Oral nucleos(t)ide analogues reduce recurrence and death in chronic hepatitis B-related hepatocellular carcinoma

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SUMMARY

Background
In patients with chronic hepatitis B (CHB)-related hepatocellular carcinoma (HCC), high viral load was associated with tumour recurrence and deaths.

Aims
To investigate the effect of nucleos(t)ide analogues (NA) on the clinical outcomes after different HCC treatments.

Methods
A territory-wide cohort study was conducted using the database from Hospital Authority. We identified CHB patients with HCC by International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis codes in 2000–2012. HCC treatments, NA use and laboratory parameters were retrieved. The primary endpoint was HCC recurrence and death. A 3-month landmark analysis was used to evaluate the primary outcome in patients with or without NA treatment.

Results
A total of 2198 CHB patients (1230 NA-untreated and 968 NA-treated) with HCC, receiving at least one type of HCC treatment were included in the analysis. At a median follow-up of 2.8 (IQR 1.4–4.9) years, tumour recurrence and death occurred in 451 (36.7%) and 578 (47.0%) untreated patients; and in 216 (22.3%) and 301 (31.1%) NA-treated patients respectively. NA therapy reduced the risk of overall HCC recurrence [adjusted sub-hazard ratio (SHR) 0.63, 95% confidence interval (CI) 0.49–0.80; \(P<0.001\)]. The effect was most obvious in patients undergoing resection (SHR = 0.58, 95% CI = 0.37–0.91, \(P = 0.018\)). The possibility of NA therapy reducing the risk of death (HR = 0.82, 95% CI = 0.64–1.03, \(P = 0.092\)), is most obvious in resection subgroup (HR = 0.64, 95% CI = 0.41–0.99, \(P = 0.050\)) but insignificant in the other treatment groups.

Conclusion
Our findings show that nucleos(t)ide analogues treatment reduces the risk of HCC recurrence in patients with chronic hepatitis B treated by surgical resection.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy in Asia due to the endemic chronic hepatitis B virus (HBV) infection.\(^1,2\) Despite advances in HCC treatment, HBV-related HCC is still associated with recurrence and therefore high mortality rate in the medium and long term.\(^3\) High HBV DNA level has been consistently shown to be associated with HCC recurrence in patients with chronic hepatitis B (CHB).\(^4\)

Oral nucleos(t)ide analogues (NA) are effective in suppressing viral replication and thus reduces the risk of HCC development.\(^5,6\) One step further, meta-analysis and numerous observational cohort studies demonstrate that NA treatment significantly reduced the risk of HCC recurrence as well as death after surgical resection.\(^7,8\) The survival benefit is due to lower chances of HCC recurrence as well as improvement in liver function after anti-viral therapy.\(^7\) Nonetheless, patients receiving local ablative therapies (LAT) were under-represented\(^7\) or even excluded\(^8\) in these studies. Furthermore, it remains uncertain whether NA treatment could benefit patients who received treatments of palliative intent such as transarterial chemoembolisation (TACE).

In this study, we aimed to investigate the impact of NA treatment on HCC recurrence and patient survival after various HCC treatments in CHB patients. The impact of NA treatment commenced before and after HCC treatment was also investigated.

METHODS

Study design

We conducted a territory-wide retrospective cohort study using the database from the Hospital Authority (HA), Hong Kong. The first visit of the included patients took place between 1 January 2000 and 31 December 2012. The accuracy and completeness of data collection based on the selection of the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes in HA database have been confirmed to be satisfactory, after the implementation of the Clinical Data Framework.\(^9\)

The in-patient and out-patient medical service provided by HA covered 70–80% Hong Kong citizens.\(^10\) Anti-viral therapy could be prescribed to CHB patients in all hospitals and specialist clinics. Full reimbursement for anti-viral therapy are available to patients with persistently high alanine aminotransferase and HBV DNA levels, cirrhosis or liver decompensation with detectable HBV DNA according to the treatment guidelines of the Asian Pacific Association for the Study of the Liver.\(^11\) The reimbursement guideline changed over time and it had become more lenient (e.g. non-invasive assessments\(^12\) might replace liver biopsy to define cirrhosis) since 2009. Patients who did not fall under the reimbursement guideline could purchase NA.\(^13\) The study protocol was approved by the local institutional review board (the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee).

Study population

Adult patients aged 18 years or above were identified from the HA database by ICD-9-CM diagnosis codes of both CHB and HCC, with or without HBsAg retrieved from laboratory parameters (Table S1). Patients with pre-existing HCC before the baseline visit, and those with hepatitis C, D and/or E virus (HCV, HDV, HEV) and human immunodeficiency virus (HIV) infection based on the diagnosis codes were excluded. Patients who received interferon after HCC treatments were not included as interferon might have been used as part of the chemotherapy regime but not as an anti-viral regime in the past.\(^14\) On the other hand, patients with other hepatic events before the baseline visit were included because a hepatic event was treated as a risk factor for death.

HCC treatments

Hepatocellular carcinoma treatments were defined by procedure codes for the following four major categories: surgical resection, liver transplantation, LAT and TACE (Table S2). Sorafenib was not reimbursed during the study period. Different procedures coded within three or 6 months landmark period from the index procedure was defined as combination treatments.

Data collection

Data were retrieved from the HA database in February 2014. Demographic data included gender and year of birth. Laboratory tests including liver biochemistry were collected annually. Other laboratory parameters were collected when CHB was coded. Baseline hepatic decompensation was defined by a diagnosis code of ascites, spontaneous bacterial peritonitis, variceal haemorrhage or hepatic encephalopathy.\(^6\)

Nucleos(t)ide analogues-treated CHB-related HCC patients were defined as those prescribed and dispensed one or more NA for CHB (i.e. lamivudine, adefovir dipivoxil, entecavir, telbivudine or tenofovir disoproxil
fumarate) within the pre-defined exposure period (3 months and 6 months) of the landmark analysis (see statistical analysis). Patients who had been taking NA before HCC treatments were also included. These medications were identified by HA’s internal drug codes (Table S3).

Clinical outcomes

HCC recurrence. Hepatocellular carcinoma recurrence was identified based on the same HCC treatment procedure codes of HCC treatments within the three or 6-month landmark period, or any HCC diagnosis codes or treatment procedure codes three or 6 months apart from the index procedure (Table S1 and S2).

Death. All-cause death was recorded. Death and its date were ascertained using data from both the HA database and Hong Kong death registry. All the events and deaths that happened during the study period from January 2000 to February 2014 were retrieved and analysed.

Statistical analyses

Continuous variables were expressed in mean ± standard deviation (s.d.) or median [interquartile range (IQR)] as appropriate, whereas categorical variables were presented as number (percentage). Qualitative and quantitative differences between groups were analysed by Chi-square or Fisher’s exact tests for categorical parameters and Student’s t-test or Mann–Whitney test for continuous parameters as appropriate (Data S1). We first determined crude incidence rates of HCC recurrence and deaths (in events/100 person-years) with 95% confidence intervals (CIs) for the NA-treated and untreated CHB-related HCC patients. To avoid immortal time bias, a 90-day landmark analysis (i.e. by eliminating patients who died during a 90-day exposure period and those who had been followed up for less than 90 days) with follow-up duration up to 5 years from the landmark date, was used to evaluate the relative risk of primary outcome in patients with or without NA treatment. In a secondary analysis, we developed propensity scores (PS), the conditional probability of receiving NA, to reduce the potential indication bias for the nonrandom treatment allocation. The propensity scores were determined by generalised boosted models. Besides evaluating the effect of NA in the whole cohort, we also investigated the effect of NA in subgroups stratified by gender, age (<60 or ≥60 years), NA use prior HCC treatments, HCC treatment modalities and individual NA drug use (lamivudine and entecavir). We carried out sensitivity analyses to vary the landmark period from 90 to 180 days to check the robustness of our results. Data were analysed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided. Statistical significance was taken as $P < 0.05$.

RESULTS

Demographic characteristics

We identified 107,800 potentially eligible CHB patients who first visited HA clinics/hospitals and who were assigned the ICD-9-CM codes of viral hepatitis. We excluded 30,972 patients who were not coded as CHB and who did not have positive HBsAg; 849 patients co-infected with HCV, HDV, HEV or HIV, respectively and 495 patients who received interferon-based treatment. From the 75,484 potentially eligible CHB patients, 6706 patients were assigned the diagnosis codes of HCC, whereas 2717 patients were assigned the procedure codes of HCC treatments. We further excluded 519 patients from the 3-month landmark analysis, as 3317 patients had HCC recurrence and 113 patients died within 3 months; one patient was younger than 18 at baseline, and 88 patients had missing laboratory data. Finally, 2198 patients were included in the study cohorts (968 patients NA-treated and 1230 NA-untreated patients) (Figure 1). For the sensitivity analysis of landmark analysis, 1992 patients (1019 NA-treated and 973 NA-untreated patients) were included in the 6-month landmark analysis (Figure S1).

Demographic characteristics, confounding drugs, comorbidities and follow-up durations of the study cohorts are presented in Table 1. The mean follow-up duration for NA-treated cohort was 2.6 ± 1.5 years, and that for NA-untreated cohort was 3.1 ± 1.7 years. The two cohorts had comparable baseline clinical characteristics. Most NA-treated patients had received either lamivudine or entecavir; adefovir and tenofovir were
reimbursed as rescue therapies for drug resistance [which was prescribed in 113 of 407 (27.7%) lamivudine-treated patients]. Most of the patients received HCC treatments of curative intent: 810 (36.9%), 0 (0%) and 1015 (46.2%) patients received surgical resection, liver transplantation and LAT, respectively, whereas 73 (3.3%) patients
Table 1 | Baseline clinical characteristics between patients with chronic hepatitis B-related hepatocellular carcinoma (HCC) did and did not receive nucleos(t)ide analogue treatment

|                        | NA-treated N = 968 | Untreated N = 1230 | P value |
|------------------------|--------------------|--------------------|---------|
| Follow-up (years) (mean, SD) | 2.6 ± 1.5          | 3.1 ± 1.7          | <0.001  |
| Male gender (n, %)       | 797 (82.3)         | 973 (79.1)         | 0.058   |
| Age (years)             | 60.8 ± 10.1        | 61.5 ± 11.0        | 0.117   |
| Sodium (mmol/L)          | 139.1 ± 3.9        | 138.4 ± 4.2        | <0.001  |
| Creatinine (μmol/L)*    | 95.0 ± 73.1        | 100.7 ± 85.7       | 0.017   |
| Albumin (g/L)           | 37.6 ± 6.6         | 35.4 ± 7.2         | <0.001  |
| Total bilirubin (μmol/L)*| 25.5 ± 56.9        | 25.9 ± 52.7        | 0.754   |
| Alanine aminotransferase (IU/L)* | 57.4 ± 100.0 | 67.4 ± 125.2 | <0.001  |

HCC treatments (n, %)
- Surgical resection: 376 (38.8) vs 434 (35.3) (P = 0.008)
- LAT: 458 (47.3) vs 557 (45.3)
- TACE: 76 (7.9) vs 146 (11.9)
- Resection & LAT: 31 (3.2) vs 42 (3.4)
- TACE & resection &/or LAT: 27 (2.8) vs 51 (4.1)

Comorbidities† (n, %)
- Coronary heart disease: 10 (1.0) vs 11 (0.9) (P = 0.740)
- Congestive heart failure: 6 (0.6) vs 25 (2.0) (P = 0.005)
- Cerebrovascular events
  - Ischaemic: 17 (1.8) vs 13 (1.1) (P = 0.161)
  - Haemorrhagic: 9 (0.9) vs 7 (0.6) (P = 0.323)
- Diabetes mellitus: 182 (18.8) vs 172 (14.0) (P = 0.002)
- Hepatic encephalopathy: 18 (1.9) vs 5 (0.4) (P = 0.001)
- Variceal bleeding: 34 (3.5) vs 21 (1.7) (P = 0.007)
- Renal failure: 13 (1.3) vs 7 (0.6) (P = 0.058)
- Spontaneous bacterial peritonitis: 14 (1.4) vs 17 (1.4) (P = 0.899)
- Ascites: 84 (8.7) vs 54 (4.4) (P < 0.001)
- Portal hypertension: 83 (8.6) vs 36 (2.9) (P < 0.001)

Concomitant drugs‡ (n, %)
- Oral hypoglycaemic agents: 170 (17.6) vs 170 (13.8) (P = 0.016)
- Metformin: 103 (10.6) vs 102 (8.3) (P = 0.060)
- Insulin: 74 (7.6) vs 68 (5.5) (P = 0.045)
- Statins: 50 (5.2) vs 35 (2.8) (P = 0.005)
- NSAID: 98 (10.1) vs 197 (16.0) (P < 0.001)

NA use (n, %)
- Lamivudine: 359 (37.1) vs 48 (3.9) (P < 0.001)
- Entecavir: 418 (43.2) vs 23 (1.9) (P < 0.001)
- Telbivudine: 29 (3.0) vs 5 (0.4) (P < 0.001)
- Adefovir dipivoxil: 133 (13.7) vs 3 (0.2) (P < 0.001)
- Tenofovir disoproxil fumarate: 20 (2.1) vs 0 (0) (P < 0.001)
- Any nucleos(t)ide: 784 (81.0) vs 76 (6.2) (P < 0.001)

Duration of NA exposure (years)§
- 1.6 ± 1.4 vs 0.6 ± 1.1 (P < 0.001)

HCC, hepatocellular carcinoma; LAT, local ablative therapy; NA, nucleos(t)ide analogues; NSAID, nonsteroidal anti-inflammatory drug; TACE, transcatheter chemoembolisation.

Alanine aminotransferase was also expressed in median (interquartile range), whereas other continuous variables were expressed in mean ± standard deviation.

* P value was calculated based on log-transformed values.
† Comorbidities were all defined based on ICD-9 diagnosis codes.
‡ All concomitant medications were represented as binary parameters.
§ Duration of exposure was measured after the landmark date.
underwent both resection and LAT. TACE were given to 222 (10.1%) patients, whereas TACE combined with resection and/or LAT were offered to 78 (3.5%) patients (Table 1).

NA treatment and HCC recurrence (also see Figure S2A)

During the study period, HCC recurrence and death occurred in 216 (22.3%) and 301 (31.1%) of the NA-treated patients; compared to 451 (36.7%) and 578 (47.0%) of NA-untreated patients respectively. The incidence (95% CI) of HCC recurrence was 10.7 (9.3–12.2) and 16.6 (15.1–18.2) per 100 person-years in NA-treated and NA-untreated patients respectively.

Nucleos(t)ide analogues-treated HCC patients had reduced risk of HCC recurrence compared to NA-untreated HCC patients [multivariable-adjusted sub-hazard ratio (SHR) = 0.63, 95% CI = 0.49–0.80, \( P < 0.001 \)] (Table 2). The beneficial effect of NA treatment was most obvious in the subgroup of patients who underwent surgical resection (adjusted SHR = 0.58, 95% CI = 0.37–0.91, \( P = 0.018 \)), but it just fell short of statistical significance in LAT subgroup (SHR = 0.68, 95% CI = 0.46–1.01, \( P = 0.058 \)). NA treatment did not reduce HCC recurrence in TACE subgroups or those receiving combination treatments (SHR ranged from 0.47 to 0.90, \( P \) ranged from 0.140 to 0.822) (Figure 3A).

NA treatment and death (also see Figure S2B)

During the study period, the death rate (95% CI) was 12.0 (10.7–13.4) and 15.1 (13.9–16.4) per 100 person-years in NA-treated and NA-untreated patients respectively (Table 2 and Figure 2).

Nucleos(t)ide analogues treatment had the tendency to reduce the risk of death (multivariable-adjusted HR = 0.82, 95% CI = 0.64–1.03, \( P = 0.092 \)) (Table 2). The benefit was again more obvious in the surgical resection subgroup (HR = 0.64, 95% CI = 0.41–0.99, \( P = 0.050 \)), but fell short of statistical significance in combined resection-LAT subgroup (HR = 0.33, 95% CI = 0.10–1.11, \( P = 0.073 \)). NA treatment did not reduce the risk death in LAT, TACE or combined TACE-resection/LAT subgroups (HR ranged from 0.80–1.62, \( P \) ranged from 0.175–0.955) (Figure 3B).

### Table 2 | Estimated crude incidence rates and adjusted hazard ratios of hepatocellular carcinoma (HCC) recurrence and death (with 95% confidence intervals) for nucleos(t)ide analogues (NA)-treated vs. untreated patients with chronic hepatitis B-related HCC

|                      | HCC recurrence* |Deaths |
|----------------------|-----------------|-------|
|                      | NA-treated      | Untreated | P |
|                      |                 |         |     |
| 3-month landmark     |                 |         |     |
| analysis \((n = 2198)\) | 216/968      | 451/1230 |       |
| No. of events/No. at risk | 10.7 (9.3–12.2) | 16.6 (15.1–18.2) | <0.001 |
| Incidence rate† (95% CI) | 0.63 (0.49–0.80) | 1 [reference] | 0.82 (0.64–1.03) | 1 [reference] | 0.092 |
| Multivariable-adjusted HR‡ (95% CI) | 0.62 (0.48–0.79) | 1 [reference] | 0.84 (0.67–1.06) | 1 [reference] | 0.146 |
| PS-weighted and multivariable-adjusted HR§ (95% CI) | 0.63 (0.48–0.84) | 1 [reference] | 0.002 | 0.85 (0.67–1.08) | 1 [reference] | 0.179 |

|                      | NA-treated      | Untreated | P |
|                      |                 |         |     |
| 6-month landmark     |                 |         |     |
| analysis \((n = 1992)\) | 216/968 | 451/1230 |       |
| Multivariable-adjusted HR‡ (95% CI) | 0.63 (0.48–0.84) | 1 [reference] | 0.002 | 0.85 (0.67–1.08) | 1 [reference] | 0.179 |

CI, confidence intervals; HCC, hepatocellular carcinoma; HR, hazard ratios; NAs, nucleos(t)ide analogues; NSAID, nonsteroidal anti-inflammatory drug; PS, propensity score.

* For the analysis of HCC recurrence, renal failure was omitted in the models due to no event in one category.
† Incidence rate was presented in per 100 person-years.
‡ Multivariable HRs were adjusted for covariates listed in Table 1 except for duration of follow-up, duration of NA exposure and individual use of NA treatments.
§ PS-weighted and multivariable HRs were adjusted for any use of NA treatments and treatments medications (oral hypoglycaemic agents, metformin, insulin, statins and NSAID).
Different age groups and male subgroups in the entire cohort but not in the female subgroups (Figure 3A). Concerning death, consistent beneficial effect of NA treatment as in the entire cohort was only observed in the subgroup of age ≥60 years; such observation was neither seen in the younger age groups nor in the gender subgroups (Figure 3B).

**Six-month landmark analysis.** When 6 months instead of 3 months were used as the landmark time, NA-treated HCC patients similarly had reduced risk of HCC recurrence compared to NA-untreated HCC patients (multivariable-adjusted SHR = 0.63, 95% CI = 0.48–0.84, P = 0.002) (Table 2). Again, the difference in death did not reach statistical significance (multivariable-adjusted HR = 0.85, 95% CI = 0.67–1.08, P = 0.179) (Table 2).

**NA treatment before HCC treatment.** Hepatocellular carcinoma recurrence was reduced only by NA treatment that started after surgical resection (SHR = 0.46, 95% CI = 0.24–0.86, P = 0.016) and not before resection (SHR = 0.87, 95% CI = 0.33–2.31, P = 0.782). In contrast, death was reduced by NA treatment that proceeded surgical resection (HR = 0.42, 95% CI = 0.21–0.83, P = 0.013) rather than followed by resection (HR = 0.85, 95% CI = 0.50–1.45, P = 0.558).

**Entecavir vs. lamivudine.** As most NA-treated patients received either lamivudine or entecavir, the impact of these treatments on HCC recurrence and death was compared. Both entecavir (SHR = 0.60, 95% CI = 0.44–0.82; P = 0.001) and lamivudine (SHR = 0.69, 95% CI = 0.49–0.96; P = 0.028) reduced HCC recurrence compared to absence of NA treatment. The benefit was more obvious in patients receiving entecavir than in those receiving lamivudine (SHR = 0.64, 95% CI = 0.46–0.89; P = 0.008) (Figure 3A). Both entecavir (SHR = 0.77, 95% CI = 0.58–1.02; P = 0.07) and lamivudine treatment (SHR = 0.78, 95% CI = 0.56–1.08; P = 0.131) tended to reduce death compared to absence of NA treatment. There was no difference in death between patients who received entecavir and those who received lamivudine (SHR = 1.07, 95% CI = 0.81–1.42; P = 0.61) (Figure 3B).

**DISCUSSION**

This is one of the largest population-based studies illustrating the beneficial effects of NA treatment in CHB patients with HCC after various HCC treatments. We demonstrated reduced risk of HCC recurrence and of death in NA-treated patients who received HCC.

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**Figure 2 | Cumulative incidence of events in nucleos(t)ide analogues (NA)-treated vs. untreated patients with chronic hepatitis B-related hepatocellular carcinoma (HCC):** (a) HCC recurrence; (b) deaths. HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues.
(a) HCC recurrence

| No. of events/No. at risk | NA-treated | Untreated |
|--------------------------|------------|-----------|
| All patients             | 216/968    | 451/1230  |
| Age, y                   |            |           |
| <60                      | 103/421    | 201/526   |
| >=60                     | 113/547    | 250/704   |
| Sex                      |            |           |
| Female                   | 36/171     | 81/257    |
| Male                     | 180/797    | 370/973   |
| Use of NA*               |            |           |
| No                       | 40/184     | 426/1154  |
| Yes                      | 176/784    | 25/76     |
| HCC treatments           |            |           |
| Tumor resection          | 54/376     | 132/434   |
| LAT                      | 123/458    | 239/557   |
| TACE                     | 17/76      | 40/146    |
| Resection & LAT          | 11/31      | 16/42     |
| TACE & resection &/or LAT| 11/27      | 24/51     |

Hazard ratio (95% CI) for interaction:

- All patients: 0.63 (0.49 to 0.80)
- Age, y <60: 0.59 (0.40 to 0.87)
- Age, y >=60: 0.63 (0.45 to 0.87)
- Sex Female: 0.86 (0.42 to 1.77)
- Sex Male: 0.58 (0.45 to 0.76)
- Use of NA* No: 0.60 (0.43 to 0.84)
- Use of NA* Yes: 0.71 (0.45 to 1.11)
- Tumor resection: 0.58 (0.37 to 0.91)
- LAT: 0.68 (0.46 to 1.01)
- TACE: 0.53 (0.22 to 1.24)
- Resection & LAT: 0.90 (0.37 to 2.20)
- TACE & resection &/or LAT: 0.47 (0.12 to 1.80)

Figure 3 | Multivariable-adjusted subgroup analyses of (a) HCC recurrence and (b) deaths according to nucleos(t)ide analogues (NA) use after HCC treatments. For the analysis of HCC recurrence, renal failure was omitted in the models due to no event in one category. *NA use prior HCC treatments. Multivariable HRs were adjusted for covariates listed in Table 1 except for duration of follow-up, duration of NA exposure and individual use of NA pre-HCC treatments. For each stratified analysis, the stratification variable was omitted from the model. CI, confidence intervals; HCC, hepatocellular carcinoma; HR, hazard ratios; LAT, local ablative therapy; NAs, nucleos(t)ide analogues; TACE, transartierial chemoembolisation.

treatments of curative intent (i.e. resection and/or LAT). The beneficial effect of NA treatment, however, was not observed in patients who received TACE or its combinations with resection and/or LAT as HCC treatments.

While surgical resection and liver transplantation are regarded as the curative treatments for HCC, LAT with radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI) are highly effective and regarded as curative alternatives for patients with small HCC.22 LATs are also considered for unresectable, solitary HCC <5 cm or two to three nodules of sizes smaller than 3 cm in Child–Pugh A to B patients when transplantation is not feasible.23 Our data showed there was no confirmed benefit from NA associated with survival in patients undergoing NA. However, while the sub-hazard ratio (SHR) just fell short of statistical significance (95% CI = 0.46–1.01, P = 0.058), a clinical effect may be pre-
sent and this requires characterisation more formally in an RCT. On the other hand, HCC patients at intermediate to advanced stages with unresectable, large and/or multifocal tumours may benefit from TACE in the presence of satisfactory liver function (Child–Pugh A/B).

Among the well-known risk factors of HCC recurrence, the most reversible risk factor is probably the high viral load. Only until recently did the Asian-Pacific guideline on HBV treatment recommend anti-viral therapy to HCC patients with HBV DNA above 2000 IU/mL before and/or after curative therapy of HCC, as in their counterparts without HCC. This recommendation was made based on the meta-analysis of nine cohort studies involving 551 patients in total. Our results also provide new data on the role of NA treatment in patients receiving other HCC therapies. We also demonstrated that potent NA likely entecavir provides more beneficial effect than lamivudine, as the latter confers the development of drug-resistant mutants and hence its benefit would be negated. Despite most of the patients who develop lamivudine-resistant mutants would be put on rescue therapy, there was also possibility that patients who develop lamivudine-resistant mutants might not been put on such rescue therapy in a timely fashion, especially in the old days. In our cohort, only 27.7% of lamivudine-treated patients received adefovir and tenofovir subsequently. This would lead to the reduced benefit in term of recurrence reduction by lamivudine.

Figure 3 | Continued.
Although the guideline recommends a treatment threshold of HBV DNA level at 2000 IU/mL, this level was not supported by existing evidence. Recent studies illustrated that even a lower level of HBV DNA would increase the risk of HCC occurrence, of HBV reactivation and even of HCC recurrence. Our recent cohort study of 432 patients with HBV-related HCC (of whom 65% received NA treatment), baseline HBV DNA level was not an independent predictor of overall survival of patients undergoing tumour resection. Because of the dynamic nature of CHB, a single HBV DNA level can never be taken as evidence of permanent immune suppression of HBV.

One of our interesting observations was that NA treatment before HCC development had different effects on HCC recurrence and death. While NA use prior HCC treatments reduced death, it did not reduce HCC recurrence. This was probably secondary to selection bias. Patients who received NA early and responded well to treatment never developed HCC and were not included in this study. In other words, patients who received NA before HCC were those who had other high risk features despite HBV DNA suppression. On the other hand, NA treatment improves survival, likely because of fewer numbers of deaths due to liver failure. There may be other drives to HCC carcinogenesis as NA treatment only reduces but not really eliminates the risk of HCC occurrence and recurrence.

Our study has the strength of a large sample size and a long follow-up duration, which increased the statistical power to detect uncommon events. Data from real-life cohorts represent a wider spectrum of patients than randomised controlled trial (RCT) results and are applicable to routine clinical practice. Nonetheless, several limitations of this study warrant comment. First, measured and unmeasured confounding factors might exist and account for differences in outcomes between NA-treated and untreated patients. We did our best to adjust to potential confounders by including various laboratory parameters, comorbidities and medications in the multivariable model. We also included the secondary analysis after adjusting the propensity scores.

Second, diagnosis coding might have been incomplete; there was also some uncertainty in the definition of HCC recurrence as it totally depended on the input from the doctors in-charge. Nonetheless, the procedure coding for HCC treatments was mostly accurate and complete. Third, even though we had retrieved the record of all the refill prescriptions; we had no information on their drug adherence such that the true beneficial impact of NA on reducing HCC recurrence and death might have been under-represented. Fourth, HA database did not provide personal information such as lifestyle, smoking, alcohol, family history of malignant diseases, body mass index, as well as HBV DNA level, presence of advanced liver fibrosis or early cirrhosis, tumour size and number, which might have contributed to HCC recurrence. However, HA drug formulary has strict regulations regarding reimbursement for nucleoside analogues that these factors should not influence if patient would receive NA treatment or not. Our recent cohort study showed that patients received NA treatment had similar size (median 3.5 cm) and proportion of multiple tumours (22.4%) compared to those did not receive NA treatment (3.8 cm and 21.2%; \(P = 0.415\) and 0.769 respectively). Fifth, we did not have the information on the cause of death, such that only all-cause mortalities rather than the liver-related ones could be studied. Sixth, only patients who received HCC treatments were included in the analysis, which in itself predicts a group that is more likely to have cancer detected earlier and more likely to be some form of specialist clinical management. These factors would lead to better outcomes. Lastly, the landmark period and definition of combination therapy within a 3-month interval was arbitrary. Our sensitivity analysis using a landmark period of 6 months, however, suggests that the findings are robust.

In conclusion, this large-scaled population-based study demonstrated that NA treatment reduces the risk of HCC recurrence and possibly death in CHB patients, particularly among those who received surgical resection.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

- **Data S1.** Detailed statistical analyses.
- **Table S1.** Diagnosis codes used as the inclusion criteria or outcomes of subjects.
- **Table S2.** Procedure codes used to define treatments for hepatocellular carcinoma (HCC).
- **Table S3.** Drug codes of nucleos(t)ide analogues used in Hospital Authority internally.
- **Figure S1.** Selection of chronic hepatitis B (CHB) patients in the 6-month landmark analysis.
- **Figure S2.** Cumulative incidence of events in nucleos(t)ide analogues (NA)-treated versus untreated patients with chronic hepatitis B related hepatocellular carcinoma (HCC) with respect to different HCC treatment modalities: (A) HCC recurrence; (B) deaths. HCC, hepatocellu-
lary carcinoma; LAT, local ablative therapy; NA, nucleos (t)ide analogues; TACE, transcatheter arterial chemoembolization.

AUTHORSHIP

Guarantor of the article: Vincent Wong.

Author contributions: Grace Wong and Yee-Kit Tse had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Grace Wong, Vincent Wong, Henry Chan were responsible for the study concept and design. All authors were responsible for the acquisition, analysis, or interpretation of data. Grace Wong, Yee-Kit Tse, Vincent Wong, Henry Chan were responsible for the drafting of the manuscript. All authors were responsible for the critical revision of the manuscript for important intellectual content.

All authors approved the final version of the manuscript.

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