantation occurred in 107 patients with an annual incidence rate of 0.77 per 100 PYs. The cumulative rates of death/transplantation were 1.7%, 3.5%, and 7.0% at 3, 5, and 10 years, respectively.

Virological Response, Landmark Analyses

A total of 2,341 (89.0%) patients achieved VR during the overall period of treatment. The cumulative VR rates by Kaplan–Meier analysis were 51.4%, 75.8%, and 90.7% at 1, 2, and 5 years of treatment, respectively (Supplementary Figure 1A).

By the 1-year landmark analysis, the VR achievement was not significantly associated with the risks of HCC and death/transplantation (P = 0.10 and P = 0.19, respectively, Supplementary Figure 2). By the 2-year landmark analysis, the VR was not significantly associated with the risk of HCC (P = 0.97, Figure 2A). However, the VR was significantly associated with a lower risk of death/transplantation (P = 0.003, Figure 2B).

ALT Normalization, Landmark Analyses

Of 2,630 cases included in the 1-year landmark analysis, 63.0% had normal ALT at 1 year of treatment. Of 2,249 cases in the 2-year landmark analysis set, 80.8% had normal ALT at 2 years of treatment. The cumulative rates of on-treatment ALT normalization were 84.7% and 90.6% at 3 and 5 years of treatment, respectively, in the entire cohort (Supplementary Figure 1B).

By the 1-year landmark analysis, ALT normalization was associated with a significantly lower risk of HCC and death/transplantation (P < 0.001 for both, Supplementary Figure 3A and B). By the 2-year landmark analysis, ALT

normalization was also associated with a significantly lower risk of HCC and death/transplantation ($P < 0.001$ for both, Figure 3).

Of the 1,675 patients who achieved VR at 1 year, 1,146 (68.4%) had normal ALT. Patients who achieved both VR and ALT normalization at 1 year were associated with a lower risk of HCC and death/transplantation compared with those achieving only VR without ALT normalization ($P < 0.001$ for both, Supplementary Figure 3).

Of the 1,657 patients with normal ALT at 1 year of treatment, 1408 (85.0%) had VR or HBV DNA levels < 2,000 IU/mL, and only 121 (7.3%) had HBV DNA ≥ 2,000 IU/mL at the same time point (Supplementary Table 1). In contrast, of the 973 patients without normal ALT at 1 year of treatment, 12.9% (a significantly higher proportion) had elevated HBV DNA levels (≥ 2,000 IU/mL, $P < 0.001$).

HBeAg Seroclearance, Landmark Analyses

A total of 1,102 (41.9%) patients achieved HBeAg seroclearance during the overall treatment period. At 2 years of treatment, 609 patients had achieved HBeAg seroclearance. The cumulative HBeAg seroclearance rates were 30.4% and 43.4% at 3 and 5 years of treatment, respectively (Supplementary Figure 1C).

By the 2-year landmark analysis, the HBeAg seroclearance was not significantly associated with the risk of HCC and death/transplantation ($P = 0.13$ and $P = 0.34$, respectively, Figure 4).

Time-Dependent Cox Analyses

In the time-dependent Cox analysis, VR achievement during the overall period of treatment was not significantly associated with the risk of HCC (adjusted hazard ratio [aHR] = 1.24, 95% CI = 0.85 - 1.81, *P* = 0.26). However, VR was independently associated with a significantly lower risk of death or transplantation (aHR = 0.61, 95% CI = 0.39 - 0.97, *P* = 0.03, Table 2).

By the multivariable time-dependent Cox analysis, ALT normalization during the overall period of treatment was an independent factor that was significantly associated with a lower risk of HCC (aHR = 0.44, 95% CI = 0.32 - 0.60, *P* < 0.001) and death/transplantation (aHR = 0.47, 95% CI = 0.30 - 0.72, *P* < 0.001, Table 2).

In contrast, HBeAg seroclearance during the overall period of treatment was not significantly associated with the risk of HCC (aHR = 1.30, 95% CI = 0.96 - 1.75, *P* = 0.09) or death/transplantation (HR = 0.97, 95% CI = 0.65 - 1.46, *P* = 0.89, Table 2).

Inverse Probability Weighting (IPW) Analyses

By the IPW analysis, VR achievement was not associated with the risk of HCC (HR = 1.16, 95% CI = 0.84 - 1.49, *P* = 0.45), but was significantly associated with

a lower risk of death/transplantation (HR = 0.65, 95% CI = 0.44 - 0.98, *P* = 0.03, Table 3). ALT normalization during the overall treatment period was again significantly associated with a lower risk of HCC (HR = 0.51, 95% CI = 0.38 - 0.67, *P* < 0.001) and death/transplantation (HR = 0.71, 95% CI = 0.53 - 0.94, *P* = 0.02). HBeAg seroclearance was not associated with the risk of HCC (HR = 1.22, *P* = 0.25) or death/transplantation (HR = 0.76, *P* = 0.33).

Subgroup Analyses by Presence of Cirrhosis

By the presence of cirrhosis, subgroup analyses were performed. In patients with cirrhosis, VR was not associated with the risk of HCC, (Supplementary Figure 4) whereas ALT normalization was independently associated with the risk of HCC by landmark analyses (Supplementary Figure 5), time-varying covariate cox model (aHR = 0.54, 95% CI = 0.38-0.78, *P* < 0.001)as well as IPW (HR = 0.62, 95% CI = 0.45-0.84, *P* = 0.002). (Supplementary table 3) Achievement of VR and ALT normalization were associated with the risk of death/transplantation in patients with cirrhosis as entire cohort. HBeAg seroclearance was not associated with the risk of HCC (aHR = 1.22, 95% CI = 0.88-1.70, *P*=0.23 by time-varying cox model; HR = 1.25, 95% CI = 0.86-1.80, *P* = 0.24 by IPW) nor death/transplantation. (aHR = 0.68, 95% CI = 0.37-1.23, *P* = 0.20 by time-varying cox model; HR = 0.68, 95% CI = 0.37-1.23, *P* = 0.20 by IPW; Supplementary Figure 6)

In patients without cirrhosis, the risk of HCC and death/transplantation did not differ regardless of achievement of VR (Supplementary Figure 7), whereas ALT normalization was independently associated with the risk of HCC and death/transplantation. (aHR = 0.12, 95% CI = 0.04-0.36, $P < 0.001$ by time-varying cox model; HR = 0.18, 95% CI = 0.06-0.55, $P = 0.002$ by IPW; Supplementary Figure 8) Consistent with cirrhotic patients, HBeAg seroclearance in patients without cirrhosis did not differ in terms of the risk of HCC or death/transplantation. (Supplementary Figure 9)

DISCUSSION

This comprehensive analysis demonstrated that on-treatment ALT normalization was a surrogate endpoint that was independently associated with the significantly lower risk of HCC and death/transplantation in treatment-naïve HBeAg-positive CHB patients who initiated treatment with ETV or TDF. On-treatment VR showed an association with significantly lower risk of death/transplantation especially in the patients with cirrhosis, but it was not associated with a lower risk of HCC. In contrast, HBeAg-seroclearance during treatment was not associated with a lower risk of HCC or death/transplantation. These results were consistently obtained by 1-year and 2-year landmark analyses, time-dependent Cox analyses, and IPW analyses, in the entire cohort and subcohorts divided by the presence of cirrhosis at baseline.

Surrogate endpoints are widely used to evaluate the treatment efficacy and to decide when to stop the treatment in the management of patients with CHB. A validated surrogate endpoint is defined as an endpoint supported by a clear mechanistic rationale and clinical data providing strong evidence that an effect on the surrogate endpoint predicts a specific clinical benefit.[17] 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 to safely discontinue the therapy.[18] Accordingly, the HBsAg seroclearance is now viewed as a validated surrogate endpoint indicating

functional cure of CHB.[1,2] However, the HBsAg seroclearance is very rarely achievable, necessitating long-term (almost indefinite) NUC therapy in most patients with CHB. Without HBsAg seroclearance, adverse clinical outcomes, especially HCC, occur even during long-term continuous treatment with highly potent NUCs, as repeatedly demonstrated by our studies and those reported by other groups.[19-23] Therefore, in this study, we focused on the validation of surrogate endpoints during continuous treatment with highly potent NUCs to provide evidence for selection of adequate monitoring strategies.

The clinical benefit of the on-treatment VR during continuous NUC therapy has been demonstrated in a randomized trial comparing lamivudine and placebo.[24] Therefore, VR is considered a key indicator of a good response to treatment.[1,2] With the current preferred NUCs (ETV, TDF, or tenofovir alafenamide [TAF]), the persistent viremia is defined as a plateau in the decline of HBV DNA and/or failure to achieve an undetectable HBV DNA level after 96 weeks of therapy.[2] Fortunately, most of the patients treated with ETV, TDF, or TAF achieve complete viral suppression with negligible risk of drug-resistance during the long-term therapy.[7,8,25,26] Thus, the most common cause of persistent viremia is patients' poor adherence to medication.[1,2] Our results shows that the patients without VR had significantly higher risk of death/transplantation are thought to reflect this point, and suggest that long-term adherence to NUC, which may be reflected in VR, needs to be encouraged to prevent hepatic decompensation. A high level of medication adherence may be ensured by

educating the patients and taking thorough histories, especially in resource-limited settings, as previously shown by a study.[27] The lack of association between VR and HCC risk may be explained by the fact that most patients without VR maintained low level viremias (HBV DNA levels < 2,000 IU/mL).

VR is often associated with normalization of ALT levels. However, our present study showed that among patients who achieved VR at 1 year, only 68.4% had normal ALT levels. The definition of normal ALT in this study (≤ 35 U/L for men and ≤ 25 for women) was based on our own previous study results,[11] which are now being recommended by the AASLD.[2] Patients who achieved both VR and ALT normalization at 1 year were associated with a lower risk of HCC and death/transplantation, but those achieving only VR without ALT normalization were not. The most likely explanation for these findings would be the persistence of concomitant liver injury by other causes, such as alcoholic or non-alcoholic fatty liver disease.[1,28,29]

Considerable attention has been given to the on-treatment ALT normalization due to the unexpected results of the two phase 3 trials comparing the efficacy between TAF and TDF.[7,8] In these trials, 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).[7,8,30] However, the correlation between ALT normalization and clinical outcomes during long-term therapy remained obscure

until recently. Similar to our results, a recent large-scale historical cohort study from Hong Kong has shown that normal on-treatment ALT in the first 12 months of antiviral treatment is associated with a significantly reduced risk of hepatic events including HCC.[31] Unfortunately, the confounding effect of VR and HBeAg seroclearance in the association between the on-treatment normal ALT and clinical outcomes was not assessed in that study. In these regards, the results of our present study provide novel information proving the association between on-treatment ALT normalization and the risk of HCC and death/transplant, which is independent of VR and HBeAg seroclearance. The biological mechanism that explains this association should be the object of future studies.

During natural course and interferon treatment of HBeAg-positive patients with CHB, HBeAg seroclearance has been associated with a reduced risk of HCC.[32,33] However, in the natural course of CHB patients as revealed from the REVEAL untreated cohort, the HBeAg seroclearance was only dependent on HBV DNA levels, and was not an independent predictor for HCC.[34,35] Furthermore, HBeAg seroclearance has not been studied as a valid surrogate endpoint associated with clinical outcomes during continuous treatment with highly potent NUCs. Our novel findings showed that HBeAg seroclearance under treatment with ETV or TDF did not alter the risk of HCC and death/transplantation.

The results of this study raise the intriguing question on the optimal on-treatment monitoring strategies for patients with CHB, especially in resource-limited settings in low- and middle-income economy countries. In 2016, the World

Health Organization (WHO) developed an ambitious strategy to eliminate viral hepatitis as a threat to public health by 2030 aiming to reduce the mortality of chronic HBV infection by 65%.[36,37] To achieve this goal, the WHO also set a global target for treatment coverage in people with chronic HBV infection eligible for antiviral therapy from 8% in 2015 to 80% in 2030.[36,37] To scale up the treatment coverage in low- and middle-income economy countries, it is essential to develop simple and validated on-treatment monitoring strategies that are feasible and affordable. 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). Nevertheless, repeated testing of HBV DNA and HBeAg during long-term treatment may be complex and unaffordable in resource-limited settings. In contrast, ALT measurement is widely available and affordable. Our results suggest that on-treatment ALT monitoring could be used as a single validated surrogate endpoint to assess long-term clinical benefit where HBV DNA and HBeAg tests are not feasible, provided that highly potent antiviral agents can be used. Indeed, among patients with normal on-treatment ALT at 1 and 2 years of treatment, only less than 8% had HBV DNA $\geq$ 2,000 IU/mL that was suspected to be related to poor medication adherence.

It should be underlined that the current study has a few limitations. First, due to the nature of historical cohort study, some biases may have been unavoidable. Because surrogate endpoints occurring at different points in time were evaluated, care was taken not to overestimate the impact of surrogate

endpoints by immortal time bias or guarantee time bias. To avoid the biases, multiple rigorous statistical methods were used, including landmark analysis at two different time points, time-dependent Cox regression analysis, and IPW analysis. Secondly, the population selected for this study included only Korean ethnicity patients infected by HBV genotype C that have a higher risk of HCC development than those infected with other genotypes.[9] Thus, the findings of this study should be carefully extrapolated to patients with CHB of other ethnicities and HBV genotypes. Finally, new emerging biomarkers for HBV infection, such as quantitative HBsAg, Hepatitis B core-related antigen, and HBV RNA levels that may help stratify the patients for the risks of clinical events were not considered herein,[38,39] because most of the patients started treatment before the availability of those markers.

In conclusion, our comprehensive analysis including a large number of HBeAg-positive patients with CHB treated with ETV or TDF demonstrated that on-treatment ALT normalization could be used as a valid surrogate endpoint for predicting HCC development and death/transplantation, independent of VR and HBeAg seroclearance. Achievement of VR was associated only with a lower risk of death/transplantation, but did not predict the risk of HCC development. In contrast, we found no association between HBeAg seroclearance and clinical outcomes. Our results suggest that on-treatment monitoring of ALT could be used to assess the treatment response and to predict long-term clinical benefit in patients under potent NUC therapy, especially in resource-limited settings where

HBV DNA and HBeAg testing is unaffordable or unfeasible. Further studies on emerging biomarkers for HBV infection are warranted to stratify the HBeAg-positive patients with CHB for the risk of clinical events during long-term antiviral treatment.

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TABLES

Table 1. Baseline characteristics of the HBeAg-positive patients with chronic hepatitis B treated with entecavir or tenofovir disoproxil fumarate

Characteristics	Subjects included in 1-year landmark analysis (n = 2,630)	Subjects included in 2-year landmark analysis (n = 2,249)
Age, mean ± SD, years	45.1 ± 11.4	44.8 ± 11.2
Men, n (%)	1,712 (65.1)	1,477 (65.7)
Cirrhosis, n (%)	1,018 (38.7)	891 (39.6)
HBV-DNA, median (IQR), log_{10}IU/mL	7.34 (6.04–8.28)	7.36 (6.04–8.28)
AST, median (IQR), IU/mL	77 (47–141)	78 (47–145)
ALT, median (IQR), IU/mL	96 (47–195)	100 (48–200)
Elevated ALT by AASLD criteria*	2,298 (87.4)	1,975 (87.8)
ALT ≥ 5 times of UNL*	820 (31.2)	725 (32.2)
Elevated ALT by local lab criteria†	2,126 (80.8)	1,833 (81.5)
Albumin, median (IQR), g/dL	3.9 (3.6–4.2)	3.9 (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	1.07 (1.01–1.16)	1.07 (1.01–1.16)
Platelets, median (IQR), 1000/mm^3	164 (120–203)	163 (121–203)
Creatinine, median (IQR), mg/dL	0.8 (0.7–1.0)	0.8 (0.7–1.0)
Entecavir/Tenofovir, n (%)	1,826/804 (69.4/30.6)	1,671/578 (74.3/25.7)
Diabetes mellitus, n (%)	139 (5.3)	111 (4.9)
CU-HCC score, median (IQR)	8.5 (4.0–23.5)	8.5 (4.0–23.5)
GAG-HCC score, median (IQR)	90.9 (76.3–113.5)	91.1 (76.4–113.8)
PAGE-B score, median (IQR)	14.0 (10.0–16.0)	14.0 (10.0–16.0)
REACH-B score, median (IQR)	12.0 (10.0–13.0)	12.0 (10.0–13.0)

ALT, alanine aminotransferase; CU, Chinese University; GAG, guide with age, gender, HBV DNA, core promoter mutations and cirrhosis; INR, international normalized ratio; IQR, interquartile range; PAGE-B based on age, gender, and platelets; REACH-B, risk estimation for hepatocellular carcinoma in chronic hepatitis B; SD, standard deviation; UNL, upper normal limit.

*Normal ALT levels by the American Association for the Study of Liver Diseases (AASLD) criteria (≤ 35 U/L for men and ≤ 25 U/L for women)

†Normal ALT levels by local laboratory criteria (≤ 40 U/L for both men and women)

Table 2. Time-dependent Cox analysis for the factors predicting hepatocellular carcinoma and death/transplantation among HBeAg-positive patients with chronic hepatitis B

Hepatocellular Carcinoma				
	Univariate analysis		Multivariate analysis*	
	HR (95% CI)	P value	HR (95% CI)	P value
Virologic response*†	1.24 (0.85–1.81)	0.26		
ALT normalization*‡	0.40 (0.29–0.53)	< 0.001	0.44 (0.32–0.60)	< 0.001
HBeAg seroclearance*	1.30 (0.92–1.84)	0.14	1.30 (0.96–1.75)	0.09
Age, per 1-year increase	1.07 (1.06–1.08)	< 0.001	1.05 (1.03–1.07)	< 0.001
Gender, men	1.47 (1.08–2.01)	0.01	2.05 (1.49–2.84)	< 0.001
HBV DNA, log (IU/mL)	0.83 (0.77–0.89)	< 0.001	0.92 (0.84–1.02)	0.11
ALT ≥ 5 times of UNL	0.25 (0.16–0.38)	< 0.001	0.46 (0.30–0.72)	< 0.001
Prothrombin time, INR	2.77 (2.26–4.11)	< 0.001	0.61 (0.28–1.33)	0.22
Albumin, g/dL	0.47 (0.39–0.57)	< 0.001	0.77 (0.59–0.99)	0.049
Total bilirubin, mg/dL	1.01 (0.95–1.06)	0.79		
Platelets, x 1000/mm^3	0.98 (0.98–0.99)	< 0.001	0.99 (0.99–0.99)	< 0.001
Cirrhosis	7.12 (5.00–10.14)	< 0.001	2.11 (1.40–3.18)	< 0.001
Diabetes	1.85 (1.20–2.87)	0.01	0.92 (0.57–1.47)	0.72
FIB-4 index	1.06 (1.04-1.07)	<0.001	0.99 (0.95-1.03)	0.63
APRI	1.00 (0.97-1.103)	0.96		
Death or Liver Transplantation				

	Univariate analysis		Multivariate analysis*	
	HR (95% CI)	P value	HR (95% CI)	P value
Virologic response*†	0.48 (0.31–0.74)	< 0.001	0.61 (0.39–0.97)	0.03
ALT normalization*‡	0.29 (0.19–0.43)	< 0.001	0.47 (0.30–0.72)	< 0.001
HBeAg seroclearance*	0.97 (0.65–1.46)	0.89		
Age, per 1–year increase	1.06 (1.04–1.08)	< 0.001	1.02 (1.00–1.05)	0.03
Sex, male	1.14 (0.77–1.69)	0.54		
HBV DNA, log (IU/mL)	0.87 (0.78–0.97)	0.02	0.92 (0.80–1.06)	0.26
ALT ≥5 times of UNL	0.51 (0.32–0.82)	< 0.001	1.03 (0.61–1.73)	0.92
Prothrombin time, INR	4.35 (3.10–6.11)	< 0.001	1.36 (0.76–2.44)	0.31
Albumin, g/dL	0.33 (0.26–0.42)	< 0.001	0.55 (0.40–0.76)	< 0.001
Total bilirubin, mg/dL	1.04 (0.98–1.10)	< 0.001	0.94 (0.85–1.04)	0.24
Platelets	0.98 (0.98–0.99)	< 0.001	0.99 (0.99–1.00)	< 0.001
Cirrhosis	7.53 (4.43–12.82)	< 0.001	2.20 (1.17–4.13)	0.01
Diabetes	3.04 (1.78–5.17)	< 0.001	1.88 (1.09–3.24)	0.02
FIB-4 index	1.07 (1.06-1.09)	<0.001	1.06 (0.99-1.15)	0.11
APRI	1.02 (1.00-1.04)	0.02	0.96 (0.89-1.03)	0.22

ALT, alanine aminotransferase; APRI, AST-to-platelet ratio; CI, confidence interval; HR, hazard ratio; INR, international normalized ratio; SD, standard deviation; UNL, upper normal limit.

*Adjusted using time-dependent Cox proportional hazards model

†Defined as serum HBV DNA levels less than 15 IU/mL

‡By American Association for the Study of Liver Diseases (AASLD) criteria (≤ 35 U/L for men and ≤ 25 U/L for women)

Table 3. Summary of analyses for the risk of clinical outcomes by achievement of on-treatment surrogate endpoints

Hepatocellular Carcinoma						
Virological response*		No. of patients		HR	95% CI	*P*
		Virological response	No virological response			
Landmark	Year 1	1,675	955	1.27	0.96–1.69	0.10
	Year 2	1,736	513	1.01	0.71–1.44	0.97
Time–varying Cox	—	2,341	289	1.24	0.85–1.81	0.26
IPW	—	—	—	1.16	0.84–1.49	0.45
ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	1,657	973	0.38	0.29–0.49	< 0.001
	Year 2	1,817	432	0.40	0.29–0.55	< 0.001
Time–varying Cox	—	2,318	312	0.44	0.32–0.60	< 0.001
IPW	—	—	—	0.51	0.38–0.67	< 0.001
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	609	1,641	1.29	0.92–1.79	0.14
Time–varying Cox	—	1,102	1,528	1.30	0.96–1.75	0.09
IPW	—	—	—	1.22	0.87–1.72	0.25
Death or Transplantation						
Virologic response		No. of patients		HR	95% CI	*P*
		Virologic response	No virologic response			
Landmark	Year 1	1,675	955	0.82	0.56–1.21	0.19
	Year 2	1,736	513	0.49	0.31–0.79	0.003
Time–varying Cox	—	2,341	289	0.61	0.39–0.97	0.03
IPW	—	—	—	0.65	0.44–0.98	0.03

ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	1,657	973	0.56	0.38–0.82	< 0.001
	Year 2	1,817	432	0.38	0.24–0.61	< 0.001
Time–varying cox	—	2,318	312	0.47	0.30–0.72	< 0.001
IPW	—	—	—	0.71	0.53–0.94	0.02
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	609	1,641	0.81	0.47–1.37	0.43
Time–varying Cox	—	1,102	1,528	0.97	0.65–1.46	0.89
IPW	—	—	—	0.76	0.44–1.32	0.33

ALT, alanine aminotransferase; CI, confidence interval; HR, hazard ratio; IPW. inverse probability weighting.

*Defined as serum HBV DNA levels less than 15 IU/mL

†By American Association for the Study of Liver Diseases (AASLD) criteria (≤ 35 U/L for men and ≤ 25 U/L for women)

FIGURES

Figure 1. Study flow diagram

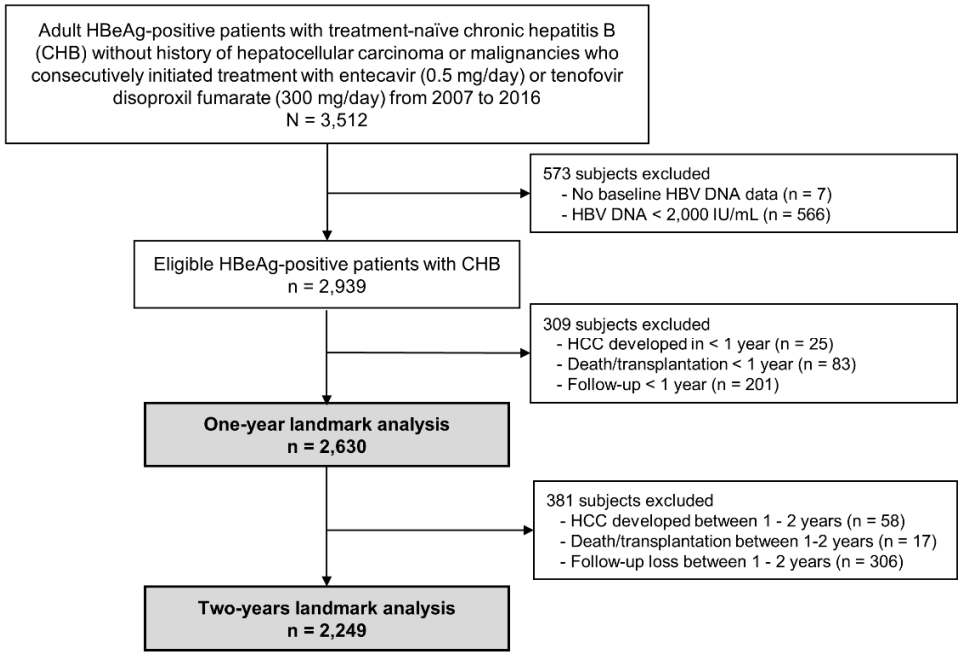
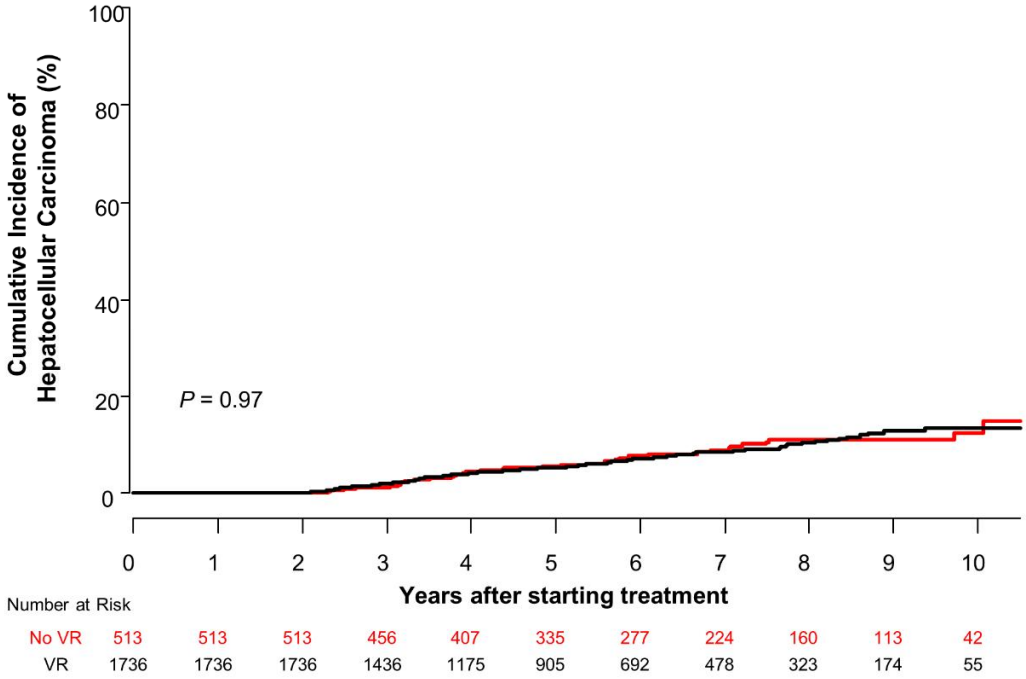


Figure 2. Landmark analysis according to virological response at 2 years of treatment

A. Cumulative incidence of hepatocellular carcinoma



B. Cumulative incidence of death / transplantation

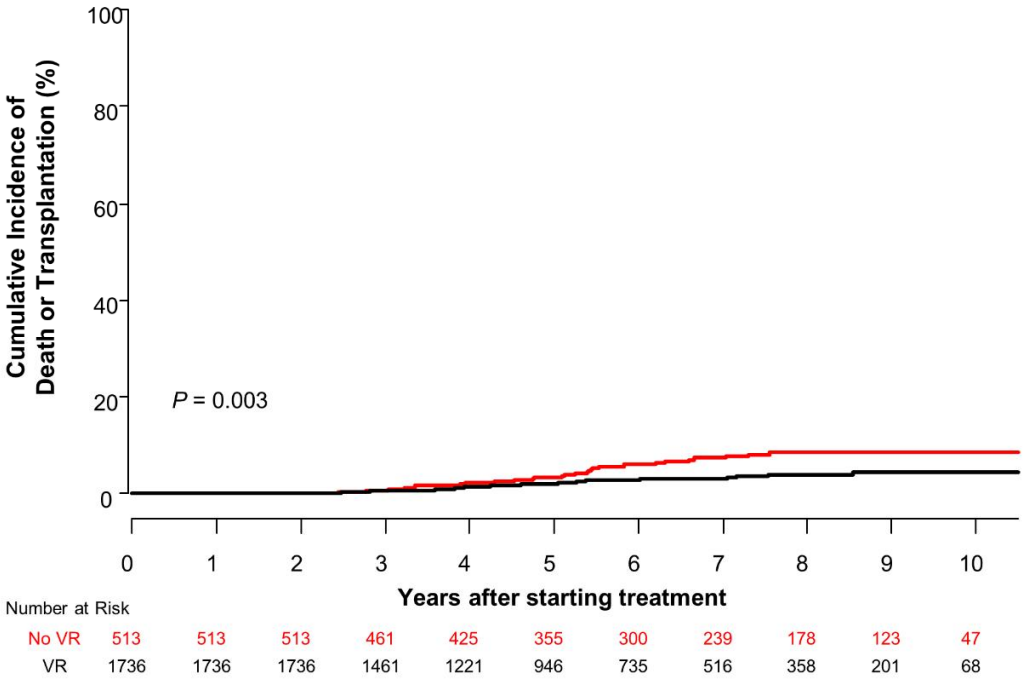
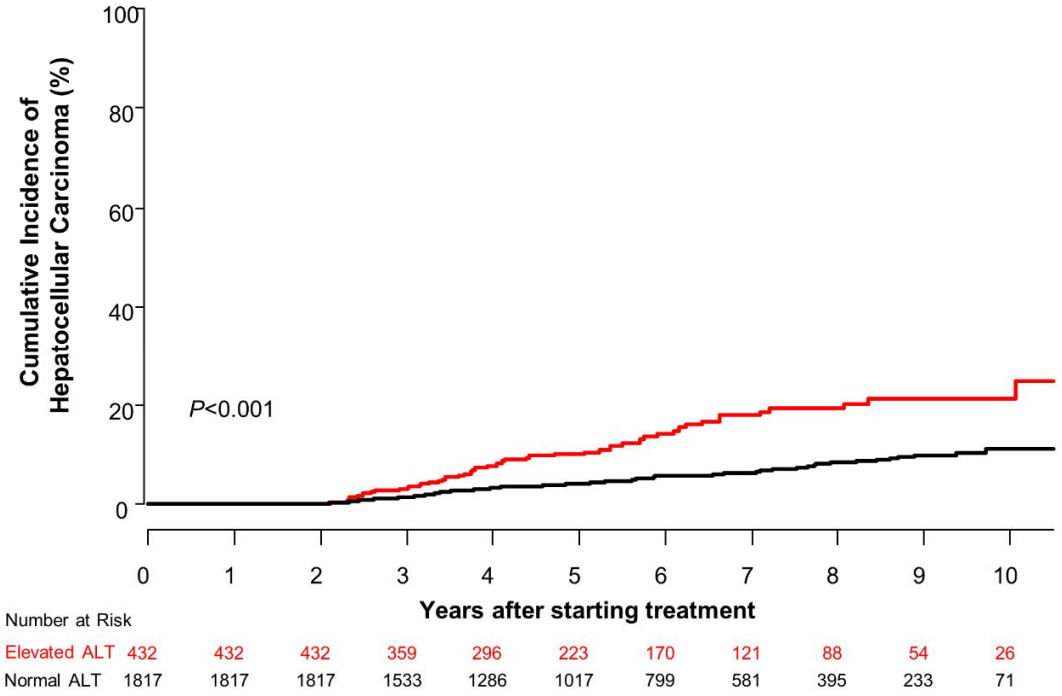


Figure 3. Landmark analysis according to ALT normalization at 2 year of treatment

A. Cumulative incidence of hepatocellular carcinoma



B. Cumulative incidence of death / transplantation

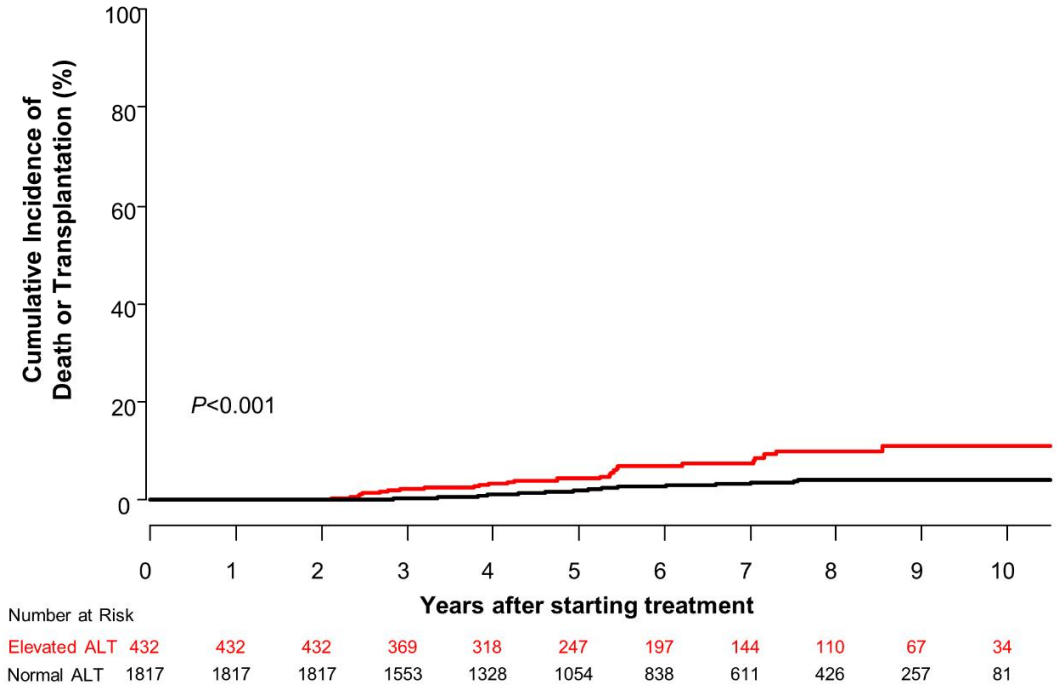
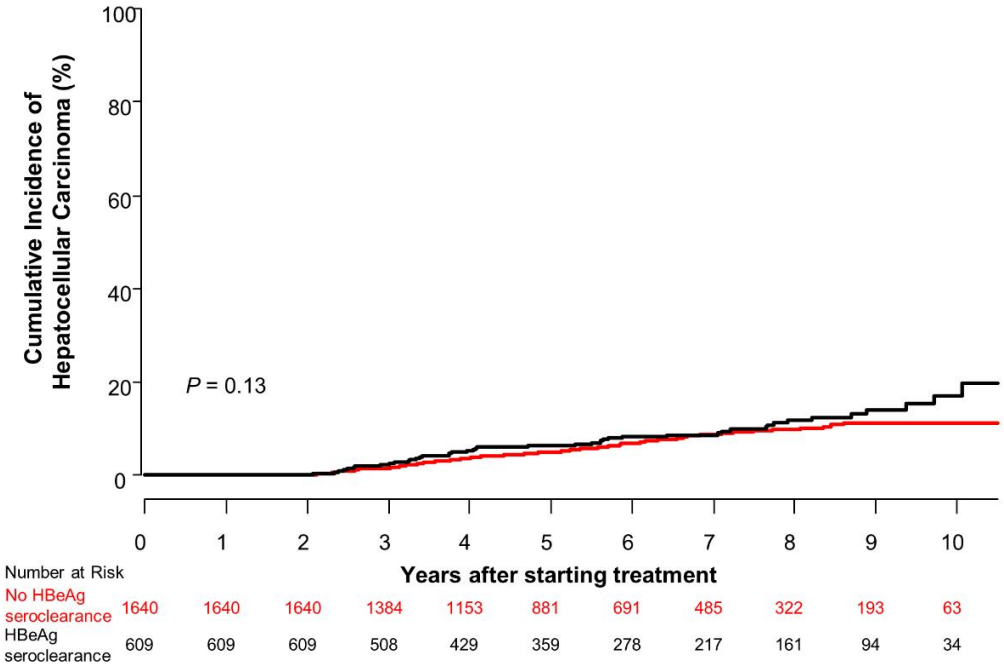
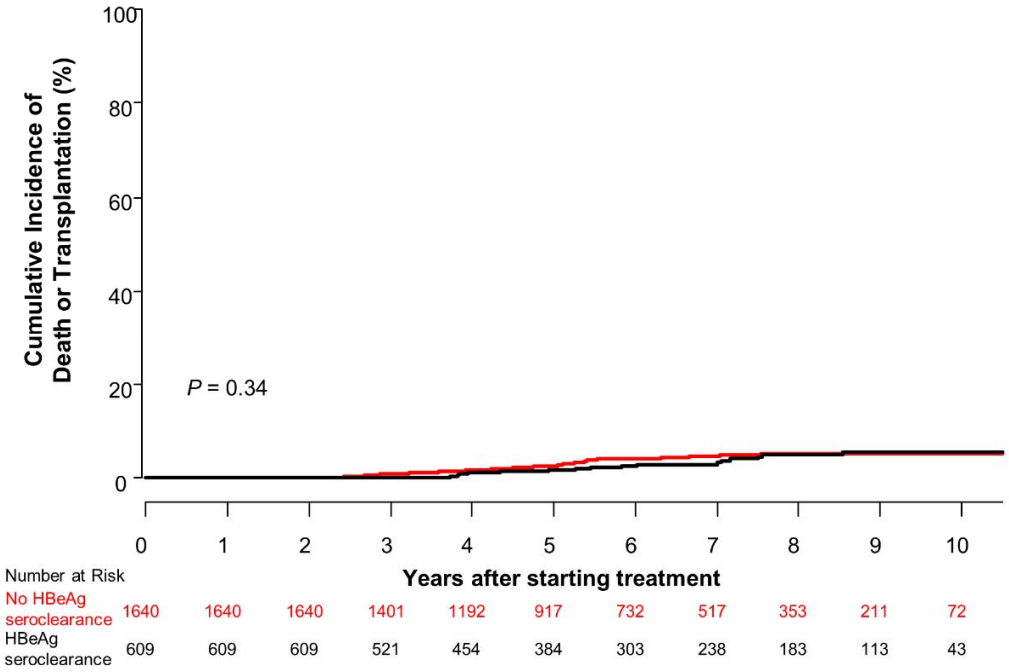


Figure 4. Landmark analysis according to HBeAg seroclearance at 2 years of treatment

A. Cumulative incidence of hepatocellular carcinoma



B. Cumulative incidence of death / transplantation



Appendix

Supplementary Table 1. Proportion of patients with virological response in those achieving ALT normalization at 1 and 2 years, respectively.

Category	Patients with VR no (%)	Patients with HBV DNA <2,000 IU/mL no (%)	Patients with persistent viremia* no (%)	*P*†	Patients with HBV DNA unavailable no (%)	Total number of patients
At 1 year						
ALT normal	1146 (69.2)	262 (15.8)	121 (7.3)	< 0.001	128 (7.7)	1657
Elevated ALT	529 (54.4)	166 (17.1)	126 (12.9)		152 (15.6)	973
At 2 years						
ALT normal	1483 (81.6)	23 (1.3)	145 (8.0)	< 0.001	166 (9.1)	1817
Elevated ALT	253 (58.6)	14 (3.2)	62 (14.4)		103 (23.8)	432

ALT, alanine aminotransferase; VR, virological response (undetectable serum HBV DNA [<15 IU/mL]).

*Defined as serum HBV DNA ≥ 2,000 IU/mL

†P values for persistent viremia after excluding patients with HBV DNA unavailable.

Supplementary Table 2. Summary of analyses for the risk of clinical outcomes by achievement of on-treatment surrogate endpoints in patients with cirrhosis

Hepatocellular Carcinoma						
Virological response*		No. of patients		HR	95% CI	*P*
		Virological response	No virological response			
Landmark	Year 1	688	330	1.01	0.74-1.37	0.96
	Year 2	697	194	0.79	0.54-1.16	0.23
Time–varying Cox	—	916	102	1.41	0.91-2.19	0.12
IPW	—	—	—			
ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	564	454	0.56	0.42-0.75	<0.001
	Year 2	665	226	0.61	0.42-0.87	0.007
Time–varying Cox	—	873	145	0.54	0.38-0.78	<0.001
IPW	—	—	—	0.62	0.45-0.84	0.002
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	273	618	1.15	0.80-1.64	0.46
Time–varying Cox	—	508	510	1.22	0.88-1.70	0.23
IPW	—	—	—	1.25	0.86-1.80	0.24
Death or Transplantation						
Virological response*		No. of patients		HR	95% CI	*P*
		Virologic response	No virologic response			
Landmark	Year 1	688	330	0.62	0.41-0.94	0.02
	Year 2	697	194	0.35	0.22-0.58	<0.001

Time–varying Cox	—	916	102	0.58	0.34-0.99	0.04
IPW	—	—	—	0.62	0.40-0.92	0.02
ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	564	454	0.92	0.61-1.39	0.69
	Year 2	665	226	0.56	0.34-0.93	0.02
Time–varying cox	—	873	145	0.58	0.36-0.93	0.02
IPW	—	—	—	0.98	0.64-1.50	0.92
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	273	618	0.62	0.35-1.11	0.11
Time–varying Cox	—	508	510	1.04	0.64-1.69	0.87
IPW	—	—	—	0.68	0.37-1.23	0.20

Abbreviations: ALT - alanine aminotransferase, CI - confidence interval, HR - hazard ratio, IPW - inverse probability weighting.

*Defined HBV DNA less than 15 IU/mL

†By American Association for the Study of Liver Diseases (AASLD) criteria (≤ 25 U/L for women and ≤ 35 U/L for men)

Supplementary Table 3. Summary of analyses for the risk of clinical outcomes by achievement of on-treatment surrogate endpoints in patients without cirrhosis

Hepatocellular Carcinoma						
Virological response*		No. of patients		HR	95% CI	*P*
		Virological response	No virological response			
Landmark	Year 1	987	625	1.82	0.90-3.70	0.09
	Year 2	1039	319	2.26	0.77-6.59	0.13
Time–varying Cox	—	1425	187	0.98	0.39-2.51	0.97
IPW	—	—	—	1.50	0.69-3.24	0.31
ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	1093	519	0.21	0.11-0.42	<0.001
	Year 2	1152	206	0.21	0.09-0.44	<0.001
Time–varying Cox	—	1445	167	0.20	0.10-0.41	<0.001
IPW	—	—	—	0.26	0.13-0.53	<0.001
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	336	1022	1.07	0.45-2.54	0.89
Time–varying Cox	—	594	1018	1.67	0.79-3.53	0.18
IPW	—	—	—	0.84	0.34-2.05	0.70
Death or Transplantation						
Virologic response*		No. of patients		HR	95% CI	*P*
		Virologic response	No virologic response			
Landmark	Year 1	987	625	1.01	0.37-2.72	0.99
	Year 2	1039	319	1.72	0.37-8.01	0.48
Time–varying Cox	—	1425	187	1.04	0.24-4.67	0.93

IPW	—	—	—	0.80	0.29-2.26	0.68
ALT normalization†		No. of patients		HR	95% CI	*P*
		ALT normalization	Elevated ALT			
Landmark	Year 1	1093	519	0.20	0.07-0.58	<0.001
	Year 2	1152	206	0.31	0.09-1.00	0.05
Time–varying cox	—	1445	167	0.12	0.04-0.36	<0.001
IPW	—	—	—	0.18	0.06-0.55	0.002
HBeAg seroclearance		No. of patients		HR	95% CI	*P*
		HBeAg clearance	No HBeAg clearance			
Landmark	Year 2	336	1022	1.09	0.29-4.11	0.90
Time–varying Cox	—	594	1018	0.83	0.23-3.07	0.77
IPW	—	—	—	0.80	0.21-3.09	0.74

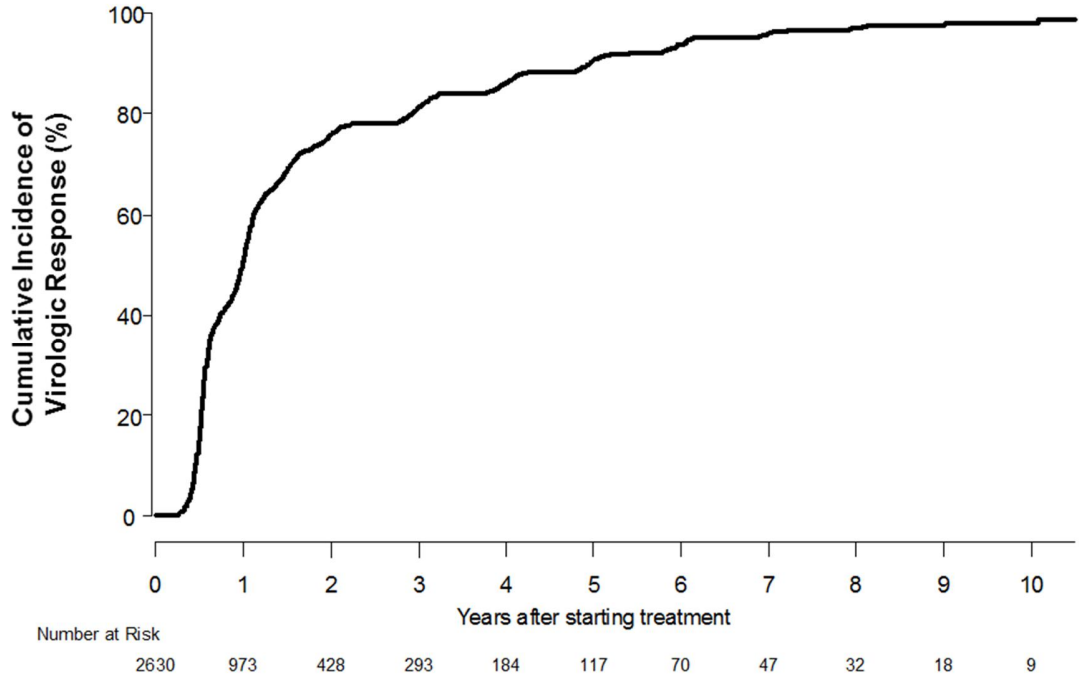
Abbreviations: ALT - alanine aminotransferase, CI - confidence interval, HR - hazard ratio, IPW - inverse probability weighting.

*Defined HBV DNA less than 15 IU/mL

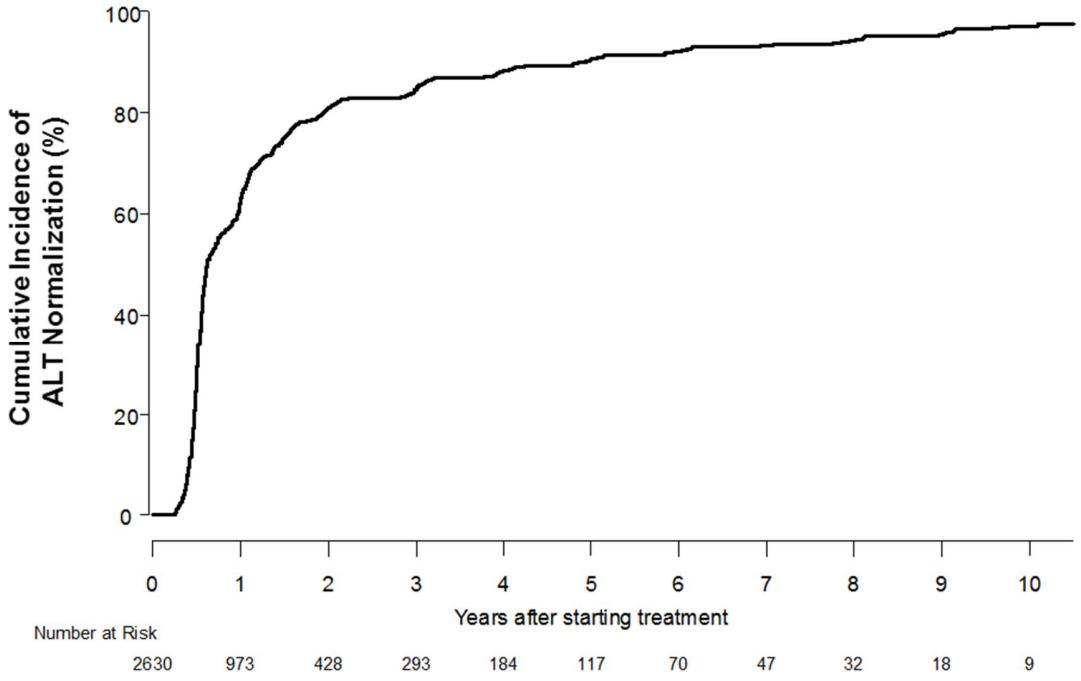
†By American Association for the Study of Liver Diseases (AASLD) criteria (≤ 25 U/L for women and ≤ 35 U/L for men)

Supplementary Figure 1. Cumulative rates of (A) virological response, (B) ALT normalization, and (C) HBeAg seroclearance during treatment with entecavir or tenofovir disoproxil fumarate in patients with chronic hepatitis B

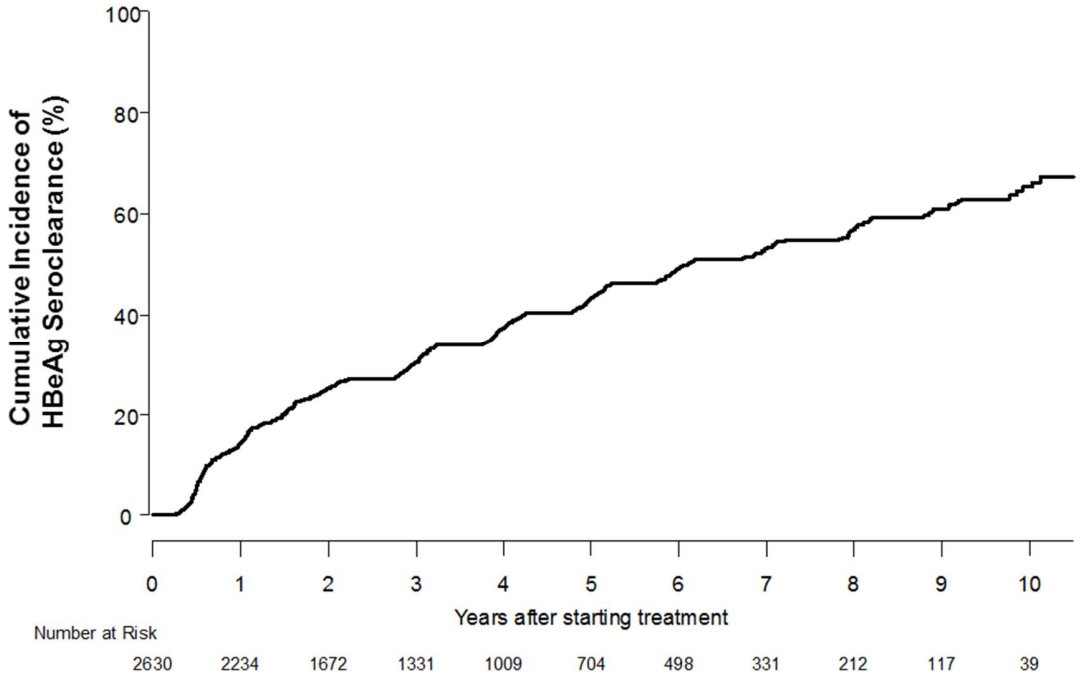
A. Virological response



B. ALT normalization

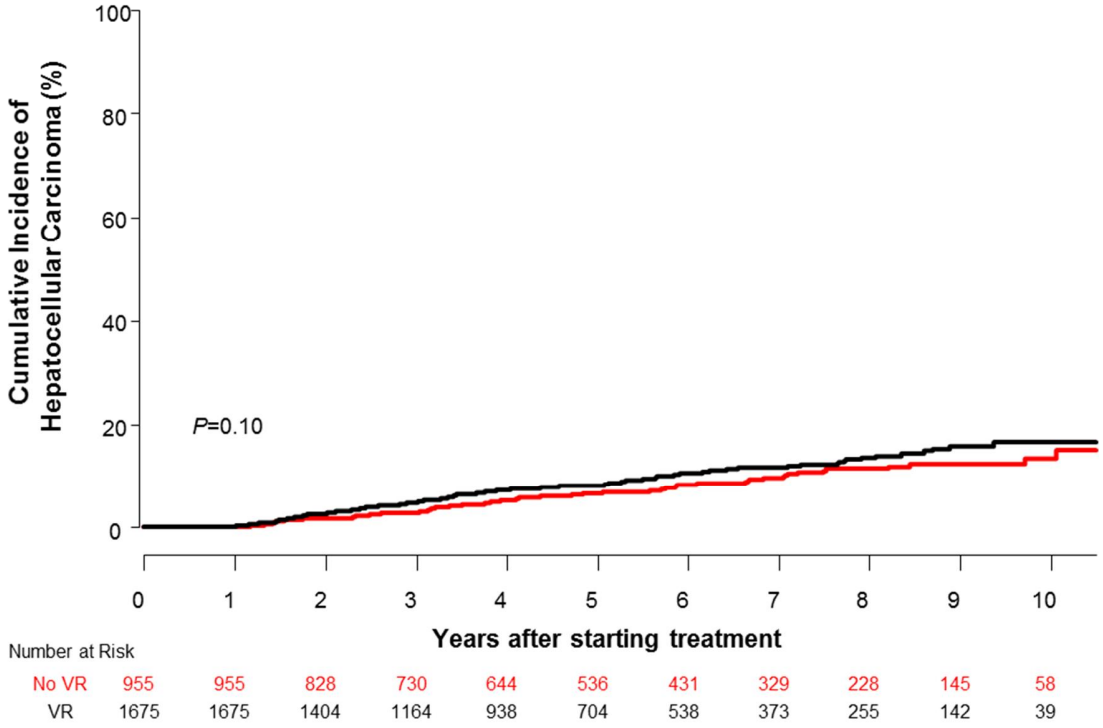


C. HBeAg seroclearance

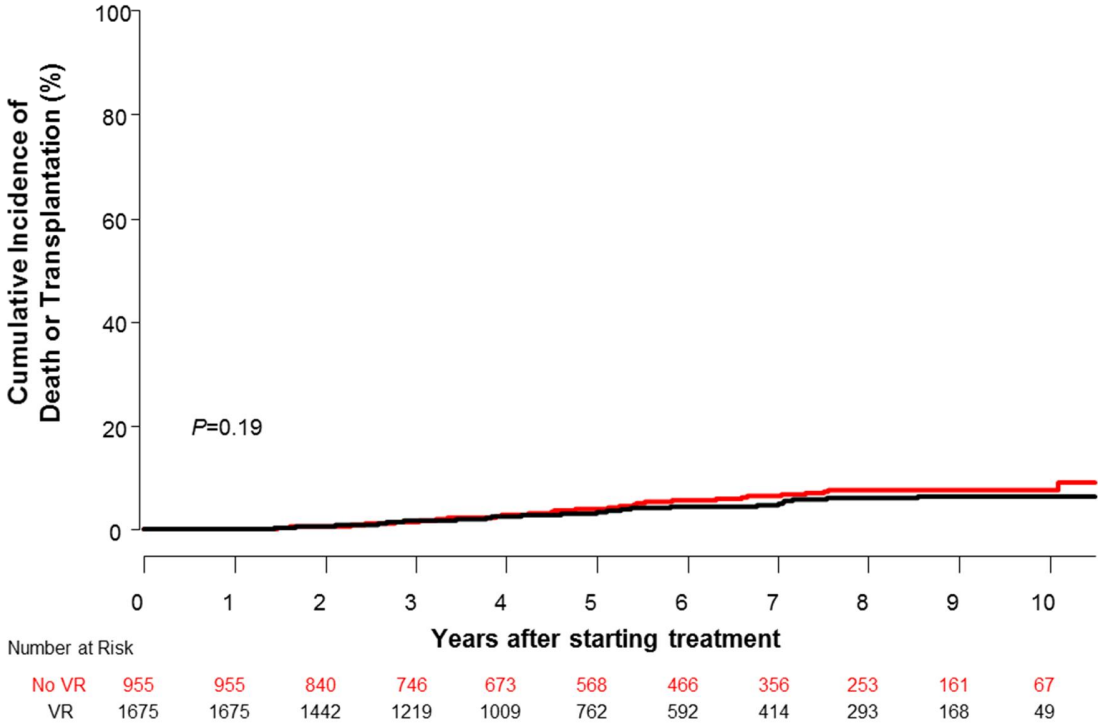


Supplementary Figure 2. Landmark analysis according to virological response at 1 year of treatment

A. Hepatocellular carcinoma

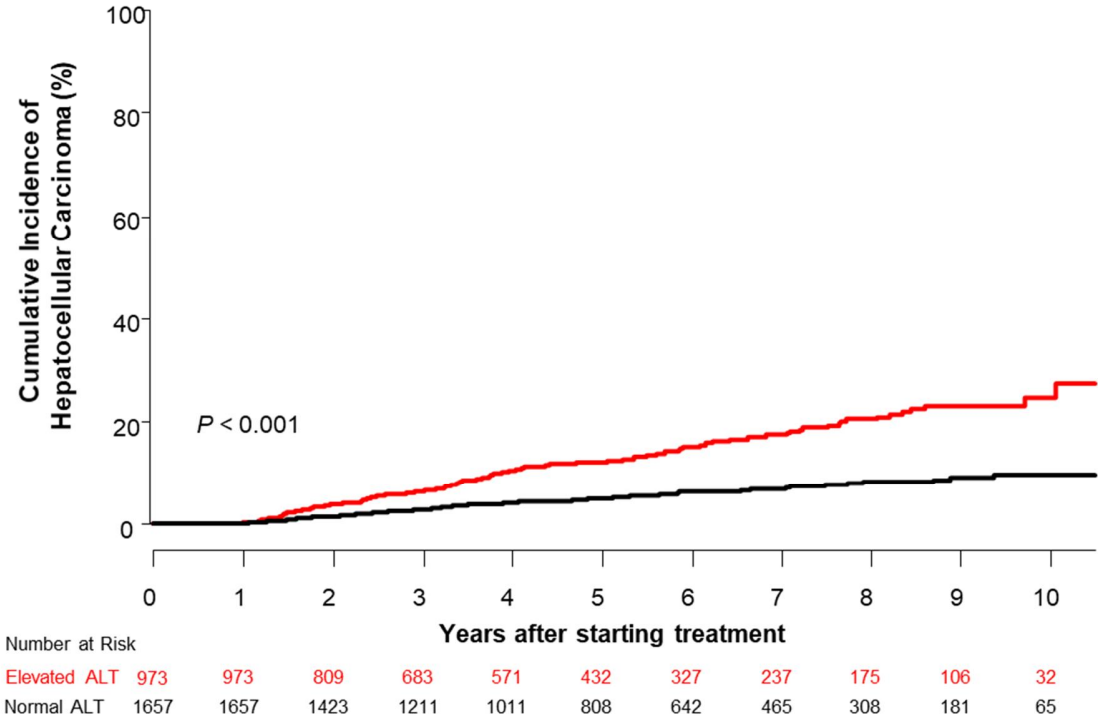


B. Death / transplantation

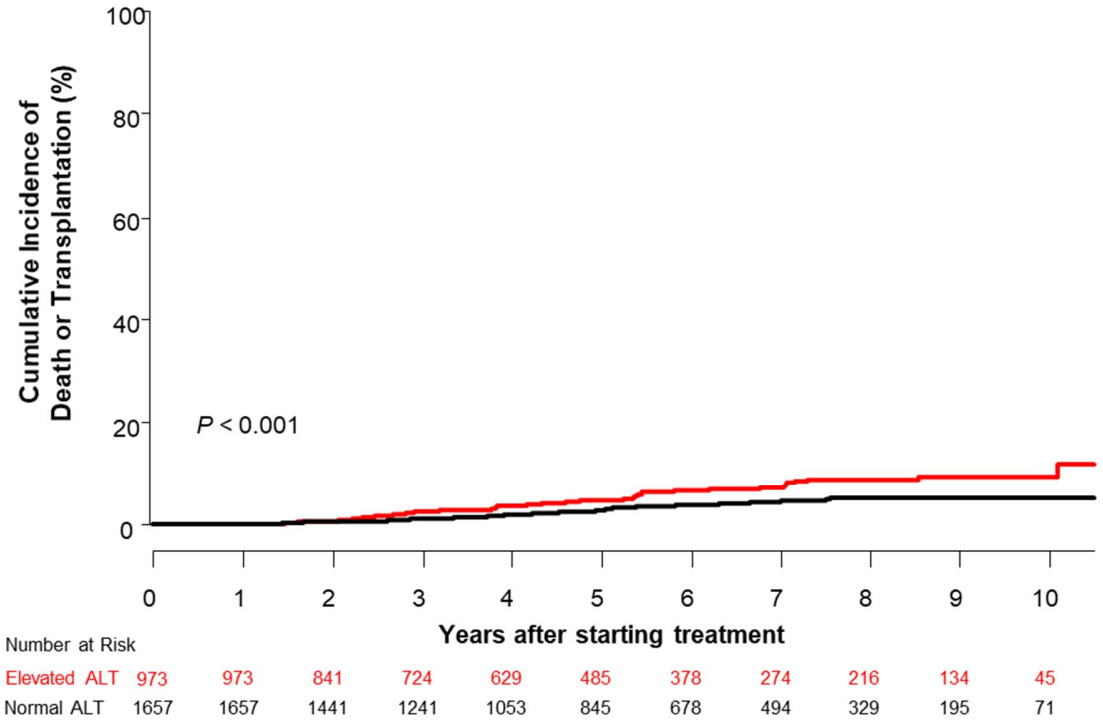


Supplementary Figure 3. Landmark analysis according to ALT normalization at 1 years of treatment

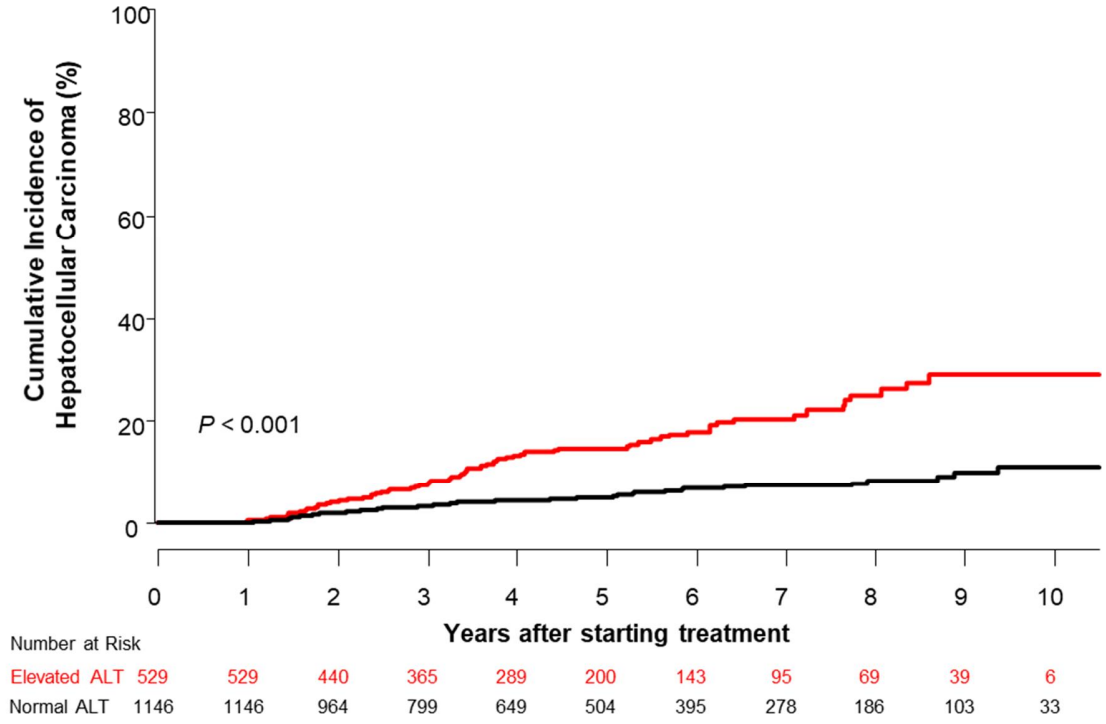
A. Hepatocellular carcinoma



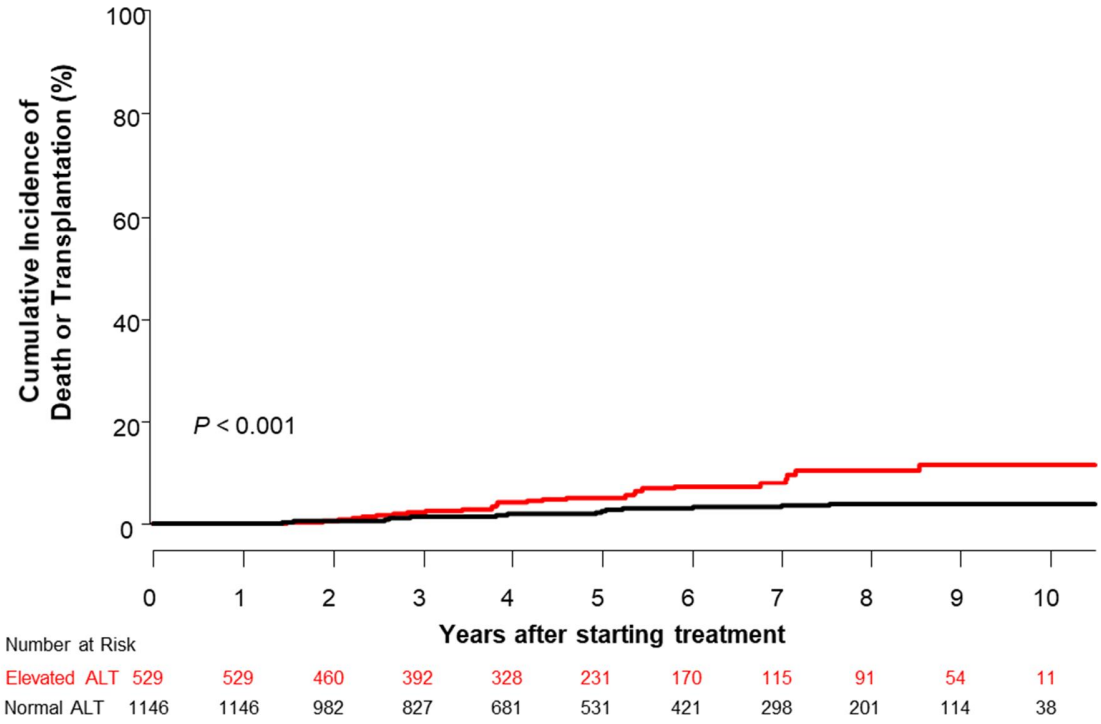
B. Death / transplantation



C. Hepatocellular carcinoma among patients who achieved virological response at 1 year of treatment

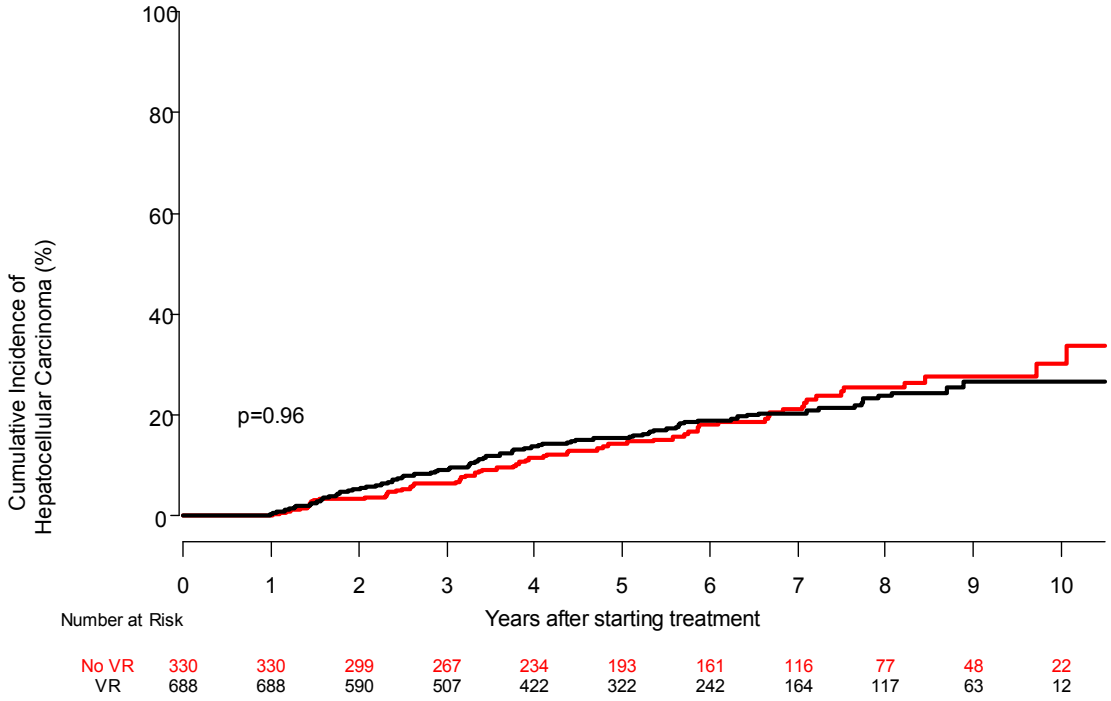


D. Death / transplantation among patients who achieved virological response at 1 year of Treatment

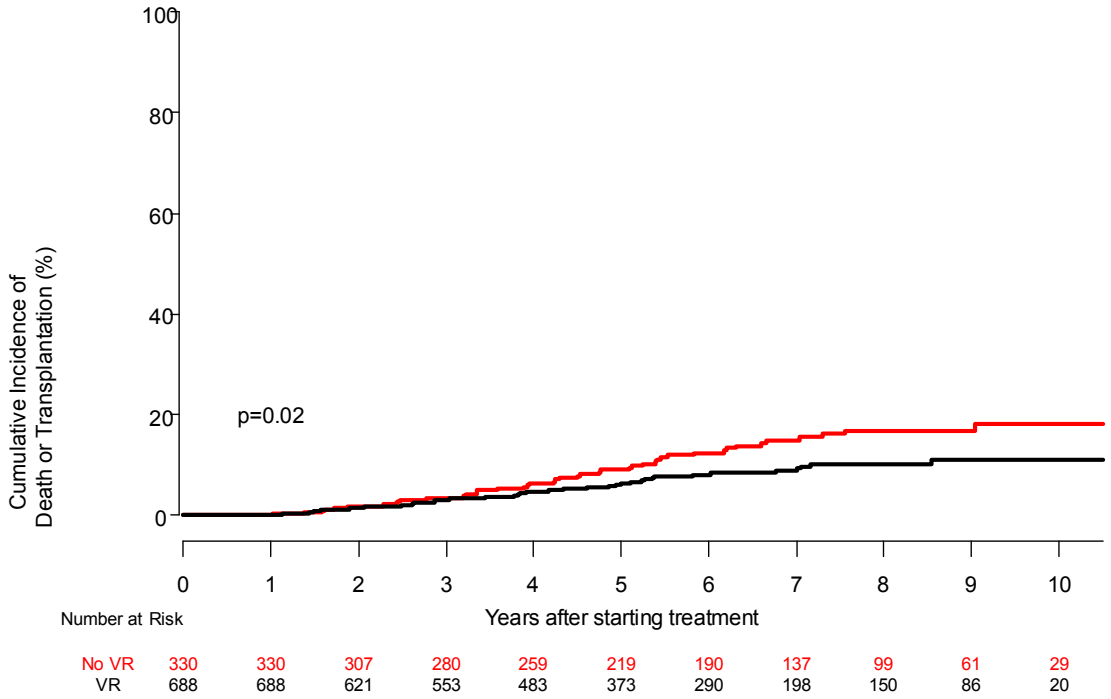


Supplementary Figure 4. Landmark analysis according to virological response in patients with cirrhosis

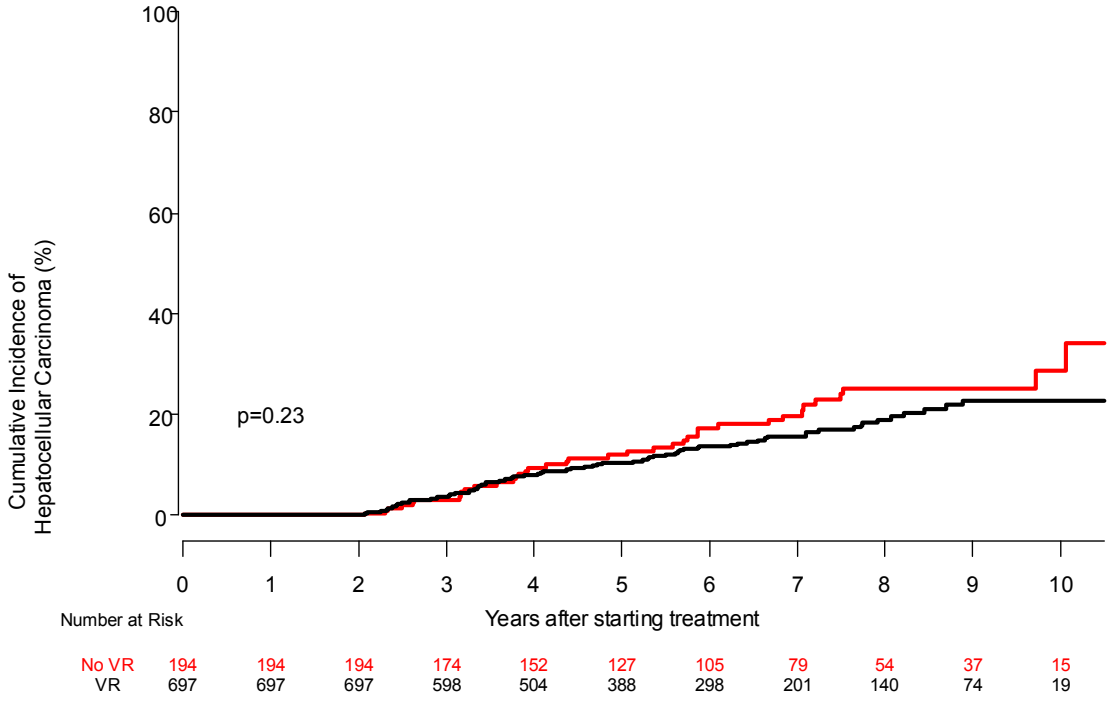
A. Hepatocellular carcinoma according to virological response at 1 year of treatment



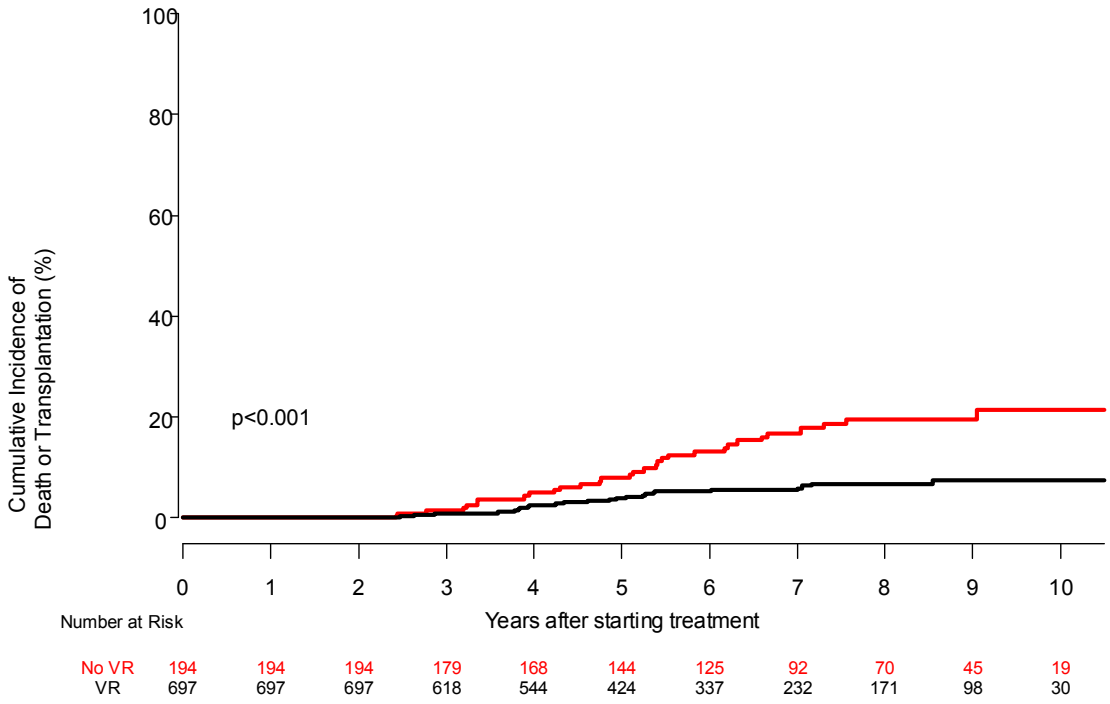
B. Death / transplantation according to virological response at 1 year of treatment



C. Hepatocellular carcinoma according to virological response at 2 years of treatment

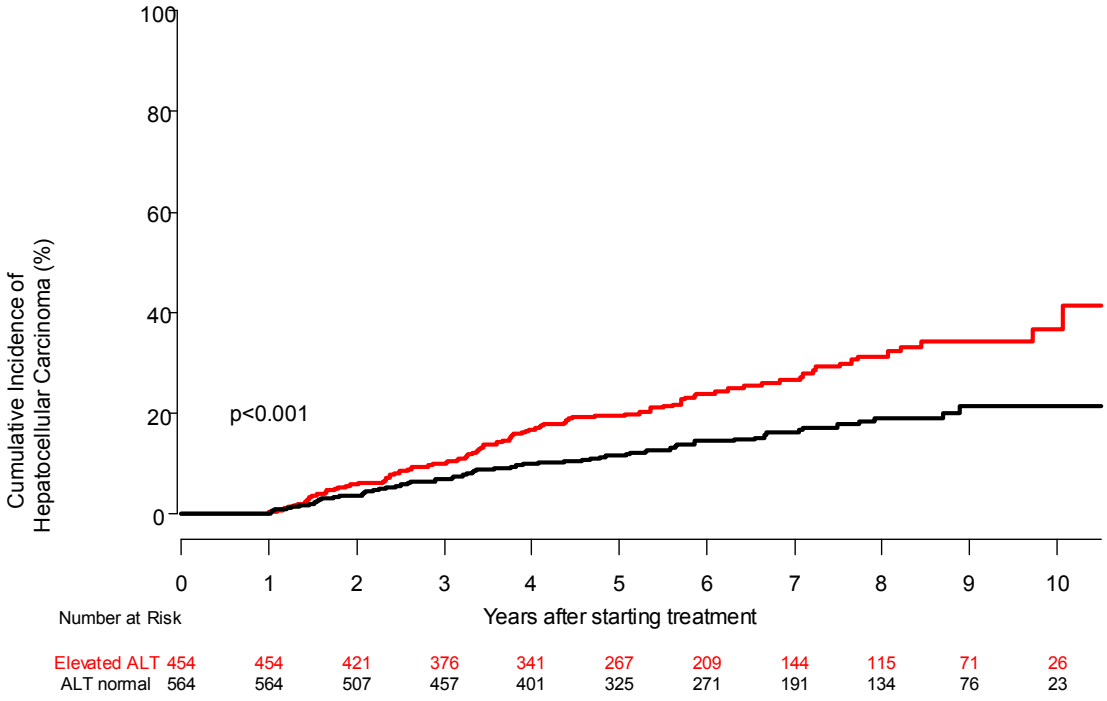


D. Death / transplantation according to virological response at 2 years of treatment

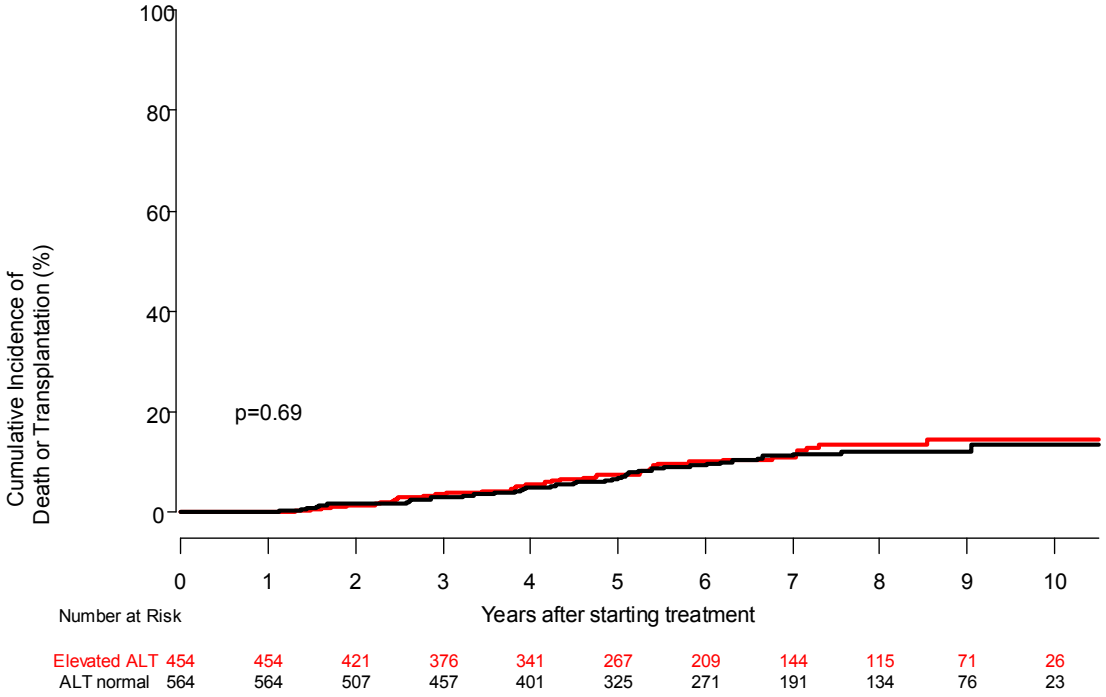


Supplementary Figure 5. Landmark analysis according to ALT normalization in patients with cirrhosis

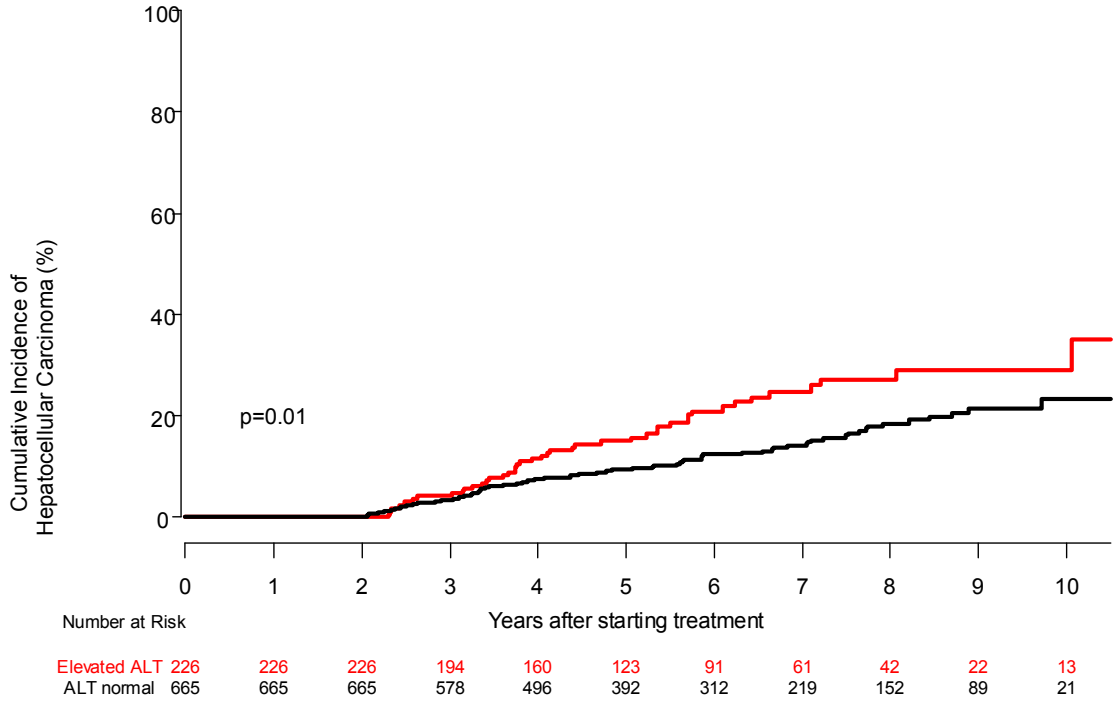
A. Hepatocellular carcinoma according to ALT normalization at 1 year of treatment



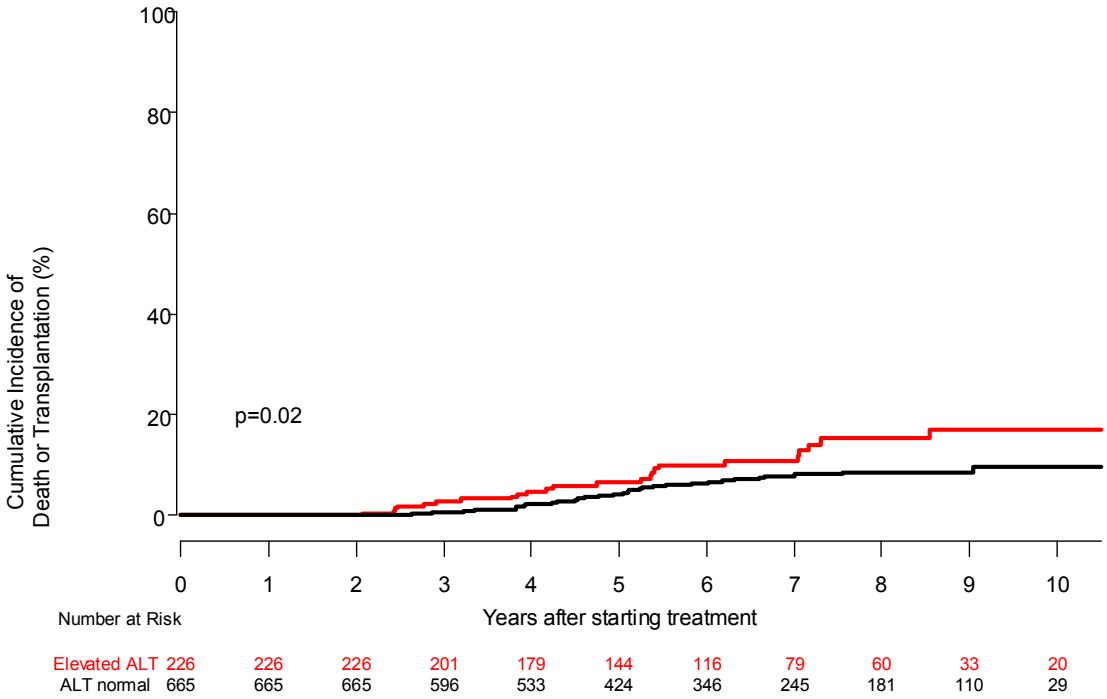
B. Death / transplantation according to ALT normalization at 1 year of treatment



C. Hepatocellular carcinoma according to ALT normalization at 2 years of treatment

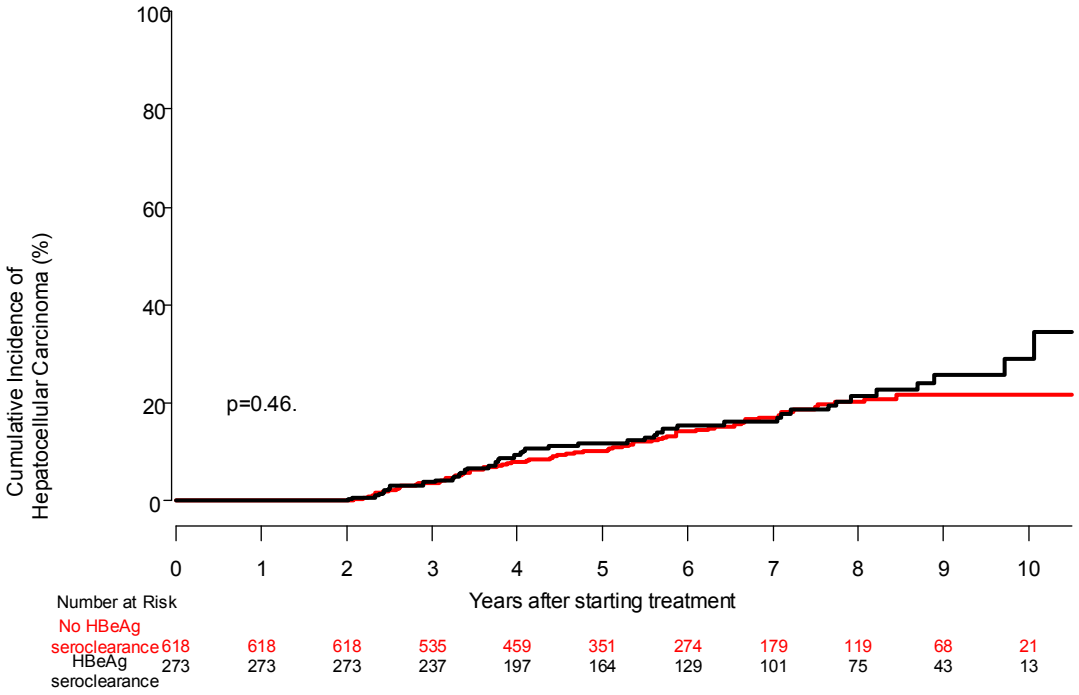


D. Death / transplantation according to ALT normalization at 2 years of treatment

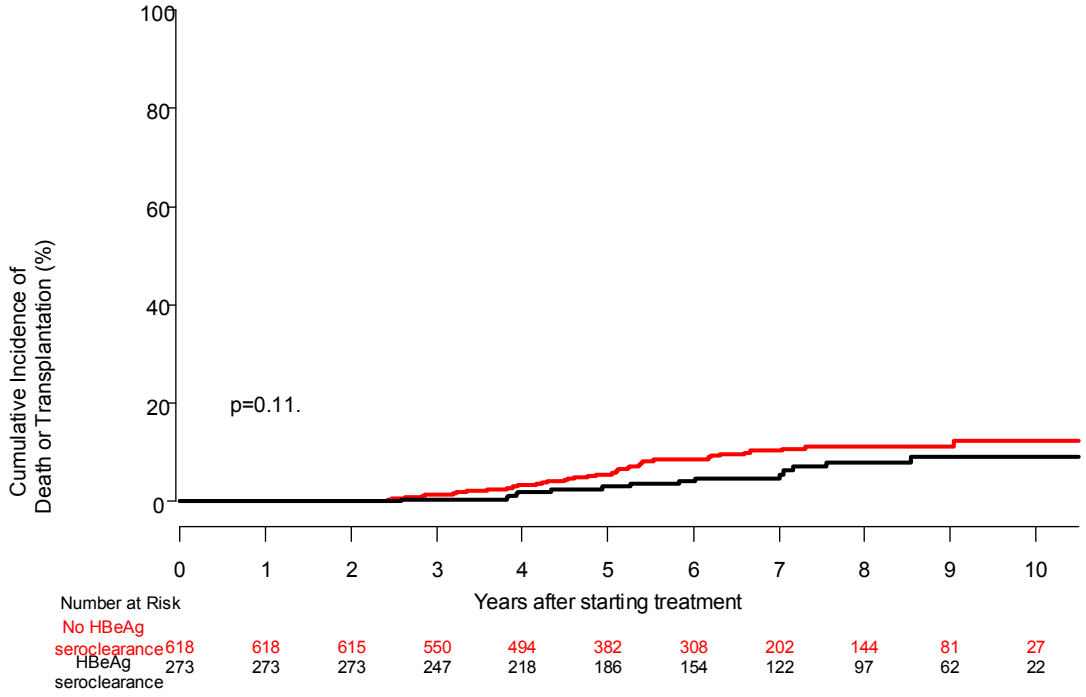


Supplementary Figure 6. Landmark analysis according to HBeAg clearance at 2 years of treatment in patients with cirrhosis

A. Hepatocellular carcinoma

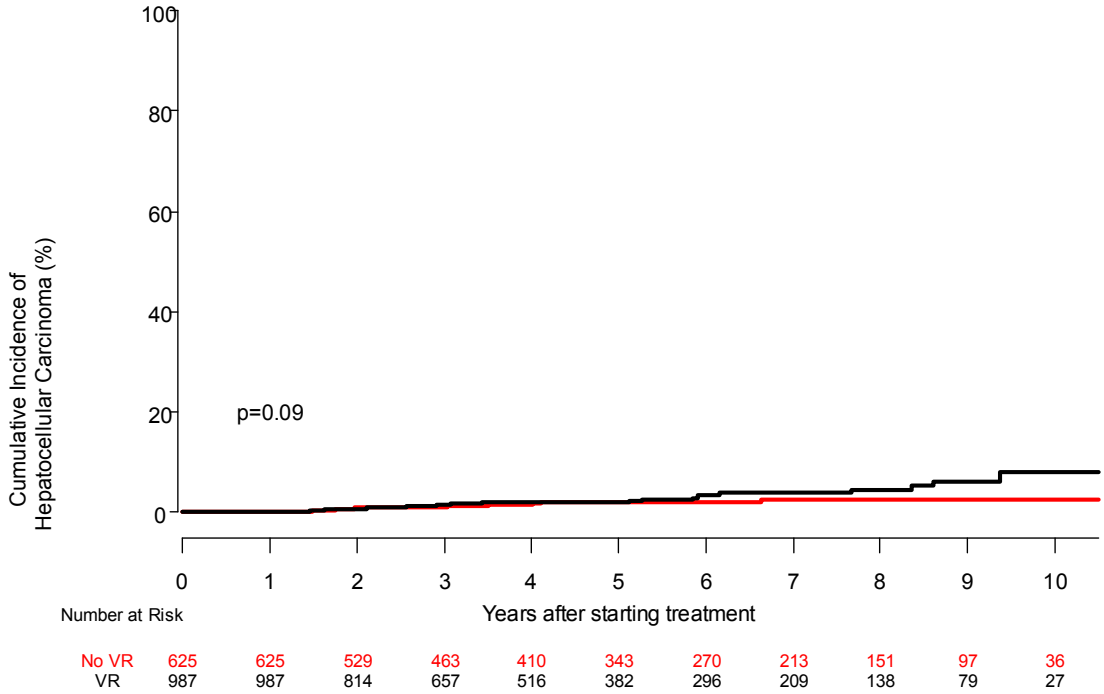


B. Death / transplantation

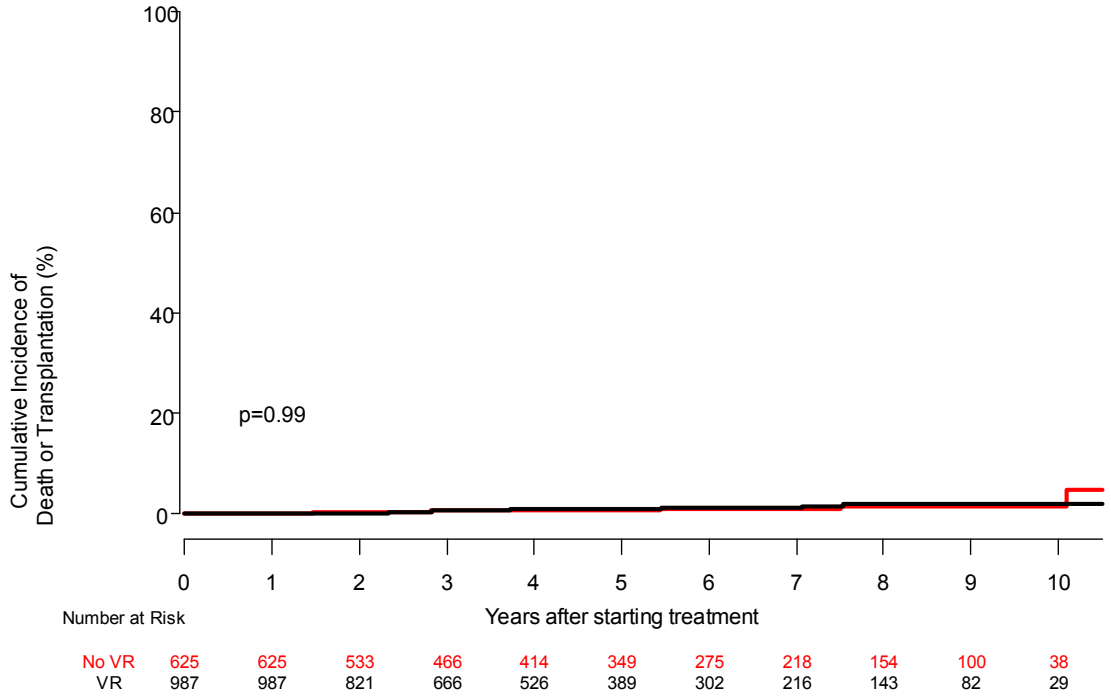


Supplementary Figure 7. Landmark analysis according to virological response in patients without cirrhosis

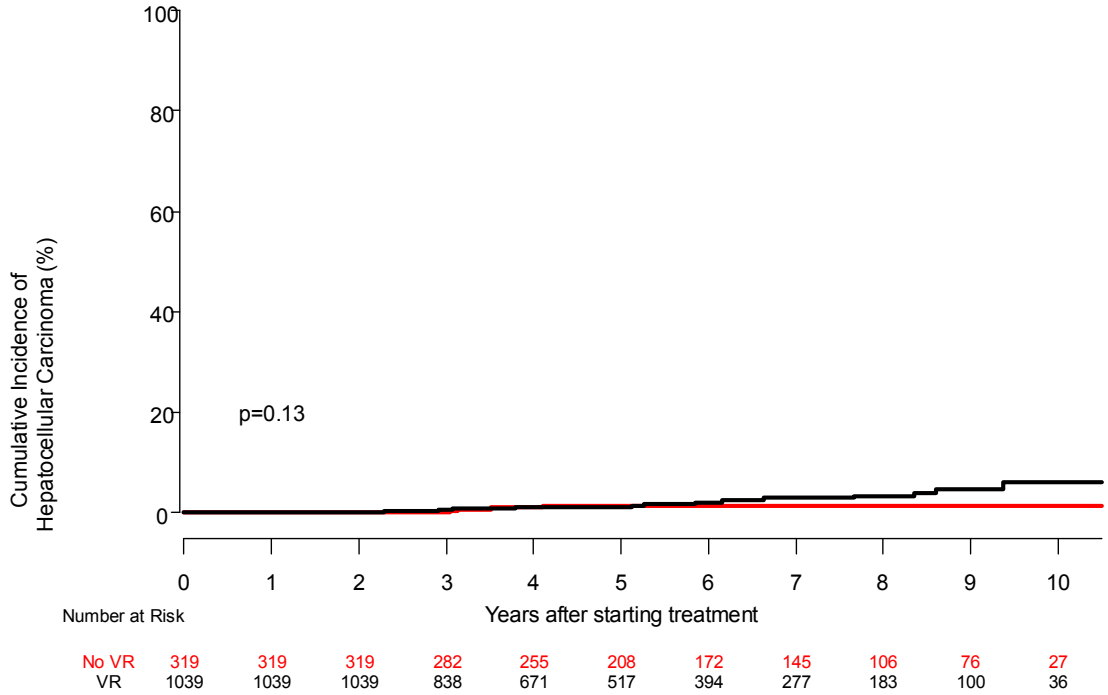
A. Hepatocellular carcinoma according to virological response at 1 year of treatment



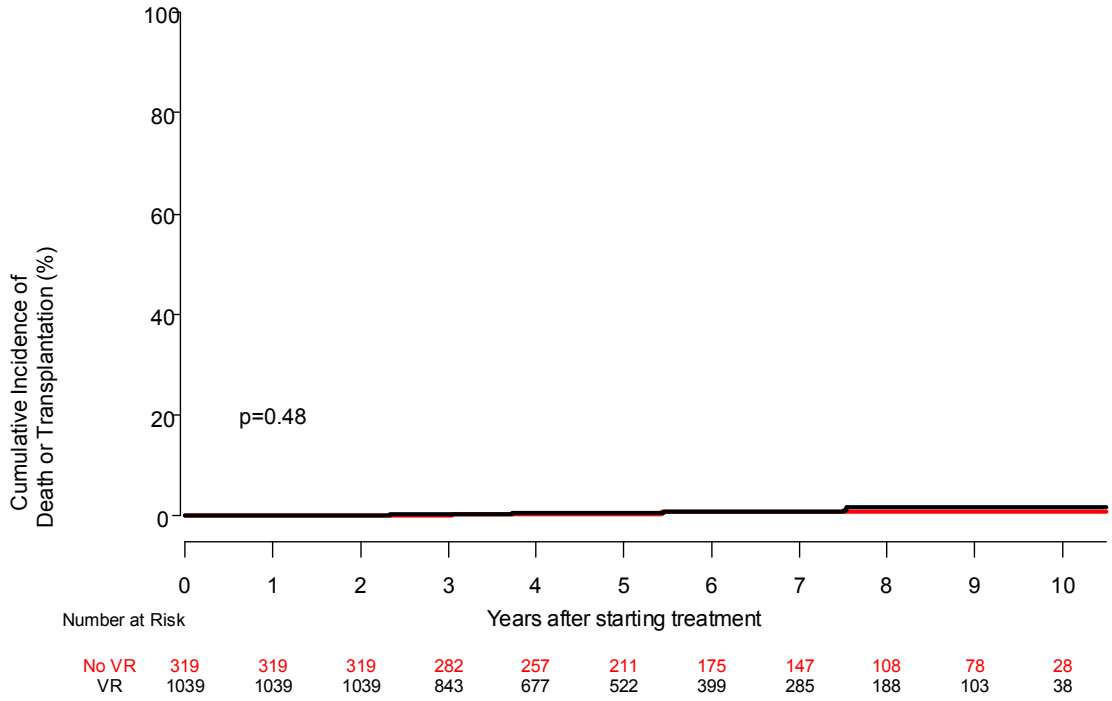
B. Death / transplantation according to virological response at 1 year of treatment



C. Hepatocellular carcinoma according to virological response at 2 years of treatment

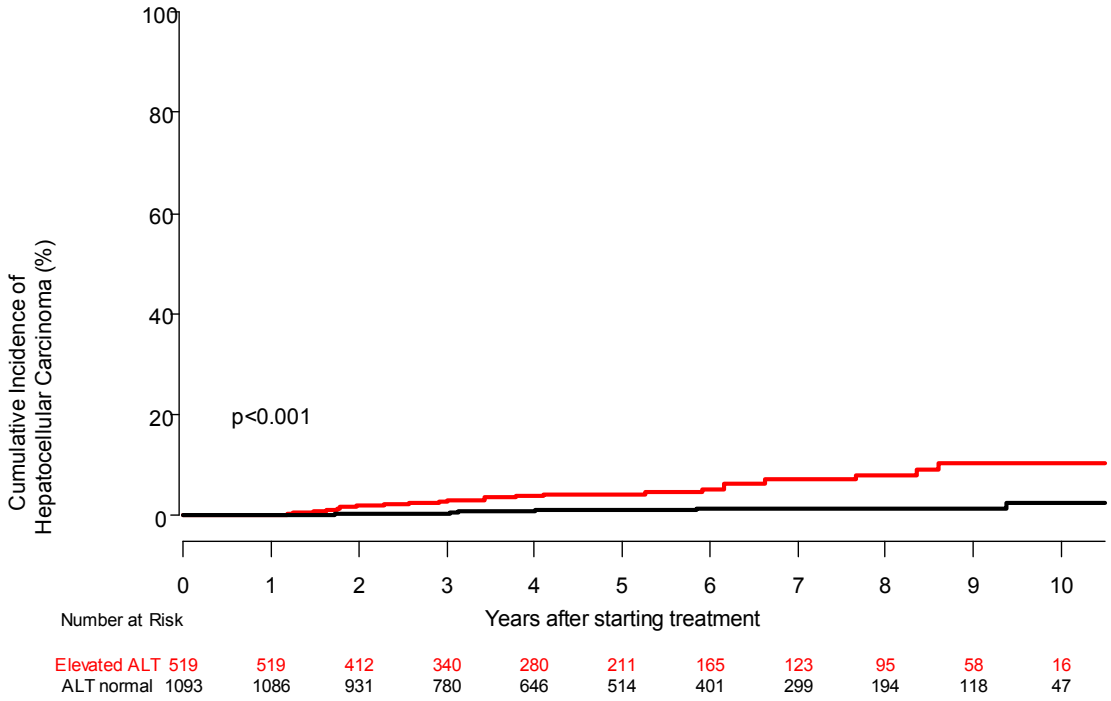


D. Death / transplantation according to virological response at 2 years of treatment

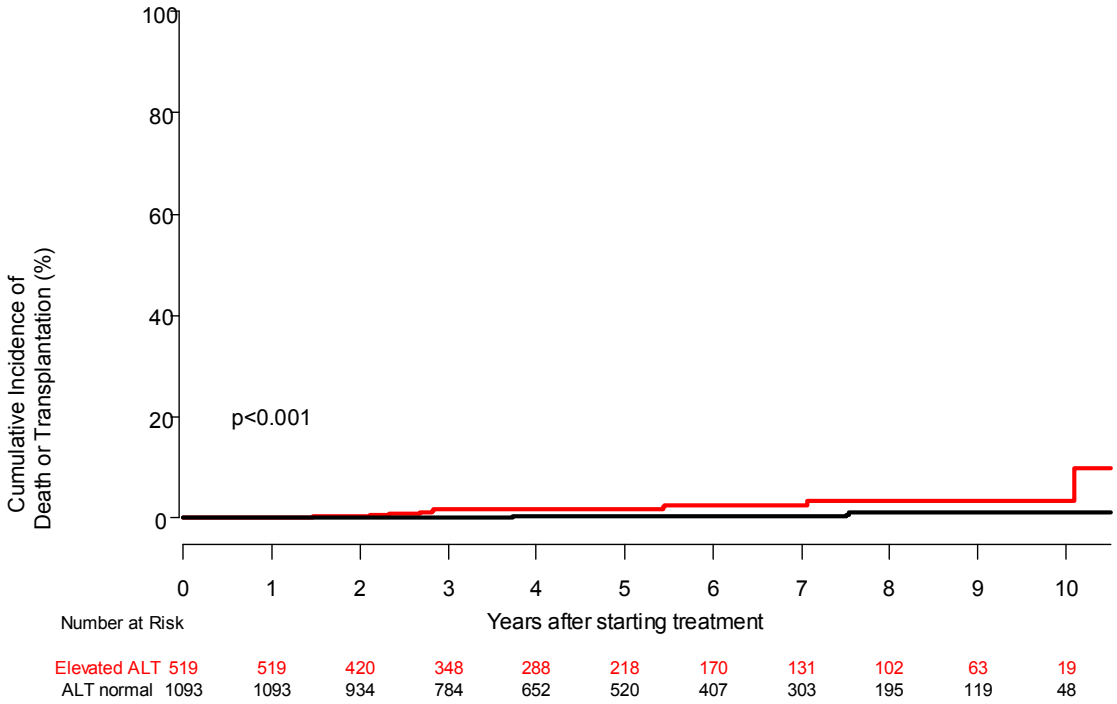


Supplementary Figure 8. Landmark analysis according to ALT normalization in patients without cirrhosis

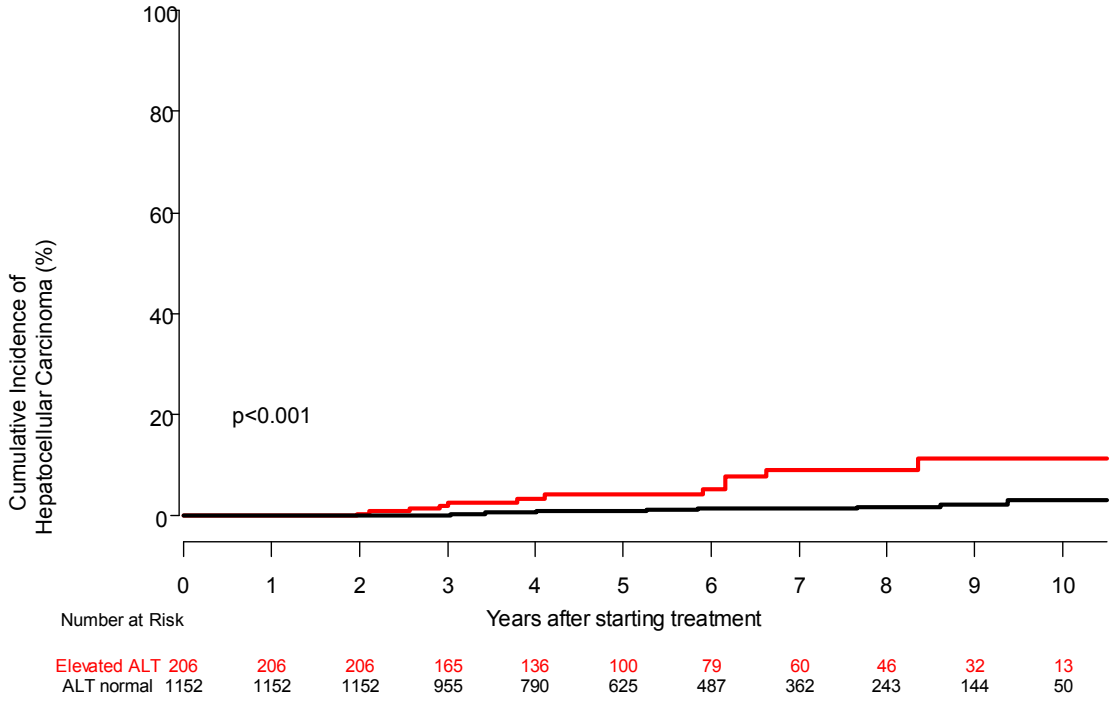
A. Hepatocellular carcinoma according to ALT normalization at 1 year of treatment



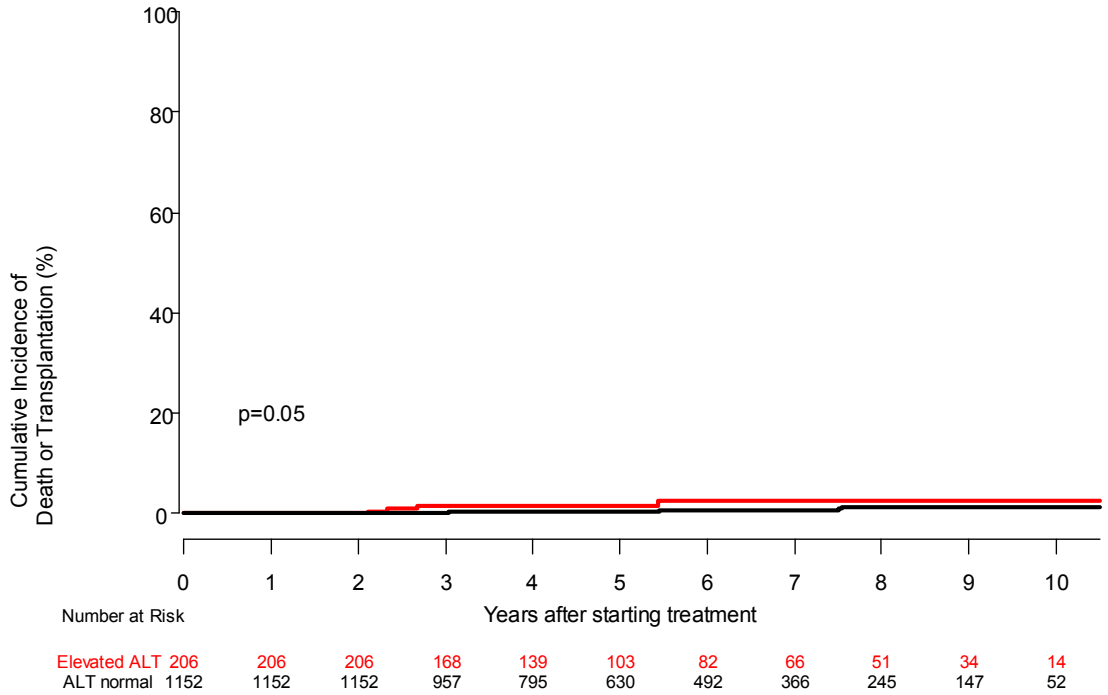
B. Death / transplantation according to ALT normalization at 1 year of treatment



C. Hepatocellular carcinoma according to ALT normalization at 2 years of treatment

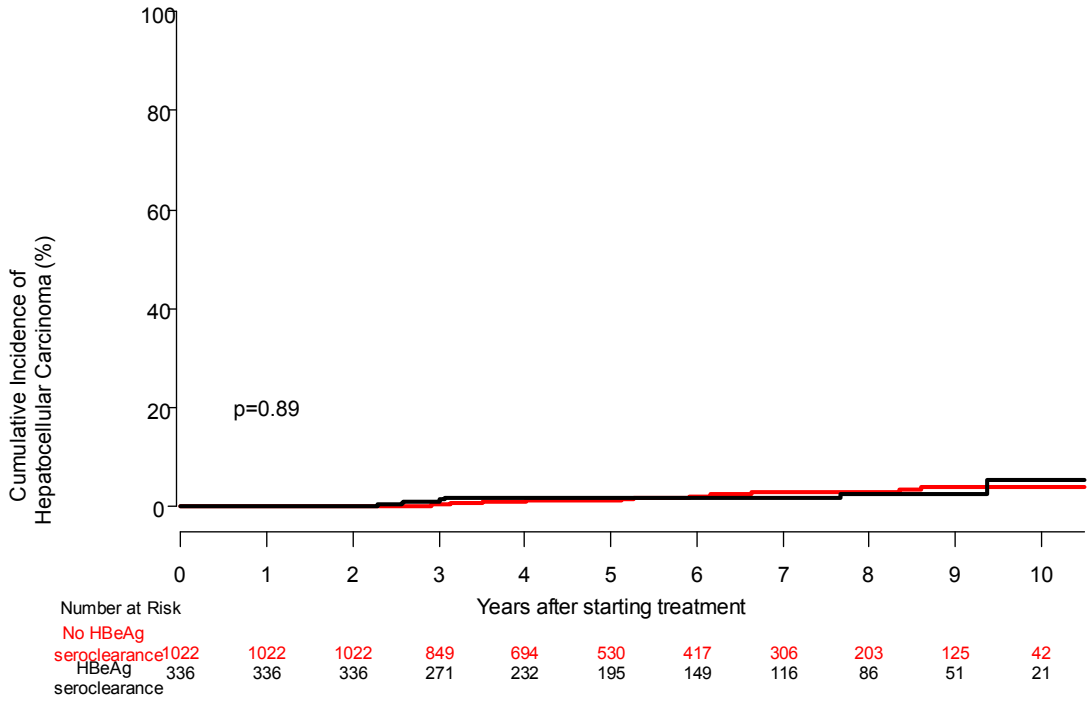


D. Death / transplantation according to ALT normalization at 2 years of treatment

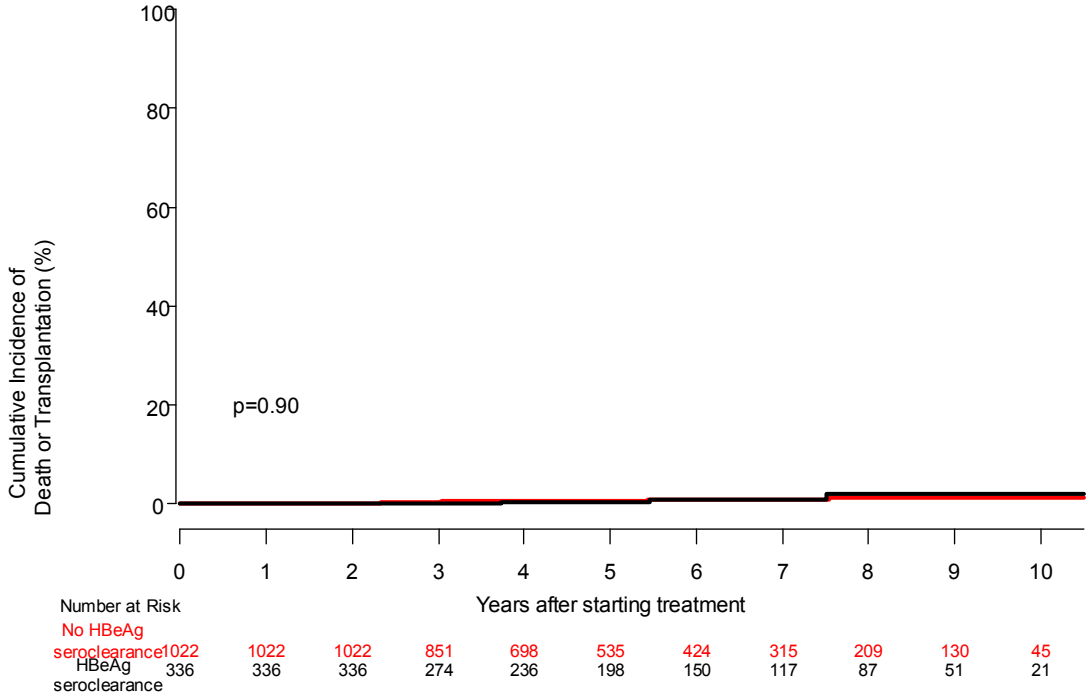


Supplementary Figure 9. Landmark analysis according to HBeAg clearance at 2 years of treatment in patients without cirrhosis

A. Hepatocellular carcinoma



B. Death / transplantation



English Abstract

ALT Normalization during Treatment of Chronic Hepatitis B Is Independently Associated with Improved Clinical Outcomes

Background & Aims: Alanine aminotransferase (ALT) normalization, virological response (VR), and HBeAg seroclearance are intermediate surrogate biomarkers associated with favorable clinical outcomes during the natural course of chronic hepatitis B (CHB), and are also commonly used to monitor treatment responses in HBeAg-positive patients. However, their correlation with clinical outcomes during highly-potent antiviral treatment remains unclear.

Methods: We analyzed a total of 2,630 HBeAg-positive CHB patients who initiated treatment with entecavir or tenofovir disoproxil fumarate by landmark and time-dependent Cox analyses. We defined VR as undetectable HBV DNA (<15 IU/mL), and normal ALT as ≤35 U/L for men and ≤25 for women.

Results: During the median period of 5.1 years of treatment, 216 patients developed HCC and 107 died or received liver transplants. ALT normalization was associated with a significantly lower risk of HCC and death/transplantation by landmark analyses at 1 and 2 years of treatment (P<0.001 for all). By 2-year landmark analysis, VR was associated with a significantly lower risk of death/transplantation (P=0.003) but not with the risk of HCC (P=0.97), while HBeAg seroclearance was not associated with the risks of HCC (P=0.13) or death/transplantation (P=0.34). On-treatment ALT normalization was

independently associated with significantly lower risks of HCC (adjusted hazard ratio [aHR]=0.44) and death/transplantation (aHR=0.47) by multivariable time-dependent Cox.

Conclusions: In HBeAg-positive CHB patients treated with highly-potent antiviral agents, ALT normalization was the sole on-treatment surrogate endpoint that was independently associated with lower risks of HCC and death/transplantation, whereas VR and HBeAg seroclearance observed during treatment were not predictive of HCC development.

Keywords: ALT normalization; HBeAg seroclearance; hepatocellular carcinoma; virological response; surrogate endpoint.