The Prognostic value of Plasma EBV DNA and Tumor Response to Neoadjuvant Chemotherapy in Advanced-Stage Nasopharyngeal Carcinoma

Li-Ting Liu, M.D.,*† Lin-Quan Tang, M.D., Ph.D.,*† Qiu-Yan Chen, M.D., Ph.D.,*† Lu Zhang, M.D.,*† Shan-Shan Guo, M.D.,*† Ling Guo, M.D., Ph.D.,*† Hao-Yuan Mo, M.D., Ph.D.,*† Chong Zhao, M.D., Ph.D.,*† Xiang Guo, M.D., Ph.D.,*† Ka-Jia Cao, M.D., Ph.D.,*† Chao-Nan Qian, M.D., Ph.D.,*† Mu-Sheng Zeng, M.D., Ph.D. * Jin-Xin Bei, M.D., Ph.D. * Ming-Huang Hong, M.D., Ph.D. *†† Jian-Yong Shao, M.D., Ph.D. *|| Ying Sun, M.D., Ph.D.,*# Jun Ma, M.D., Ph.D.,*# and Hai-Qiang Mai, M.D., Ph.D.,*†

Author Affiliations:

* Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, China

† Department of Nasopharyngeal Carcinoma, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

†† Good Clinical Practice center, Sun Yat-sen University Cancer Center, Guangzhou 510060, P. R. China;

|| Department of Molecular Diagnostics, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

Department of Radiation Oncology, Sun Yat-sen University Cancer Center, Guangzhou 510060, P. R. China.

Li-Ting Liu and Lin-Quan Tang contributed equally to this article.

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Corresponding author: Hai-Qiang Mai, M.D., Ph.D., Department of Nasopharyngeal Carcinoma, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou 510060, P. R. China; E-mail: maihq@sysucc.org.cn; **Tel.:** +86-20-87343643; **Fax:** +86-20-87343392

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Authors' Disclosures of Potential Conflicts of Interest

The authors have no potential conflicts of interest to declare.

Key words: nasopharyngeal carcinoma, EBV DNA, tumor response

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Abstract

Purpose

To explore the prognostic value of the plasma load of Epstein-Barr viral (EBV) DNA and the tumor response to neoadjuvant chemotherapy (NACT) in advanced-stage nasopharyngeal carcinoma (NPC).

Patients and Methods

A total of 185 consecutive patients with Stage III-IVb NPC treated with NACT followed by concurrent chemoradiotherapy (CCRT) were prospectively enrolled. The primary endpoint was progression-free survival (PFS), and the secondary endpoints included locoregional relapse-free survival (LRFS) and distant metastasis-free survival (DMFS).

Results

EBV DNA was detected in 165 (89%) patients before treatment but was undetectable in 127 (69%) patients after NACT. Detectable EBV DNA levels after NACT were correlated with poor prognosis (3-year PFS, 71.8% VS 85.2%, $P = 0.008$ and 3-year DMFS, 82.5% VS 92.3%, $P = 0.013$). An unsatisfactory tumor response (SD/PD) after NACT was also correlated with poor clinical outcome (3-year PFS, 71.1% VS 85.9%, $P = 0.005$ and 3-year LRFS, 82.7% VS 93.5%, $P = 0.012$). Multivariate analysis showed that the EBV DNA level

after NACT (HR=2.31, 95%CI=1.18-4.54, $P=0.015$) and the tumor response to NACT (HR=2.84, 95%CI=1.42-5.67, $P=0.003$) were both significant prognostic factors for PFS. Multivariate analysis also showed that EBV DNA after NACT was the only significant predictor of DMFS (HR=2.99, 95%CI=1.25-7.15, $P=0.014$) and that tumor response to NACT was the only significant predictor of LRFS (HR=3.31, 95%CI=1.21-9.07, $P=0.020$).

Conclusion

Detectable EBV DNA levels and an unsatisfactory tumor response (SD/PD) after NACT serve as predictors of poor prognosis for advanced-stage NPC patients. These findings will facilitate further risk stratification and/or early treatment modification before CCRT.

Introduction

Nasopharyngeal carcinoma (NPC) is an endemic malignancy in South China and South Asia that is distinct from other head and neck cancers based on its epidemiology, biological characteristics and treatment modalities (1,2). Radiation therapy (RT) is the primary treatment for NPC. For non-metastatic loco-regionally advanced NPC, previous studies have demonstrated that concurrent chemoradiotherapy (CCRT) is more effective than RT alone (3,4). CCRT has been established as the standard of care for advanced NPC, but recently, the addition of neoadjuvant chemotherapy (NACT) to the previously established regimen has been found to be an attractive multidisciplinary approach. Now, evidence supporting the use of NACT prior to definitive CCRT is increasing, and this approach is associated with decreased risks of distant metastases, translating to improved clinical outcomes (5-11). Despite the survival benefit obtained from modern treatment modalities and techniques, 20%-30% of patients die due to distant and/or loco-regional relapse(12). If patients at high risk of treatment failure could be identified, they could be treated with an intense treatment strategy. Alternatively, patients in the low-risk group could be spared the toxicities related to excessive treatment.

The plasma level of Epstein-Barr virus (EBV) DNA, as measured by real-time

quantitative polymerase chain reaction (PCR), has been reported to be associated with NPC (13). Previous studies showed that the plasma level of EBV DNA can be used for clinical and molecular surveillance of NPC patients (14). EBV DNA levels before treatment (pre-DNA), EBV DNA levels after CCRT/RT (post-DNA) and the fluctuation of the EBV DNA levels in NPC patients treated with RT have significant prognostic value with respect to clinical outcome (15-19). EBV DNA load has been observed to display a downward trend over the course of treatment. Likewise, gross tumor volume exhibited a shrinking trend during treatment. Previous studies of other cancer types have also reported that the response to initial chemotherapy is related to outcome (20-23). Given such observations, we assumed that patients could be separated into high- and low-risk groups after NACT based on EBV DNA level and/or the tumor response. The high-risk group was hypothesized to be correlated with residual post-CCRT DNA, residual post-CCRT lesions and poor clinical outcome. Therefore, EBV DNA level along with tumor response to NACT may be a new prognostic tool to help improve the prognostic stratification of advanced-stage NPC patients and may be used to tailor subsequent therapy after completion of NACT. This prognostic tool may help in the design and modification of the CCRT regimen.

Materials and Methods

Patients

A total of 185 previously untreated, biopsy-proven NPC patients with stage

III-IVb disease (according to the 7th edition of the AJCC/UICC criteria) and WHO 2-or 3-type disease were prospectively enrolled between January 2009 and December 2012 (Table 1). The eligibility criteria were as follows: (1) no evidence of distant metastases, (2) ECOG performance status grade of 0 or 1, (3) age of 18 years or older, (4) absence of secondary malignancy, pregnancy or lactation, and (5) consistent hematologic sample results. All patients were evaluated via a complete physical examination, magnetic resonance imaging (MRI) of the nasopharynx and neck, chest X-ray, abdominal sonography, electrocardiography, bone scan, fiber-optic nasopharyngoscopy and complete blood sampling, including differential cell counts, biochemical profile, and EBV serology. This study was approved by the Clinical Research Committee of the study institute, and patients were required to provide written informed consent before entering the study.

Chemotherapy and Radiation Therapy

All patients underwent NACT, consisting of cisplatin (80-100mg/m² D1) and 5-fluorouracil (800-1000 mg/m², 120 hours continuous intravenous infusion) administered every 3 weeks for 2 cycles. This was followed by radiation therapy using the intensity-modulated radiation therapy (IMRT) technique and concurrent cisplatin (80-100mg/m²) chemotherapy administered on days 1, 22, and 43 of the radiation therapy (3,24). The design of the IMRT plan was based on previous studies (25-27) (Supplementary Materials). All patients were treated according to the principles of treatment for NPC patients at the study

institute.

Quantification of Plasma EBV DNA Levels and Tumor Response Assessment

Plasma EBV DNA sample collection and plasma EBV DNA copy number measurement via real-time quantitative polymerase chain reaction (PCR) were performed before the initiation of treatment, after the completion of NACT and 1 month post-CCRT therapy. The cutoff level of EBV DNA after NACT was scored based on a detectable/undetectable scale (a cutoff value of 0 copies per milliliter), as no validation dataset was included in the present study. The median EBV DNA concentration decreased to 0 copies per milliliter after the completion of CCRT, thus, the post-CCRT DNA cutoff value was also based on a detectable/undetectable cutoff (a cutoff value of 0 copies per milliliter), while the pre-DNA cutoff value (4000 copies per milliliter) had been previously established (16,28). No attempt was made to perform repeated analyses using alternative cutoffs. All patients received a 2nd MRI after NACT and a 3rd MRI after CCRT to evaluate tumor response. Then, the patients were classified by the investigator according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (29) as complete response (CR), partial response (PR), stable disease (SD), disease progression (PD) or not assessable. If the primary tumor exhibited a CR or a PR to NACT, we considered the patient to be a favorable responder.

Outcome and Follow-up

Our primary study endpoint was progression-free survival (PFS), defined as the time to first failure at any site or death from any cause or patient censoring at the date of the last follow-up. Secondary endpoints included locoregional relapse-free survival (LRFS), defined as the period from the first day of treatment to the date of loco-regional relapse or patient censoring at the date of the last follow-up, and distant metastasis-free survival (DMFS), defined as the period from the date of treatment to the date of distant relapse or patient censoring at the date of the last follow-up. The patients were evaluated at least once every 3 months during the first 3 years after the completion of treatment and every 6 months thereafter until death. Nasopharyngoscopy, MRI of the head and neck, chest radiography, abdominal sonography, and plasma EBV DNA measurement were routinely performed annually or upon clinical indication of tumor relapse.

Statistical Analysis

The relationship between tumor response and the plasma load of EBV DNA was evaluated by the χ^2 test. Kaplan–Meier survival curves were used to analyze the time-to-event endpoints, and the log-rank test was used to compare the differences between the two subsets. A Cox proportional hazard model was used for the multivariate analyses of the following variables: EBV DNA after NACT, T stage, N stage, age and gender. Because EBV DNA load after NACT and tumor response to NACT were significantly correlated, a separate multivariate analysis was performed, including tumor response to

NACT, T stage, N stage, age and gender. The analyses were performed using the statistical software package SPSS 17.0 (SPSS, Chicago, IL). All statistical tests were two-sided, and a P value of less than 0.05 was considered significant.

Results

One hundred and eighty-five patients were prospectively enrolled between January 2009 and December 2012. Seven patients died, 22 patients exhibited distant metastasis, and 17 patients developed local or regional recurrence (Table 4). The median follow-up duration was 33 months (range: 8-49 months). Additionally, 165 (89%) of the patients displayed detectable (>0 copies per milliliter) EBV DNA levels before NACT. However, after NACT, a total of 127 patients displayed undetectable ($=0$ copies per milliliter) EBV DNA levels, among whom 99 patients exhibited CR/PR and 28 exhibited SD/PD to NACT. Among the other 58 patients who displayed detectable EBV DNA levels after NACT, 26 patients exhibited CR/PR and 32 patients exhibited SD/PD to NACT. For all patients, the response to NACT was as follows: two (1%) patients exhibited a CR, 123 (66%) patients exhibited a PR, 59 (32%) patients exhibited SD, and 1 (1%) patient exhibited PD. A detectable EBV DNA level (>0 copies per milliliter) after NACT was significantly correlated with an unsatisfactory tumor response to NACT ($P<0.001$) (Table 2). After the completion of CCRT, eight patients had residual post-DNA and 40 patients had a residual lesion in either the nasopharynx or a lymph node. For patients

displaying detectable or undetectable EBV DNA after NACT, the proportion of patients displaying detectable post-CCRT DNA (>0 copies per milliliter) was 6/58 and 2/127 after CCRT, respectively ($P=0.018$). The EBV DNA level after NACT predicted the post-CCRT EBV DNA level in 75% of cases (Table 2). For the patients who did or did not exhibit an unsatisfactory response (SD/PD) to NACT, the proportion of patients who exhibited a post-CCRT residual lesion was 22/60 (36.6%) and 18/125 (14.4%), respectively ($P=0.001$). The patients who exhibited an unsatisfactory response to NACT were significantly more likely to exhibit residual disease after completion of treatment (CCRT).

Relationship between EBV DNA level after NACT and clinical outcome

The 3-year PFS and 3-year DMFS of patients with a detectable EBV DNA level after NACT were significantly worse than those of patients with a undetectable EBV DNA level after NACT [3-year PFS, 71.8% (95%CI =60.0%-83.5%) VS 85.2% (95%CI =78.5%-91.9%), $P=0.008$ (Fig 1) and 3-year DMFS, 82.5% (95%CI =72.7%-92.3%) VS 92.3% (95%CI =87.4%-97.2%), $P=0.013$ (Fig 1)]. However, 3-year LRFS was not significantly different between these two patient groups [86.9% (95%CI =77.9%-95.9%) VS 91.3% (95%CI =86.0%-96.6%), $P=0.333$ (Fig 1)].

Relationship between tumor response to NACT and clinical outcome

Unsatisfactory tumor response also predicted worse 3-year PFS and 3-year LRFS compared to a satisfactory tumor response [3-year PFS, 71.1% (95%CI =59.5%-82.7%) VS 85.9% (95%CI =79.4%-92.4%), $P=0.005$ (Fig 1); 3-year

LRFS, 82.7% (95%CI =72.9%-92.5%) VS 93.5% (95%CI =88.8%-98.2%), $P=0.012$ (Fig 1)]. However, 3-year DMFS was not significantly different between these two patient groups [86.6% (95%CI = 78.0%-95.2%) VS 90.6% (95%CI= 85.3%-95.9%), $P=0.270$ (Fig 1)].

Univariate analysis revealed that the levels of EBV DNA pre-treatment (HR=2.79, 95%CI=1.27-6.13, $P=0.010$), post-CCRT therapy (HR=5.74, 95%CI=2.20-14.95, $P<0.001$) and after NACT (HR=2.35, 95%CI=1.22-4.51, $P=0.011$) and the tumor response to NACT (HR=2.46, 95%CI=1.28-4.74, $P=0.007$) were significantly associated with PFS. Univariate analysis also demonstrated that the levels of EBV DNA post-CCRT therapy (HR=5.31, 95%CI= 1.54-18.26, $P=0.008$) and after NACT (HR=2.77, 95%CI=1.20-6.40, $P=0.018$) were significantly associated with DMFS and that tumor response to NACT (HR=3.23, 95%CI=1.23-8.50, $P=0.017$) was significantly associated with LRFS. As our goal was to identify a new time point during treatment before CCRT to evaluate the efficacy of EBV DNA level as a prognostic indicator, the levels of EBV DNA at pre-treatment and post-CCRT therapy, which had been studied previously, were excluded from the multivariate analysis. In addition, as mentioned above, EBV DNA level after NACT was significantly correlated with tumor response to NACT ($P<0.001$) (Table 2), meaning that these two factors modify each other's effect on the risk of poor outcome. EBV DNA level after NACT and tumor response to NACT were incorporated in multivariate analyses separately. A multivariate Cox

proportional-hazards model was generated that incorporated the following variables: EBV DNA level after NACT, T stage, N stage, age and gender. In the above Cox regression analysis, the EBV DNA level after NACT was the only significant prognosticator associated with PFS (HR=2.31, 95%CI=1.18-4.54, $P=0.015$) and DMFS (HR=2.99, 95%CI=1.25-7.15, $P=0.014$). A separate multivariate Cox regression analysis incorporated tumor response to NACT, T stage, N stage, age and gender and demonstrated that tumor response to NACT was the only significant prognosticator associated with PFS (HR=2.84, 95%CI=1.42-5.67, $P=0.003$) and LRFS (HR=3.31, 95%CI=1.21-9.07, $P=0.020$) (Table 4).

Discussion

To the best of our knowledge, no previous study has explored the prognostic value of EBV DNA levels combined with tumor response after the course of NACT for NPC patients. This lack of understanding hinders early intervention using a risk-adapted treatment strategy for patients treated with both NACT and CCRT. Due to the development of modern treatment modalities, such as IMRT, tomotherapy, targeted therapy and biotherapy, individualized treatment has become particularly important, as these treatments are expensive and somewhat toxic. Thus, it is necessary to integrate various biomarkers and diagnostic imaging assays to determine the risk of poor outcome in individual patients. The goal of our study was to use EBV DNA level and tumor response after NACT as outcome predictors to identify the subpopulation that was

sensitive to chemotherapy as early as possible in order to guide the treatment strategy before the implementation of CCRT and improve survival. Intriguingly, according to our findings, after NACT, patients who had an undetectable EBV DNA level had significantly reduced risks of disease progression and distant metastasis compared to those with a detectable EBV DNA level. Meanwhile, patients who exhibited a CR/PR after NACT had lower risks of disease progression and locoregional relapse than those who exhibited SD/PD. It is inspiring to see that EBV DNA level after NACT and tumor response to NACT are two complementary factors for the stratification of patients with high risk of treatment failure. To be more specific, plasma EBV DNA level represents tumor DNA level and can originate from necrotic or apoptotic cancer cells. The presence of viral DNA in viral-related tumors offers a distinct marker for detection in the blood, thus, patients with detectable EBV DNA levels after NACT most frequently experienced distant failures. While, tumor response to chemotherapy mainly represented the change in gross tumor volume, the tumor volume of patients who exhibited SD/PD to NACT shrunk insignificantly or increased. Therefore, this subgroup of patients seemed insensitive to chemotherapy and, thus, was the subgroup that experienced the most locoregional failures. Early identification of higher-risk patients may be critical for improving outcomes and will be useful for risk stratification before CCRT. Specifically, patients with detectable EBV DNA post-NACT most frequently experienced distant failure; therefore, this subgroup requires intense treatment

after NACT: (1) the administration of an additional target agent, such as bevacizumab, which is a known chimeric monoclonal antibody that targets VEGF (30), (2) the inclusion of adjuvant chemotherapy, or (3) the addition of immunotherapy. For example, the EBV viral antigens expressed by NPC tumor cells have become attractive targets for immunotherapy as another form of 'targeted therapy' in this era of personalized management (31,32). We are initiating a phase II study of adoptively transferred tumor-infiltrating lymphocyte (TIL) immunotherapy following CCRT in patients with high risk of treatment failure (33). Adoptive immunotherapy is a potential avenue for patients in the high-risk group. Patients who do not achieve a CR/PR post-NACT most frequently experienced locoregional failures; therefore, they require the following: (1) an increased dose of radiation therapy, (2) combined use of a second antitumor drug, such as paclitaxel, to enhance the radiosensitizing effect of CCRT, or (3) the administration of an EGFR inhibitor during CCRT. For the low-risk group, the treatment intensity could be reduced to avoid unnecessary toxicities and cost. As the initial treatment of NPC is very important, determining the appropriate radiation dose and course of chemotherapy is crucial, and EBV DNA load after the completion of NACT and tumor response to NACT are ideal markers for providing an adequate basis for determining the optimal treatment regime and radiation plan. Selecting the treatment strategy based on EBV DNA level and tumor response to NACT may enable further improvement in NPC prognosis. However, the effect of NACT on

NPC remains controversial. The National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (version 2.2014) recommends NACT followed by CCRT for stage II-IVb NPC, and this recommendation is supported by level III evidence. Hui EP, et al. reported a phase II study that revealed a positive impact of NACT combined with CCRT on survival (6). Bossi et al. also suggested that for patients with advanced stage nasopharyngeal carcinoma in endemic areas, NACT followed by high-dose cisplatin IMRT is a promising treatment strategy (8). Mario Airolidi et al. showed that NACT ensured excellent loco-regional disease control and overall survival with a low incidence of distant metastases (7). A meta-analysis carried out by OuYang PY et al. showed that NACT can effectively enhance OS and reduce DMFS (9). Chua et al. analyzed two Phase III studies and showed that NACT followed by RT was associated with a modest but significant decrease in relapse and an improvement in disease-specific survival in advanced-stage NPC; however, no improvement in overall survival was observed (34). Fountzilas et al. reported that NACT followed by CCRT did not significantly improve response rate and/or survival compared to CCRT alone (35). The preliminary results of trial NPC-0501 indicate that the benefit of changing to an induction-concurrent sequence remains uncertain (24). Thus, whether NACT should be included in the standard treatment protocol remains uncertain. Despite all of these findings, the potential advantages of NACT include better tolerability and early

eradication of micro-metastases. Furthermore, early tumor shrinkage could help attain better coverage of the gross tumor and minimize the changes in the contour of the neck during the RT course. A previous study demonstrated that NACT consisting of cisplatin–fluorouracil could significantly reduce gross tumor volume and the volume that failed to reach 70 Gy of radiation, leading to a significant increase in tumor control probability (25). In everyday clinical practice, treating loco-regionally advanced NPC with cisplatin-based NACT/CCRT is relatively safe and has long-term efficacy (10). Therefore, our results can be applied to patients who receive both NACT and CCRT.

The first limitation of our study is that the data were obtained exclusively from one center; therefore, these results must be validated by other datasets. The second limitation is that our study was performed from 2009 to 2012, and the median follow-up duration was 33 months (range: 8-48 months). A longer follow-up period is necessary to evaluate the long-term outcomes of these patients and to validate our results.

In conclusion, our results demonstrate that detectable EBV DNA and SD/PD after NACT are correlated with poor clinical outcome. EBV DNA level and tumor response after NACT may complement each other for assessing the outcome of NPC patients who received both NACT and CCRT. Therefore, these markers could be used to identify patients at high risk of treatment failure and to perform further risk stratification and/or early treatment modification before implementation of CCRT. Further investigation is necessary to confirm

our findings.

References

1. Choa G. Nasopharyngeal carcinoma. Some observations on the clinical features and technique of examination. *Pacific medicine and surgery* 1967;75:172-174.
2. Cao SM, Simons MJ, Qian CN. The prevalence and prevention of nasopharyngeal carcinoma in china. *Chinese journal of cancer* 2011;30:114-119.
3. Al-Sarraf M, LeBlanc M, Giri PG, et al . Chemoradiotherapy versus radiotherapy in patients with advanced nasopharyngeal cancer: Phase iii randomized intergroup study 0099. *J Clin Oncol* 1998;16:1310-1317.
4. Lin JC, Jan JS, Hsu CY, et al. Phase iii study of concurrent chemoradiotherapy versus radiotherapy alone for advanced nasopharyngeal carcinoma: Positive effect on overall and progression-free survival. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2003;21:631-637.
5. Hong RL, Ting LL, Ko JY, et al. Induction chemotherapy with mitomycin, epirubicin, cisplatin, fluorouracil, and leucovorin followed by radiotherapy in the treatment of locoregionally advanced nasopharyngeal carcinoma. *J Clin Oncol* 2001;19:4305-4313.
6. Hui EP, Ma BB, Leung SF, et al. Randomized phase ii trial of concurrent cisplatin-radiotherapy with or without neoadjuvant docetaxel and cisplatin in advanced nasopharyngeal carcinoma. *J Clin Oncol* 2009;27:242-249.
7. Airoidi M, Gabriele P, Gabriele AM, et al. Induction chemotherapy with carboplatin and taxol followed by radiotherapy and concurrent weekly carboplatin + taxol in locally advanced nasopharyngeal carcinoma. *Cancer chemotherapy and pharmacology* 2011;67:1027-1034.
8. Bossi P, Orlandi E, Bergamini C, et al. Docetaxel, cisplatin and 5-fluorouracil-based induction chemotherapy followed by intensity-modulated radiotherapy concurrent with cisplatin in locally advanced ebv-related nasopharyngeal cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2011;22:2495-2500.
9. OuYang PY, Xie C, Mao YP, et al. Significant efficacies of neoadjuvant and adjuvant chemotherapy for nasopharyngeal carcinoma by meta-analysis of published literature-based randomized, controlled trials. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2013;24:2136-2146.
10. Boscolo-Rizzo P, Tirelli G, Mantovani M, et al. Non-endemic locoregionally advanced nasopharyngeal carcinoma: Long-term outcome after induction plus concurrent chemoradiotherapy in everyday clinical practice. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* 2014.
11. Sun X, Zeng L, Chen C, et al. Comparing treatment outcomes of different chemotherapy sequences during intensity modulated radiotherapy for advanced n-stage nasopharyngeal carcinoma patients. *Radiation oncology (London, England)* 2013;8:265.
12. 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. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 2014;110:398-403.

13. Lo YM, Chan LY, Lo KW, et al. Quantitative analysis of cell-free epstein-barr virus DNA in plasma of patients with nasopharyngeal carcinoma. *Cancer research* 1999;59:1188-1191.
14. Chan KC, Hung EC, Woo JK, et al. Early detection of nasopharyngeal carcinoma by plasma epstein-barr virus DNA analysis in a surveillance program. *Cancer* 2013;119:1838-1844.
15. Lin JC, Wang WY, Chen KY, et al. Quantification of plasma epstein-barr virus DNA in patients with advanced nasopharyngeal carcinoma. *The New England journal of medicine* 2004;350:2461-2470.
16. Chan AT, Lo YM, Zee B, et al. Plasma epstein-barr virus DNA and residual disease after radiotherapy for undifferentiated nasopharyngeal carcinoma. *J Natl Cancer Inst* 2002;94:1614-1619.
17. Hou X, Zhao C, Guo Y, et al. Different clinical significance of pre- and post-treatment plasma epstein-barr virus DNA load in nasopharyngeal carcinoma treated with radiotherapy. *Clinical oncology* 2011;23:128-133.
18. Wang WY, Twu CW, Chen HH, et al. Long-term survival analysis of nasopharyngeal carcinoma by plasma epstein-barr virus DNA levels. *Cancer* 2013;119:963-970.
19. Wang WY, Twu CW, Chen HH, et al. Plasma ebv DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2010;16:1016-1024.
20. Visser JH, Wessels G, Hesselning PB, et al. Prognostic value of day 14 blast percentage and the absolute blast index in bone marrow of children with acute lymphoblastic leukemia. *Pediatric hematology and oncology* 2001;18:187-191.
21. Picci P, Rougraff BT, Bacci G, et al. Prognostic significance of histopathologic response to chemotherapy in nonmetastatic ewing's sarcoma of the extremities. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1993;11:1763-1769.
22. Bacci G, Longhi A, Versari M, et al. Prognostic factors for osteosarcoma of the extremity treated with neoadjuvant chemotherapy: 15-year experience in 789 patients treated at a single institution. *Cancer* 2006;106:1154-1161.
23. Rosen G, Caparros B, Groshen S, et al. Primary osteogenic sarcoma of the femur: A model for the use of preoperative chemotherapy in high risk malignant tumors. *Cancer investigation* 1984;2:181-192.
24. Lee AW, Ngan RK, Tung SY, et al. Preliminary results of trial npc-0501 evaluating the therapeutic gain by changing from concurrent-adjuvant to induction-concurrent chemoradiotherapy, changing from fluorouracil to capecitabine, and changing from conventional to accelerated radiotherapy fractionation in patients with locoregionally advanced nasopharyngeal carcinoma. *Cancer* 2014.
25. Lee AW, Lau KY, Hung WM, et al. Potential improvement of tumor control probability by induction chemotherapy for advanced nasopharyngeal carcinoma. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* 2008;87:204-210.
26. Lin S, Lu JJ, Han L, et al. J. Sequential chemotherapy and intensity-modulated radiation therapy in the management of locoregionally advanced nasopharyngeal carcinoma: Experience of 370 consecutive cases. *BMC cancer* 2010;10:39.
27. Yu Z, Luo W, Zhou QC, et al. Impact of changing gross tumor volume delineation of intensity-modulated radiotherapy on the dose distribution and clinical treatment outcome after induction chemotherapy for the primary locoregionally advanced nasopharyngeal carcinoma. *Chinese journal of cancer* 2009;28:1132-1137.

28. Leung SF, Chan KC, Ma BB, et al. Plasma epstein-barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma. *Ann Oncol* 2014;25:1204-1208.
29. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised recist guideline (version 1.1). *European journal of cancer (Oxford, England : 1990)* 2009;45:228-247.
30. Lee NY, Zhang Q, Pfister DG, et al. Addition of bevacizumab to standard chemoradiation for locoregionally advanced nasopharyngeal carcinoma (rtog 0615): A phase 2 multi-institutional trial. *The Lancet Oncology* 2012;13:172-180.
31. Masmoudi A, Toumi N, Khanfir A, et al. Epstein-barr virus-targeted immunotherapy for nasopharyngeal carcinoma. *Cancer Treat Rev* 2007;33:499-505.
32. Gottschalk S, Heslop HE, Rooney CM. Adoptive immunotherapy for ebv-associated malignancies. *Leukemia & lymphoma* 2005;46:1-10.
33. Li J, Chen QY, He J, et al. Phase i trial of adoptively transferred tumor-infiltrating lymphocyte immunotherapy following concurrent chemoradiotherapy in patients with locoregionally advanced nasopharyngeal carcinoma. *Oncoimmunology* 2015;4:e976507.
34. Chua DT, Ma J, Sham JS, et al. Long-term survival after cisplatin-based induction chemotherapy and radiotherapy for nasopharyngeal carcinoma: A pooled data analysis of two phase iii trials. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005;23:1118-1124.
35. Fountzilas G, Ciuleanu E, Bobos M, et al. Induction chemotherapy followed by concomitant radiotherapy and weekly cisplatin versus the same concomitant chemoradiotherapy in patients with nasopharyngeal carcinoma: A randomized phase ii study conducted by the hellenic cooperative oncology group (hecog) with biomarker evaluation. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2012;23:427-435.

Figure legends

Fig1: Kaplan-Meier curves of progression-free survival, distant metastasis-free survival and locoregional relapse-free survival in NPC patients with undetectable/detectable EBV DNA level after NACT (A, C, E) and satisfactory/unsatisfactory response to NACT (B, D, F).

Figure 1

DNA=0 after NACT	127	123	106	36	3
DNA>0 after NACT	58	52	41	18	1

CR/PR after NACT	125	123	105	40	3
SD/PD after NACT	60	52	52	14	1

DNA=0 after NACT	127	125	112	38	3
DNA>0 after NACT	58	53	44	18	1

CR/PR after NACT	125	122	107	41	3
SD/PD after NACT	60	56	49	15	1

DNA=0 after NACT	127	124	111	36	3
DNA>0 after NACT	58	54	47	20	2

CR/PR after NACT	125	124	110	40	3
SD/PD after NACT	60	54	48	16	2

Tables

Table 1. Characteristics of the patients

Characteristic	Undetectable EBV DNA	Detectable EBV DNA	PR/CR after	SD/PD after
	levels after NACT*	levels after NACT*	NACT	NACT
	No. of patients	No. of patients	No. of patients	No. of patients
Total	127	58	125	60
Gender				
Female	29	14	31	12
Male	98	44	94	48
Age				
Median	44	46.5	43	47
Range	18-68	19-77	18-77	18-72
T stage				
T1	2	0	2	0
T2	15	5	14	6
T3	60	25	65	20
T4	50	28	44	34
N stage				
N0	12	1	9	4
N1	48	21	47	22

N2	55	24	51	28
N3	12	12	18	6
Stage group				
III	71	24	71	24
IVa	44	22	36	30
IVb	12	12	18	6

*Undetectable/detectable EBV DNA levels after NCAT is based on a cutoff value of 0 copies per milliliter.

Table 2. The relationship between the EBV DNA levels post-therapy, the tumor response to NACT and the EBV DNA levels after NACT

	Undetectable EBV DNA levels after NACT*	Detectable EBV DNA levels after NACT*	P value
CR/PR after NACT	99	26	<0.001
SD/PD after NACT	28	32	
Undetectable EBV DNA levels post-CCRT therapy [#]	125	52	0.018
Detectable EBV DNA levels post-CCRT therapy [#]	2	6	

*Undetectable/detectable EBV DNA levels after NCAT is based on a cutoff value of 0 copies per milliliter.

[#]Undetectable/detectable EBV DNA levels post-CCRT therapy is based on a cutoff value of 0 copies per milliliter.

Table 3. Incidence and site of initial treatment failure

	No. of patients %		
	Locoregional only	Distant only	Locoregional and distant
Detectable EBV DNA levels after NACT (n=58)*	7(12.1%)	12(20.7%)	0
Undetectable EBV DNA levels after NACT(n=127)*	10(8%)	10(8%)	0
SD/PD after NACT (n=60)	10(16.7%)	9(15.0%)	0
CR/PR after NACT (n=125)	7(5.6%)	13(10.4%)	0

*Undetectable/detectable EBV DNA levels after NCAT is based on a cutoff value of 0 copies per milliliter.

Table 4. Multivariate analyses of the independent significance of the experimental intervention on clinical outcome: Hazard Ratios, 95% Confidence Intervals, and P Values

Analysis*	PFS	DMFS	LRFS
	HR(95%CI)	HR(95%CI)	HR(95%CI)
	P	P	P
EBV DNA level after NACT, [#] >0 copies/ml VS =0 copies/ml [#]	2.31(1.18-4.54) 0.015	2.99(1.25-7.15) 0.014	1.52(0.57-4.08) 0.408
PR/CR VS SD/PD after NACT	2.84(1.42-5.67)	1.99(0.81-4.88)	3.31(1.21-9.07)

0.003

0.134

0.020

*The analyses were adjusted for other significant covariates, including T stage, N stage, sex and age.

#Undetectable/detectable EBV DNA levels after NCAT is based on a cutoff value of 0 copies per milliliter.