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Association of Nucleos(t)ide Analogue Therapy with Reduced Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B—a Nationwide Cohort Study

Short title: HBV therapy and HCC incidence

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Abbreviations: Hepatitis B virus (HBV), hepatocellular carcinoma (HCC), chronic hepatitis B (CHB), National Health Insurance Research Database (NHIRD)

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Authors' Contribution:

Chun-Ying Wu, Jaw-Ching Wu and Jaw-Town Lin were responsible for designing and conducting the entire study. Chun-Ying Wu was responsible for drafting of the manuscript. Jaw-Ching Wu and Jaw-Town Lin were responsible for organizing the research team, obtaining NHIRD data and participating in data interpretation. Hsiu J. Ho managed the database and conducted statistical analyses. Chien-Wei Su, Teng-Yu Lee, Shen-Yung Wang, and Chuhui Wu provided essential intellectual contents and participated in data interpretation. All authors have seen and approved the final version of the manuscript. All authors had full access to all of the data and take responsibility for the integrity and accuracy of the data.

ABSTRACT

Background & Aims: Treatment for hepatitis B virus (HBV) infection reduces the risk of hepatocellular carcinoma (HCC). However, the long-term protective effects for subgroups of patients with chronic hepatitis B (CHB) patients are unclear.

Methods: We conducted a retrospective nationwide cohort study using data from Taiwan's National Health Insurance Research Database (from January 1, 1997 through December 31, 2010). Cumulative incidences were calculated and multivariable analyses were carried out after adjusting for competing mortality. Propensity scores were used to match 21,595 patients with CHB who received nucleoside analogue therapy for at least 90 days (treated cohort) with 21,595 untreated patients with CHB (controls), who received hepatoprotectants for at least 90 days. Data were collected from the treated cohort for a mean period of 3.46 years and from controls for 5.24 years.

Results: The treated cohort had a significantly lower 7-year incidence of HCC (7.32%; 95% confidence interval [CI], 6.77%–7.87%) than controls (22.7%; 95% CI, 22.1%–23.3%; *P*<.001). After adjusting for competing mortality and other confounders, nucleos(t)ide analogue treatment was associated with a reduced risk of HCC, with an adjusted hazard ratio of 0.37 (95% CI, 0.34–0.39, *P*<.001). Sensitivity analyses confirmed the association between nucleos(t)ide analogue treatment and reduced risk of HCC. Age, sex, cirrhosis, and diabetes mellitus modified this association.

Conclusion: Based on a retrospective, nationwide study in Taiwan, nucleoside analogue therapy use is associated with reduced risk of HCC in patients with chronic hepatitis B virus infection.

KEYWORDS: hepatoma; HBV; antiviral agent; NHIRD

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-related mortality. It is estimated that more than 748,000 new HCC cases and about 700,000 deaths occur annually worldwide[1,2]. Many risk factors contribute to the development of HCC, including hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, non-alcoholic steatohepatitis, and metabolic syndrome[3]. Globally and in Asia particularly, chronic HBV infection is the most frequent underlying cause of HCC and accounts for approximately half of HCC cases[4,5]. Vaccines against HBV have successfully reduced the incidence of HBV in the younger generations; however, there are still more than 350 million patients infected with HBV worldwide[6]. Chronic hepatitis B (CHB) infection not only causes hepatitis, but also leads to hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC)[1,3,6,7]. HBV replication has been identified as a major element of immune-mediated liver tissue injury and disease progression. Higher HBV DNA levels are associated with increased risk of HCC development and recurrence[4,8,9].

Nucleos(t)ide analogue therapy effectively suppresses HBV replication by inhibiting HBV polymerase[10,11]. Treatment with nucleos(t)ide analogues has been reported to delay disease progression in CHB patients[12,13]. Regression of liver cirrhosis has been observed with long-term use of nucleos(t)ide analogues[14,15]. In addition, nucleos(t)ide analogue therapy has been reported to be associated with reduced risk of HCC development and recurrence[16-18]. In a meta-analysis that pooled 5 studies and a total of 2289 CHB patients, the risk of HCC was reduced by 78% among those receiving nucleos(t)ide analogues therapy relative to controls that did not received nucleos(t)ide analogue[19]. In another systematic review of 21 studies involving based on 3881 treated and 534 untreated patients, the protective roles of

nucleos(t)ide analogues in the development of HCC were further confirmed[20]. In the CALM study, nucleos(t)ide analogue therapy attenuated 51% risk of HCC in patients with CHB[12]. However, some literatures reporting no beneficial effects for nucleos(t)ide analogue to reduce HCC risk among CHB patients. In a recent cohort study, the risk of HCC development in patients with oral antiviral therapy is still significantly higher than patients with inactive CHB[21]. In another study, oral nucleos(t)ide analogue reduces the incidence of cirrhosis and risk of complications, but not the development of HCC in cirrhotic patients[22]. With these controversial evidences, a long-term population-based nationwide study will be helpful to investigate the association between nucleos(t)ide analogue treatment and risk of HCC in HBV infected patients. In the present study, we conducted a nationwide cohort study to examine whether nucleos(t)ide analogue use is associated with reduced risk of HCC in CHB patients. Furthermore, we examined the number needed to be treated (NNT) for one less HCC development.

METHODS

Study design:

We conducted this nationwide cohort study based on Taiwan's National Health Insurance Research Database (NHIRD). The NHIRD was set up in 1997 when the National Health Insurance (NHI) program, a compulsory universal health insurance program for nearly all 23.7 million residents in Taiwan, was established. Comprehensive health care information, including diagnoses, prescriptions, and laboratory check-up items can be retrieved from the NHIRD, which has been described in detail in our previous studies[18, 23-24]. International classification of diseases-9 (ICD-9) codes are used to define diseases in this database. The

accuracy of diagnosis of major diseases, such as ischemic stroke and acute coronary syndrome, has been validated[25, 26]. This study has been approved by the ethical review board of the National Health Research Institutes, Taiwan.

Study subjects:

CHB patients in the NHIRD were defined as those meeting the following two criteria: (1) diagnosed with CHB (ICD-9 codes: 070.2, 070.3, and V02.61) three times in outpatient clinic or admitted with a diagnosis of HBV infection between January 1, 1997 and December 31, 2010 and (2) having used nucleos(t)ide analogues or hepatoprotective agents (e.g. silymarin, liver hydrolyste, and choline bitartrate). These CHB patients were followed up from the time of diagnosis of CHB until the development of HCC, death or December 31, 2010. We first excluded patients not using nucleos(t)ide analogues or hepatoprotective agents for at least 90 days. Nucleos(t)ide analogues have been covered under the NHI program for CHB patients since October 1, 2003. However, reimbursement for nucleos(t)ide analogues requires patients to fulfill certain criteria, such as twice-elevated serum aminotransferase (ALT $\geq$ 2X) and elevated HBV DNA titer (> 2000 IU/mL). The reimbursement duration ranges from 18 months to 36 months[18]. The reimbursement for hepatoprotective agents requires only elevated aminotransferase level (ALT $\geq$ 1X). Hepatoprotective agents have been reimbursed since the beginning of Taiwan's NHI program in 1997. Next, we excluded patients with HCV (ICD-9 codes: 070.41, 070.44, 070.51, 070.54, and V02.62), human immunodeficiency virus (ICD-9 code: 042), other viral hepatitis (ICD-9 code: V02.69), and malignant tumors (ICD-9 codes: 104-208).

Study cohorts:

Among the eligible patients, the treated patients were defined as those who received

nucleos(t)ide analogues for at least 90 days. Those who did not used nucleos(t)ide analogues for at least 90 days were defined as non-treated patients. The demographic data of these two groups were first compared. Then, propensity scores for estimating the probability of receiving nucleos(t)ide analogue therapy were developed using the logistic regression model to estimate the differences in baseline characteristics between the treated patients and the untreated patients. The propensity score approach used in our study was described in Dehajia RH, et al[27]. We matched each treated patient with one untreated patient, based on propensity scores. Histograms before and after matching were examined to assess the success of the propensity scores in balancing the two groups. The index date of follow-up was the first date of nucleos(t)ide analogue prescription for the treated cohort and the first date of hepatoprotective agent prescription for the untreated cohort.

Hepatocellular carcinoma risk analysis:

HCC diagnosis was defined according to the major diagnosis of admission (ICD-9 codes: 155.0 and 155.2) or enrollment in the Registry for Catastrophic Illness Patient Database (RCIPD), a subset of the NHIRD. Patients were registered in the RCIPD if their diagnoses were confirmed by pathological reports or typical imaging presentations. The first date of admission or enrollment in the RCIPD was defined as the date of HCC development. We excluded patients with a diagnosis of HCC in the first 90 days after start of nucleos(t)ide analogue therapy or hepatoprotective agents. Cumulative incidences (CIs) of HCC were analyzed after adjusting for competing mortality.

Since death usually results from underlying comorbidities that may also impact HCC risk, its occurrence leads to informative censoring in calculating HCC incidence. Therefore, death prior to HCC development was considered a competing risk event on survival analysis.

Death-adjusted cumulative incidences in competing risk data ratios were analyzed using modified Kaplan-Meier method and Gray method[28,29]. The R package "cmprsk" (http://cran.r-project.org/web/packages/cmprsk/index.html) was used in the competing risk analyses.

Multivariable analyses:

To determine whether nucleos(t)ide analogue use was independently associated with reduced risk of HCC, Cox proportional hazards model was developed. Since the case number in the present study was large enough, all the parameters defined a prior were used in the model to exclude as many confounders as possible.

To examine potential heterogeneity of treatment effect in relation to confounders, we performed interaction analyses by adding interaction terms between treatment and potential confounding factors, including age, sex, cirrhosis, liver decompensation, comorbidities, use of use of statins, use of NSAIDs, and use of metformin in the multivariable analyses. Since the sample size is quite large in the present study, we defined the alpha level at 0.05 as significantly different for the interaction instead of using alpha level at 0.10. Once a factor is found significantly interacted with treatment, multivariable stratified analyses were conducted to examine the associations of nucleos(t)ide use and risk of HCC in CHB patients with these factors.

Sensitivity analyses:

Nucleos(t)ide analogues have been covered under the NHI program since October 1, 2003, midway through the follow-up period. Therefore, the follow-up durations in the two treatment groups differed significantly. The impact of reimbursement of antivirals in 2003

may induce several other unmeasured factors, such as different screening policy, different accessibility of health care, and different health consciousness of patients, etc. To address the differential follow-up in the two treatment groups, we conducted sensitivity analyses with fixed duration by limiting the index date of follow-up to between October 1, 2003 and September 30, 2005 and the follow-up duration to 5 years.

To examine the impact of other potential unmeasured confounders on the estimated treatment effect, we performed sensitivity analyses with add-on of an unmeasured confounder according to the method of Lin et al. using the R package "obsSens"[30]. In the sensitivity analyses, we added another hypothetical unmeasured confounder with a similar favorable protective effect as our antiviral agents. Then we examined how this added unmeasured factor confounded our observations with different prevalence in the treated and untreated groups.

Statistical analysis:

The demographic characteristics of the treated and untreated patients were compared using the χ^2 test and Student's t-test. The number of patients who needed to be treated for one less HCC occurrence was defined as NNT and calculated by the inverse of the absolute risk reduction. The confidence intervals of NNT were calculated according to the method of Altman et al. in a survival setting[31]. All data management was performed using SAS 9.1 software (SAS Institute, Cary, NC). Cumulative incidences were analyzed using the survival and epitools packages of R.

RESULTS

Demographic data:

Between1997 and 2010, a total of 199,451 patients were diagnosed with CHB. Among them, 81,823 patients had used nucleos(t)ide analogues or hepatoprotectants for at least 90 days. Patients with hepatitis C, other hepatitis, human immunodeficiency virus, and those with malignancies before the use of nucleos(t)ide analogues or hepatoprotectants were excluded. Among the remaining 72,458 patients, 47,611 patients were in the untreated group and 24,847 patients were in the treated group. These two groups had significant differences in their demographic data (Supplemental Table 1). To estimate the probability of receiving nucleos(t)ide analogue therapy, age, gender, comorbidities (in separate terms for individual comorbidities), use of statins, use of NSAIDs, and use of metformin were used to calculate propensity scores. The original propensity scores of the untreated patients (mean=0.29) were significantly lower when compared with the treated cohort (mean=0.44) ($P<0.001$). We used propensity score to match one patient in the treated cohort with one patient in the untreated cohort. The histograms of propensity score before and after matching are shown in Supplemental Figure 1. Before matching, the untreated patients had significantly lower propensity scores (black bars in the histogram) compared with the treated patients (gray bars in the histogram) (Supplemental Figure 1A). After matching, the two groups had comparable distributions of propensity scores (Supplemental Figure 1B). Finally, we recruited 21,595 patients into the treated cohort and 21,595 patients into the untreated cohort (Figure 1). In the treated cohort, a total of 19,063 patients received only one nucleos(t)ide analogue, including 12,938 patients who received lamivudine, 5,748 patients who received entecavir, and 377 patients who received telbivudine. The remaining 2,532 patients received more than one

nucleos(t)ide analogue.

As shown in Table 1, the mean age of these two cohorts was 43.5 and about three-fourths of the patients were male. The mean follow-up durations for the treated and untreated cohorts were 3.46 and 5.24 years, respectively. The mean sonography and alpha-fetoprotein (AFP) screening frequencies (number per year) were 2.08 and 2.88 for the treated cohort, 1.90 and 1.81 for the untreated cohorts, respectively. The mean duration of nucleos(t)ide analogue use in the treated cohort was 1.44 years. The mean duration of hepatoprotectant use in the untreated cohort was 1.24 years. The treated cohort had significantly lower incidence of HCC (N=992, 4.6%) when compared with the untreated cohort (N=4,454, 20.6%). Competing mortality (death before the development of HCC) was significantly lower in the treated cohort (N=1,036, 4.8%) than in the untreated cohort (N=2,256, 11.8%). Overall mortality in the treated cohort (N=1,406, 6.5%) was also significantly lower than in the untreated cohort (N=4,778, 22.1%).

Seven-year cumulative incidences of HCC for treated and untreated cohorts:

Cumulative incidences of HCC after adjustment for competing mortality are shown in Figure 2. Patients in the treated cohort were associated with significantly lower risk of HCC (7-year cumulative incidence: 7.32%; 95%CI, 6.77-7.87%) than those in the untreated cohort (22.70%; 95%CI, 22.11-23.30%) (P<0.001). On average, the annual incidences of HCC in treated and untreated cohorts were 1.05% and 3.24%, respectively. The unadjusted NNT associated with one less HCC development within 7 years was 7 (95% CI, 6.2-6.9). This suggests that use of nucleos(t)ide analogues in 7 CHB patients is associated with one less HCC development within 7 years.

Multivariable analysis

Without controlling for other factors, nucleos(t)ide analogue treatment was associated with reduced risk of HCC development (HR= 0.34; 95% CI, 0.32-0.37, P<0.001). After adjusting for competing mortality and other confounders, we found that nucleos(t)ide analogues treatment is associated with a significantly lower risk of HCC (HR=0.37; 95%CI, 0.34-0.39, P<0.001). Older age, male gender, and liver cirrhosis were found to be risk factors for increased HCC risk. Patients with comorbidities including liver decompensation, hypertension, chronic obstructive pulmonary disease (COPD), acute coronary syndrome, and cerebral vascular diseases were found to be associated with reduced risk of HCC due to higher competing mortality. Use of statin and use of NSAIDs or aspirin were associated with significantly lower risk of HCC in CHB patients (Table 2).

To examine whether significant heterogeneity of treatment effect exists in relation to age, sex, cirrhosis, liver decompensation, diabetes, and other potential confounders, we added interaction terms to the multivariable analyses (Supplemental Table 2). On the interaction analysis, we found statistically significant interactions between nucleos(t)ide use and age, gender, liver cirrhosis, and diabetes. Since the interactions are statistically significant, we cannot interpret the main effect of treatment. Instead, we examined the effect of treatment within each level of the factors, including age, gender, liver cirrhosis, and diabetes. In Figure 3, we conducted multivariable subgroup analyses. We found that the treated cohort was associated with a reduced risk of HCC in all subgroups. The beneficial effect of nucleoside analogues was especially significant among younger patients (<40 years old: HR=0.13; 40-50 years old: HR=0.30; ≥ 50 years old: HR=0.49), patients without cirrhosis (non-cirrhosis vs. cirrhosis: HR=0.27; vs. HR=0.72), and patients without diabetes (non-diabetes vs. diabetes:

HR=0.34 vs. HR=0.69).

Sensitivity analysis

In the sensitivity analysis with fixed duration, we only identified CHB patients between October 1, 2003 and September 30, 2005 because of three following reasons. First, nucleos(t)ide analogues were covered under the NHI program since October 1, 2003. Second, we wish to follow these patients up to 5 years until the end of 2010. Third, these patients much used nucleos(t)ide analogues or hepatoprotectants for at least 90 days. Propensity scores were used to match each treated patient with one untreated patient. Finally, we identified 4,545 patients in the treated cohort and 4,545 patients in the untreated cohort. The demographic characteristics and outcomes are shown in Supplemental Table 3. Patients in the treated cohort were associated with significantly lower risk of HCC development (5-year cumulative incidence: 6.62%; 95%CI, 5.90-7.35%) than those in the untreated cohort (19.08%; 95%CI, 17.93-20.22%) (P<0.001) (Supplemental Figure 2). On multivariable analysis, the treated cohort was associated with reduced risk of HCC development (adjusted HR=0.31, 95%CI, 0.27-0.53, P<0.001) (Supplemental Table 4).

In Supplemental Figure 3, we used sensitivity analysis to examine the trend of estimates of the treated hazard on covariate-adjusted Cox model with add-on of an unmeasured confounder with relative hazard of 0.3. When all the subjects in untreated group have the add-on unmeasured confounder (prevalence of the confounder in the untreated group is 1.0) and none of subjects in treated group has this unmeasured confounder (prevalence of the confounder in the treated group is 0.0), then the impact of antiviral therapy would be beneficial (HR=0.1, the bottom line in Supplemental Figure 3). On the contrary, when none of subjects in the untreated group has the add-on unmeasured confounder (P0=0.0) and all

subjects in treated group have this confounder; then the impact of antiviral therapy would be not protective (HR=1.2, the top line in Figure 3). In most situations, patients who received nucleos(t)ide analogues had lower risk of HCC occurrence relative to those who did not, even if a favorable unmeasured confounder exists.

DISCUSSION

This population-based cohort study demonstrated that use of nucleos(t)ide analogues is associated with reduced long-term risk of HCC in CHB patients. After adjusting for death as the competing cause of risk and for multiple confounding factors, we found that use of nucleos(t)ide analogues is associated with an adjusted HR of 0.37 for HCC occurrence in CHB patients. The association between nucleos(t)ide analogues use and lower risk of HCC was found in all subgroups of CHB patients, especially in younger patients, patients without liver cirrhosis, patients without liver decompensation, and patients without diabetes. We further validated our observations by sensitivity analyses.

In the present study, the association between use of nucleos(t)ide analogues and risk of HCC in CHB patients diminished with age. The HRs associated with use of nucleos(t)ide analogues were 0.13, 0.30 and 0.49 for patients younger than 40, aged between age 40-50, and older than 50, respectively. Several reasons may explain this observation. First, this interaction resulted from the rising probability of HCC in CHB patients with advanced age. In the REVEAL study, which investigated the natural history of CHB patients in Taiwan, Chen et al. found that the risk of HCC in CHB patients remains low before age 40, starts to rise in the forties, and significantly increases after age 50[7]. The rising probability of HCC with advanced age may be reflected in the absolute risk reduction by use of nucleos(t)ide

analogues in different age groups. The 7-year HCC absolute risk reductions for patients younger than 40, aged 40-50, and older than 50 were 7.92%, 18.23% and 18.68%, respectively. Second, older patients may have more comorbidities, which leads to higher risk of competing mortality. In the present study, we found that patients with hypertension, COPD, acute coronary syndrome, and cerebral vascular diseases have higher competing mortality and lower HCC risk after adjusting for competing mortality. Third, the starting time for nucleos(t)ide analogue use may be too late to rescue the carcinogenesis of HCC. However, we need more evidence to support this hypothesis.

For non-cirrhotic CHB patients, the average annual incidences of HCC were 0.68% and 2.97% for the treated and the untreated cohorts, respectively. The adjusted HR was 0.25 for the use of nucleos(t)ide analogues. Our observations of the treated group were comparable with those of previous reports, but the annual HCC incidence in the untreated non-cirrhotic patients in the present study was higher than in previous studies.[13,20,32]. In a meta-analysis based on five studies comparing patients treated with nucleos(t)ide analogues with controls, Sung et al. reported that the risk of HCC after nucleos(t)ide analogue treatment is reduced by 78%[19]. In another systematic review of 21 studies by Papatheodoridis et al., HCC developed in 2.8% and 6.4% of nucleos(t)ide analogue-treated and untreated CHB patients, respectively, during a 46 month period[20]. In a retrospective cohort study based on 377 CHB patients (17% with cirrhosis), annual HCC incidences were 0.4% for nucleos(t)ide analogue-treated group and 2.5% for control group, respectively[33]. The higher annual HCC incidence in our untreated non-cirrhotic patients may be due to the requirement for higher baseline aminotransferase levels to obtain reimbursement for hepatoprotective agents.

For cirrhotic CHB patients, the average annual incidences of HCC in the present study

were 3.90% and 4.94% for treated and untreated cohorts, respectively. The adjusted HR was 0.72 for the use of nucleos(t)ide analogue. The annual incidence of our treated cohort was slightly higher than that of previous studies. In a retrospective cohort study based on CHB patients with cirrhosis, the annual incidences of HCC in nucleos(t)ide analogue treated and untreated groups were 1.02% and 6.0%, respectively[34]. In the CALM study, a randomized trial based on CHB patients with cirrhosis or advanced fibrosis, nucleos(t)ide analogue use was found to reduce risk of HCC development over a median duration of 32.4 months of therapy (3.9% vs. 7.4%, P=0.047)[12]. A possible explanation for the higher annual incidences in the treated cohort in the present study is the strict NHI regulations regarding nucleos(t)ide analogue reimbursement[18]. Only high-risk populations, including patients with higher baseline HBV viral load, higher ALT level, or higher prevalence of liver decompensation are eligible for reimbursement. These higher risk populations may contribute to the higher annual incidences.

The chemopreventive effect of nucleos(t)ide analogue therapy in the present study was significantly higher in non-diabetic patients when compared with diabetic patients (adjusted HRs: 0.34 versus 0.69). In our recent nationwide case-control study, we found that diabetes is independently associated with increased risk of HCC development (OR=2.25)[35]. In the United States, diabetes has also been found to be associated with 2-3 fold increase in the risk of HCC, regardless of other HCC risk factors, such as viral hepatitis[36,37]. Several factors may explain the association between HCC and diabetes, such as increased risks of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in diabetic patients. Metformin has been fond to be associated with a decreased risk of HCC in diabetes patients, via inhibition of hepatoma cell proliferation and induction of cell cycle arrest at G0/G1 phase[35]. In the era of nucleos(t)ide

analogue therapy, more studies are needed to investigate the role of diabetes in the carcinogenesis of HCC to further decrease HCC incidence.

Recently, several HCC-score calculators have been introduced[38-40]. Unfortunately, we did not have information on baseline ALT and HBV DNA levels, which have been shown to be associated with HCC risk in these HCC-score calculators. Given that reimbursement for nucleos(t)ide analogues requires twice-elevated aminotransferase and higher HBV DNA levels (>2000 IU/mL), while reimbursement for hepatoprotective agents (control group) requires only elevated aminotransferase level (ALT ≥ 1X), the higher baseline HBV DNA levels and aminotransferase levels in the treated cohort may have led to a more conservative estimation of the protective effect of nucleos(t)ide analogues.

In the present study, we used many methods to prevent potential confounders. However, some unmeasureable bias may still exist. Propensity score matching was used to select comparable controls to imitate a randomized clinical trial. Although we used all potential confounders in the model to create propensity score, there were some significant differences in the distributions, such as for gender and liver cirrhosis. The large sample size in the present study may be the reason for the statistical significance. For examples, the differences between 24.5% female in the treated cohort vs. 23.1% female in the untreated cohort, and the differences of 13.2% vs. 14.0% cirrhosis in the treated and untreated cohorts are statistically significant, but may be not clinically significant. Reimbursement for nucleos(t)ide analogues began in 2003, midway through the follow-up period, which may also have confounded our observations. However, protective role of nucleos(t)ide analogue uses in HCC risk was found on sensitivity analyses with fixed duration.

In conclusion, nucleos(t)ide analogue use is associated with reduced risk of HCC in

CHB patients. Age, gender, liver cirrhosis, and diabetes mellitus modify this association. More studies are needed to explore the wider use of nucleos(t)ide analogues for prolonged periods to further decrease the incidences of HCC.

FIGURE LEGENDS:

Figure 1. Study patient selection flow diagram. *A case may be excluded due to more than one criterion. Therefore, the total excluded cases in each step may outnumber the sum of case numbers excluded by individual criteria.

Figure 2. Cumulative incidences of HCC after adjustment for competing mortality. Calculation and comparison of cumulative incidences in competing risk data ratios were conducted using modified Kaplan-Meier method and Gray's method. Patients who developed HCC during the first three months were excluded. Abbreviations: Untreated: CHB patients not receiving nucleos(t)ide analogues; Treated: CHB patients receiving nucleos(t)ide analogues.

Figure 3. Multivariable stratified analyses. Among chronic CHB patients, nucleoside analogue use (treated cohort) is associated with reduced risk of HCC development in all subgroups. All P values were significant.

REFERENCES

1. Jemal A, Bray F, Center MM, et al: Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. El-Serag HB, Rudolph KL: Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557-2576.
3. El-Serag HB: Hepatocellular carcinoma. N Engl J Med 2011;365:1118-1127.
4. Liu CJ, Kao JH: Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. J Chin Med Assoc 2007;70:141-145.
5. Sherman M, Llovet JM: Smoking, hepatitis B virus infection, and development of hepatocellular carcinoma. J Natl Cancer Inst 2011;103:1642-1643.
6. Liaw YF, Chu CM: Hepatitis B virus infection. Lancet 2009;373:582-592.
7. Chen CJ, Yang HI: Natural history of chronic hepatitis B REVEALed. J Gastroenterol Hepatol 2011;26:628-638.
8. 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.
9. Wu JC, **Huang YH**, **Chau GY**, et al: Risk factors for early and late recurrence in hepatitis B-related hepatocellular carcinoma. J Hepatol 2009;51:890-897. (Author names in bold designate shared co-first authors.)
10. Liaw YF: Antiviral therapy of chronic hepatitis B: opportunities and challenges in Asia. J Hepatol 2009;51:403-410.
11. Yuen MF, Lai CL: Treatment of chronic hepatitis B: Evolution over two decades. J Gastroenterol Hepatol 2011;26 Suppl 1:138-143.
12. Liaw YF, Sung JJ, Chow WC, et al: Lamivudine for patients with chronic hepatitis B and

advanced liver disease. N Engl J Med 2004;351:1521-1531.

13. Liaw YF: Impact of hepatitis B therapy on the long-term outcome of liver disease. Liver Int 2011;31 Suppl 1:117-121.
14. Chang TT, Liaw YF, Wu SS, et al: Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886-893.
15. Marcellin P, Gane E, Buti M, et al: Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468-475.
16. Lai CL, Yuen MF: Prevention of hepatitis B virus-related hepatocellular carcinoma with antiviral therapy. Hepatology 2013;57:399-408.
17. Sherman M: Does hepatitis B treatment reduce the incidence of hepatocellular carcinoma? Hepatology 2013;58: 18-20.
18. Wu CY, **Chen YJ**, Ho HJ, et al: Association between nucleos(t)ide analogues and risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver resection. JAMA 2012;308:1906-1914. (Author names in bold designate shared co-first authors.)
19. Sung JJ, Tsoi KK, Wong VW, et al: Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther 2008;28:1067-1077.
20. Papatheodoridis GV, Lampertico P, Manolakopoulos S, et al: Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. J Hepatol 2010;53:348-356.
21. Cho JY, Paik YH, Sohn W, et al.: Patients with chronic hepatitis B treated with oral antiviral therapy retain a higher risk for HCC compared with patients with inactive stage

disease. Gut 2014 March 13; Epub ahead of print.

22. Niro GA, Ippolito AM, Fontana R, et al.: Long-term outcome of hepatitis B virus-related Chronic Hepatitis under protracted nucleos(t)ide analogues. J Viral Hepat; 2013;20:502-507.
23. Wu CY, Wu MS, Kuo KN, et al: Effective reduction of gastric cancer risk with regular use of nonsteroidal anti-inflammatory drugs in Helicobacter pylori-infected patients. J Clin Oncol 2010;28:2952-2957.
24. Wu CY, Kuo KN, Wu MS, et al: Early Helicobacter pylori eradication decreases risk of gastric cancer in patients with peptic ulcer disease. Gastroenterology 2009;137:1641-1648 e1-2.
25. Wu CY, **Chan FK**, Wu MS, et al: Histamine2-receptor antagonists are an alternative to proton pump inhibitor in patients receiving clopidogrel. Gastroenterology 2010;139:1165-1171. (Author names in bold designate shared co-first authors.)
26. Cheng CL, Kao YH, Lin SJ, et al: Validation of the National Health Insurance Research Database with ischemic stroke cases in Taiwan. Pharmacoepidemiol Drug Saf 2011;20:236-242.
27. Dehejia RH and Wahba S: Propensity score-matching methods for nonexperimental causal studies, The review of Economics and Statistics 2002;84:151-161.
28. Prentice RL, Kalbfleisch JD, Peterson AV, Jr., et al: The analysis of failure times in the presence of competing risks. Biometrics 1978;34:541-554.
29. Gray RJ: A class of K-sample tests for comparing the cumulative incidence of a competing risk. The Annals of Statistics 1988;16:1141-1154.
30. Lin DY, Psaty BM, Kronmal RA: Assessing the sensitivity of regression results to

unmeasured confounders in observational studies. Biometrics 1998;54:948-963.

31. Altman DG and Andersen PK: Calculating the number needed to treat for trials wehre the outcomes is time to an event. BMJ 1999;319:1492-1495.

32. Papatheodoridis GV, Manolakopoulos S, Touloumi G, et al: Virological suppression does not prevent the development of hepatocellular carcinoma in HBeAg-negative chronic hepatitis B patients with cirrhosis receiving oral antiviral(s) starting with lamivudine monotherapy: results of the nationwide HEPNET. Greece cohort study. Gut 2011;60:1109-1116.

33. Matsumoto A, Tanaka E, Rokuhara A, et al: Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. Hepatol Res 2005;32:173-184.

34. Eun JR, Lee HJ, Lee SH, et al: The effect of lamivudine and adefovir dipivoxil on preventing hepatocellular carcinoma in hepatitis B virus-related liver cirrhosis. Hepatology 2007;46:664A-665A.

35. Chen HP, **Shieh JJ**, Chang CC, et al: Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. Gut 2012;62;606-615. (Author names in bold designate shared co-first authors.)

36. El-Serag HB, Tran T, Everhart JE: Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460-468.

37. Davila JA, Morgan RO, Shaib Y, et al: Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 2005;54:533-539.

38. Wong VW, **Chan SL**, Mo F, et al. Clinical scoring system to predict hepatocellular

carcinoma in chronic hepatitis B carriers. J Clin Oncol 2010;28:1660–1665. (Author names in bold designate shared co-first authors.)

39. Yang HI, Sherman M, Su J, et al. Nomograms for risk of hepato- cellular carcinoma in patients with chronic hepatitis B virus infec- tion. J Clin Oncol 2010;28:2437–2444.
40. Yang HI, Yuen MF, Chan HL, et al. Risk estimation for hepato- cellular carcinoma in chronic hepatitis B (REACH-B): develop- ment and validation of a predictive score. Lancet Oncol 2011; 12:568 –574.

Table 1. Baseline demographic characteristics and outcomes of study cohorts

	Untreated* (N=21595)	Treated* (N=21595)	P value[#]
	Number (%)	Number (%)	
Age (mean±SD)[+]	43.52±13.38	43.53±13.61	0.935
Gender			0.001
Female	4982 (23.1)	5281 (24.5)	
Male	16613 (76.9)	16314 (75.5)	
Follow-up years (mean±SD)^			
Mean±SD	5.24 ± 2.17	3.46 ± 2.2	<.001
Median (IQR)	6.51 (3.54-7.00)	3.34 (1.40-5.50)	<.001
Sonography Screening (number / year)			
Mean±SD	1.90±1.58	2.08±1.61	<.001
Median (IQR)	1.54 (0.83-2.48)	1.85 (1.16-2.63)	<.001
AFP Screening (number / year)			
Mean±SD	1.81±1.69	2.88±2.29	<.001
Median (IQR)	1.38 (0.56-2.55)	2.41 (1.38-3.84)	<.001
Nucleos(t)ide analogue therapy duration (years)			
Mean±SD		1.44±0.74	
Median (IQR)		1.42 (1.02-1.68)	
Hepatoprotective agents (years)			
Mean±SD	1.24±1.28	0.78±1.14	<.001
Median (IQR)	0.77 (0.43-1.55)	0.36 (0.08-0.98)	<.001
Concomitant drug users[++]			
Statin	1413 (6.5)	1474 (6.8)	0.248
NSAIDs or Aspirin	11996 (55.5)	11903 (55.1)	0.373
Metformin	1880 (8.7)	2000 (9.3)	0.045
Major coexisting diseases			
Cirrhosis	3016 (14.0)	2847 (13.2)	0.018
Liver decompensation	1646 (7.6)	1695 (7.8)	0.387
Hypertension	1827 (8.5)	1893 (8.8)	0.265
Diabetes	1574 (7.3)	1590 (7.4)	0.782
Chronic obstructive pulmonary disease	486 (2.3)	462 (2.1)	0.450
Acute coronary syndrome	674 (3.1)	654 (3.0)	0.596
Cerebral vascular disease	506 (2.3)	499 (2.3)	0.848
Renal failure	389 (1.8)	414 (1.9)	0.393
Hypercholesterolemia	206 (1.0)	201 (0.9)	0.842
Charlson's Score			
Mean±SD	0.80±1.53	0.79±1.52	0.308
Median (IQR)	0 (0-1)	0 (0-1)	0.165
Propensity Score[##]			
Mean±SD	0.42±0.16	0.42±0.16	0.476
Median (IQR)	0.42 (0.3-0.53)	0.42 (0.3-0.53)	0.397
Events			

HCC occurrence	4454 (20.6)	992 (4.6)	<.001
Death before HCC occurrence	2556 (11.8)	1036 (4.8)	<.001
Overall death	4778 (22.1)	1406 (6.5)	<.001

*Untreated: not receiving nucleos(t)ide analogues; Treated: receiving nucleos(t)ide analogues

#: P values were compared using the χ^2 test and Student's t-test.

+: Age is treated as a continuous variable

^: Follow-up is defined as the time of nucleos(t)ide analogue or hepatoprotective treatment.

++: Drug users indicate patients using drugs at least one day per month on average.

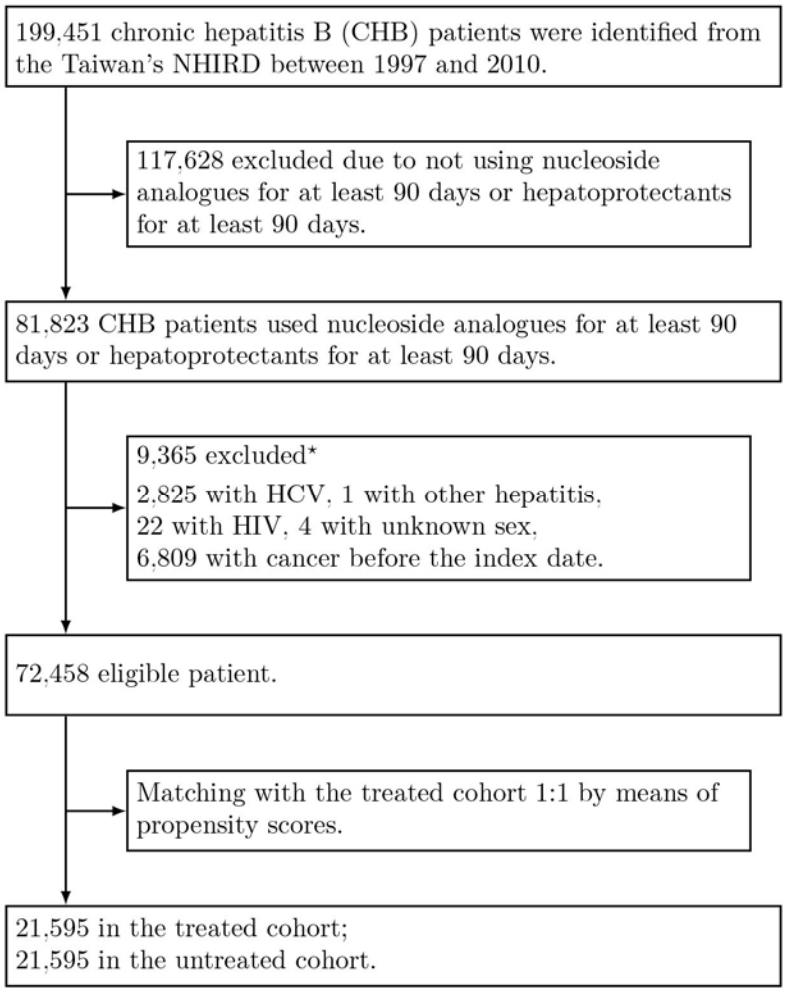
##: Age, gender, acute coronary syndrome, cerebral vascular diseases, COPD, diabetes, cirrhosis, liver decompensation, renal failure, hypertension, hypercholesterolemia, use of statins, use of NSAIDs or aspirin or COXIBs, and use of metformin were included in the propensity score calculation.

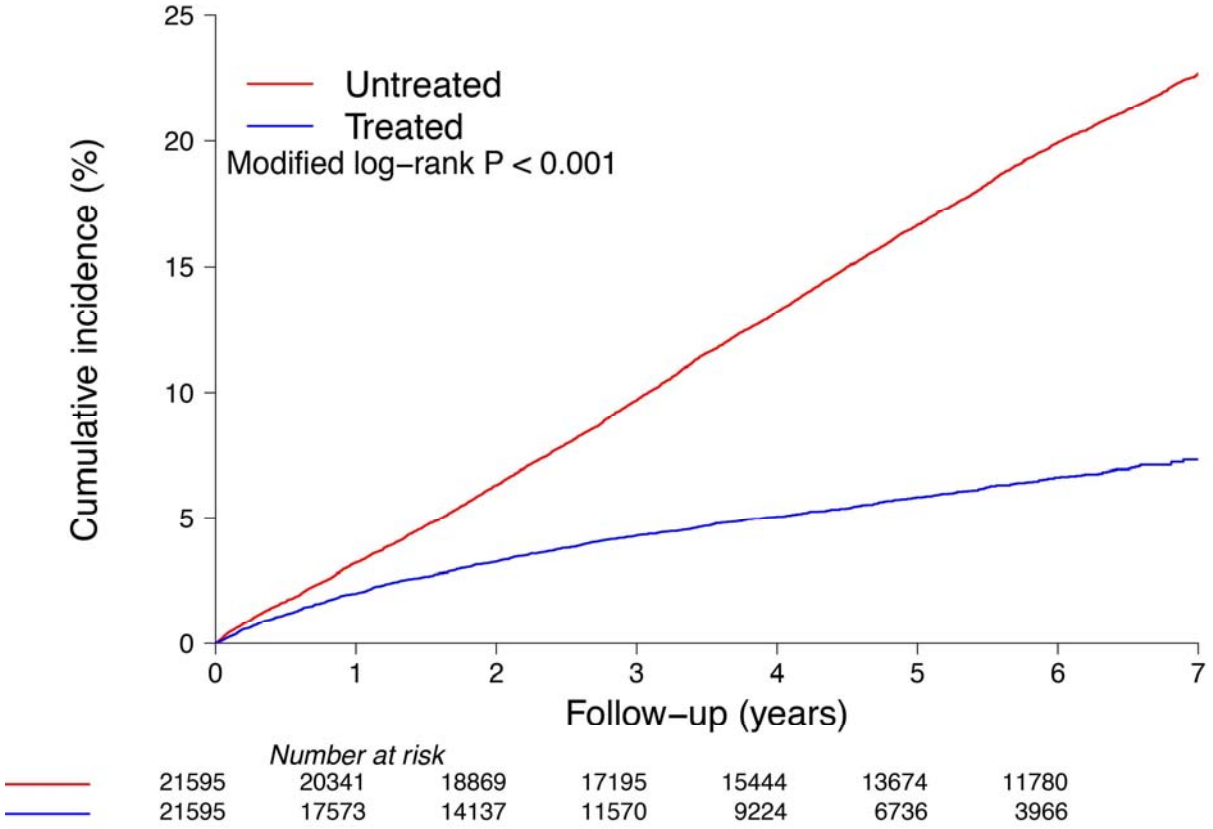
Abbreviations: N, number; SD: standard deviation; IQR: interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs

Table 2. Multivariable Cox proportional hazards model analysis for risk of HCC occurrence after adjusting for competing mortality

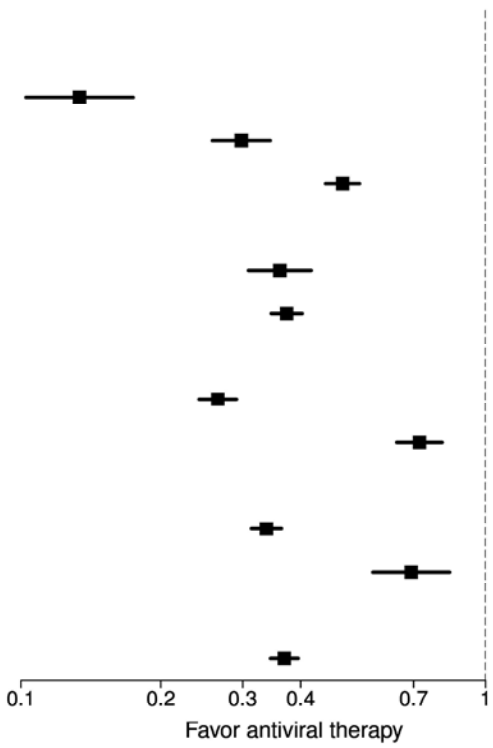
	Hazards Ratio[¶]	95% CI	p-value
Treated vs. untreated	0.37	0.34 – 0.39	<0.001
Age, per each incremental year	1.06	1.06 – 1.06	<0.001
Male	1.52	1.42 – 1.63	<0.001
Cirrhosis	1.92	1.77 – 2.08	<0.001
Liver Decompensation	0.87	0.78 – 0.97	0.013
Hypertension	0.81	0.72 – 0.90	<0.001
Diabetes	1.05	0.93 – 1.17	0.450
COPD	0.71	0.59 – 0.87	0.001
ACS	0.66	0.55 – 0.79	<0.001
CVA	0.50	0.40 – 0.64	<0.001
Renal failure	0.84	0.68 – 1.04	0.110
Hypercholesterolemia	0.87	0.61 – 1.23	0.430
Statin use	0.55	0.47 – 0.63	<0.001
NSAIDs or Aspirin use	0.62	0.58 – 0.65	<0.001
Metformin use	0.97	0.879-1.073	0.570

[¶]Adjusted for covariate factors, including age, gender, comorbidities, use of statins, use of NSAIDs or aspirin and use of metformin.





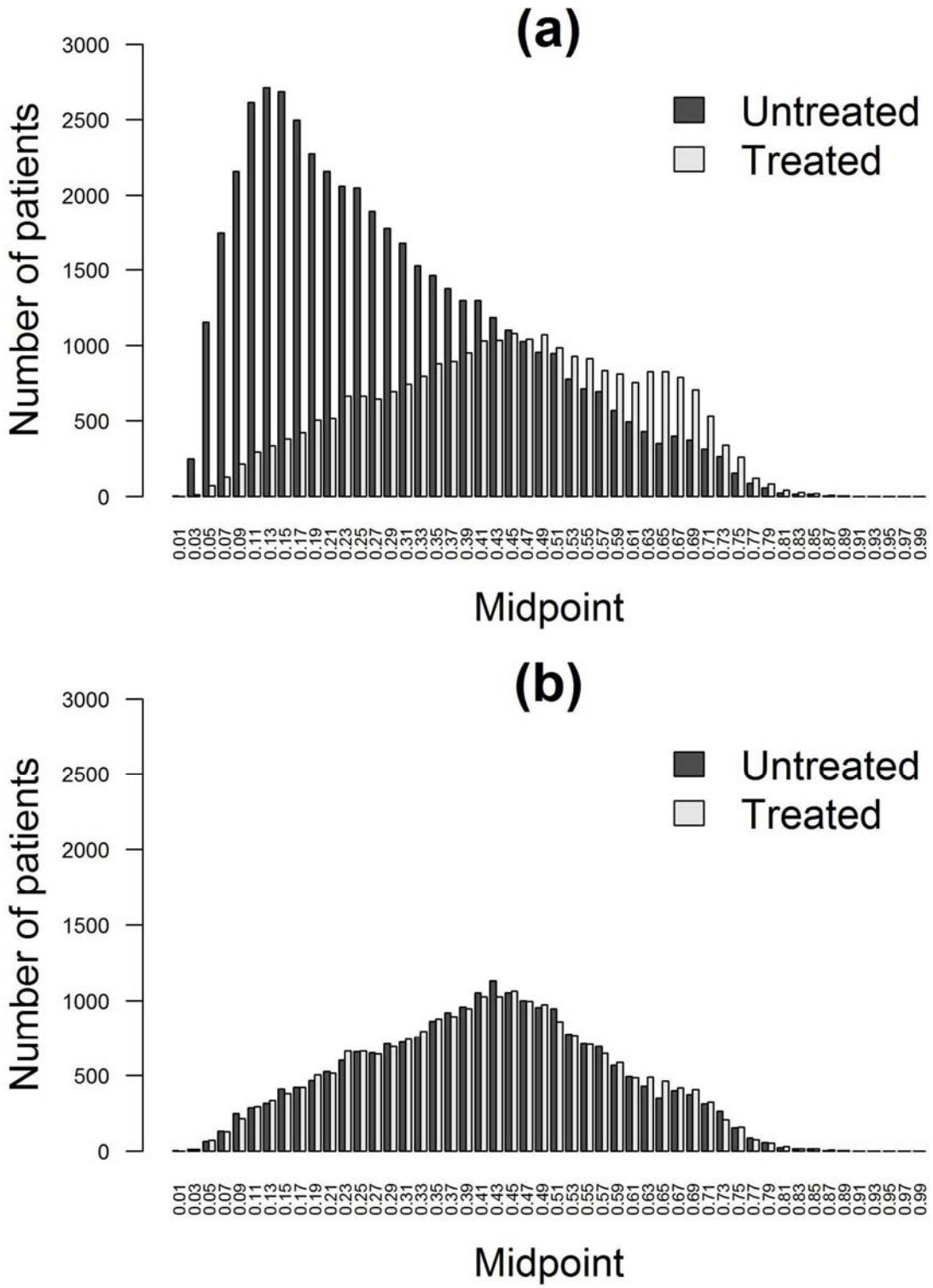
	Untreated		Treated		
Subgroup	**N**	**HCC**	**N**	**HCC**	**HR (95% C.I.)**
Age					
(0,40]	8945	713	9390	61	0.13 (0.10–0.17)
(40,50]	6039	1410	5321	223	0.30 (0.26–0.34)
(50,100)	6611	2331	6884	708	0.49 (0.45–0.54)
Gender					
Female	4982	976	5281	195	0.36 (0.31–0.42)
Male	16613	3478	16314	797	0.37 (0.35–0.40)
Cirrhosis					
No	18579	3478	18748	521	0.27 (0.24–0.29)
Yes	3016	976	2847	471	0.72 (0.64–0.81)
Diabetes					
No	20021	4081	20005	824	0.34 (0.31–0.36)
Yes	1574	373	1590	168	0.69 (0.57–0.84)
Total	21595	4454	21595	992	0.37 (0.34–0.40)

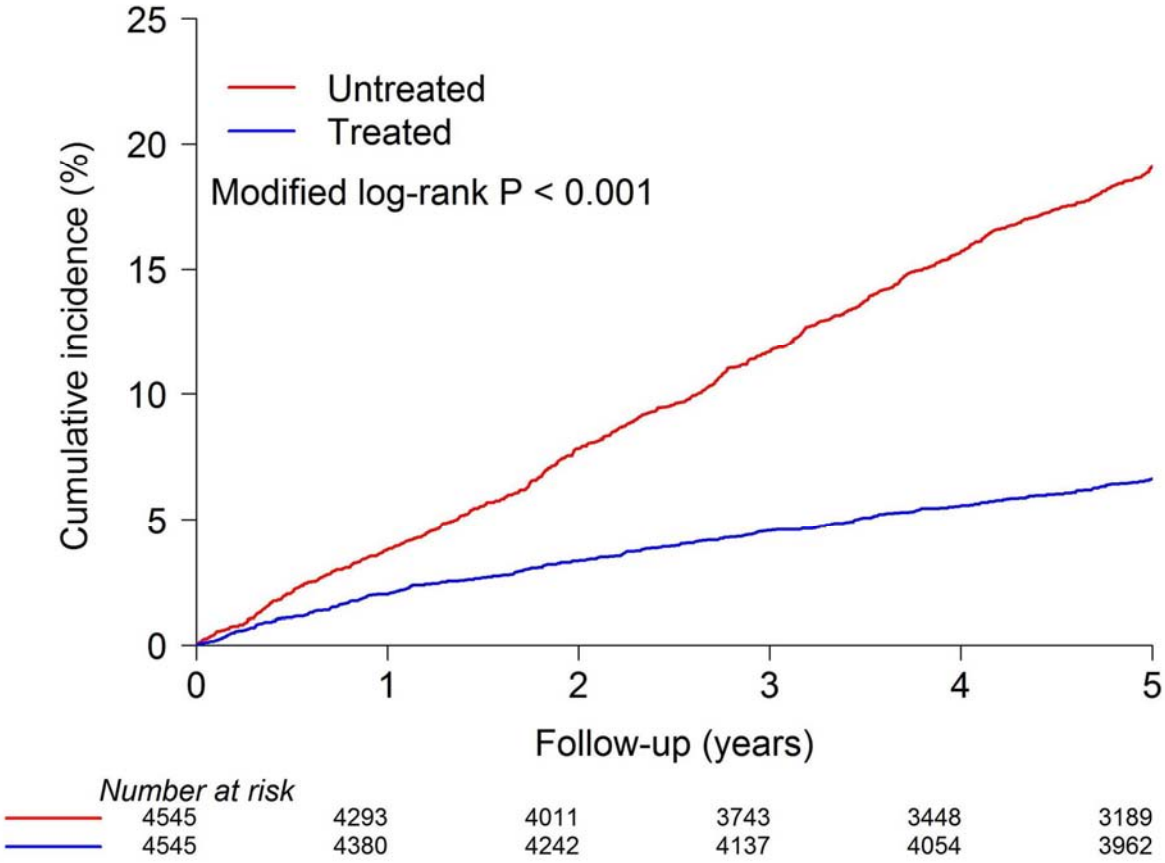


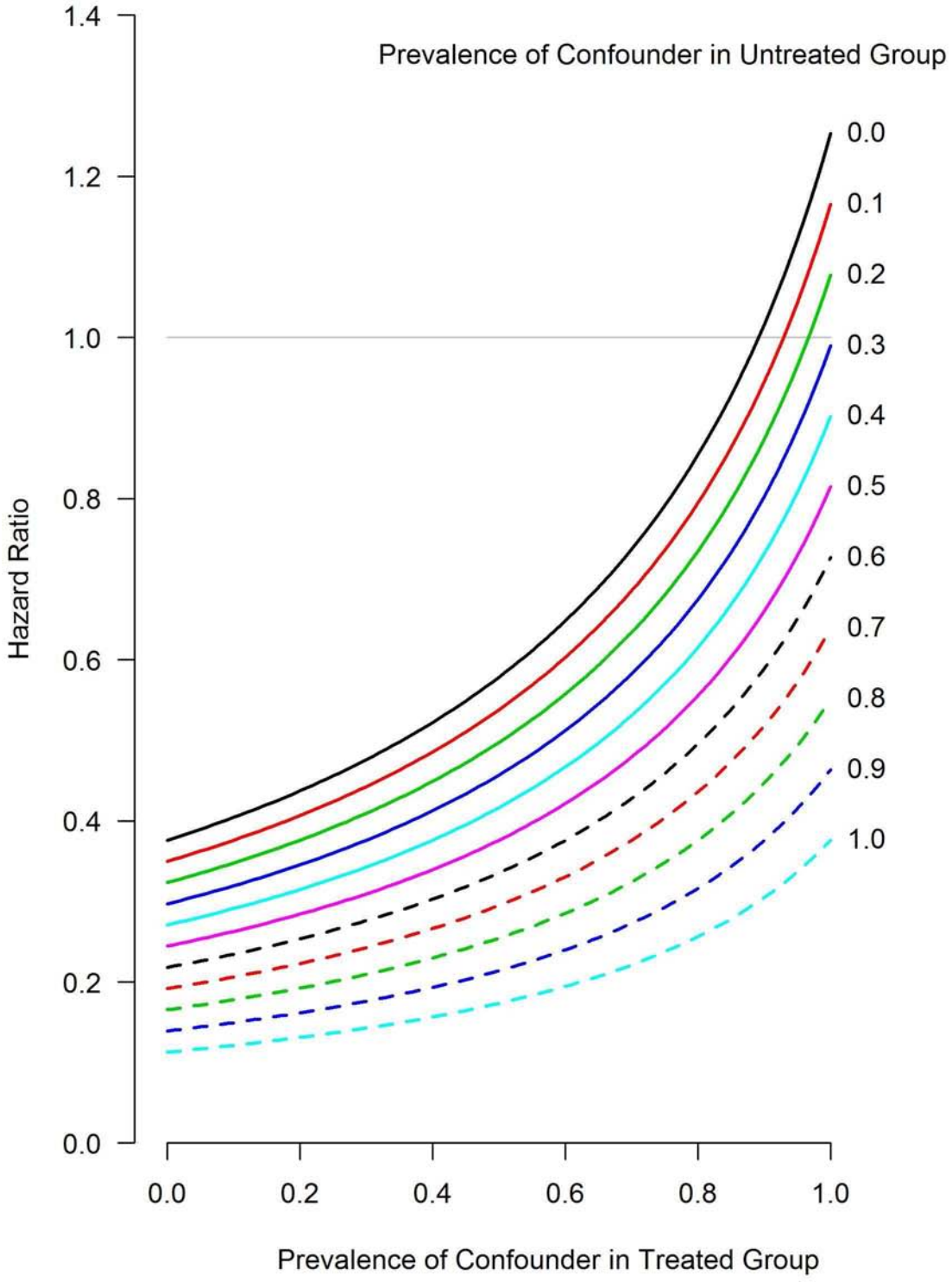
Supplemental Figure 1. Histograms of propensity score before and after matching. Part A: histogram of propensity scores before matching; part B; histogram of propensity scores after matching; Abbreviations: Untreated: CHB patients not receiving nucleos(t)ide analogues; Treated: CHB patients receiving nucleos(t)ide analogues.

Supplemental Figure 2. Sensitivity analysis with fixed duration. Since nucleos(t)ide analogues began to be covered by insurance on October 1, 2003, we conducted a sensitivity analysis by limiting the index date of follow-up to between October 1, 2003 and September 30, 2005 and limiting the follow-up duration to 5 years. Calculation and comparison of cumulative incidences in competing risk data ratios were conducted using modified Kaplan-Meier method and Gray's method. Abbreviations: Untreated: CHB patients not receiving nucleos(t)ide analogues; Treated: CHB patients receiving nucleos(t)ide analogues.

Supplemental Figure 3. Sensitivity analysis with add-on of an unmeasured confounder. This figure displays the trend of estimates for the treated hazard on covariate-adjusted Cox proportional hazards model. For example, when all the subjects in untreated group have the add-on unmeasured confounder (the prevalence of the confounder in the untreated group is 1.0) and none of subjects in treated group has this unmeasured confounder (the prevalence of the confounder in the treated group is 0.0), the impact of antiviral therapy would be beneficial (HR=0.1, the bottom line in figure). On the contrary, when none of subjects in the untreated group has the add-on unmeasured confounder and all subjects in treated group have this confounder; then the impact of antiviral therapy would be not protective (HR=1.2, the top line in the figure).


(a)
Number of patients
3000
2500
2000
1500
1000
500
0
Untreated
Treated
0.01 0.03 0.05 0.07 0.09 0.11 0.13 0.15 0.17 0.19 0.21 0.23 0.25 0.27 0.29 0.31 0.33 0.35 0.37 0.39 0.41 0.43 0.45 0.47 0.49 0.51 0.53 0.55 0.57 0.59 0.61 0.63 0.65 0.67 0.69 0.71 0.73 0.75 0.77 0.79 0.81 0.83 0.85 0.87 0.89 0.91 0.93 0.95 0.97 0.99
Midpoint
(b)
Number of patients
3000
2500
2000
1500
1000
500
0
Untreated
Treated
0.01 0.03 0.05 0.07 0.09 0.11 0.13 0.15 0.17 0.19 0.21 0.23 0.25 0.27 0.29 0.31 0.33 0.35 0.37 0.39 0.41 0.43 0.45 0.47 0.49 0.51 0.53 0.55 0.57 0.59 0.61 0.63 0.65 0.67 0.69 0.71 0.73 0.75 0.77 0.79 0.81 0.83 0.85 0.87 0.89 0.91 0.93 0.95 0.97 0.99
Midpoint




Prevalence of Confounder in Untreated Group
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Hazard Ratio
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Prevalence of Confounder in Treated Group

Supplemental Tables:

Supplemental Table 1:

Table 1. Baseline demographic characteristics and outcomes of study cohorts before propensity score matching

	Untreated* (N=47611)	Treated* (N=24847)	P value[#]
	Number (%)	Number (%)	
Age (mean±SD)[+]	51.62±14.34	41.95±13.70	<.001
Gender			<.001
Female	13021 (27.3)	6087 (24.5)	
Male	34590 (72.7)	18760 (75.5)	
Follow-up years (mean±SD)^			
Mean±SD	5.18 ± 2.17	3.52 ± 2.2	<.001
Median (IQR)	6.32 (3.48-7.00)	3.44 (1.47-5.55)	<.001
Nucleos(t)ide analogue therapy duration (years)			
Mean±SD		1.42±0.73	
Median (IQR)		1.41 (1.00-1.63)	
Hepatoprotective agents (years)			
Mean±SD	1.41±1.44	0.74±1.11	<.001
Median (IQR)	0.87 (0.46-1.80)	0.34 (0.07-0.93)	<.001
Concomitant drug users[++]			
Statin	6260 (13.1)	1529 (6.2)	<.001
NSAIDs or Aspirin	34615 (72.7)	12395 (49.9)	<.001
Metformin	11004 (23.1)	2017 (8.1)	<.001
Major coexisting diseases			
Cirrhosis	5179 (10.9)	3172 (12.8)	<.001
Liver decompensation	2087 (4.4)	2121 (8.5)	<.001
Hypertension	5133 (10.8)	2055 (8.3)	<.001
Diabetes	4415 (9.3)	1713 (6.9)	<.001
Chronic obstructive pulmonary disease	1829 (3.8)	491 (2.0)	<.001
Acute coronary syndrome	2214 (4.7)	676 (2.7)	<.001
Cerebral vascular disease	2535 (4.9)	506 (2.0)	<.001
Renal failure	676 (1.4)	496 (2.0)	<.001
Hypercholesterolemia	747 (1.6)	209 (0.8)	<.001
Charlson's Score			
Mean±SD	0.76±1.43	0.77±1.51	0.294
Median (IQR)	0 (0-1)	0 (0-1)	<.001
Propensity Score[##]			
Mean±SD	0.29±0.17	0.44±0.17	<.001
Median (IQR)	0.25 (0.15-0.41)	0.45 (0.32-0.58)	<.001
Events			
HCC occurrence	11574 (24.3)	1059 (4.3)	<.001
Death before HCC occurrence	5972 (12.5)	1185 (4.8)	
Overall death	11865 (24.9)	1583 (6.4)	<.001

*Untreated: not receiving nucleos(t)ide analogues; Treated: receiving nucleos(t)ide analogues

[#]: P values were compared using the χ^2 test and Student's t-test.

[+]: Age is treated as a continuous variable

^: Follow-up is defined as the time of nucleos(t)ide analogue or hepatoprotective treatment.

[++]: Drug users indicate patients using drugs at least one day per month on average.

[##]: Age, gender, acute coronary syndrome, cerebral vascular diseases, COPD, diabetes, cirrhosis, liver decompensation, renal failure, hypertension, hypercholesterolemia, use of statins, use of NSAIDs or aspirin or COXIBs, and use of metformin were included in the propensity score calculation.

Abbreviations: N, number; SD: standard deviation; IQR: interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs

Supplemental Table 2:

Interaction analysis: Multivariable Cox proportional hazards model analysis for risk of HCC occurrence after adding interactions between therapy and subgroup factors.

	Hazard Ratio	95% CI	p-value
Covariates			
Treated vs. untreated	0.08	0.06 – 0.11	<.001
Age, per year	1.05	1.05 – 1.06	<.001
Male gender	1.48	1.34 – 1.55	<.001
Cirrhosis	1.60	1.46 – 1.76	<.001
Liver Decompensation	0.84	0.74 – 0.95	0.005
Hypertension	0.81	0.72 – 0.92	0.001
Diabetes	0.99	0.87 – 1.13	0.900
COPD	0.71	0.57 – 0.88	0.002
Acute coronary syndrome	0.67	0.55 – 0.83	<.001
Cerebral vascular disease	0.41	0.30 – 0.55	<.001
Renal failure	0.89	0.70 – 1.13	0.330
Hypercholesterolemia	0.83	0.55 – 1.24	0.360
Statin use	0.54	0.46 – 0.64	<.001
NSAIDs or Aspirin use	0.60	0.57 – 0.64	<.001
Metformin use	0.92	0.82 – 1.03	0.150
Interactions with Treatment			
Age (per year)*treatment	1.02	1.01 – 1.02	<.001
Male*treatment	1.31	1.09 – 1.58	0.004
Cirrhosis*treatment	2.06	1.69 – 2.50	<.001
Liver Decompensation*treatment	1.21	0.96 – 1.52	0.110
Hypertension*treatment	0.98	0.76 – 1.27	0.880
Diabetes*treatment	1.33	1.02 – 1.73	0.034
COPD*treatment	0.94	0.61 – 1.44	0.760
Acute coronary syndrome*treatment	0.91	0.58 – 1.43	0.690
Cerebral vascular disease*treatment	1.56	0.93 – 2.60	0.090
Renal failure*treatment	0.85	0.50 – 1.43	0.530
Hypercholesterolemia*treatment	1.19	0.52 – 2.70	0.690
Statin use*treatment	0.92	0.65 – 1.31	0.650
NSAIDs or Aspirin use*treatment	1.09	0.94 – 1.26	0.270
Metformin use*treatment	1.12	0.87 – 1.43	0.380

Abbreviations: COPD, chronic obstructive pulmonary disease; NSAIDs, non-steroidal anti-inflammatory drugs

Supplemental Table 3:

Sensitivity analysis with fixed duration: baseline demographic characteristics and outcomes

Since nucleoside analogues have been reimbursed under the NHI program since October 1, 2003, we conducted sensitivity analysis by limiting index date of follow-up to between October 1, 2003 and September 30, 2005 and limiting the follow-up duration to 5 years.

	Untreated* (N=4545)	Treated* (N=4545)	P value[#]
	Number (%)	Number (%)	
Age (mean±SD)[+]	44.81±12.82	44.67±12.99	0.609
Gender			0.660
Female	1117 (24.6)	1098 (24.2)	
Male	3428 (75.4)	3447 (75.5)	
Follow-up years (mean±SD)^			
Mean±SD	4.26 ± 1.37	4.63 ± 1.10	<.001
Median (IQR)	5.00 (4.1-5.00)	5.00 (5.00-5.00)	<.001
Nucleoside analogue therapy duration (years)			
Mean±SD		1.46±0.84	
Median (IQR)		1.44 (1.02-1.53)	
Hepatoprotective agents (years)			
Mean±SD	1.09±1.02	0.92±1.25	<.001
Median (IQR)	0.71 (0.42-1.37)	0.47 (0.13-1.19)	<.001
Concomitant drug users[++]			
Statin	409 (9.0)	420 (9.2)	0.716
NSAIDs or Aspirin	2869 (63.1)	2851 (62.7)	0.712
Metformin	529 (11.6)	548 (12.1)	0.559
Major coexisting diseases			
Cirrhosis	517 (11.4)	517 (11.4)	1.000
Liver decompensation	269 (5.9)	284 (6.2)	0.539
Hypertension	381 (8.4)	352 (7.7)	0.281
Diabetes	323 (7.1)	305 (6.7)	0.482
Chronic obstructive pulmonary disease	107 (2.4)	94 (2.1)	0.392
Acute coronary syndrome	125 (2.8)	119 (2.6)	0.746
Cerebral vascular disease	105 (2.3)	89 (2.0)	0.276
Renal failure	82 (1.8)	79 (1.7)	0.874
Hypercholesterolemia	53 (1.2)	45 (1.0)	0.477
Charlson's Score			
Mean±SD	0.76±1.45	0.70±1.41	0.031
Median (IQR)	0 (0-1)	0 (0-1)	0
Propensity Score[##]			
Mean±SD	0.54±0.20	0.54±0.20	0.451
Median (IQR)	0.55 (0.41-0.70)	0.55 (0.41-0.70)	0.368
Events			
HCC occurrence	867 (19.1)	301 (6.6)	<.001

Death before HCC occurrence	489 (10.8)	282 (6.2)	<.001
Overall death	884 (19.4)	405 (8.9)	<.001

*Untreated: not receiving nucleoside analogues; Treated: receiving nucleoside analogues

[#]: P values were compared using the χ^2 test and Student's t-test.

[+]: Treating age as a continuous variable

^: Follow-up is defined as the period of nucleoside analogue or hepatoprotective treatment.

[++]: Drug users indicate patients using drugs for at least one day per month on average.

[##]: Age, gender, acute coronary syndrome, cerebral vascular diseases, COPD, diabetes, cirrhosis, liver decompensation, renal failure, hypertension, hypercholesterolemia, use of statins, use of NSAIDs, aspirin or COXIBs, and use of metformin were included in the propensity score calculation.

Abbreviations: N, number; SD: standard deviation; IQR: interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs

Supplemental Table 4:

Sensitivity analysis with fixed duration: Multivariable Cox proportional hazards model analysis for risk of HCC occurrence

	Hazards Ratio[¶]	95% CI	p-value
Treated vs. untreated	0.31	0.27 – 0.35	<0.001
Age, per each incremental year	1.06	1.06 – 1.07	<0.001
Male	1.80	1.56 – 2.09	<0.001
Cirrhosis	1.81	1.52 – 2.15	<0.001
Liver Decompensation	0.89	0.70 – 1.13	0.340
Hypertension	0.74	0.58 – 0.94	0.014
Diabetes	1.04	0.83 – 1.31	0.740
COPD	0.65	0.42 – 1.00	0.047
Acute coronary syndrome	0.76	0.51 – 1.15	0.190
Cerebral vascular disease	0.54	0.34 – 0.85	0.008
Renal failure	1.12	0.72 – 1.74	0.620
Hypercholesterolemia	0.52	0.24 – 1.12	0.094
Statin use	0.64	0.50 – 0.81	<0.001
NSAIDs or Aspirin use	0.61	0.54 – 0.69	<0.001
Metformin use	1.19	0.99 – 1.43	0.060

[¶]Adjusted for covariate factors, including age, gender, comorbidities, use of statins, use of NSAIDs or aspirin and use of metformin.

Abbreviations: COPD, chronic obstructive pulmonary disease; NSAIDs, non-steroidal anti-inflammatory drugs