Development of a risk classification system combining TN-categories and circulating EBV DNA for non-metastatic NPC in 10,149 endemic cases

Fo-Ping Chen, Li Lin, Jin-Hui Liang, Sze Huey Tan, Enya H.W. Ong, Ying-Shan Luo, Luo Huang, Adelene Y.L. Sim, Hai-Tao Wang, Tian-Sheng Gao, Bin Deng, Guan-Qun Zhou, Jia Kou, Melvin L.K. Chua and Ying Sun

Abstract

Background: The objective of this study was to construct a risk classification system integrating cell-free Epstein-Barr virus (cfEBV) DNA with T- and N- categories for better prognostication in nasopharyngeal carcinoma (NPC).

Methods: Clinical records of 10,149 biopsy-proven, non-metastatic NPC were identified from two cancer centers; this comprised a training (N=9,259) and two validation cohorts (N=890; including one randomized controlled phase 3 trial cohort). Adjusted hazard ratio (AHR) method using a two-tiered stratification by cfEBV DNA and TN-categories was applied to generate the risk model. Primary clinical endpoint was overall survival (OS). Performances of the models were compared against American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) 8th edition TNM-stage classification and two published recursive partitioning analysis (RPA) models, and were validated in the validation cohorts.

Results: We chose a cfEBV DNA cutoff of \( \geq 2,000 \) copies for optimal risk discretization of OS, disease-free survival (DFS) and distant metastasis-free survival (DMFS) in the training cohort. AHR modeling method divided NPC into six risk groups with significantly disparate survival (\( p < 0.001 \) for all): AHR1, T1N0; AHR2A, T1N1/T2-3N0 cfEBV DNA < 2,000 (EBV\(_\text{low}\)); AHR2B, T1N1/T2-3N0 cfEBV DNA \( \geq 2,000 \) (EBV\(_\text{high}\)) and T1-2N2/T2-3N1 EBV\(_\text{low}\); AHR3, T1-2N2/T2-3N1 EBV\(_\text{high}\) and T3N2/T4N0 EBV\(_\text{low}\); AHR4, T3N2/T4 N0-1 EBV\(_\text{high}\) and T1-3N3/T4N1-3 EBV\(_\text{low}\); AHR5, T1-3N3/T4 N2-3 EBV\(_\text{high}\). Our AHR model outperformed the published RPA models and TNM stage with better hazard consistency (1.35 versus 3.98–12.67), explained variation (0.248 versus 0.438–0.749), and C-index (0.707 versus 0.662–0.700). In addition, our AHR model was superior to the TNM stage for risk stratification of OS in two validation cohorts (\( p < 0.001 \) for both).

Conclusion: Herein, we developed and validated a risk classification system that combines the AJCC/UICC 8th edition TN-stage classification and cfEBV DNA for non-metastatic NPC. Our new clinicomolecular model provides improved OS prediction over the current staging system.

Keywords: adjusted hazard ratio, Epstein-Barr virus DNA, nasopharyngeal carcinoma, risk stratification, TNM stage

Introduction

Epstein-Barr virus (EBV) is invariably linked with the endemic form of nasopharyngeal carcinoma (NPC). In these EBV-associated NPC tumors, the virus-encoded genomic region is ubiquitously expressed in most tumor cells.\(^1,2\) In addition to
the detection of EBV within the tumor, small genomic fragments of the virus, which are presumably released by circulating NPC tumor cells, can be detected using ultrasensitive polymerase chain reaction (PCR)-based assays. Hence, several studies have examined and reported on the clinical utility of these circulating cell-free EBV DNA (cfEBV DNA) molecular assays for population screening of NPC\(^3\) and disease surveillance.\(^4,5\) Apart from its advantages for early detection, quantification of cfEBV DNA load has also been investigated as a biomarker of tumor burden, and circulating viral load has been shown to correlate to clinical stage of disease.\(^6\) To this point, studies have shown that pretreatment cfEBV DNA load is complementary to conventional TNM staging for clinical prognostication,\(^7,8\) which would suggest that this biomarker provides additional biological information that is not captured by T- and N-classification.

However, despite its potential prognostic significance, the existing American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) 8th edition TNM-stage classification does not consider pretreatment EBV DNA for risk stratification. This is related to several factors, including poor inter-laboratory concordance in cfEBV DNA quantification by the PCR assay, use of different EBV DNA thresholds for risk stratification,\(^2,9\) poor inter-laboratory concordance in cfEBV DNA quantification, and limited cohort sample sizes for robust model development.\(^2,9\) On this note, Tang et al.\(^7\) presented a prognostic nomogram for disease-free survival (DFS) using several known prognostics variables, including cfEBV DNA. In their nomogram, cfEBV DNA was considered as a continuous variable with an assigned weightage. Nonetheless, this is impractical for routine clinical use, since the system developed was non-intuitive, and it did not seem to impact on the clinical management of NPC. Recently, two published recursive partitioning analysis (RPA)-based risk stratification system classified NPC patients into five and four clinico-molecular risk groups using cfEBV DNA, T- and N-categories.\(^8,10\) But owing to the limited sample sizes in both studies (1,529 cases [training 979 patients, validation 550 patients]; and 518 cases, respectively), the discordant models still require validation in larger cohorts.

Here, we aimed to construct a robust clinico-molecular model by combining pretreatment cfEBV DNA titer with T- and N-categories that is superior for risk stratification than the 8th edition TNM-stage classification, using a large dataset of 9,259 patients who were treated at an academic center. We also investigated the performance of our model in multiple internal (including patients from a prospective phase 3 randomized controlled trial) and external validation cohorts.

**Materials and methods**

**Patient selection**

The study cohort comprised 10,149 patients with histologically proven, non-metastatic (M0) NPC from two academic institutions. This comprised a training cohort (\(N=9,259\)) for model development, which was identified from the NPC-specific database embedded within the big-data intelligence platform at the Sun Yat-Sen University Cancer Center (SYSUCC) (Supplementary Materials, online only). An independent prospective cohort (NCT01245959, Supplementary Materials, online only) with 237 patients from the same center,\(^11\) and an external cohort from the Wuzhou Red Cross Hospital (WZRCH, \(N=653\)) were enrolled for validation. Overall, the training and validation cohorts were diagnosed and treated between 2009 and 2015. The inclusion and exclusion criteria, and detail procedures of patient selections were illustrated in Figure 1. The institutional ethical review boards of all included hospitals approved this retrospective analysis of anonymized data (IRB reference No.: [SYSUCC] YB2020-338-01; [WZRCH] LL2019-16). Informed consent was obtained for all patients from the SYSUCC-TPF trial cohort; while requirement for informed consent was waived by the ethical review boards for the SYSUCC-Training and WZRCH cohorts, given the retrospective nature of this study.

**Diagnosis, treatment, and follow-up**

All 10,149 patients were diagnosed, treated, and followed-up according to the respective institutional guidelines for NPC in the academic centers (Supplementary Materials, online only). All patients in each cohort were restaged by two radiation oncologists (GQZ and YS [SYSUCC-Training, SYSUCC-TPF]; JHL and TSG [WZRCH]) who are specialized in head and neck cancer in accordance with the AJCC/UICC 8th edition TNM-staging system,\(^12\) with discordance resolved by consensus.
The primary endpoint was overall survival (OS), which was calculated from start of treatment to date of death from any cause, or date of last follow-up visit. Secondary endpoints were DFS, locoregionally recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS), calculated from start of treatment to date of first relapse, locoregional recurrence, and distant metastasis, respectively. Patients who were alive at end of study period were censored at the date of last follow-up visit. Survival analyses were performed using the Kaplan–Meier method and compared by the log-rank test. Cox proportional hazards regression was used for hazard ratio (HR) estimation in the multivariable analyses.

Determination of cfEBV DNA cutoff for risk discretization, and combinatorial cfEBV DNA and TN-category model construction
The relationship between cfEBV DNA titer and outcomes was calculated using Cox proportional hazards regression model through restricted cubic splines (RCS). RCS allows threshold identification of cfEBV DNA on outcomes as described in previous studies. Adjusted hazard ratios (AHRs) method was used to derive the risk classification model combining TN-status and cfEBV DNA. The performance of the AHR model in predicting OS was assessed and compared against the AJCC/UICC 8th edition TNM-stage classification and two published RPA models.

Validation of proposed clinicomolecular risk stratification system in three independent cohorts
Validation of the proposed clinicomolecular risk stratification model (TN + cfEBV DNA) was performed in the SYSUCC-TPF and WZRCH cohorts by evaluating the performances of this model for prognostication of OS. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the accuracy of AHR model for survival prediction against AJCC/UICC 8th edition TNM-staging system. Decision curve analysis (DCA) was used to compare the efficacy of survival prediction between AHR model and TNM-stage classification.

All statistical tests were two-sided, and a p value of <0.05 was considered significant. Statistical analyses were performed in R version 3.4.4 (http://www.r-project.org/), Stata 14.2 software (StataCorp, College Station, TX), and SPSS 23.0 software (SPSS Inc, IL).

Results
Patient characteristics and treatment outcomes
The characteristics of patients from the training and validation cohorts are shown in Table 1.

\[\text{Table 1. Patient Characteristics and Treatment Outcomes}\
\]

**Statistical analysis**

**Validation of proposed clinicomolecular risk stratification system in three independent cohorts**

**Results**

**Patient characteristics and treatment outcomes**

The characteristics of patients from the training and validation cohorts are shown in Table 1.
Table 1. General characteristics of patients with nasopharyngeal carcinoma in the training and validation cohorts.

|                                | Training cohort | Validation cohorts |
|--------------------------------|-----------------|--------------------|
|                                | SYSUCC-training | SYSUCC-TPF         |
|                                | (n = 9,259)     | (n = 237)          |
|                                |                 | WZRCH (n = 653)    |
| Age (years)                    |                 |                    |
| Median                         | 45              | 41                 |
| IQR                            | 38–53           | 35–48              |
| Sex, n (%)                     |                 |                    |
| Male                           | 6,784 (73.3)    | 193 (81.4)         |
| Female                         | 2,475 (26.7)    | 44 (18.6)          |
| WHO histologic type, n (%)     |                 |                    |
| Keratinizing                   | 238 (2.6)       | 0 (0)              |
| Nonkeratinizing                | 9,021 (97.4)    | 237 (100)          |
| Tumor category, n (%)          |                 |                    |
| T1                             | 1,533 (16.6)    | 5 (2.1)            |
| T2                             | 1,508 (16.2)    | 14 (5.9)           |
| T3                             | 4,294 (46.4)    | 145 (61.2)         |
| T4                             | 1,924 (20.8)    | 73 (30.8)          |
| Node category, n (%)           |                 |                    |
| N0                             | 1,449 (15.6)    | 0 (0)              |
| N1                             | 4,664 (50.2)    | 129 (54.4)         |
| N2                             | 2,004 (21.6)    | 91 (38.4)          |
| N3                             | 1,160 (12.5)    | 17 (7.2)           |
| Stage, n (%)                   |                 |                    |
| I                              | 514 (5.6)       | 0 (0)              |
| II                             | 1,644 (17.8)    | 0 (0)              |
| III                            | 4,249 (45.9)    | 152 (64.1)         |
| IVA                            | 2,852 (30.8)    | 85 (35.9)          |
| cfEBV DNA, copy/mL             |                 |                    |
| Median                         | 2,050           | 5,630              |
| IQR                            | 0–17,000        | 652–33,200         |
| Chemotherapy, n (%)            |                 |                    |
| None                           | 1,250 (13.5)    | 0 (0)              |
| NACT ± ACT                     | 906 (9.8)       | 0 (0)              |
| CCRT ± ACT                     | 3,412 (36.9)    | 122 (51.5)         |
| NACT + CCRT ± ACT              | 3,691 (39.9)    | 115 (48.5)         |

ACT, adjuvant chemotherapy; CCRT, concurrent chemoradiotherapy; cfEBV DNA, cell-free Epstein-Barr virus DNA; IQR, interquartile range; NACT, neoadjuvant chemotherapy; SYSUCC, Sun Yat-Sen University Cancer Center; TPF, docetaxel/cisplatin/fluorouracil chemotherapy regimen; WHO, World Health Organization; WZRCH, Wuzhou Red Cross Hospital.
Median follow-up of these cohorts was 66.1 (interquartile range: 53.6–81.5) months, 82.1 (71.2–89.8) months, and 60.9 (47.0–67.8) months, respectively. The breakdown of the sites of relapses of these cohorts is detailed in Supplementary Table 1 (online only). Estimated 5-year OS rates were 86.1% (95% confidence interval [CI] 85.7%–86.5%) for SYSUCC-Training cohort, 81.4% (95% CI 78.9%–83.2%) for SYSUCC-TPF, 78.7% (77.0%–80.4%) for WZRCH cohort, respectively.

Prognostic effect of cfEBV DNA on survivals
We observed a consistent relationship between cfEBV DNA (log-scale) and OS, DFS, and DMFS (Supplementary Figure 1, online only), but not for LRFS in cfEBV DNA higher than 2,000 copies (log[cfEBV DNA] 3.32–3.34). This may be explained by the fact that our cohort was exclusively treated using intensity-modulated radiotherapy (IMRT), and thus lessened the association of conventional clinical prognostic variables with LRFS. We also performed a sensitivity analysis to determine the optimal cutoff value for cfEBV DNA by testing for association with survival outcomes in subgroups dichotomized by 2,000, 20,000, 200,000, and 2,000,000 copies of cfEBV DNA (per 10-fold increase). Stable HRs were observed for OS, DFS, and DMFS with the different cfEBV DNA titers cutoffs (HROS 2.36 [2,000 copies] versus 2.33 [20,000], 2.33 [200,000], 2.88 [2,000,000]; HRDFS 2.17 versus 2.10, 2.09, 2.49; HRDMFS 2.54 versus 2.44, 2.48, 2.68; Supplementary Figure 1, online only). We therefore conclude that cfEBV DNA of 2,000 copies is a stable and robust cutoff for risk stratification in non-metastatic NPC.

Prognostic performance of current 8th edition TNM stage
The performance of the 8th edition TNM stage for prognostication in the training cohort is illustrated in Supplementary Figure 2 (online only); we tested the intra-group consistency of each TN-category in the SYSUCC-Training cohort by splitting the patients using TN-category and cfEBV DNA titers. Interestingly, we observed significant heterogeneity among patients with stage II to IVA NPC (p < 0.001 for all comparisons; Supplementary Figure 2, online only). In particular, stages III and IVA patients harbored the widest heterogeneity for OS between the subgroups, and thus we deduced that the current 8th edition TN-categories can be subdivided into finer groupings with improved homogeneity of OS within each risk group.

Performances of clinicomolecular risk stratification system against 8th edition TNM and published RPA models
Next, we compared the AHR model against the AJCC/UICC 8th edition TNM-stage classification and two published RPA models,8,10 Table 2
Therapeutic Advances in Medical Oncology 13

summarizes the performance of all the models in the SYSUCC-Training cohort. The AHR model was the most superior among all the different methods in terms of hazard consistency, hazard discrimination, explained variation, balance, C-index, Somers’D, Akaike information criterion, and Bayesian information criteria. The HRs for risk of death of the four risk classifications are presented in Supplementary Table 4 (online only); our AHR model outperformed TNM-stage classification and the published RPA models for prognostication. We therefore selected our AHR model for validation.

Validation of AHR model for prognostication in three independent cohorts

Figure 3 shows the OS outcomes of our AHR model in the two validation cohorts (N=890); AHR risk classification system yielded clear separation for the different AHR risk groups in SYSUCC-TPF and WZRCH cohorts. This validates the reproducibility.
Table 2. Performance evaluation of AHR, RPA, and 8th edition AJCC/UICC TNM stage schema for nasopharyngeal carcinoma.

|                         | Proposed model | Published models |
|-------------------------|----------------|-----------------|
|                         | AHR            | AJCC 8th        | RPA_Guo | RPA_Lee |
| Hazard consistency      | 1.35           | 8.25            | 3.98    | 12.67   |
| Score                   | 0              | 0.609           | 0.232   | 1       |
| Rank                    | 1              | 3               | 2       | 4       |
| Hazard discrimination   | 5.29           | 6.69            | 11.90   | 13.35   |
| Score                   | 0              | 0.175           | 0.821   | 1       |
| Rank                    | 1              | 2               | 3       | 4       |
| Explained variation     | 0.248          | 0.201           | 0.225   | 0.164   |
| Score                   | 0              | 0.561           | 0.275   | 1       |
| Rank                    | 1              | 3               | 2       | 4       |
| Likelihood difference   | 119.33         | **133.63**      | 131.10  | 92.37   |
| Score                   | 0.347          | 0               | 0.062   | 1       |
| Rank                    | 3              | 1               | 2       | 4       |
| Balance                 | 0.385          | 0.534           | 0.438   | 0.749   |
| Score                   | 0              | 0.408           | 0.146   | 1       |
| Rank                    | 1              | 3               | 2       | 4       |
| Overall score           | 0.347          | 1.753           | 1.535   | 5.000   |
| Overall rank            | 1              | 3               | 2       | 4       |
| C-indexa                | **0.707**      | 0.677           | 0.700   | 0.662   |
| Score                   | 0              | 0.669           | 0.158   | 1.000   |
| Rank                    | 1              | 3               | 2       | 4       |
| Somers’D                | **0.413**      | 0.353           | 0.399   | 0.323   |
| Score                   | 0              | 0.669           | 0.158   | 1.000   |
| Rank                    | 1              | 3               | 2       | 4       |
| AIC                     | **25195**      | 25387           | 25265   | 25510   |
| Score                   | 0              | 0.608           | 0.223   | 1       |
| Rank                    | 1              | 3               | 2       | 4       |
| BIC                     | **25245**      | 25422           | 25308   | 25546   |
| Score                   | 0              | 0.589066        | 0.209497| 1       |
| Rank                    | 1              | 3               | 2       | 4       |

AHR, adjusted hazard ratio; AIC, Akaike information criterion; AJCC, American Joint Committee on Cancer; BIC, Bayesian information criteria; RPA, recursive partitioning analysis; UICC, Union for International Cancer Control.

*aAdjusted for age and gender.
of the AHR groupings in external cohorts, with independent cfEBV DNA testing and different clinical and cfEBV DNA parameters. In addition, AHR model achieved superior accuracy for survival prediction than TNM-staging system in SYSUCC-Training (AUC AHR 0.681 [95% CI 0.668–0.695] versus AUCTNM 0.641 [0.627–0.655]; Supplementary Figure 4A), SYSUCC-TPF (AUC AHR 0.726 [0.657–0.794] versus AUCTNM 0.666 [0.590–0.741]; Supplementary Figure 4B), and WZRCH (AUC AHR 0.731 [0.689–0.773] versus AUCTNM 0.683 [0.643–0.723]; Supplementary Figure 4C), which were also validated by DCA analyses (Supplementary Figure 4D-F).

Finally, we present the clinical impact of our AHR model against the existing AJCC/UICC 8th edition TNM classification (Table 3). Our AHR risk stratification system was able to re-classify patients from every TN-stage group (other than for stage I) in the SYSUCC-Training cohort, thereby highlighting the intra-group heterogeneity in terms of OS-likelihood by the current stage classification system.

**Discussion**

Conventional TNM-stage classification represents a sound system for the clinical stratification of patients to inform on prognosis for NPC. Nonetheless, it is limited by the simplistic consideration of primary tumor extent and regional nodal burden, which may not capture the biological complexity of NPC.19–21 Novel prognostic tools integrating clinical and molecular (cfEBV DNA) parameters for NPC are not yet implemented in

---

**Table 3.** Distribution of patients in the AHR groups, compared with the 8th edition TNM classification system in the training cohort.

| 8th UICC/AJCC | N   | Risk group |
|---------------|-----|------------|
| Stage I       | 514 (5.6%) | AHR1: 514 (100%) |
| Stage II      | 1,644 (17.8%) | AHR2A: 641 (39.0%) |
|               |       | AHR2B: 696 (42.3%) |
|               |       | AHR3: 307 (18.7%) |
| Stage III     | 4,249 (45.9%) | AHR2A: 415 (9.8%) |
|               |       | AHR2B: 1,527 (35.9%) |
|               |       | AHR3: 1,657 (39.0%) |
|               |       | AHR4: 650 (15.3%) |
| Stage IVA     | 2,852 (30.8%) | AHR3: 115 (4.0%) |
|               |       | AHR4: 1,461 (51.3%) |
|               |       | AHR5: 1,276 (44.7%) |

AHR, adjusted hazard ratio; AJCC, American Joint Committee on Cancer; UICC, Union for International Cancer Control.
the clinic, partly because of model impracticality and limited sample size of these studies. To address this unmet need, we adopted a big-data approach by assembling the largest dataset reported to date of 10,149 NPC cases, all of whom had pre-treatment cfEBV DNA quantification and diagnostic staging that were centrally performed. We defined a robust cutoff of ≥2,000 EBV DNA copies for risk discretization and applied an intuitive two-tiered classification schema to integrate cfEBV DNA titer and conventional T- and N-categories in the SYSUCC-Training cohort of 9,259 NPC patients. Apart from using a biostatistical approach of classifying patients, we also considered the clinical principles of the disease that underpin the development of the current AJCC/UICC 8th edition TNM-stage classification, and divided patients into six risk groupings that are more homogeneous in terms of risk of death within each subgroup. We identified that the AHR model was most superior for prognostication against the published RPA models, and the TNM-stage classification. Our proposed AHR risk stratification criteria showed comparable performance for prognostication of OS in two separate cohorts; this is impressive considering that these cohorts varied in terms of clinical characteristics and treatment parameters. Moreover, the ability to stratify patients in the validation cohorts was observed, despite using cfEBV DNA readings that were derived using assays performed at different institutions (the SYSUCC was harmonized with the international standard testing method). Notably, our model was also validated in a subset of 237 high-risk patients from a prospective clinical trial of induction TPF that was exclusively conducted in high-risk, locoregionally advanced NPC patients (5-year OS ranging from 56.3% [AHR5] to 98.0% [AHR2B]). Based on these findings, we have presented a new risk classification system combining conventional TN-categories and baseline cfEBV DNA titer for non-metastatic NPC that outperforms the existing stage classification system using the largest dataset reported to date.

Contrary to cfEBV DNA quantification at the mid-point and conclusion of treatment, the proposition to incorporate pretreatment cfEBV DNA for prognostication in NPC is contentious for several reasons; this includes the reporting of different cutoffs for risk discretization, which is further compounded by the possibility of inter-laboratory variation. To address these issues, we relied on a large dataset of 9,259 cases for which cfEBV DNA was quantified at a single clinical laboratory. Our PCR-based assay had high sensitivity (>90% detection at 500 copies) and limited within-run (<10%) and between-day (<20%) variation, and was recently validated under the premise of a global harmonization effort. Next, we observed a similar linear dose (cfEBV DNA load)-response relationship for HR\textsubscript{OS}, HR\textsubscript{DFS}, HR\textsubscript{DMFS} and coincidentally derived comparable cutoffs of 3.32–3.34 lg(cfEBV DNA) for HR > 1.0 for the respective endpoints. The choice of 2,000 copies as a threshold is further corroborated by our sensitivity analysis showing the stability of HR\textsubscript{OS} (2.33–2.88) when using cutoffs ranging from 2,000 to 2,000,000 copies. Taken together, our data addressed the perennial issues that hinder the mainstream incorporation of pretreatment cfEBV DNA for prognostication.

Ultimately, our work begs the question regarding the implications of our new and more refined risk classification system on the treatment of NPC patients. Currently, the National Comprehensive Cancer Network (NCCN) guidelines propose concurrent chemoradiotherapy (CCRT), CCRT + adjuvant chemotherapy (ACT) or neoadjuvant chemotherapy (NACT) + CCRT as reasonable treatment options for TNM stage II to IVA patients, but the choice of appropriate chemotherapy intensity to combine with RT remains contentious. While it extends beyond the scope of our study findings, we propose that the new system using cfEBV DNA and TNM stage potentially helps to optimize clinical trial design and patient recruitment to better streamline treatment recommendations for NPC patients. Here, we proposed clinical trials to compare efficacy of RT alone versus CCRT for patients with AHR 2A to establish an optimal treatment strategy for this low-risk subgroup to avoid over-treatment, while considering trials comparing CCRT versus NACT + CCRT/CCRT + ACT among patients with AHR 2B and AHR 3 to ensure adequate intensity of treatment for this intermediate-risk subgroup. In addition, it is notable that the survival of patients with AHR 4 and AHR5 remains unsatisfactory; we propose conducting of clinical trials for investigations of new drugs or therapies to improve the prognosis of these patients.

Several caveats of our findings ought to be highlighted. Foremost, treatment regimens were not included as covariates for AHR model construction. This was because interventions are not baseline attributes, and there was no control over the allocation of interventions (such as randomization), which would confound any comparative analyses.
between the different AHR groups. It is also based on this reasoning that we did not investigate the association of our AHR risk groups with treatment efficacy. This analysis is beyond the scope of our study, especially given the potential treatment biases. Prospective clinical trials are needed to investigate the appropriate treatment strategy for each AHR risk group. Next, we acknowledged the clinical heterogeneity between our training and validation cohorts. Of note, the cfEBV DNA levels in the WZRCH cohort were lower than the levels observed in the SYSUCC-Training cohort (median cfEBV DNA: 500 copies versus 2,050 copies). This variation could be explained by protocol variations between laboratories, but regardless, we were able to show that the cutoff of 2,000 copies was still able to identify two risk groups with disparate survival in the WZRCH cohort (Supplementary Figure 5A–D). This indirectly supports the robustness of our proposed cfEBV DNA cutoff of 2,000 copies, even with different cfEBV DNA molecular assays.

In conclusion, we successfully defined an optimal cfEBV DNA cutoff at baseline and combined the biomarker with conventional TN-categories to construct a new AHR risk classification system for M0 NPC. Our model stratifies patients into six risk groups with improved intra-group homogeneity for OS compared with the existing AJCC/UICC 8th edition TNM-staging system. This new system could be the basis for future strategies of clinical trial designing and patient recruitment for better streamlining treatments in NPC patients.

Acknowledgements
Fo-Ping Chen, Li Lin, Jin-Hui Liang, Sze Huey Tan, and Enya H.W. Ong contributed equally to this work. Melvin L.K. Chua and Ying Sun are co-senior authors.

Author contributions
Conception and design: Ying Sun, Melvin L.K. Chua, Fo-Ping Chen
Financial support: Ying Sun, Melvin L.K. Chua
Administrative support: Ying Sun, Melvin L.K. Chua
Provision of study materials or patients: Fo-Ping Chen, Ying-Shan Luo
Collection and assembly of data: Fo-Ping Chen, Guan-Qun Zhou, Ying-Shan Luo, Li Lin, Jia Kou, Jin-Hui Liang, Tian-Sheng Gao, Bin Deng, Sze Huey Tan, Enya H.W. Ong, Luo Huang
Data analysis and interpretation: Fo-Ping Chen, Ying-Shan Luo, Guan-Qun Zhou, Sze Huey Tan, Enya H.W. Ong, Hai-Tao Wang, Adelene Y.L. Sim

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authenticity of this article has been validated by uploading the key raw data onto the Research Data Deposit public platform (www.researchdata.org.cn), with the approval number as RDDA2020001517.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We thank Yiducloud (Beijing) Technology Ltd. for supporting part of the data extraction and processing. This study was supported by grants from the National Natural Science Foundation of China (81930072), Key-Area Research and Development Program of Guangdong Province (2019B020230002), the Health & Medical Collaborative Innovation Project of Guangzhou City, China (NO. 201604020003, 201803040003), the Special Support Program of Sun Yat-sen University Cancer Center (16zxxtle06), the Natural Science Foundation of Guangdong Province (No. 2017A030312003), the National Key R&D Program of China (2016YFC0902000), and Innovation Team Development Plan of the Ministry of Education (No. IRT_17R110). MLKC is supported by the National Medical Research Council Singapore Clinician Scientist Award (NMRC/CSA-INV/0027/2018), National Research Foundation Proton Competitive Research Program (NRF-CRP17-2017-05), Ministry of Education Tier 3 Academic Research Fund (MOE2016-T3-1-004), the Duke-NUS Oncology Academic Program Goh Foundation Proton Research Programme, NCCS Cancer Fund, and the Kua Hong Pak Head and Neck Cancer Research Programme.

Role of the funding source
The sponsors had no role in shaping study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to the data and had final responsibility for the decision to submit the manuscript for publication.
References

1. Lin JC, Wang WY, Chen KY, et al. Quantification of plasma Epstein-Barr virus DNA in patients with advanced nasopharyngeal carcinoma. N Engl J Med 2004; 350: 2461–2470.

2. Li YQ, Khin NS and Chua MLK. The evolution of Epstein-Barr virus detection in nasopharyngeal carcinoma. Cancer Biol Med 2018; 15: 1–5.

3. Chan KCA, Woo JKS, King A, et al. Analysis of plasma Epstein-Barr virus DNA to screen for nasopharyngeal cancer. N Engl J Med 2017; 377: 513–522.

4. Lo YM, Chan LY, Chan AT, et al. Quantitative and temporal correlation between circulating cell-free Epstein-Barr virus DNA and tumor recurrence in nasopharyngeal carcinoma. Cancer Res 1999; 59: 5452–5455.

5. Wang WY, Twu CW, Lin WY, et al. Plasma Epstein-Barr virus DNA screening followed by 18F-fluoro-2-deoxy-D-glucose positron emission tomography in detecting posttreatment failures of nasopharyngeal carcinoma. Cancer 2011; 117: 4452–4459.

6. Lo YM, Leung SF, Chan LY, et al. Plasma cell-free Epstein-Barr virus DNA quantitation in patients with nasopharyngeal carcinoma. Correlation with clinical staging. Ann N Y Acad Sci 2000; 906: 99–101.

7. Tang LQ, Li CF, Li J, et al. Establishment and validation of prognostic nomograms for endemic nasopharyngeal carcinoma. J Natl Cancer Inst 2016; 108: djw291.

8. Guo R, Tang LL, Mao YP, et al. Proposed modifications and incorporation of plasma Epstein-Barr virus DNA improve the TNM staging system for Epstein-Barr virus-related nasopharyngeal carcinoma. Cancer 2019; 125: 79–89.

9. Leung SF, Zee B, Ma BB, et al. Plasma Epstein-Barr viral deoxyribonucleic acid quantitation complements tumor-node-metastasis staging

prognostication in nasopharyngeal carcinoma. J Clin Oncol 2006; 24: 5414–5418.

10. Lee VH, Kwong DL, Leung TW, et al. The addition of pretreatment plasma Epstein-Barr virus DNA into the 8th edition of nasopharyngeal cancer TNM stage classification. Int J Cancer 2019; 144: 1713–1722.

11. Sun Y, Li WF, Chen NY, et al. Induction chemotherapy plus concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in locoregionally advanced nasopharyngeal carcinoma: a phase 3, multicentre, randomised controlled trial. Lancet Oncol 2016; 17: 1509–1520.

12. Pan JJ, Ng WT, Zong JF, et al. Proposal for the 8th edition of the AJCC/UICC staging system for nasopharyngeal cancer in the era of intensity-modulated radiotherapy. Cancer 2016; 122: 546–558.

13. Therneau TM and Grambsch PM. Modeling survival data: extending the Cox model. New York: Springer, 2000.

14. Molinari N, Daures JP and Durand JF. Regression splines for threshold selection in survival data analysis. Stat Med 2001; 20: 237–247.

15. Heinzl H and Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. Comput Methods Programs Biomed 1997; 54: 201–208.

16. Xu W, Shen XW, Su J, et al. Refining evaluation methodology on TNM stage system: assessment on HPV-related oropharyngeal cancer. Austin Biom and Biostat 2015; 2: 1014.

17. Sun X, Su S, Chen C, et al. Long-term outcomes of intensity-modulated radiotherapy for 868 patients with nasopharyngeal carcinoma: an analysis of survival and treatment toxicities. Radiother Oncol 2014; 110: 398–403.

18. Setton J, Han J, Kannarunimit D, et al. Long-term patterns of relapse and survival following definitive intensity-modulated radiotherapy for non-endemic nasopharyngeal carcinoma. Oral Oncol 2016; 53: 67–73.

19. Ganz P, Heidecker B, Hveem K, et al. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. JAMA 2016; 315: 2532–2541.

20. Fraser M, Sabelnykova VY, Yamaguchi TN, et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature 2017; 541: 359–364.

21. Li YY, Chung GT, Lui VW, et al. Exome and genome sequencing of nasopharynx cancer
identifies NF-κB pathway activating mutations. *Nat Commun* 2017; 8: 14121.

22. Ly JW, Chen YP, Zhou GQ, *et al.* Liquid biopsy tracking during sequential chemo-radiotherapy identifies distinct prognostic phenotypes in nasopharyngeal carcinoma. *Nat Commun* 2019; 10: 3941.

23. Le QT, Zhang Q, Cao H, *et al.* An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. *Clin Cancer Res* 2013; 19: 2208–2215.

24. National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology. Head and neck cancer, version 1, https://www.nccn.org/professionals/physician_gls/f_guidelines.asp (2021, accessed 9 November 2020).

25. Chen YP, Ismaila N and Chua MLK, *et al.* Chemotherapy in combination with radiotherapy for definitive-intent treatment of stage II-IVA nasopharyngeal carcinoma: CSCO and ASCO guideline. *J Clin Oncol* 2021; 39: 840–859.

26. Chua MLK, Wee JTS, Hui EP, *et al.* Nasopharyngeal carcinoma. *Lancet* 2016; 387: 10022.

27. Zhang Y, Chen L, Hu GQ, *et al.* Gemcitabine and cisplatin induction chemotherapy in nasopharyngeal carcinoma. *N Engl J Med* 2019; 381: 1124–1135.