의학박사 학위논문

만성 B형 간염 환자에서 경구용 항바이러스제 사용이 간 외 장기에 미치는 잠재적 발암성의 독성학적 평가에 대한 고찰

Toxicological assessment of potential extrahepatic carcinogenicity for oral nucleoside/nucleotide analogues in patients with chronic hepatitis B

울산대학교 대학원

의학과

임지혜

Toxicological assessment of potential extrahepatic carcinogenicity for oral nucleoside/nucleotide analogues in patients with chronic hepatitis B

지도교수 이한주

이 논문을 의학박사 학위논문으로 제출함

2021년 2월

울산대학교 대학원

의학과

임지혜

임지혜의 의학박사학위 논문을 인준함

심사위원장 심주현 인

심사위원 이한주 인

삼사위원 김강모 인

심사위원 송기원 인

심사위원 안지현 인

울산대학교 대학원

2021년 2월

Abstract

Background and Aims: Evidence on the carcinogenicity of oral nucleos(t)ide analogues (NAs) is still inconclusive lacking tenofovir disoproxil fumarate (TDF) data in patients with chronic hepatitis B (CHB). We aimed to provide confirmatory results for the relevant issue using a large set of CHB patients with data on all major NA drugs.

Methods: The study population consists of 10,331 patients with CHB receiving primary NA treatment longer than 6 months and 24,836 untreated controls followed at least during the period based on the International Council of Harmonization guidelines. Using an inverse-probability-of-treatment-weighted (IPTW) method, cumulative incidence of extrahepatic cancers was compared between treated and untreated patients and across the cyclopentane (entecavir), L-nucleoside (clevudine, lamivudine, and telvibudine), and acyclic phosphonate categories of NAs (adefovir, besifovir, and tenvofir). Analyses of individual cancers as sub-endpoints were additionally performed.

Results: During averages of 4.1 ± 3.1 years and 6.8 ± 5.5 years in the respective pairs, extrahepatic cancers occurred in 208 treated and 1,014 untreated patients. Cumulative incidence of overall extrahepatic malignancies did not differ between the two groups in the IPTW cohort (HR 1.002, 95% CI [0.859-1.169], p=0.977). Similar statistical trends were observed in analyses across the three NA chemical subsets and untreated set. Per-cancer analyses indicated that NA treatment was significantly associated with increased risks of colorectal/anal cancers and lymphoma (IPTW-adjusted HRs [95% CI], 1.538 [1.175-2.013]; and 1.784 [1.196-2.662]). Inversely, breast cancer and prostate cancer were less prevalent in the NA-treated group,

compared to the counterpart (IPTW-adjusted HRs [95% CI], 0.669 [0.462-0.967]; and 0.521 [0.329-0.825]).

Conclusions: We found that long NA treatment had carcinogenic risks for colorectal/anal and lymphoid tissues in CHB patients, although it did not affect most of extrahepatic organs. Protective effects of NAs on breast and prostate cancers should be further confirmed.

Key words: carcinogenesis; hepatitis B virus; nucleic acids, nucleotides, and nucleosides; toxicology

Contents

List of Figures and Tables

Introduction

Hepatitis B virus (HBV) infection has been a major global concern for public health causing acute and chronic liver disease worldwide over the last several decades [1, 2]. Since lamivudine (LAM) was used as a first-generation drug in 1998 [3], effective suppression of HBV replication by the landmark oral antivirals including entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide has prevented disease progression and reduced the risk of HCC occurrence [4-6].

In the process of preclinical development of these nucleos(t)ide analogues (NAs), an issue that their mechanism of action could confer the potential to interfere with human genomic or mitochondrial DNA synthesis has been raised with the rodent findings [7-10]. Meanwhile, it was presumed that ETV, TDF, and telbivudine (LdT) had a carcinogenic potency, and LAM, adefovir dipivoxil (ADV), and besifovir were also unequivocally genotoxic to predict potential carcinogenicity [7, 11]. Two prior studies from Hong Kong and Korea mainly focusing on ETV did not find notable increased risk of non-hepatocellular cancers of any type in NA users when compared with untreated controls [12, 13]. Such evidence with lacking data on TDF, the most recent agent, was neither complete nor conclusive. Moreover, given that the chemical structure of pharmaceuticals could determine tumorigenesis at a specific target organ, there is a substantial need for the relevant analyses according to the molecular scaffolds for oral antivirals [14-16].

As serious concerns of relevance increase with therapeutic duration of NAs, particularly ETV and TDF, since the end of the 20th century, we embarked on the

present large-scale, long-term comprehensive study using an inverse-probability-of-treatment-weighted (IPTW) method to confirm extrahepatic carcinogenesis by organ site with respect to human safety of the 3 chemical classes of oral NAs in patients with CHB.

Methods

Study population

In this retrospective cohort study at the Asan Medical Center, a research-driven hospital designated by the Korean ministry of Health and Welfare, eligible patients' data were retrieved from the own Clinical Research Data Warehouse (CRDW) system providing research data extracted from hospital Electronic Medical Records (EMR) with full protection of patient privacy (i.e., Asan BiomedicaL research Environment, ABLE) [17]. We first identified 94,792 patients 18 years or older and diagnosed with chronic hepatitis B (CHB) having hepatitis B surface antigen positivity over >6 months in our hospital between January 2000 and December 2019. Among these HBV-infected patients, we next excluded a part of subjects from the study based on the following criteria: 1) 16,781 patients were loss to follow up or dead within 6 months after enrollment; 2) 34,321 patients were diagnosed with any kinds of malignancy before, or within 6 months after enrollment; 3) 1,586 patients underwent solid or hematopoietic stem cell transplantation before, or within 6 months after enrollment; 4) 2,511 patients were infected with human immunodeficiency virus, or treated with immunosuppressive agents or high dose steroid; 5) 3,858 patients used primary oral antiviral agents less than 6 months: The International Council of Harmonization (ICH) guidelines require cancer warnings for drugs continuously used during a minimum period of 6 months [18]; 7) 586 patients used more than two kinds of antiviral agents at the same time. A total of 35,167 patients were finally included in the study analysis: 10,331 NA-treated and 24,836 untreated participants (Figure 1). The study was approved by the institutional review board at our center (IRB No. 2020-0449).

Definition of the study groups

The 'treated group' was defined as patients on NA-treatment at least for half a year, and divided into 3 categories according to the chemical structure and therapeutic mechanism of used NAs: 1) cyclopentane group with ETV; 2) L-nucleoside group with LAM, LdT, or clevudine; and 3) acyclic phosphonate group with TDF, ADV, or besifovir [19, 20]. The 'untreated group' was not administered any NA during the entire observation period.

Clinical data collection

All data at baseline (at the beginning of NA therapy for treated patients; and in the first visit to our hospital for untreated patients) and during follow-ups were obtained from the hospital EMR. Gender, age, hyperglycemia (fasting plasma glucose ≥126 mg/dL, or HbA1C ≥6.5%), dyslipidemia (fasting plasma total cholesterol ≥240 mg/dl, triglyceride ≥200 mg/dL, LDL cholesterol ≥160mg/dL, or HDL <40mg/dL), fatty liver, and hepatitis C virus (HCV) infection were utilized as common risk factors for cancer in the IPTW analysis [21-26]. In addition, HBV-related factors such as hepatic function panel, serum HBV DNA titer, ascites, and liver cirrhosis, together with coagulation tests, were collected and included in the sensitivity analysis.

Clinical outcomes

To primarily verify the carcinogenic risk of NAs by chemical class, we measured outcomes referring to occurrence of extrahepatic cancers of any type during the follow-up period. Intrahepatic cholangiocarcinoma (CCA), in addition to

hepatocellular carcinoma (HCC), that could be partly prevented by NAs *per se* due to its HBV-mediated pathogenesis were excluded from extrahepatic malignant types induced by NA treatment [27, 28]. Secondarily, individual incidence of each cancer type was also examined in the entire population. Cancer events were identified by ICD-10 system and classified by organ system on a topographic basis [29, 30]. As national health insurance service covers most of health care costs for patients with cancer who are charged co-payments of only 5% of the total costs in South Korea, diagnostic coding of cancer is seldom missed out in all new cases [31].

Statistical analysis

In order to compare traditional cancer risk and liver-related parameters at baseline between NA-treated and untreated groups, Chi-square test for categorical variables and two sample t-test for continuous variables were used as appropriate, in which the Markov chain Monte Carlo method of multiple imputation was used for variables with missing values [32]. The propensity score approach was applied to make accurate causal inferences between oral NA treatment and occurrence of extrahepatic malignancies. As covariates included in the estimation of propensity scores, traditional cancer risk factors, which are common potential confounders for any malignant development such as sex, age, hyperglycemia, dyslipidemia, fatty liver, and chronic HCV infection, were used [23-26]. In the first analysis for comparing the incidence rate of extrahepatic cancers between NA-treated and untreated groups (i.e., Model 1), propensity scores were conventionally estimated by applying multiple logistic regression analysis. A multinomial propensity score method using generalized boosted model from the R package twang was applied for the next analysis targeting

across the four groups (i.e., cyclopentane, L-nucleoside, acyclic phosphonate, and untreated groups; Model 2) [33]. After estimating the propensity scores, we checked the goodness-of-fit and balance of them and then confirmed that the models satisfied all assumptions. Cox proportional hazards regression analyses were used to estimate hazard ratios (HRs), and inferential statistical analysis was performed to test significance of differences in the incidence of cancer events between the groups. Each inference was performed using not only univariate and multivariate regression methods but an inverse probability treatment weighting (IPTW)-adjusted approach. The events in the first half year were fairly censored in the treated and untreated groups in order to evaluate the effects at least after 6 months of NA treatment. The end date is the date of each extrahepatic cancer diagnosis (i.e., event); or last follow-up, death/organ transplantation, or switch to a second-line NA (i.e., censorship). We also conducted the statistical power analysis to evaluate the significance of HRs for cancer incidence between the two groups, and ultimately expected to infer statistical significance of HRs ≥1.2 based on our sample size in order to detect the cancer incidence rate of 4% vs. 2% for the corresponding groups with power of >80%, using a two-sided log-rank test at the 5% significance level.

Results

Baseline characteristics of the population

The patients' demographic characteristics at the baseline are shown in Table 1. The mean age was 46.3 years (standard deviation [SD], 12.1), and over half of the patients (55.8%) were men. HCV was co-infected in 1.0% of the patients, and hyperglycemia, dyslipidemia, and fatty liver were observed in 12.9%, 8.4%, and 5.1%, respectively. 3,502, 4,052, and 2,777 patients belonged to the cyclopentane, L-nucleoside, and acyclic phosphonate groups, respectively. NA-treated patients were younger (mean ± SD, 46.1 ± 11.0 vs. 46.4 ± 12.5 years; $p<0.001$); and had more male gender (66.0% vs. 51.6%; $p<0.001$), as did not dyslipidemia (5.5% vs. 9.6%; $p<0.001$). Data on baseline hepatic parameters of the entire patients were shown in Supplementary table 1. After the individual IPTW adjustments in model 1 with NA-treated and untreated cohorts; and model 2 with the three NA groups and untreated control, all standard mean differences were between ± 0.1 which reflected that the covariates of two or four groups were balanced (Supplementary table 2) [34].

NA treatment and extrahepatic cancers

The treated group (n=10,331) was followed for an average of 4.1 ± 3.1 years, while the untreated group (n=24,836) was followed for an average of 6.8 ± 5.5 years. Extrahepatic cancers were newly developed in a total of 1,222 cases (208 NA-treated [2.0%] and 1,014 untreated patients [4.0%]) in the pooled cohort during the observation period. Among them, 13 treated patients and 41 controls experienced more than one type of the extrahepatic cancers with a maximum of three disease in one control. The Cox proportional hazards regression revealed that antiviral treatment

did not increase the incidence of overall extrahepatic malignancies for crude analysis (HR 0.992, 95% CI [0.850-1.158], p=0.919), which was maintained after the multivariable-adjusted analysis (HR 0.994, 95% CI [0.851-1.161], p=0.941) as well as IPTW analysis in Model 1 (HR 1.002, 95% CI [0.859-1.169], p=0.977) (Table 2). The relevant Kaplan-Meier curves for the unweighted and IPTW cohorts were shown in Figure 2: the cumulative incidence of the entire extrahepatic cancers was 2.5 % vs. 2.5 %, 5.7 % vs. 5.9 %, and 10.0 % vs. 14.7 % respectively at 5, 10, and 15 years between the untreated and NA-treated groups with IPTW adjustment.

In model 2 for investigating the effect of NA category on overall cancer development outside the liver, we found no significant differences in IPTW-adjusted cancer incidence rate between the cyclopentane, L-nucleoside, and acyclic phosphonate groups and the untreated set (HRs [95% CI], 1.084 [0.879-1.335]; 1.189 [0.942-1.501]; and 0.825 [0.584-1.166], respectively), which was reproduced in multivariate regression settings (HRs [95% CI], 1.064 [0.872-1.298]; 1.002 [0.768-1.300]; and 0.810 [0.578-1.136], respectively; Table 2 and Figure 3). Additional between-group analyses also revealed comparable outcomes across the NA categories (HRs [95% CI], 1.313 [0.889-1.939] for cyclopentane vs. acyclic phosphonate group; and 1.441 [0.965-2.152] for L-nucleoside vs. acyclic phosphonate group; 0.911 [0.678-1.225] for cyclopentane vs. L-nucleoside group; Table 2 and Figure 3).

Organ-specific cancer occurrence

We additionally explored the antiviral effects on occurrence of specific cancers on a per-organ basis as sub-endpoints. The incidence of extrahepatic

malignancies was 0.593% person-years in the entire study population: 0.616% person-years in the untreated group and 0.501% person-years in the treated group (Table 3 and Supplementary table 3). Colorectal and anal cancers (IPTW-adjusted HR [95% CI], 1.538 [1.175-2.013]; p=0.002) and lymphoma (1.784 [1.196-2.662]; p=0.005) were more prevalent in NA-treated patients (Figure 4).

On the contrary, NA treatment was associated with significantly lower incidence rates of breast cancers in women (IPTW-adjusted HR [95% CI], 0.669 [0.462-0.967]; p=0.033) and prostate cancer in men (0.521 [0.329-0.825]; p=0.005; Figure 5).

Discussion

Given the preclinical data from animal models, there have been serious worries for NAs targeting HBV likely to be genotoxic or carcinogenic after drug accumulation in affected patients [7-10]. In this study using adjustment and weighting of established risk confounders, our 20-year follow-up results demonstrated that oral NA agents did not increase the overall incidence of extrahepatic cancers other than both HCC and intrahepatic CCA with potential benefits from anti-HBV therapy in treatment-naïve patients with CHB [35], and the trend was maintained regardless of the chemical type of NAs. However, it was importantly noted that the rates of colorectal/anal (1.0 % vs. 0.5 % at 10 years) and lymphoid malignancies (0.3 % vs. 0.2% at 10 years) were exceptionally increased after NA treatment.

In fact, the ICH guidelines formally require genetic toxicologic and carcinogenic studies for NAs developed against HBV, as the drugs interfere with human nucleotide metabolism and are continuously used for a long duration ≥6 months, which may lead to DNA damage-associated malignancies [18, 36]. There are a few plausible explanations that antiviral agents affect host nucleic acid biosynthesis: 1) antivirals cause the severe imbalance of intracellular deoxynucleotide triphosphate pools impacting DNA production [37]; 2) incorporate into host DNA strand and induce DNA single-strand break [10]; and 3) inhibit human mitochondrial DNA replication increasing reactive oxygen species vulnerable to cellular damage [9, 38]. Among anti-HBV NAs known to have genotoxicity, in particular TDF, ETV, and LdT have been proven to be actually tumorigenic in animal studies, albeit at extremely higher doses during shorter periods compared to clinical human exposure. In detail, rodent and

primate models showed that TDF, ETV, and LdT markedly increased the incidence of malignant or benign tumors in duodenum and uterus; in lung, vascular system, salivary gland, and brain; and in pancreas, adrenal gland and mammary gland, respectively [7].

There are still limited reports with a lack of TDF data regarding carcinogenic effects of oral antivirals in patients with CHB. A 3-year landmark analysis with PS weighting from Hong Kong did not find remarkable increments in risk of common eight non-hepatocellular cancers by NA uses without ETV-specific effect in 4,782 treated patients, when compared with 39,712 untreated counterparts. However, the NA group took the drugs for only average 2.2 years after the landmark period (3 years) with non-HCC cancer incidence of 1.5% (non-HCC/ICC cancer incidence of 3.4% in our treated series), and individual antiviral-specific effects were not analyzed [13]. A subsequent Korean study of 2,400 treated and 7,467 untreated patients with a median follow-up of about 4 years, in which more than half of the patients were treated by ETV with 5.2% in the TDF arm, also did not reveal direct relationships between the exposure to NAs and development of seven types of non-HCC malignancies [12]. In addition, the known protective effect of antiviral treatment on intrahepatic CCA development calculated as extrahepatic events other than HCC in the two prior studies could biasedly underestimate the cancer risk in NA-treated cases [27, 28]. Considering the low incidence and time-dependency of cancer events, we believe that this larger and longer study including a number of participants on ETV (n=3,502) and TDF (n=2,571) with the strongest suspicion of carcinogenicity provided more conclusive evidence on the relevant issue. Furthermore, comparative assessment across chemical classes of

NAs rather than individual drugs may guarantee more reliable prediction and interpretation of pharmaceutical carcinogenicity under the concept of the ‘Structure Activity Relationship’ that are basic to toxicological investigations [14-16].

An interesting per-cancer finding in our investigation was that treated patients had increased risks of bowel cancer and lymphoma. A prior Hong Kong study also suggested that oral NA treatment appeared to increase colorectal cancer risk in CHB patients, albeit not conclusive due to a small absolute number of the events during the study period [13]. We think that our considerable size of the colorectal disease in patients receiving NA therapy could justify the preference of more accurate and frequent colorectal cancer screening in NA-treated subjects. On the other hand, it is speculated that the potential of NA in lymphomagenesis might be associated with nucleotide alteration in the susceptible cytokine genes as a biological driver of the lymphoid disease, together with ETV-induced plasma cell hyperplasia in rodent and mammalian models [39-41].

Surprisingly, we identified the novel inhibitory potential of NAs for prostate in men and breast cancers in women. First, recent evidence indicates that HBV is tropic for breast tissues even where the viruses replicate. Possible roles of occult HBV infection in breast carcinogenesis have been presumably proposed by the following mechanisms: (1) deactivation followed by long-term raise of free estrogen due to prolonged hepatic necroinflammation by the virus; (2) breast-specific oncogenic effects of hepatitis B X-interacting protein; and (3) cyclin D1 overexpression caused by HBx [42, 43]. These observations may support decreased rate of the female breast

disease in our antiviral set. In terms of prostate cancer, the possible cross-talk between HBV replication and androgen stimulation responsible for prostate cancer may explain such anti-cancer effect of antiviral therapy [44].

A significant limitation of our and previous Asian studies was not to consider the effect of racial disparity on NA-mediated carcinogenesis[12, 13]. Given the different susceptibility to various cancer types probably determined by genetic and behavior/environmental factors, further validation of our results using non-Asian data are needed [45]. Another consideration is between-individual variances in behaviors and intervals of health checkup or cancer screening. However, our long follow-ups and severity of cancers *per se* may attenuate such effects on study outcomes. Lastly, in line with previous retrospective researches, information on alcohol consumption, smoking, and family history of cancer as another traditional cancer risk factors were unavailable and thus not adjusted in our IPTW analyses [12, 13, 46].

In conclusion, this comparative study based on data of full spectra of NA and cancer series indicates that we do not need to be worried about the carcinogenic risk of long-term NA treatment at most of extrahepatic sites in CHB patient care. However, patients exposed to oral NAs should be extra cautious about possibilities of colorectal and anal cancers and lymphoma. Pathogenic evidence on the protective role of oral NAs in developing breast and prostate cancers is further required.

References

1. Korean Association for the Study of the L: **KASL clinical practice guidelines for management of chronic hepatitis B**. *Clin Mol Hepatol* 2019, **25**(2):93-159.
2. **Hepatitis B** [https://www.who.int/news-room/fact-sheets/detail/hepatitis-b]
3. **EPIVIR (lamivudine)** [https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/020564s37_020596s036lbl.pdf]
4. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS, Jr., Bzowej NH, Wong JB: **Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance**. *Hepatology* 2018, **67**(4):1560-1599.
5. **EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection**. *J Hepatol* 2017, **67**(2):370-398.
6. Yim HJ, Kim JH, Park JY, Yoon EL, Park H, Kwon JH, Sinn DH, Lee SH, Lee J-H, Lee HW: **Comparison of clinical practice guidelines for the management of chronic hepatitis B: When to start, when to change, and when to stop**. *Clin Mol Hepatol* 2020, **26**(4):411-429.
7. Friedrich A, Olejniczak K: **Evaluation of carcinogenicity studies of medicinal products for human use authorised via the European centralised procedure (1995-2009)**. *Regul Toxicol Pharmacol* 2011, **60**(2):225-248.
8. Brambilla G, Mattioli F, Robbiano L, Martelli A: **Studies on genotoxicity and carcinogenicity of antibacterial, antiviral, antimalarial and antifungal drugs**. *Mutagenesis* 2012, **27**(4):387-413.
9. Kayaaslan B, Guner R: **Adverse effects of oral antiviral therapy in chronic hepatitis B**. *World Journal of Hepatology* 2017, **9**(5).
10. Jiang L, Wu X, He F, Liu Y, Hu X, Takeda S, Qing Y: **Genetic Evidence for Genotoxic Effect of Entecavir, an Anti-Hepatitis B Virus Nucleotide Analog**. *PLoS One* 2016, **11**(1):e0147440.
11. **Medication detail information of besifovir dipivoxil maleate** [http://www.health.kr/searchDrug/result_drug.asp?drug_cd=2017051600001]
12. Kim SS, Ahn EK, Cho SY, Park RW, Cho HJ, Kim J-H, Kim HG, Lee GR, Hwang SH, Yang MJ *et al*: **Impact of nucleos(t)ide analog treatment on the development of malignancy in patients with chronic hepatitis B**. *Medicine* 2018, **97**(24).

13. Wong GL, Tse YK, Yip TC, Chan HL, Tsoi KK, Wong VW: **Long-term use of oral nucleos(t)ide analogues for chronic hepatitis B does not increase cancer risk - a cohort study of 44 494 subjects**. *Aliment Pharmacol Ther* 2017, **45**(9):1213-1224.

14. Carrasquer CA, Malik N, States G, Qamar S, Cunningham SL, Cunningham AR: **Chemical structure determines target organ carcinogenesis in rats**. *SAR QSAR Environ Res* 2012, **23**(7-8):775-795.

15. Combes RD: **The use of structure-activity relationships and markers of cell toxicity to detect non-genotoxic carcinogens**. *Toxicol In Vitro* 2000, **14**(4):387-399.

16. Cunningham AR, Carrasquer CA, Qamar S, Maguire JM, Cunningham SL, Trent JO: **Global structure-activity relationship model for nonmutagenic carcinogens using virtual ligand-protein interactions as model descriptors**. *Carcinogenesis* 2012, **33**(10):1940-1945.

17. Shin S-Y, Kim WS, Lee J-H: **Characteristics desired in clinical data warehouse for biomedical research**. *Healthc Inform Res* 2014, **20**(2):109-116.

18. **ICH Topic S 1 A; The Need for Carcinogenicity Studies of Pharmaceuticals** [https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s-1-need-carcinogenicity-studies-pharmaceuticals-step-5_en.pdf]

19. Ghany MG, Doo EC: **Antiviral resistance and hepatitis B therapy**. *Hepatology* 2009, **49**(S5):S174-S184.

20. Yim HJ, Hwang SG: **Options for the management of antiviral resistance during hepatitis B therapy: reflections on battles over a decade**. *Clin Mol Hepatol* 2013, **19**(3):195-209.

21. **2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020**. *Diabetes Care* 2020, **43**(Suppl 1):S14-s31.

22. **Korean Guideline for the Management of Dyslipidemia 4th Ed** [https://www.lipid.or.kr/file/guidlines.pdf]

23. Zhu T, Gao Y, Wang J, Li X, Shang S, Wang Y, Guo S, Zhou H, Liu H, Sun D *et al*: **CancerClock: A DNA Methylation Age Predictor to Identify and Characterize Aging Clock in Pan-Cancer**. *Frontiers in Bioengineering and Biotechnology* 2019, **7**(388).

24. Chang SC, Yang WV: **Hyperglycemia, tumorigenesis, and chronic inflammation**. *Crit Rev Oncol Hematol* 2016, **108**:146-153.

25. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA: **Obesity, Inflammation, and Cancer**. *Annual Review of Pathology: Mechanisms of Disease* 2016, **11**(1):421-449.

26. Nickels JT, Jr.: **New links between lipid accumulation and cancer progression**. *J Biol Chem* 2018, **293**(17):6635-6636.

27. Jeong S, Tong Y, Sha M, Gu J, Xia Q: **Hepatitis B virus-associated intrahepatic cholangiocarcinoma: a malignancy of distinctive characteristics between hepatocellular carcinoma and intrahepatic cholangiocarcinoma**. *Oncotarget* 2016, **8**(10).

28. Lee TY, Hsu YC, Yu SH, Lin JT, Wu MS, Wu CY: **Effect of Nucleos(t)ide Analogue Therapy on Risk of Intrahepatic Cholangiocarcinoma in Patients With Chronic Hepatitis B**. *Clin Gastroenterol Hepatol* 2018, **16**(6):947-954 e944.

29. **International Statistical Classification of Diseases and Related Health Problems 10th Revision** [https://icd.who.int/browse10/2019/en]

30. **Korean Standard Classification of Diseases** [http://www.koicd.kr/]

31. **Health insurance system of Korea about cancer patients** [https://www.nhis.or.kr/nhis/policy/wbhada15400m01.do]

32. Schafer JL: **Multiple imputation: a primer**. *Statistical Methods in Medical Research* 1999, **8**(1):3-15.

33. McCaffrey DF, Griffin BA, Almirall D, Slaughter ME, Ramchand R, Burgette LF: **A tutorial on propensity score estimation for multiple treatments using generalized boosted models**. *Statistics in Medicine* 2013, **32**(19):3388-3414.

34. Zhang Z, Kim HJ, Lonjon G, Zhu Y, written on behalf of AMEB-DCTCG: **Balance diagnostics after propensity score matching**. *Ann Transl Med* 2019, **7**(1):16-16.

35. Lee TY, Hsu YC, Yu SH, Lin JT, Wu MS, Wu CY: **Effect of Nucleos(t)ide Analogue Therapy on Risk of Intrahepatic Cholangiocarcinoma in Patients With Chronic Hepatitis B**. *Clin Gastroenterol Hepatol* 2018, **16**(6):947-954.e944.

36. **ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use** [https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-s2-r1-genotoxicity-testing-data-interpretation-pharmaceuticals-intended-human-use-step_en.pdf]

37. Tang H, Griffin J, Innaimo S, Lehman-Mckeeman L, Llamoso C: **The Discovery and Development of a Potent Antiviral Drug, Entecavir, for the Treatment of Chronic Hepatitis B**. *J Clin Transl Hepatol* 2013, **1**(1):51-58.

38. Pol S, Lampertico P: **First-line treatment of chronic hepatitis B with entecavir or tenofovir in 'real-life' settings: from clinical trials to clinical practice**. *J Viral Hepat* 2012, **19**(6):377-386.

39. Assis-Mendonca GR, Lourenco GJ, Delamain MT, de Lima VCC, Colleoni GWB, de Souza CA, Soares FA, Lima CSP, Vassallo J: **Single-nucleotide variants in TGFB1, TGFBR2, IL17A, and IL17F immune response genes contribute to follicular lymphoma susceptibility and aggressiveness**. *Blood Cancer J* 2020, **10**(10):97.

40. **Entecavir** [https://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/21797_BARACLUDE_pharmr.PDF]

41. Giannuzzi D, Marconato L, Cascione L, Comazzi S, Elgendy R, Pegolo S, Cecchinato A, Bertoni F, Aresu L, Ferraresso S: **Mutational landscape of canine B-cell lymphoma profiled at single nucleotide resolution by RNA-seq**. *PLOS ONE* 2019, **14**(4):e0215154.

42. Adhikari VP, Lu L-J, Kong L-Q: **Does hepatitis B virus infection cause breast cancer?** *Chinese Clinical Oncology* 2016, **5**(6):81-81.

43. Qin B, Zhao K, Wei J, Wang X, Xu M, Lang J, Sun H, Jin K: **Novel evidence indicates the presence and replication of hepatitis B virus in breast cancer tissue**. *Oncol Rep* 2020, **43**(1):296-305.

44. Wu MH, Ma WL, Hsu CL, Chen YL, Ou JHJ, Ryan CK, Hung YC, Yeh S, Chang C: **Androgen Receptor Promotes Hepatitis B Virus-Induced Hepatocarcinogenesis Through Modulation of Hepatitis B Virus RNA Transcription**. *Sci Transl Med* 2010, **2**(32).

45. Ozdemir BC, Dotto GP: **Racial Differences in Cancer Susceptibility and Survival: More Than the Color of the Skin?** *Trends Cancer* 2017, **3**(3):181-197.

46. Katzke VA, Kaaks R, Kuhn T: **Lifestyle and Cancer Risk**. *Cancer J* 2015, **21**(2):104-110.

Table 1. Baseline traditional cancer risk factors of study subjects according to nucleos(t)ide analogue treatment

Variables*	Untreated n=24,836	NA-treated n=10,331	SMD	p-value	Cyclopentane group† n=3,502	L-nucleoside group† n=4,052	Acyclic phosphonate group† n= 2,777
Age (years)	46.4±12.5	46.1±11.0	-0.097	<.0001	48.1±10.7	43.2±10.9	47.6±10.7
Male gender	12,804 (51.6)	6,814 (66.0)	0.320	<.0001	2,258 (64.5)	2,865 (70.7)	1,691 (60.9)
Co-infection of HCV	250 (1.0)	97 (0.9)	-0.012	0.559	41 (1.2)	30 (0.7)	26 (0.9)
Hyperglycemia	3,174 (12.8)	1,365 (13.2)	0.027	0.260	518 (14.8)	559 (13.8)	289 (10.4)
Dyslipidemia	2,373 (9.6)	563 (5.5)	-0.262	<.0001	205 (5.9)	189 (4.7)	169 (6.1)
Fatty liver	1,240 (5.0)	557 (5.4)	0.015	0.122	158 (5.0)	254 (6.3)	145 (5.2)

Values are expressed as the mean ± standard deviation, or frequency (percentage).

*The numbers of missing data in untreated group, 8.6%, 1.5%, 0.5%, and 9.5% for HCV infection, hyperglycemia, dyslipidemia, and fatty liver respectively.

†Cyclopentane, L-nucleoside, and Acyclic phosphonate groups consisted of patient sets were treated with entecavir; clevudine, lamivudine or telbivudine; and adefovir, tenofovir, or besifovir, respectively.

HCV, hepatitis C virus; NA, Nucleos(t)ide analogue; SDM, standardized mean difference

Table 2. Comparisons of the incidence rates of extrahepatic malignancies according to nucleos(t)ide analogue treatment in chronic hepatitis B patients

	Univariate analysis		Multivariate analysis		IPTW analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Model 1*						
NA-treated vs. Untreated	0.992 (0.850-1.158)	0.919	0.994 (0.851-1.161)	0.941	1.002 (0.859-1.169)	0.977
Model 2*						
†Cyclopentane vs. Untreated	1.143 (0.938-1.394)	0.186	1.064 (0.872-1.298)	0.542	1.084 (0.879-1.335)	0.451
†L-nucleoside vs. Untreated	0.868 (0.666-1.130)	0.292	1.002 (0.768-1.300)	0.988	1.189 (0.942-1.501)	0.145
†Acyclic vs. Untreated	0.835 (0.596-1.100)	0.296	0.810 (0.578-1.136)	0.222	0.825 (0.584-1.166)	0.276
Cyclopentane vs. Acyclic	1.197 (0.854-1.678)	0.104	1.313 (0.900-1.916)	0.158	1.313 (0.889-1.939)	0.172
L-nucleoside vs. Acyclic	1.039 (0.687-1.571)	0.857	1.237 (0.817-1.871)	0.315	1.441 (0.965-2.152)	0.074
Cyclopentane vs. L-nucleoside	1.152 (0.885-1.501)	0.292	1.062 (0.774-1.455)	0.710	0.911 (0.678-1.225)	0.538

*Model 1: IPTW-adjusted analysis for the comparison between untreated and NA-treated groups; and Model 2: IPTW-adjusted analysis for the comparisons between untreated controls and three NA groups

†Cyclopentane, L-nucleoside, and Acyclic phosphonate groups consisted of patient sets were treated with entecavir; clevudine, lamivudine or telbivudine; and adefovir, tenofovir, or besifovir, respectively.

CI, Confidence interval; HR, Hazard ratio; IPTW, inverse probability of treatment weighting; NA, Nucleos(t)ide analogue

Table 3. Incidences and hazard ratios of extrahepatic malignancies (more than 30 events) in chronic hepatitis B patients with and without antiviral treatment

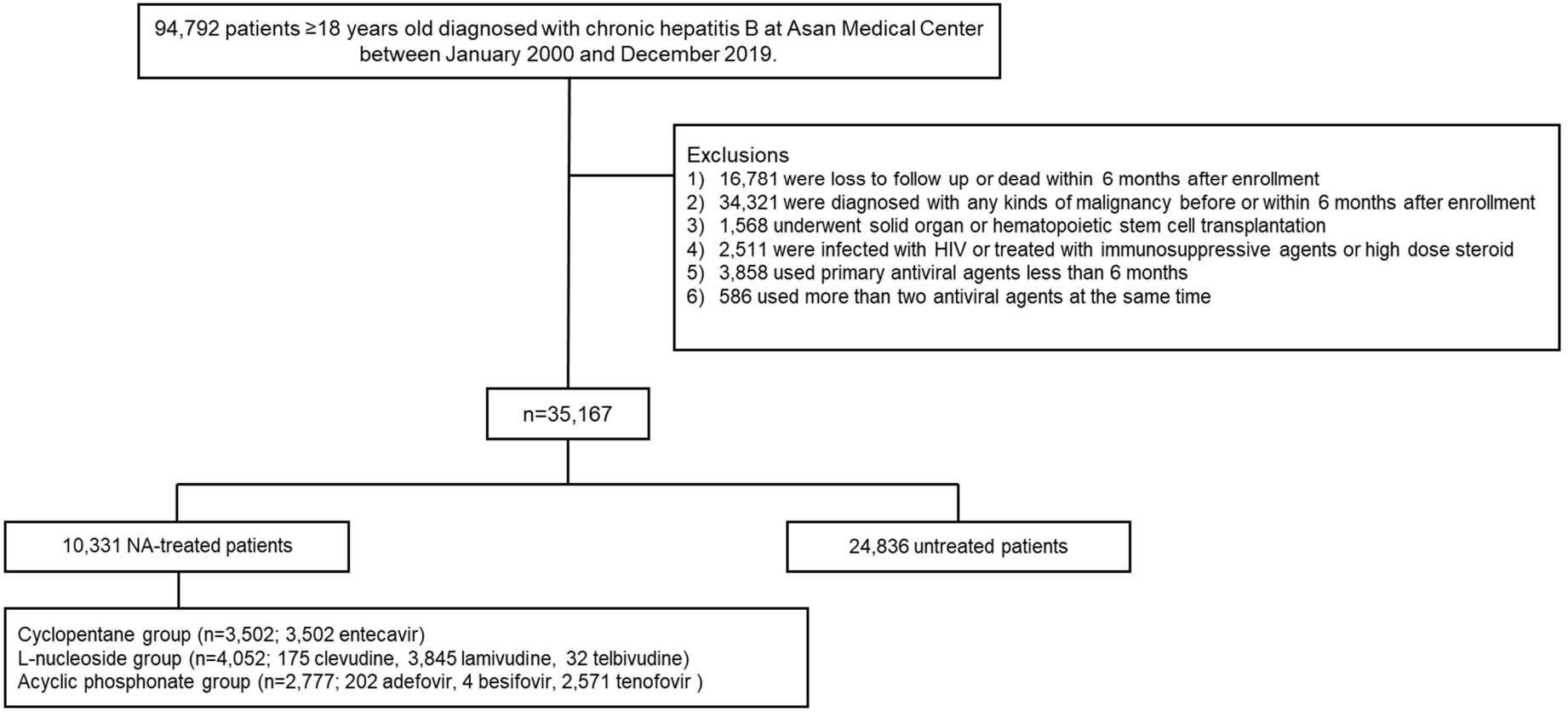
	Percentage per person-years (Number of cancers)			IPTW analysis	
	Total N=35,167	Untreated n=24,836	NA-treated n=10,331	HR (95% CI)	p-value
Thyroid cancer	0.114 (240)	0.121 (203)	0.088 (37)	0.893 (0.722-1.104)	0.296
Stomach cancer	0.094 (197)	0.093 (157)	0.095 (40)	1.161 (0.924-1.459)	0.200
Lung and pleural cancers	0.063 (132)	0.068 (114)	0.043 (18)	0.874 (0.636-1.202)	0.408
Colorectal and anal cancers	0.062 (131)	0.059 (99)	0.076 (32)	1.538 (1.175-2.013)	0.002
Urinary organ cancer	0.043 (90)	0.044 (75)	0.036 (15)	0.903 (0.632-1.290)	0.575
Prostate cancer*	0.072 (82)	0.086 (74)	0.029 (8)	0.521 (0.329-0.825)	0.005
Lymphoma	0.029 (61)	0.027 (46)	0.036 (15)	1.784 (1.196-2.662)	0.005
Female genital cancer*	0.048 (46)	0.043 (35)	0.077 (11)	1.592 (0.987-2.567)	0.057
Breast cancer*	0.044 (42)	0.042 (34)	0.056 (8)	0.669 (0.462-0.967)	0.033
Bone, skin, and soft tissue cancers	0.019 (41)	0.019 (32)	0.021 (9)	1.33 (0.801-2.209)	0.270
Pancreas cancer	0.018 (39)	0.021 (35)	0.010 (4)	0.595 (0.312-1.137)	0.116

Extrahepatic biliary cancer	0.018 (37)	0.018 (31)	0.014 (5)	1.012 (0.574-1.782)	0.968
Head and neck cancers	0.016 (34)	0.017 (28)	0.014 (6)	0.954 (0.522-1.743)	0.878

*Breast and female genital cancers evaluated only in women; and prostate cancer only in men

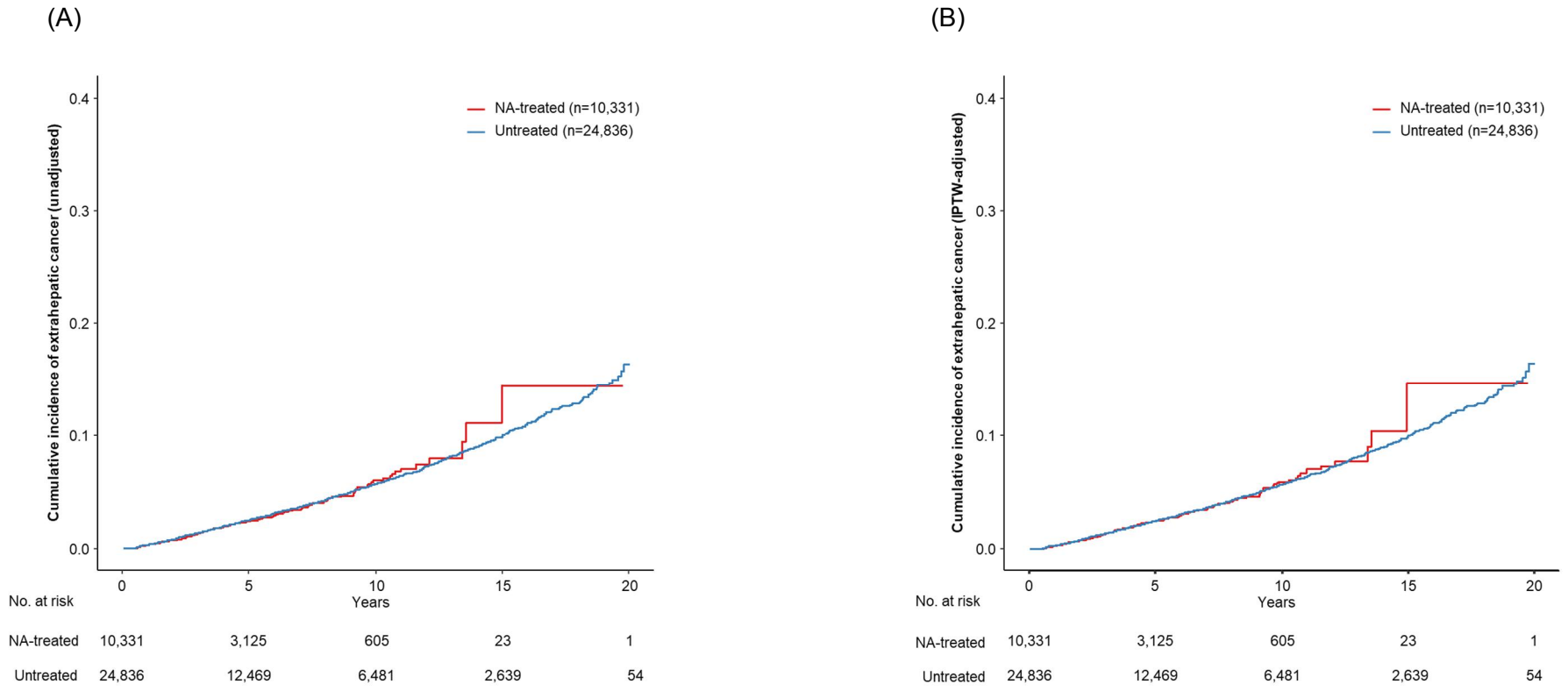
CI, Confidence interval; HR, Hazard ratio; IPTW, inverse probability of treatment weighting; NA, Nucleos(t)ide analogue

Figure 1. Flowchart describing the eligible patients



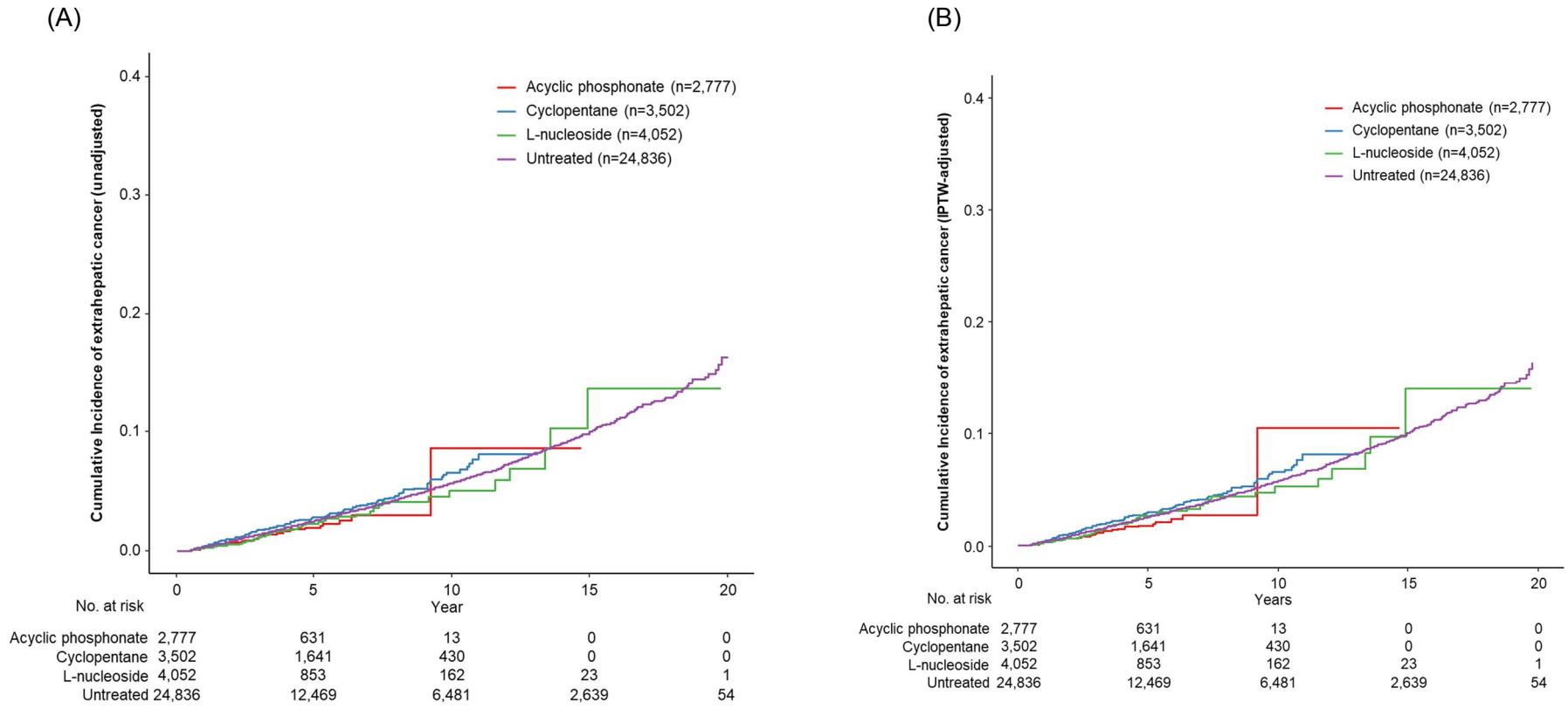
HIV, human immunodeficiency virus; NA, Nucleos(t)ide analogue

Figure 2. (A) Crude and (B) IPTW-adjusted Kaplan-Meier analyses of developing extrahepatic cancers between NA-treated and untreated patients



Comparison of extrahepatic cancer incidence rates between NA-treated and untreated patients revealed no statistical differences in unadjusted analysis as well as IPTW-adjusted analysis (log-rank test: $P > 0.05$).

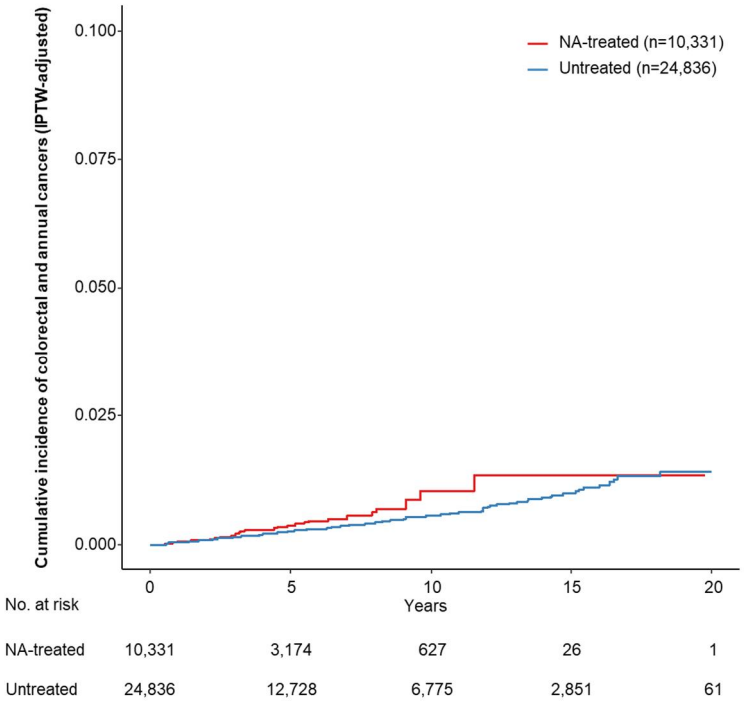
Figure 3. (A) Crude and (B) IPTW-adjusted Kaplan-Meier analyses of developing extrahepatic cancers between three NA groups and untreated controls



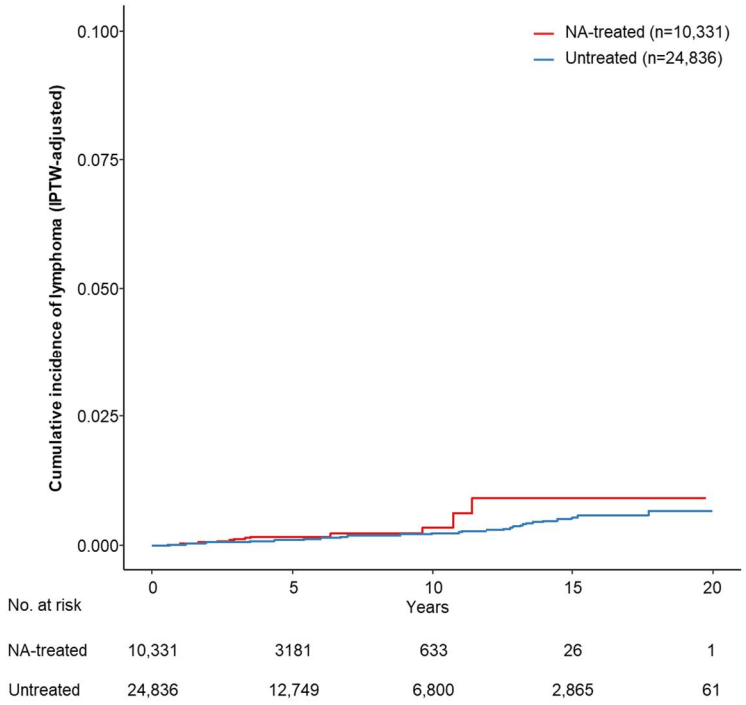
The crude and IPTW-adjusted extrahepatic cancer developments were not different between cyclopentane, L-nucleoside, and acyclic phosphonate groups and the untreated set.

Figure 4. IPTW–adjusted Kaplan-Meier analyses of developing (A) colorectal/anal cancers and (B) lymphoma between NA-treated and untreated patients

(A)



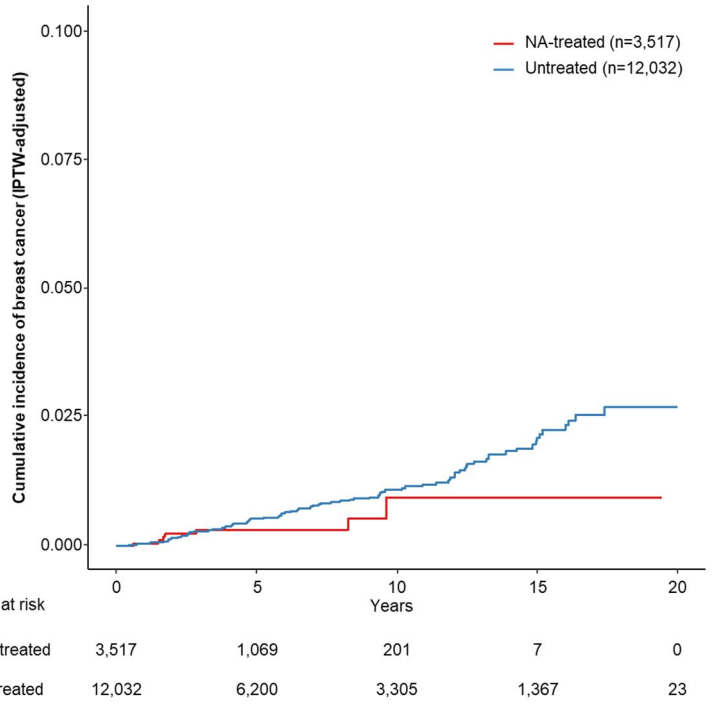
(B)



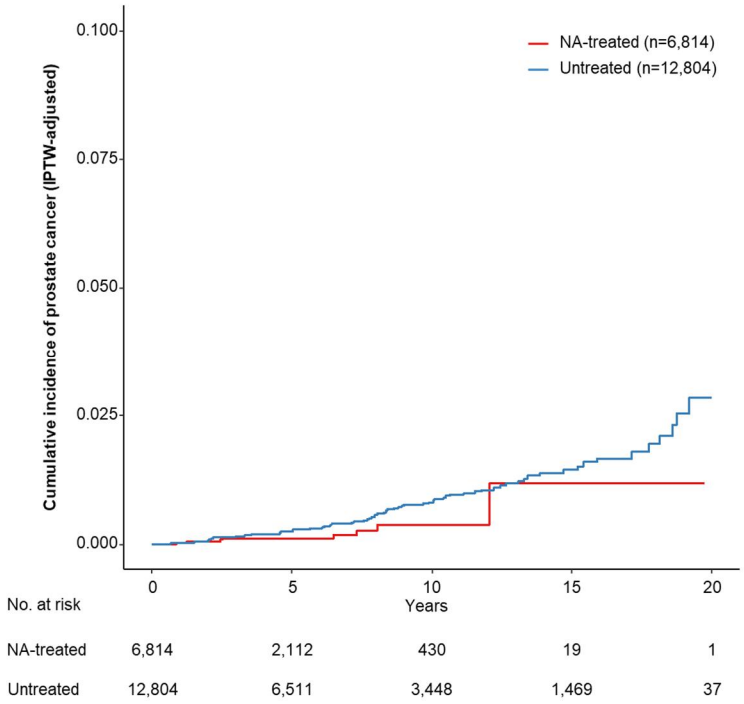
The cumulative incidences of colorectal/anal cancers and lymphoma were significantly increased in NA treated patients. The IPTW-adjusted HRs were higher in NA-treated group (HRs [95% CI] p-value, 1.538 [1.175-2.013] p=0.002; and 1.784 [1.196-2.662] p=0.005).

Figure 5. IPTW–adjusted Kaplan-Meier analyses of developing (A) breast cancer between NA-treated and untreated women and (B) prostate cancer between NA-treated and untreated men

(A)



(B)



Development of breast cancer for women was less prevalent in NA-treated patients (IPTW-adjusted HR [95% CI], 0.669 [0.462-0.967]; p=0.033). Likewise, prostate cancer for men was less occurred in NA-treated patients (0.521 [0.329-0.825]; p=0.005).

Supplementary table 1. Baseline hepatic factors of study subjects according to nucleos(t)ide analogue treatment

Variables*	Untreated n=24,836	NA-treated n=10,331	p-value	Cyclopentane group† n=3,502	L-nucleoside group† n=4,052	Acyclic phosphonate group† n=2,777
PT (INR)	1.1±0.2	1.2±0.2	<.0001	1.2±0.2	1.2±0.3	1.1±0.2
Albumin (g/dL)	4.3±0.5	4.5±0.4	<.0001	4.5±0.4	4.5±0.4	4.4±0.3
Bilirubin (g/dL)	1.0±1.2	1.6±2.9	<.0001	1.6±3	1.8±3.2	1.2±2
Platelet (×10³/uL)	207.6±65.9	156±67.6	<.0001	152.7±67.1	148.3±65.4	171.3±68.7
ALT (IU/L)	42.6±144.3	103.7±289.4	<.0001	102.9±336.8	114.8±249.9	88.5±277.6
HBV DNA (IU/mL)	$5.5x10^2$ ($7.8x10^5$)	$6.7x10^4$ ($2.0x10^9$)	<.0001	$6.3x10^5$ ($9.9x10^{10}$)	$1.8x10^3$ ($6.8x10^7$)	$3.1x10^5$ ($8.8x10^{10}$)
Ascites	843 (3.4)	1206 (11.7)	<.0001	432 (12.3)	494 (12.2)	280 (10.1)
Cirrhosis	2604 (10.5)	4431 (42.9)	<.0001	1622 (46.3)	1598 (39.4)	1211 (43.6)

Values are expressed as the mean ± standard deviation, median (interquartile range), or frequency (percentage).

*The numbers of missing data in untreated group, 26.5%, 0.3%, 0.7%, 0.7%, 0.3%, and 63.6% for PT, albumin, bilirubin, platelet, ALT, and HBV DNA in the blood, respectively.

*The numbers of missing data in NA-treated group, 7.4%, <0.1%, <0.1%, <0.1%, <0.1%, and 6.7% for PT, albumin, bilirubin, platelet, ALT, and HBV DNA in the blood, respectively.

†Cyclopentane, L-nucleoside, and Acyclic phosphonate groups consisted of patient sets were treated with entecavir; clevudine, lamivudine or telbivudine; and adefovir, tenofovir, or besifovir, respectively.

ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid; NA, Nucleos(t)ide analogue; PT, prothrombin time; SDM, standardized mean difference

Supplementary table 2. Baseline traditional cancer risk factors in chronic hepatitis B patients with and without antiviral treatment after IPTW adjustment

	Model 1*			**Model 2***				
	Untreated	NA-treated	SMD	Untreated	Cyclopentane group†	L-nucleoside group†	Acyclic phosphonate group†	SMD
	n=24,836	n=10,331		n=24,836	n=3,502	n=4,052	n=2,777	
Age (years)	46.3±12.5	46.5±11.1	0.015	46.3±12.1	46.4±11.7	46.2±11.8	46.3±11.8	0.010
Male gender	13,856 (55.8)	5,771 (55.9)	0.001	13,850 (55.8)	1,920 (56.3)	2,289 (56.5)	1,565 (56.3)	0.007
Co-infection of HCV	246 (1.0)	104 (1.0)	0.001	245 (1.0)	33 (1.0)	34 (0.8)	24 (0.9)	0.002
Hyperglycemia	3,206 (12.9)	1,335 (12.9)	0.001	3,195 (12.9)	436 (12.8)	521 (12.9)	332 (11.9)	0.009
Dyslipidemia	2,072 (8.3)	846 (8.2)	0.006	2,078 (8.4)	265 (7.8)	299 (7.4)	217 (7.8)	0.010
Fatty liver	1,273 (5.1)	539 (5.2)	0.004	1,269 (5.1)	172 (5.0)	203 (5.0)	141 (5.1)	0.001

*Model 1: IPTW-adjusted analysis for the comparison between untreated and NA-treated groups; and Model 2: IPTW-adjusted analysis for the comparisons between untreated controls and three NA groups

†Cyclopentane, L-nucleoside, and Acyclic phosphonate groups consisted of patient sets were treated with entecavir; clevudine, lamivudine or telbivudine; and adefovir, tenofovir, or besifovir, respectively.

HCV, hepatitis C virus; NA, Nucleos(t)ide analogue; SDM, standardized mean difference

Supplementary table 3. Incidences and hazard ratios of extrahepatic malignancies (less than 30 events) in chronic hepatitis B patients with and without antiviral treatment

	Percentage per person-years (Number of cancers)			IPTW analysis	
	Total N=35,167	Untreated n=24,836	NA-treated n=10,331	HR (95% CI)	p-value
Esophgeal cancer	0.005 (10)	0.004 (6)	0.01 (4)	1.837 (0.556-6.075)	0.319
Small bowel cancer	0.005 (10)	0.005 (8)	0.005 (2)	1.249 (0.469-3.327)	0.657
Myeloid cancer	0.004 (9)	0.003 (5)	0.01 (4)	4.048 (1.491-10.9)	0.006
Other cancers*	0.004 (9)	0.004 (7)	0.005 (2)	1.085 (0.403-2.921)	0.905
Centeral nervous system cancers	0.004 (8)	0.004 (7)	0.002 (1)	0.536 (0.136-2.104)	0.371

*Other cancers included thymic cancer, primary liver cancers other than hepatocellular carcinoma and intrahepatic cholangiocarcinoma, and malignancy of unknown origins.

CI, Confidence interval; HR, Hazard ratio; IPTW, inverse probability of treatment weighting; NA, Nucleos(t)ide analogues.

국문요약

제목: 만성 B형 간염 환자에서 경구용 항바이러스제 사용이 간 외 장기에 미치는 잠재적 발암성의 독성학적 평가에 대한 고찰

연구 배경 및 목적: 만성 B 형 간염 환자에게 경구용 항바이러스제가 널리 사용되고 있다. 그러나 경구용 항바이러스제의 발암 가능성에 대해서는 근거가 부족하여 아직 결론을 내리지 못하고 있다. 특히 2008 년 미국 식품의약국의 승인을 받고 최근 1 차 치료제로 널리 사용되는 테노포비어(tenofovir)의 발암성에 대한 평가는 더욱 부족하다. 이 연구는 대규모 만성 B 형 간염 환자의 코호트를 이용하여 주요 경구용 항바이러스제의 발암성을 평가하고자 한다.

연구 방법: 국제의약품구제조화위원회(International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)에서는 임상적 사용이 6개월 이상 지속될 것으로 예측되는 약물에 대해서는 발암성 평가를 하도록 하고 있다. 이에 따라 첫 치료로 사용한 경구용 항바이러스제를 6개월 이상 유지한 10,331 명의 치료군과 같은 기간 동안 약제를 복용하지 않은 24,836 명의 대조군을 대상으로 하였다. 치료군은 사용한 약제의 구조에 따라 사이클로펜테인(cyclopentane), L-뉴클레오사이드(L-nucleoside), 아세클릭인산염(acyclic phosphonate groups)의 3 군으로 분류하였다. 역확률 가중치(inverse probability of treatment weighting) 방법을 이용하여 치료군과 대조군 간, 그리고 약제 투약한 3군과 대조군 간 외 장기의 누적 암 발생률을 비교하였다. 그리고 각 장기 별 암 발생에 대하여 추가적으로 분석하였다.

결과: 치료군은 4.1 ± 3.1년, 대조군은 6.8 ± 5.5 동안 추적하였고, 치료군에서 208 명, 대조군에서 1,014 명의 환자에서 간 외 암이 발생하였다. 역 확률 가중치 법으로 분석하였을 때 양 군의 간 외 암 발생에 유의미한 차이는 없었다. 마찬가지로 약제 구조별 3군과 대조군 사이의 비교에서 간 외 암 발생에 대한 차이는 없었다. 장기 별 암 발생 분석 결과 경구용 항바이러스제 치료는 대장, 항문암과 림프종의 위험도를 증가시키고, 유방암과 전립선암의 발생 빈도는 낮추는 것으로 나타났다.

고찰: 만성 B형 간염 환자의 장기간 경구용 항바이러스제의 사용은 대장, 항문 암 및 림프종을 제외하면 기타 간 외 장기에 발암성이 없다는 것을 확인하였다. 경구용 항바이러스제가 유방암과 전립선암의 발생을 줄이는 것에 대한 추가 연구가 필요하겠다.

중심단어: 발암성; 만성 B 형 간염, 경구용 항바이러스제