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Antiviral Therapy Substantially Reduces Hepatocellular Carcinoma Risk in Chronic Hepatitis B Patients in the Indeterminate Phase

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Abbreviations: CHB, chronic hepatitis B; ALT, alanine transaminase; HBV, hepatitis B virus, AASLD**,** American Association for the Study of Liver Diseases; ASD, absolute standardized difference; ULN, upper limit of normal; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface Ag; HBeAg, hepatitis B e antigen; SD, standard deviation; IQR, interquartile range; HRs, hazard ratios; aHR, adjusted hazard ratio; CI, confidence interval; IPTW, inverse probability of treatment weighting

Ethics approval: This study was approved by the institutional review board at Stanford University (Palo Alto, CA, USA) (IRB 13927) and each participating center. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul.

Graphical abstract
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ABSTRACT

Background & Aims: Hepatocellular carcinoma (HCC) risk in chronic hepatitis B (CHB) is higher in the indeterminate phase compared to the inactive phase. However, it is unclear if antiviral therapy reduces HCC risk in this population. We aimed to evaluate the association between antiviral therapy and HCC risk in the indeterminate phase.

Approach & Results: We analyzed 855 adult (59% male), treatment-naïve CHB patients without advanced fibrosis in the indeterminate phase at 14 centers (U.S., Europe, and Asia). Inverse probability of treatment weighting (IPTW) was used to balance the treated (n=405) and untreated (n=450) groups. The *primary outcome* was HCC development. The mean age was 46 ±13 years, the median ALT was 38 (IQR, 24 – 52) U/L, the mean HBV DNA was 4.5 ±2.1 $\log_{10}$ IU/ml and 20% were HBeAg positive. The two groups were similar after IPTW. After IPTW (n=819), the 5-, 10- and 15-year cumulative HCC incidence was 3%, 4%, and 9% among treated patients (n=394) versus 3%, 15%, and 19%, among untreated patients(n=425), respectively (*P*=0.02), with consistent findings in subgroup analyses for age>35 years, males, HBeAg positive, HBV DNA >1,000 IU/mL, and ALT<upper limit of normal. In multivariable Cox proportional hazards analysis adjusted for age, sex, HBeAg, HBV DNA, ALT, diabetes, and platelets, antiviral therapy remained an independent predictor of reduced HCC risk (adjusted HR 0.3, 95%CI 0.1 – 0.6, *P*=0.001).

Conclusion: Antiviral therapy reduces HCC risk by 70% among indeterminate phase CHB patients. These data have important implications for the potential expansion of CHB treatment criteria.

INTRODUCTION

Liver cancer is the 6th most common cancer worldwide but is the 3rd leading cause of cancer death [1]. The leading cause of liver cancer globally is chronic hepatitis B (CHB) which contributed to 40% of the world's liver cancer deaths in 2019 [2,3]. Emerging data suggest that a substantial proportion of patients with chronic hepatitis B (CHB) do not fall into any of the defined phases (immune-tolerant, immune-active, and inactive) and hence are considered indeterminate [4]. Patients who remain in the indeterminate phase have a 14-fold higher risk of hepatocellular carcinoma (HCC) compared to those who remain in the inactive phase[3]. Current practice guidelines do not recommend antiviral therapy for the treatment of CHB in the indeterminate phase [5-9]. Hence, most patients in the indeterminate phase remain untreated [10,11].

Data are limited for the clinical utility of antiviral therapy in the indeterminate phase. Multiple studies have shown antiviral therapy reduced the risk of HCC in CHB patients in the immune-active phase[12-17]. However, the impact of antiviral therapy on HCC development in CHB patients in the indeterminate phase is unknown. Therefore, we aimed to examine the association between antiviral therapy and HCC risk in patients with CHB in the indeterminate phase in a large, multi-center, multi-national study within the REAL-B[13] consortium.

METHODS

Study Design

This is a retrospective cohort study of CHB patients (HBsAg+ or HBV DNA+ ≥ 6 months) who were evaluated and monitored as part of their routine care between August 1992 and November

2021 at 14 sites in the U.S., Europe, and Asia (**Supplemental Figure 1**). All clinical, laboratory and imaging data were collected from a review of individual patient medical records using a standardized case report form and unified data variable definition at each study center [13]. De-identified data were transmitted to and analyzed at the REAL-B Data Coordinating Center at Stanford University Medical Center (Palo Alto, CA, USA). This study was approved by the institutional review board at Stanford University (Palo Alto, CA, USA, IRB 13927) and each participating center, and received a waiver of consent. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul.

Inclusion and exclusion criteria

Patients ≥18 years of age who were treatment naïve with CHB and in the indeterminate phase were included in this study. Included patients were confirmed to be in the indeterminate phase by two consecutive sets of alanine aminotransferase (ALT) and HBV DNA, along with HBeAg within 12 months of the study. Patients with any of the following criteria were excluded from the study: patients in the immune tolerant, immune active, and inactive phases of chronic hepatitis B based on the AASLD practice guidance and treatment algorithm [5], liver transplant recipients, history of malignancy apart from HCC, less than 1-year of follow-up, HCC development within six months of the baseline date, coinfections with hepatitis C or HIV, advanced fibrosis/cirrhosis (assessed by FIB-4 in all patients and other methods as defined below) and insufficient laboratory data for assessment of baseline clinical characteristics (ALT, AST, HBV DNA, HBeAg, platelet count and the presence of diabetes) (**Supplemental Figure 1**).

Definitions

We defined the clinical phases as per the American Association for the Study of Liver Diseases (AASLD) guidelines (**Table 1**)[18]. Patients were considered to be in the indeterminate phase when

they did not meet the standard criteria for any of the clinical phases (immune-tolerant, immune-active, and inactive) based on the American Association for the Study of Liver Diseases guidance and treatment algorithm, as previously described [4,5] (**Table 1**). For example, patients who were negative for HBeAg, had ALT<ULN, and HBV DNA >20,000 IU/ml were classified as indeterminate as they did not fall into any of the standard phases. Patients with documented significant fibrosis (transient elastography > 8 kPA or magnetic resonance elastography > 2.99[19]) and HBV DNA >2,000 IU/ml were not included as these patients were considered to be immune active.

We assessed the FIB-4 score in all patients, and ascertained the presence of advanced fibrosis/cirrhosis by FIB-4>3.25 [19] or by any of the following criteria where available: liver stiffness by transient elastography >12.5 kPA [19], liver stiffness by magnetic resonance elastography > 4.63 kPA[20], fibrosure/fibrotest <0.32 [19], liver biopsy showing stage 3 or 4 fibrosis, nodular liver contour on imaging, splenomegaly or hepatic decompensation and HCC by cytology, histology, or non-invasive criteria based on the AASLD guideline [21]. All patients with advanced fibrosis were excluded.

We defined the baseline date among untreated patients as the date of the second set of ALT and HBV DNA confirming they were in the indeterminate phase, and among antiviral-treated patients, as the date antiviral therapy was initiated. Antiviral-treated patients were confirmed to be in the indeterminate phase by HBeAg and two sets of ALT and HBV DNA within 12 months and remained in the indeterminate phase up to the point of initiation of antiviral therapy. These antiviral treatment patients were maintained on antiviral therapy till the end of follow-up.

Study outcomes

The *primary outcome* was the development of HCC. *Secondary outcomes* were factors associated with HCC development.

Statistical analysis

Continuous variables were described using mean ± standard deviation (SD) or median (interquartile range [IQR]) and assessed using the Student's *t*-test if variables were normally distributed or the Wilcoxon rank-sum test when assumptions of normality were not met. Categorical variables were described using proportions and assessed using the Chi-squared test.

Follow-up was censored at the date of the last follow-up, development of advanced fibrosis/cirrhosis, HCC, death, or once the patient migrated to a different phase (such as the immune active phase) from indeterminate (for untreated patients), whichever came first. All antiviral-treated patients remained in the indeterminate phase up to the point of initiation of antiviral therapy and remained on antiviral therapy till the end of follow-up.

Inverse probability of treatment weighting (IPTW) analysis was performed to balance baseline characteristics between the two study groups (antiviral-treated versus untreated) for age, sex, ALT, AST, DNA, HBeAg, platelet count, presence of diabetes, and follow-up time. The balance between the two study groups was assessed using the absolute standardized differences (ASDs), with ASDs below 0.1 indicative of good balance. The cumulative HCC incidence was compared between antiviral treated versus untreated indeterminate patients who remained indeterminate for the IPTW cohort using the Kaplan-Meier method and the log-rank test. We performed subgroup analysis by median age, sex, HBeAg status, upper limit of normal ALT (ULN, >35 U/L for males, >25 U/L for females), and HBV DNA. We also performed a sensitivity analysis using the age cutoff of 35 years as previously suggested for higher-risk patients[7]. Univariable and multivariable Cox proportional hazards regression were utilized to estimate hazard ratios (HRs)

for factors associated with HCC development, using the backward stepwise method to select variables for multivariable Cox regression model if the *P*-value in the univariable analysis was < 0.1 or if the association has been previously reported. All authors had access to the study data and reviewed and approved the final manuscript. Statistical analysis was completed using STATA version 15 (College Station, TX), and a two-tailed *P*-value ≤ 0.05 was considered to be statistically significant.

RESULTS

Study population

Of the total of 2,228 patients with indeterminate phase CHB who did not have a history of HCC, liver decompensated, or liver transplant at baseline (**Supplemental Figure 1),** 855 patients (405 treated, 450 untreated) met our study inclusion criteria and were included in the study. At baseline, the cohort mean (±SD) age was 46.4 (±13.1) years, and 59% were male (**Table 2A**). Most patients (94%) were Asian, and 12.2% had type 2 diabetes. The median (IQR) ALT was 38 (24 – 52) U/L, 174 patients (20.4%) were HBeAg positive, and the mean (±SD) HBV DNA was 4.46 (±2.1) $\log_{10}$ IU/ml. About two-thirds of patients (67.4%) had a FIB-4<1.45, while the remainder had a FIB-4 from 1.45 – 3.25. In total, there were 4,714.3 person-years of follow-up. In the total study population, antiviral-treated patients were more likely males (65.7% versus 52.9%, *P*<0.001), more likely to have positive HBeAg (28.4% versus 13.1%, *P*<0.001), higher

HBV DNA (5.2 $\log_{10}$ IU/ml versus 3.8 $\log_{10}$ IU/ml, *P* <0.0001), higher ALT (43 U/L versus 33 U/L, *P*<0.0001) but shorter follow-up time (1,916.92 person-years versus 2,797.42 person-years, *P*<0.0001) compared with untreated patients (**Table 2A**). The median (IQR) time between ALT labs and HBV DNA was 6.9 (4.6 – 8.4) months and 7.4 (6.1-9.1) months, respectively. A total of 8 (1.7%) and 9 (2.0%) treatment-naïve indeterminate phase patients transitioned to the immune active phase by year-5 and year-10 of follow-up, respectively, and were censored at the point of phase transition. Following IPTW, baseline characteristics were similar, including age, sex, presence of diabetes, HBeAg status, HBV DNA, and follow-up time (**Table 2B**). All ASDs were <0.1, except for baseline ALT levels (40 U/L versus 35 U/L, ASD 0.13 in the antiviral-treated versus untreated patients) (**Table 2B**). The IPTW cohort included 819 patients (394 treated, 425 untreated).

Impact of antiviral therapy on HCC risk in the indeterminate phase (IPTW cohort)

In the IPTW cohort, there were a total of 38 cases of HCC, 11 among antiviral-treated patients, and 27 among untreated patients. The 5-, 10- and 15-year HCC incidence was 2.5%, 3.9%, and 9.4%, among antiviral-treated patients versus 2.7%, 14.7%, and 19.1% among untreated patients, respectively (**Figure 1A**, *P*=0.02). Landmark analysis by follow-up time determined that the reduction in HCC risk occurred after 5 years (**Figure 1B,** *P*=0.67 for the first 5 years and 0.007 for years 5 and after). In unadjusted analysis, antiviral therapy (HR 0.4, 95% CI 0.2 – 0.9, *P*=0.02) was associated with reduced HCC risk, while age > 45 years (HR 2.4, 95% CI 1.2 – 5.0, *P*=0.02) male sex (HR 33.2, 95% CI 3.6 – 309.0, *P*=0.002) and positive HBeAg (HR 3.18, 95% CI 1.69 – 6.01, *P*<0.001) were associated with greater HCC risk. After multivariable adjustment for age, sex, presence of diabetes, platelet count, ALT, HBV DNA, and HBeAg status, antiviral therapy remained a strong predictor of reduced HCC risk (adjusted HR [aHR] 0.3, 95% CI 0.1 –

0.6, *P*=0.001) (**Table 3**), while age > 45 years (aHR 4.6, 95% CI 2.1 – 10.1, *P*<0.001), male sex (aHR 32.5, 95% CI 3.5 – 304.4, *P*=0.002), ALT ≥ ULN (aHR 2.4, 95% CI 1.0 – 5.6, *P*=0.05) and positive HBeAg status (aHR 3.7, 95% CI 1.8 – 7.7, *P*<0.001) were independent predictors of HCC development.

Subgroup analyses (IPTW cohort)

Among patients 45 years of age or older, HCC incidence was significantly lower at 5-, 10- and 15-years among antiviral-treated patients (2.6%, 5.0%, and 14.7%, respectively) as compared to untreated patients (5.0%, 19.0%, and 24.0%, respectively) (**Figure 2A,** *P*=0.04). However, in patients younger than 45 years, there was no statistically significant difference between the two groups (**Figure 2B,** *P*=0.13).

Among males, HCC incidence was also significantly lower in treated compared to untreated patients (**Supplemental Figure 2A,** *P*=0.03), but there was only one case of HCC among untreated patients and none among antiviral-treated patients for the female subgroup, resulting in no significant difference treated and untreated patients in the female subgroup (*P*=0.40).

By ALT level, HCC incidence was lower in treated compared to untreated patients in both subgroups of patients with ALT above and below the upper limit of normal (ULN) (≥35 U/L for males and ≥25 U/L for females) and ALT<ULN (**Figure 2C** and **Figure 2D,** *P*=0.05 and 0.04, respectively).

By HBV DNA levels, HCC incidence was lower among antiviral-treated patients compared to untreated patients among patients with HBV DNA >1,000 IU/mL (**Figure 2E**, *P*=0.007), but not in those with HBV DNA ≤1,000 IU/mL (**Figure 2F,** *P*=0.96). Similarly, HCC incidence was lower in treated compared to untreated only among patients that were HBeAg positive,

(**Supplemental Figure 2B,** *P*=0.0005), but not among those who were HBeAg negative (**Supplemental Figure 2C,** *P*=0.83).

Sensitivity analysis (IPTW cohort)

Using a lower age cutoff of 35 years as a sensitivity analysis, we found significantly lower HCC incidence among treated patients who were older than 35 as compared to their untreated counterparts (**Supplemental Figure 2D,** *P*=0.005). On multivariable analyses adjusting for age, sex, diabetes, platelet count, ALT, HBeAg, and HBV DNA levels, antiviral therapy was also associated with a 70% reduction in HCC risk (aHR 0.3, 95% CI 0.2-0.7, *P*=0.003) (**Supplemental Table 1**). Age >35 years was associated with 3 times higher HCC risk, though this association did not reach the conventional level for statistical significance (aHR 3.0, 95% CI 0.9-9.5, *P*=0.06).

DISCUSSION

In this large, multinational study consisting of indeterminate phase CHB patients without advanced fibrosis from 14 sites in the U.S., Europe, and Asia, we determined that antiviral therapy was associated with a 70% reduction in the incidence of HCC after adjusting for relevant confounders using comparative groups that were well balanced by IPTW. In the IPTW cohort, the cumulative HCC incidence in treated versus untreated patients at 5-, 10- and 15-years were 3%, 4%, and 9% versus 3%, 15%, and 19%, respectively, with the benefits only apparent after five years of antiviral therapy as determined by our landmark analysis, consistent with previous literature[14]. Lower HCC incidence was observed in several patient subgroups that received antiviral therapy: male patients, those older than 35 years, those with positive HBeAg, and those with HBV DNA >1,000 IU/mL. Notably, significantly lower HCC incidence was also observed in treated patients with ALT<ULN as compared to untreated patients.

These data have important clinical implications. Given that a substantial proportion of patients with CHB are in the indeterminate phase, this represents a major proportion of CHB patients at increased risk of HCC [4] that can be mitigated by well-tolerated antiviral therapy as shown in this study. Therefore, treatment indications for CHB may be expanded to include indeterminate patients at higher risk such as male patients, those older than 35 years, positive HBeAg, and

patients with HBV DNA>1,000 IU/mL regardless of ALT levels, especially given the favorable side effect profile and efficacy of current first-line nucleos(t)ide agents [10,22-28]. Additionally, these data may be helpful to simplify the management of CHB, an unmet need that has been highlighted by a recent systematic review of the literature by the World Health Organization[27]. Inconsistencies between CHB guidelines and complicated treatment algorithms may be burdensome for care providers and likely contribute to suboptimal linkage to care for patients with CHB[24,25].

A recent multicenter study of 3,624 patients with treatment naïve CHB determined that the proportions of patients who developed HCC outside treatment recommendation according to APASL, AASLD, and EASL criteria were 64.0%, 46.0% and 33.5%, respectively[29]. Another multicenter study of 271 treatment-ineligible untreated patients and 514 treatment-eligible treated patients found that patients that never met standard AASLD 2018 criteria had a higher risk of developing cirrhosis and/or HCC when compared to treatment-eligible and treated patients[30]. The current study fulfills an important gap in the literature by providing evidence that antiviral therapy for indeterminate phase patients is associated with a substantial reduction in HCC risk. Further studies are required to refine the selection of patients in the indeterminate phase that should be treated including additional studies with a larger sample size of lower-risk patients in our study such as females and HBeAg-negative patients, as well as the cost-effectiveness of such a strategy.

This multicenter study is the first to describe the impact of antiviral therapy on HCC risk in non-cirrhotic patients in the indeterminate phase. Its strengths include large sample size, multinational representation, long follow-up, rigorous balancing of baseline characteristics between groups by IPTW and robust adjustment for confounders during multivariable regression

analysis, and detailed subgroup sensitivity analysis. Since advanced fibrosis/cirrhosis is a major risk factor for HCC, we calculated FIB-4 for all patients and excluded those with FIB-4>3.25. We did not include any patients with documented significant fibrosis based on elastography who had an HBV DNA >2,000 IU/mL as these patients were considered immune active. However, this study is not without limitations. Data were not available for why certain patients in the indeterminate phase received antiviral therapy while others did not, and the decision for antiviral therapy was based on the local practice guidelines and their physician's discretion. However, we believe that this reflects the heterogeneity in current real-world clinical practice. Most of our patients were Asians, so these data may not be generalizable for patients of other ethnicities. The study was retrospective and subject to the biases associated with retrospective studies. However, a prospective clinical trial in this area may not be feasible due to the large number of patients required and the long follow-up duration. A substantial proportion of patients in the indeterminate phase were excluded from the analyses due to missing data or insufficient follow-up; therefore, the findings require cautious interpretation. Although we performed IPTW for multiple variables to balance the baseline characteristics between the two study groups, there may be residual unmeasured confounders, such as a family history of HCC, that were not accounted for. Additional biomarker data, such as quantitative HBsAg, HBcrAg, and HBV RNA may have provided useful information, but were not available for this study. We acknowledge that there may be some overlap between the indeterminate phase and situations where the AASLD guidance recommends initiation of therapy, such as when ALT > ULN and HBV DNA is persistently >2,000 IU/ml or > 20,000 IU/ml when HBeAg is negative and positive, respectively. However, we determined that antiviral therapy was associated with reduced HCC risk in the indeterminate phase in subgroup analyses beyond the criteria in the treatment

algorithm, such as when ALT <ULN, and when HBV DNA >1,000 IU/ml. These data suggest that patients beyond current treatment indications may derive substantial benefits from antiviral therapy.

Conclusion

Utilizing real-world data from a multinational consortium from both East and West, we determine that antiviral therapy for patients with CHB in the indeterminate phase is associated with a substantial reduction in the risk of HCC. These data have important implications for the potential expansion of CHB treatment criteria. Further studies are needed to evaluate the cost-effectiveness of such a strategy.

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Table 1. Study definitions for the various phases of chronic hepatitis B

	HBeAg	HBV DNA (IU/mL)	ALT (U/L)*	Histology/ non-invasive test for fibrosis
Immune tolerant	+	>1 million	<1x ULN	Minimal inflammation; No fibrosis
Immune active**	+	≥20,000	≥2x ULN	FIB-4>3.25, ≥ Stage 2 fibrosis on histology, liver stiffness by transient elastography >8.0kPA[19,31], liver stiffness by magnetic resonance elastography >2.99 kPA[20], fibrosure<0.58[19], APRI >1.5
	-	≥2,000		
Inactive***	-	<2,000	<1x ULN	Absence of significant inflammation or fibrosis
Indeterminate	Any patient that does not fit the above criteria			

*ALT ULN (U/L): 35 (male); 25 (female)
**Based on the AASLD guidance algorithm for treatment and management of HBsAg-positive persons without cirrhosis
***HBeAg (-) and Anti-HBe (+)
Abbreviations: HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase

Table 2. Baseline characteristics of indeterminate patients by treatment status (A) overall and (B) after inverse probability of treatment weighting (IPTW)

(A) Before IPTW	**Overall cohort (n=855)**	**No treatment (n=450)**	**Treated (n = 405)**	***P*-value**	**ASD**
Mean age (years)	46.4 ± 13.1	45.6 ± 14.1	47.4 ± 11.8	0.05	0.13
Male sex (n, %)	504 (59.0%)	238 (52.9%)	266 (65.7%)	<0.001	0.26
Diabetes (n, %)	104 (12.2%)	61 (13.6%)	43 (10.6%)	0.19	0.09
Alcohol (%)	24.2%	21.7%	26.2%	0.27	--
HBeAg positive (n, %)	174 (20.4%)	59 (13.1%)	115 (28.4%)	<0.001	0.38
HBV DNA (log_{10} IU/ml)	4.5 ± 2.1	3.8 ± 1.8	5.2 ± 2.1	<0.0001	0.73
% with DNA <2,000	27.4%	36.0%	17.8%	<0.001	--
% with DNA ≥ 2000-20,000	25.5%	33.3%	16.8%		
% with DNA >20,000	47.1%	30.7%	65.4%		
Median ALT (IQR)[U/L]	38 (24 – 52)	33 (20 – 45)	43 (31 – 62)	<0.0001	0.28
% ALT <1X ULN*	33.7%	44.7%	21.5%	<0.001	--
% with 1 ≤ ALT < 1.5	33.7%	31.6%	36.1%		
% with 1.5 ≤ ALT < 2	17.0%	12.4%	22.0%		
% with ALT ≥ 2	15.7%	11.3%	20.5%		
FIB-4 <1.45, n (%)	576 (67.4%)	325 (72.2%)	251 (62.0%)	0.001	--
FIB-4 1.45 – 3.25, n (%)	279 (32.6%)	125 (27.8%)	154 (38.0%)		
Time between ALT labs (months)	6.9 (4.6 – 8.5)	7.5 (6.1 – 8.9)	5.5 (3.1 – 8.1)	<0.0001	--
(B) After IPTW	**Overall cohort (n=819)**	**No treatment (n=425)**	**Treated (n = 394)**	***P*-value**	**ASD**
Mean age (years)	46.9 ± 13.3	46.6 ± 14.8	47.2 ± 11.5	--	0.04
Male sex (n, %)	475 (58.0%)	243 (57.2%)	232 (58.9%)	--	0.03
BMI (kg/m^2)	23.7 ± 3.6	23.8 ± 3.7	23.5 ± 3.4	--	--
Diabetes (n, %)	99 (12.1%)	51 (12.0%)	49 (12.4%)	--	0.02
Alcohol (%)	24.3%	25.7%	23.4%	--	--
HBeAg positive (n, %)	184	95	89	--	0.006

	(22.5%)	(22.4%)	(22.6%)		
HBV DNA (log_{10} IU/ml)	4.4 ± 2.1	4.4 ± 2.0	4.5 ± 2.1	--	0.06
% with DNA <2,000	27.6%	28.6%	26.4%	--	--
% with DNA ≥ 2000-20,000	27.1%	29.7%	24.3%		
% with DNA >20,000	45.4%	41.7%	49.3%		
ALT (IQR)[U/L]	37 (24 – 50)	35 (21 – 48)	40 (27 – 55)	--	0.13
% ALT <1X ULN*	34.9%	42.4%	26.9%	--	--
% with 1 ≤ ALT < 1.5	34.0%	30.9%	37.3%		
% with 1.5 ≤ ALT < 2	17.4%	14.7%	20.3%		
% with ALT ≥ 2	13.8%	12.1%	15.6%		
FIB-4 <1.45, n (%)	558 (68.1%)	293 (68.9%)	265 (67.3%)	--	--
FIB-4 1.45 – 3.25, n (%)	261 (31.9%)	132 (31.1%)	129 (32.7%)		
Time between ALT labs (months)	6.9 (4.6 – 8.4)	7.2 (6.0 – 8.6)	6.0 (3.2 – 8.2)	--	--

Values presented as number (%), mean ± standard deviation or median (IQR)
IPTW matched for age, sex, ALT, AST, DNA, HBeAg, platelet count, presence of diabetes, and follow-up time.
Abbreviations: Inverse probability of treatment weighting, IPTW; ASD; absolute standardized difference; BMI, body mass index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; IQR, inter-quartile range; ULN, upper limit of normal.

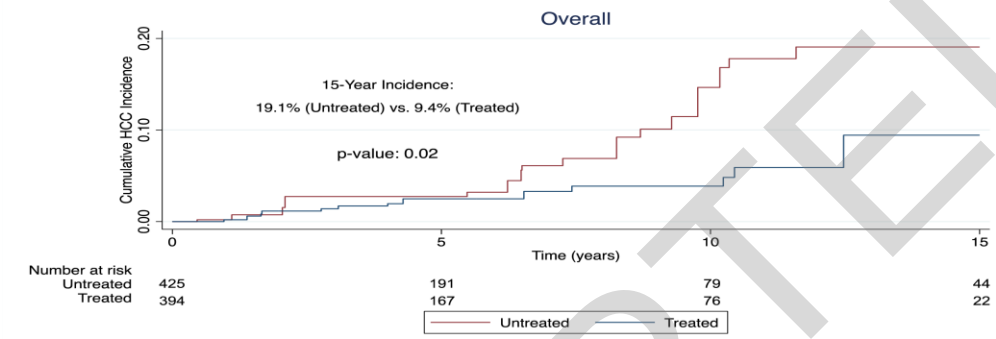
Table 3. IPTW cohort: Predictors of hepatocellular carcinoma in treatment naïve patients who remained indeterminate versus treated indeterminate patients

Variables	Events	Univariable Analysis		Multivariable Analysis**	
		HR (95% CI)	*P*-value	Adjusted HR* (95% CI)	*P*-value
Treatment status					
Not treated	27	Referent		Referent	
Treated	11	0.4 (0.2 – 0.9)	0.02	0.30 (0.10 – 0.60)	0.001
Age					
≤45 years	10	Referent		Referent	
>45 years	28	2.4 (1.2 – 5.0)	0.02	4.6 (2.1 – 10.1)	<0.001
Sex					
Female	1	Referent		Referent	
Male	37	33.2 (3.6 – 309.0)	0.002	32.5 (3.5 – 304.4)	0.002
Diabetes					
Not Present	29	Referent		Referent	
Present	9	1.5 (0.7 – 3.1)	0.33	1.6 (0.7 – 3.5)	0.26
Platelet					
≥ 150	31	Referent		Referent	
< 150	7	2.02 (0.9 – 4.6)	0.09	2.0 (0.8 – 4.8)	0.11
ALT					
< 1 × ULN*	8	Referent		Referent	
≥1 × ULN*	30	1.9 (0.9 – 4.2)	0.09	2.4 (1.0 – 5.6)	0.05
HBeAg					
Negative	20	Referent		Referent	
Positive	18	3.2 (1.7 – 6.0)	<0.001	3.7 (1.8 – 7.7)	<0.001
HBV DNA					
≤1,000 IU/ml	9	Referent		Referent	
>1,000 IU/ml	29	1.1 (0.5 – 2.4)	0.77	1.6 (0.7 – 4.0)	0.27

*ALT ULN: ≤35 U/L for males and ≤25 U/L for females
****Adjusted for treatment status, age, sex, diabetes, platelet count, ALT, HBeAg, and HBV DNA
IPTW matched for age, sex, ALT, AST, DNA, HBeAg, platelet count, presence of diabetes, and follow-up time.
Abbreviations: Inverse probability of treatment weighting, IPTW; HBeAg: hepatitis B e-antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; IPTW, inverse probability of treatment weighting

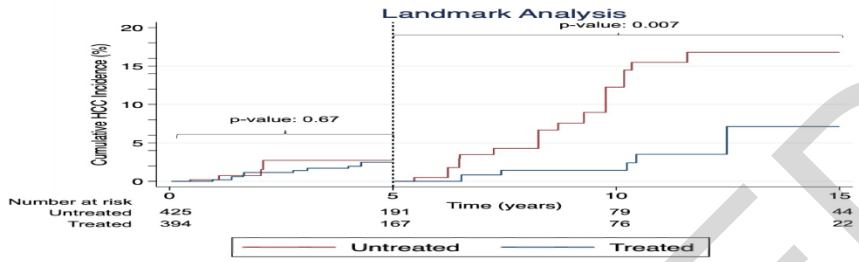
Figure 1. IPTW cohort: Hepatocellular carcinoma incidence in untreated patients who remained indeterminate throughout follow-up versus antiviral-treated patients who were indeterminate before treatment, (A) overall, (B) with landmark analysis.

Fig:A



Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	2,310	27	11.6 (7.0 – 20.9)	2.7% (1.4 – 5.4)	14.7% (9.6 – 22.0)	19.1% (12.9 – 27.6)
Treated	2,160	11	5.2 (3.0 – 9.9)	2.5% (1.1 – 5.4)	3.9% (1.9 – 7.8)	9.4% (4.5 – 19.3)

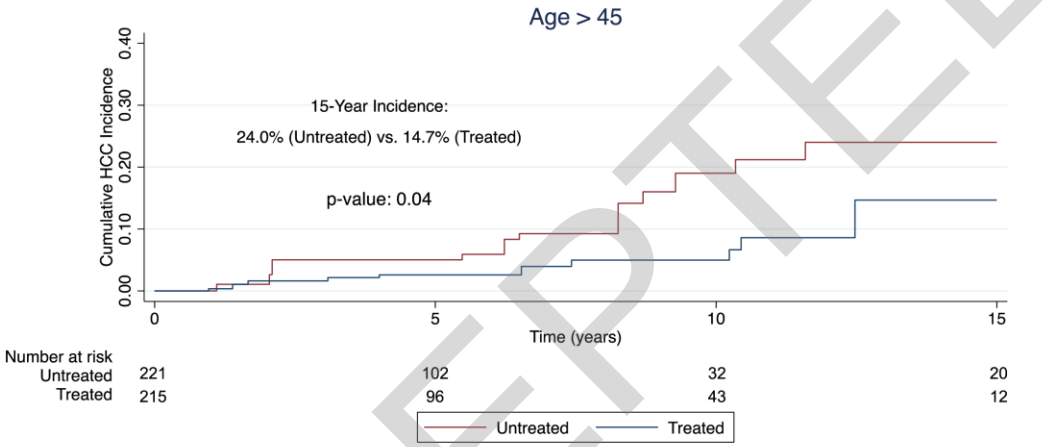
Fig:B



Abbreviations: IPTW, inverse probability of treatment weighting; HCC, hepatocellular carcinoma
IPTW was balanced for age, sex, ALT, AST, DNA, HBeAg, platelet count, presence of diabetes, and follow-up time.

Figure 2. IPTW cohort: Hepatocellular carcinoma incidence in untreated patients who remained indeterminate throughout follow-up versus antiviral-treated patients who were indeterminate before treatment, subgroup analysis for (A) Age > 45 years, (B) Age ≤ 45 years, (C) ALT ≥ ULN, (D) ALT < ULN, (E) HBV DNA > 1,000 IU/ml, (F) HBV DNA ≤ 1,000 IU/ml

Fig:A

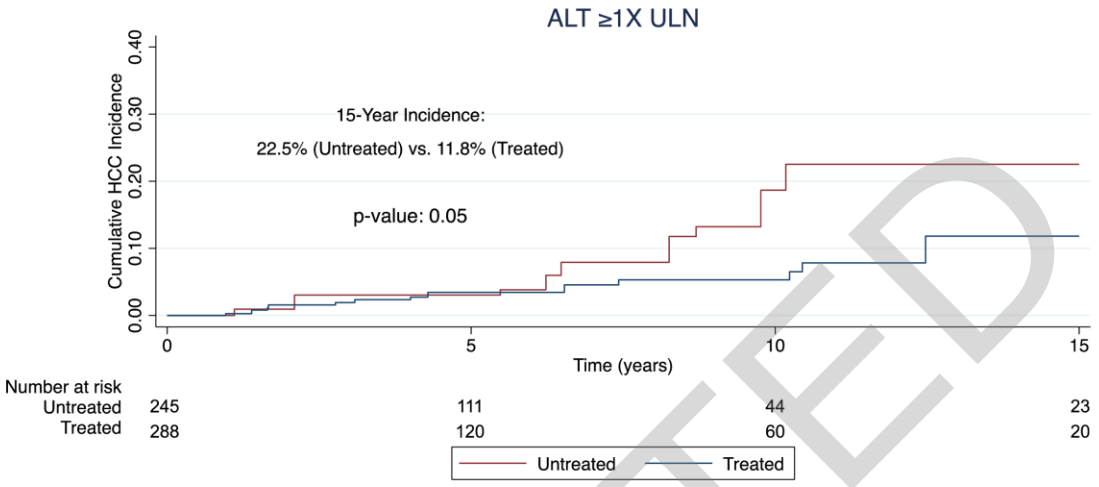


Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	1,138	18	15.9 (8.5 – 33.3)	5.0% (2.5 – 10.1)	19.0% (11.4 – 30.7)	24.0% (14.8 – 37.7)
Treated	1,217	9	7.4 (3.9 – 15.6)	2.6% (1.0 – 6.7)	5.0% (2.1 – 11.4)	14.7% (6.6 – 31.0)

Fig:B

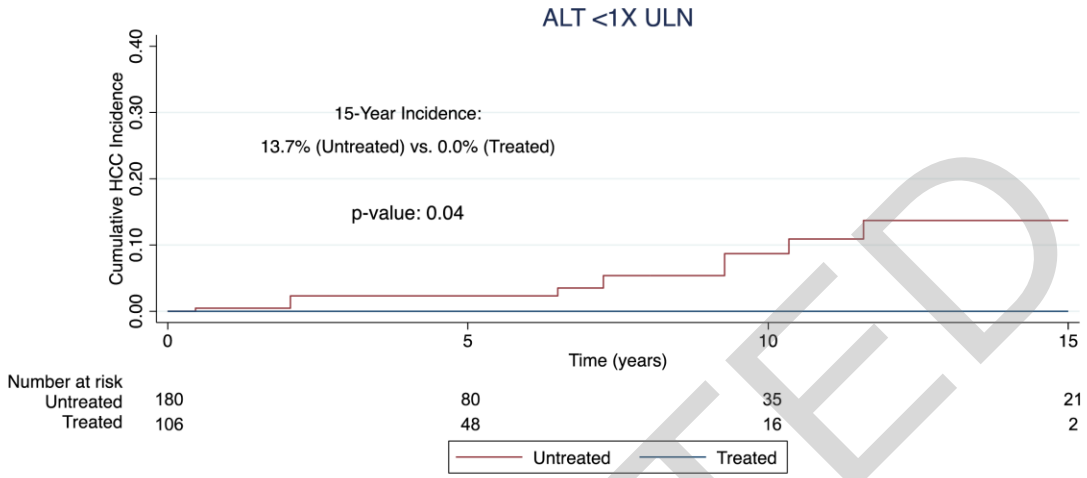
Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	1,172	9	7.5 (3.0 – 25.2)	0.4% (0.0 – 3.6)	10.2% (4.8 – 21.0)	14.1% (7.3 – 26.3)
Treated	943	2	2.4 (0.7 – 12.8)	2.4% (0.6 – 8.7)	2.4% (0.6 – 8.7)	2.4% (0.6 – 8.7)

Fig:C



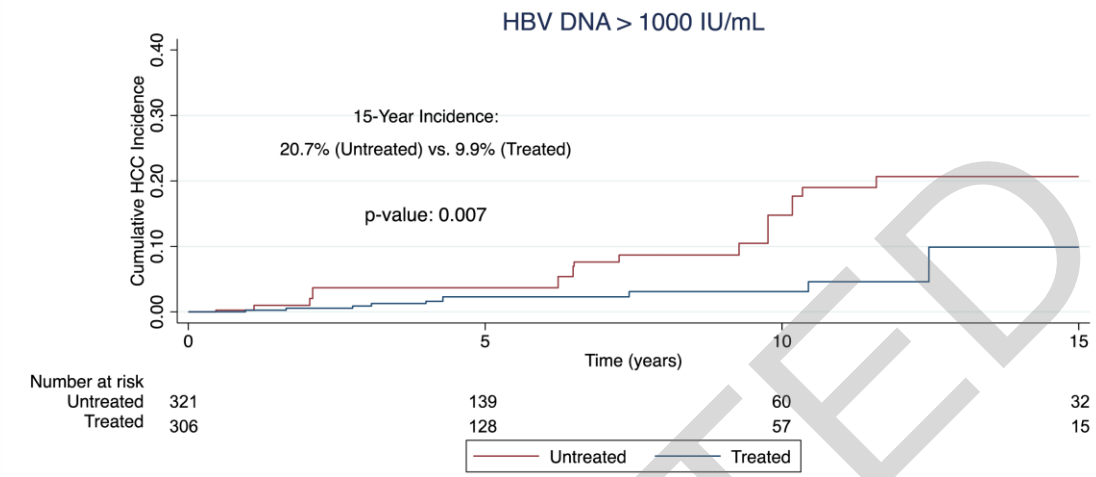
Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	1,323	19	14.2 (7.5 – 30.8)	3.0% (1.3 – 7.0)	18.7% (11.5 – 29.5)	22.5% (14.3 – 34.5)
Treated	1,590	11	7.1 (4.0 – 14.0)	3.5% (1.6 – 7.5)	5.3% (2.6 – 10.7)	11.8% (5.9 – 23.1)

Fig:D



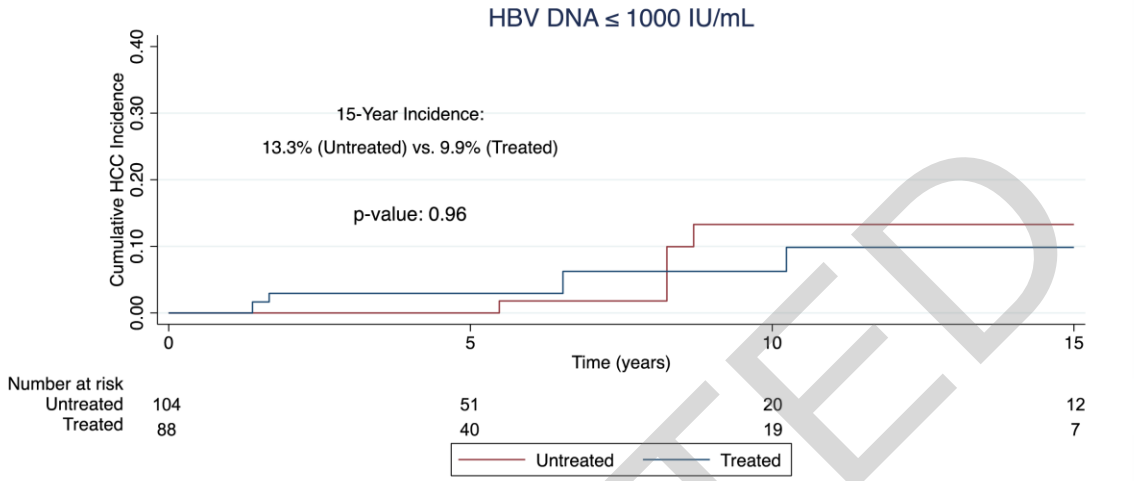
Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	987	8	8.2 (3.8 – 21.5)	2.3% (0.7 – 7.1)	8.7% (3.7 – 19.6)	13.7% (6.5 – 27.5)
Treated	571	0	0 (N/A)	0 (N/A)	0 (N/A)	0 (N/A)

Fig:E



Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	1,705	22	13.1 (7.5 – 25.2)	3.7% (1.9 – 7.2)	14.8% (9.1 – 23.4)	20.7% (13.4 – 31.1)
Treated	1,631	7	7.1 (2.2 – 9.8)	2.3% (0.9 – 6.1)	3.1% (1.2 – 7.8)	9.9% (3.8 – 24.5)

Fig:F



Antiviral Status	Person years	Cases	Incidence per 1,000 person years (95% CI)	5-year incidence (%) (95% CI)	10-year incidence (%) (95% CI)	15-year incidence (%) (95% CI)
Untreated	605	5	7.5 (1.7 – 65.6)	0 (N/A)	13.3% (5.4 – 30.5)	13.3% (5.4 – 30.5)
Treated	529	4	7.8 (2.7 – 29.7)	2.9% (0.8 – 10.4)	6.2% (2.0 – 18.5)	9.9% (3.3 – 27.3)

Abbreviations: IPTW, inverse probability of treatment weighting; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; HBV DNA, hepatitis B virus DNA; ULN, upper limit of normal (>35 U/L for males, >25 U/L for females)
IPTW was balanced for age, sex, ALT, AST, DNA, HBeAg, platelet count, presence of diabetes, and follow-up time.