VOLUME 36 · NUMBER 31 · NOVEMBER 1, 2018

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Analysis of Plasma Epstein-Barr Virus DNA in Nasopharyngeal Cancer After Chemoradiation to Identify High-Risk Patients for Adjuvant Chemotherapy: A Randomized Controlled Trial

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Published at jco.org on July 10, 2018.

Clinical trial information: NCT00370890.

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0732-183X/18/3631w-3091w/$20.00

ABSTRACT

Purpose
The contribution of adjuvant chemotherapy after chemoradiation therapy (CRT) in nasopharyngeal cancer (NPC) remains controversial. Plasma Epstein-Barr virus (EBV) DNA is a potential biomarker of subclinical residual disease in NPC. In this prospective, multicenter, randomized controlled trial, we used plasma EBV DNA to identify patients with NPC at a higher risk of relapse for adjuvant chemotherapy.

Patients and Methods
Eligible patients with histologically confirmed NPC of Union for International Cancer Control stage IIB to IVB, adequate organ function, and no locoregional disease or distant metastasis were screened by plasma EBV DNA at 6 to 8 weeks after radiotherapy (RT). Patients with undetectable plasma EBV DNA underwent standard surveillance. Patients with detectable plasma EBV DNA were randomly assigned to either adjuvant chemotherapy with cisplatin and gemcitabine for six cycles (arm 1) or observation (arm 2). Patients were stratified for primary treatment (RT *v* CRT) and stage (II/III *v* IV). The primary end point was relapse-free survival (RFS).

Results
Seven hundred eighty-nine patients underwent EBV DNA screening. Plasma EBV DNA was undetectable in 573 (72.6%) and detectable in 216 (27.4%); 104 (13.2%) with detectable EBV DNA were randomly assigned to arms 1 (n = 52) and 2 (n = 52). After a median follow-up of 6.6 years, no significant difference was found in 5-year RFS rate between arms 1 and 2 (49.3% *v* 54.7%; $P = .75$; hazard ratio for relapse or death, 1.09; 95% CI, 0.63 to 1.89). The level of post-RT plasma EBV DNA correlated significantly with the hazards of locoregional failure, distant metastasis, and death.

Conclusion
In patients with NPC with detectable post-RT plasma EBV DNA, adjuvant chemotherapy with cisplatin and gemcitabine did not improve RFS. Post-RT plasma EBV DNA level should be incorporated as the selection factor in future clinical trials of adjuvant therapy in NPC.

J Clin Oncol 36:3091-3100. © 2018 by American Society of Clinical Oncology

INTRODUCTION

Nasopharyngeal cancer (NPC) is endemic in southern China, including Hong Kong, where nearly all patients harbor the Epstein-Barr virus (EBV) in their tumor cells.[1,2] Most patients with localized NPC will achieve complete clinical remission after curative-intent radiotherapy (RT) or chemoradiation therapy (CRT). Despite a high rate of initial control, disease subsequently will relapse in 30% to 40% of patients either at the local site or, more commonly, with distant metastasis, which prevents long-term cure. In the study conducted by the Prince of Wales and Queen Elizabeth Hospitals in Hong Kong, concurrent cisplatin and RT improved the 5-year overall survival (OS) rate from 58.6% to 70.3%, which confirms this treatment as a standard option in advanced-stage NPC.[3] Although the addition of adjuvant plus concurrent chemotherapy to RT conferred superior survival over RT

ASSOCIATED CONTENT

Appendix
DOI: https://doi.org/10.1200/JCO.2018.77.7847

Data Supplement
DOI: https://doi.org/10.1200/JCO.2018.77.7847

DOI: https://doi.org/10.1200/JCO.2018.77.7847

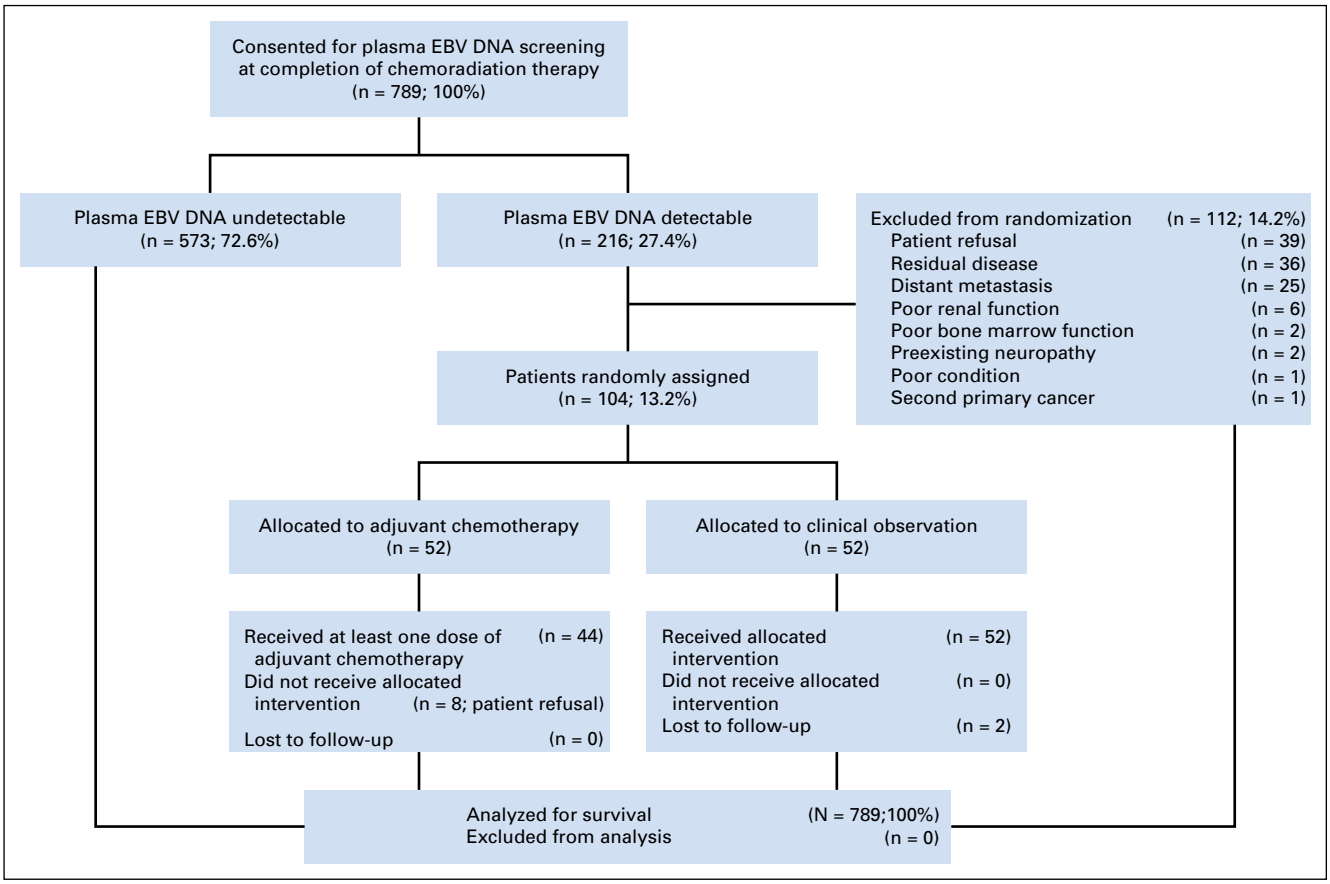


Fig 1. CONSORT diagram of study populations. EBV, Epstein-Barr virus.

alone in the Intergroup 0099 study,[4] the relative contribution of the concurrent and adjuvant components of chemotherapy has been inadequately assessed,[5] and the extent to which adjuvant chemotherapy can affect OS in NPC remains uncertain.

Adjuvant chemotherapy after RT alone did not improve OS in two previous phase III trials.[6,7] In the most recent phase III trial, 508 patients with advanced NPC were randomly assigned to adjuvant chemotherapy (cisplatin and fluorouracil) or observation after CRT, with no significant improvement in 5-year failure-free survival rate with adjuvant chemotherapy versus CRT alone (75% *v* 71%; hazard ratio [HR], 0.88; 95% CI, 0.64 to 1.22).[8,9] In the updated Meta-Analysis of Chemotherapy in Nasopharynx Cancer of 4,806 patients, the addition of concurrent chemotherapy to RT significantly improved OS in locally advanced NPC.[10] However, the interaction between treatment effect on OS and the timing of chemotherapy was significant in favor of both concurrent CRT with adjuvant chemotherapy (HR, 0.65; 95% CI, 0.56 to 0.76) and CRT without adjuvant chemotherapy (HR, 0.80; 95% CI, 0.70 to 0.93) but not adjuvant chemotherapy or induction chemotherapy alone. The observation of a small difference in the HRs in favor of CRT followed by adjuvant chemotherapy when indirectly compared with CRT without adjuvant chemotherapy in a network meta-analysis suggested that administration of more chemotherapy to patients with locally advanced NPC may achieve a better result.[11] Not all patients likely will benefit from adjuvant chemotherapy after CRT, so the challenge is in patient selection to maximize the magnitude of benefit.

EBV DNA detected in cell-free plasma has higher specificity and sensitivity for EBV-positive disease compared with EBV DNA in peripheral blood mononuclear cells.[12] We and others have shown that plasma EBV DNA level is an independent prognostic biomarker for NPC.[13-15] Post-RT plasma EBV DNA has an even stronger correlation with prognosis and has been used to monitor recurrence during post-treatment surveillance.[16,17] Elevated plasma EBV DNA level has been shown to predate clinical recurrence by 3 to 7 months.[18] A detectable or high level of post-RT plasma EBV DNA can predict a poor progression-free survival or OS and represents a biomarker of subclinical residual disease.[16]

We hypothesized that by selecting high-risk patients with NPC with a significant likelihood of harboring occult distant metastasis (defined by detectable post-RT plasma EBV DNA) and using the more active chemotherapy regimen of cisplatin and gemcitabine with superior efficacy for metastatic disease[19] administered earlier in the adjuvant setting, we can reduce distant metastasis and significantly improve relapse-free survival (RFS) by eradicating subclinical micrometastasis. Correlation of post-RT plasma EBV DNA with clinical outcome is a secondary end point of the study.

PATIENTS AND METHODS

Study Design and Participants

The Hong Kong NPC Study Group 0502 trial is a prospective, open-label, multicenter, randomized controlled trial conducted at all six

Table 1. Baseline Characteristics of Study Population

Characteristic	Patients, No. (%)				Randomly Assigned Patients, No. (%)		
	A: All With Plasma EBV DNA	B: With Undetectable Plasma EBV DNA	C: With Detectable Plasma EBV DNA	*P* (B *v* C)	C1: Adjuvant Chemotherapy	C2: Observation	*P* (C1 *v* C2)
No. of patients	789	573	216		52	52	
Median age, years (range)	51.0 (19 to 81)	51.0 (19 to 80)	51.5 (20 to 81)	.330	50.0 (33 to 79)	52.0 (20 to 68)	.890
Sex				.084			.200
Male	593 (75.2)	440 (76.8)	153 (70.8)		33 (63.5)	39 (75.0)	
Female	196 (24.8)	133 (23.2)	63 (29.2)		19 (36.5)	13 (25.0)	
ECOG PS	—	—	—	—			.540
0					47 (90.4)	45 (86.5)	
1					5 (9.6)	7 (13.5)	
UICC T stage				.034			.430
T1	181 (22.9)	135 (23.6)	46 (21.2)		14 (26.9)	10 (19.2)	
T2	187 (23.7)	137 (23.9)	50 (23.2)		12 (23.1)	17 (32.7)	
T3	291 (36.9)	220 (38.4)	71 (32.9)		16 (30.8)	15 (28.9)	
T4	130 (16.5)	81 (14.1)	49 (22.7)		10 (19.2)	10 (19.2)	
UICC N stage				< .001			.120
N0	97 (12.3)	80 (14.0)	17 (7.9)		8 (15.4)	2 (3.9)	
N1	296 (37.5)	226 (39.4)	70 (32.4)		16 (30.8)	25 (48.1)	
N2	323 (40.9)	228 (39.8)	95 (44.0)		19 (36.5)	18 (34.6)	
N3	73 (9.3)	39 (6.8)	34 (15.7)		9 (17.3)	7 (13.5)	
UICC overall stage				< .001			.920
IIB	222 (28.1)	170 (29.7)	52 (24.1)		15 (28.9)	14 (26.9)	
III	381 (48.3)	294 (51.3)	87 (40.3)		18 (34.6)	21 (40.4)	
IVA	113 (14.3)	70 (12.2)	43 (19.9)		10 (19.2)	10 (19.2)	
IVB	73 (9.3)	39 (6.8)	34 (15.7)		9 (17.3)	7 (13.5)	
Treatment modality				.150			.590
RT alone	146 (18.5)	113 (19.7)	33 (15.3)		7 (13.5)	9 (17.3)	
CRT	643 (81.5)	460 (80.3)	183(84.7)		45 (86.5)	43 (82.7)	
Neoadjuvant chemotherapy				< .001			.180
Concurrent chemotherapy alone	481 (74.8)	363 (78.9)	118 (64.5)		31 (68.9)	35 (81.4)	
Neoadjuvant chemotherapy	162 (25.2)	97 (21.1)	65 (35.5)		14 (31.1)	8 (18.6)	
Median post-RT EBV DNA (IQR)	0 (0 to 13)	0 (0 to 0)	61 (22.5 to 251)	—	42.5 (17.0 to 111.5)	44.5 (18.5 to 148.5)	.590
Post-RT EBV DNA, copies/mL				—			.730
0	573 (72.6)	573 (100)	0		0	0	
1-49	100 (12.7)	0	100 (46.3)		31 (59.6)	27 (51.9)	
50-499	72 (9.1)	0	72 (33.3)		16 (30.8)	19 (36.5)	
≥ 500	44 (5.6)	0	44 (20.4)		5 (9.6)	6 (11.5)	
Post-RT PET/CT* (baseline)	—	—	—	—			.880
Negative					38 (80.9)	39 (79.6)	
Positive					9 (19.1)	10 (20.4)	
Median follow-up, years (95% CI)	6.27 (5.98 to 6.54)	6.17 (5.79 to 6.51)	6.65 (6.24 to 7.08)	.420	6.6 (5.3 to 7.6)	6.5 (5.5 to 7.5)	.940

NOTE. ECOG PS of 0 and 1 correspond to asymptomatic performance and symptomatic but ambulatory performance, respectively. *P* values were calculated by *t* test for continuous data, χ^2 test for categorical data, Mann-Whitney *U* test for age and plasma EBV DNA, and log-rank test for median duration of follow-up.

Abbreviations: CRT, chemoradiation therapy; EBV, Epstein-Barr virus; ECOG PS, Eastern Cooperative Oncology Group performance status; IQR, interquartile range; PET/CT, positron emission tomography/computed tomography; RT, radiotherapy; UICC, Union for International Cancer Control.

*A PET/CT report was not available for eight patients (five in adjuvant chemotherapy arm, three in observation arm) who did not complete a second PET/CT study.

oncology centers in Hong Kong. Eligible patients were age ≥ 18 years with a histologic diagnosis of locoregionally advanced NPC of Union for International Cancer Control (UICC; 6th edition) stage IIB, III, IVA, or IVB; no clinical evidence of persistent locoregional disease or distant metastasis after completion of primary RT or CRT; Eastern Collaborative Oncology Group performance status of 0 to 1; and adequate bone marrow and organ function. Exclusion criteria were second primary malignancy, > 12 weeks after completion of primary RT, and peripheral or ototoxicity greater than grade 2 (Appendix, online only).

Eligible patients were consented for plasma EBV DNA screening at 6 to 8 weeks post-RT. Patients with undetectable plasma EBV DNA underwent standard surveillance. Patients with detectable plasma EBV DNA were consented for restaging work-up and random assignment to adjuvant chemotherapy versus observation.

The study was conducted in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonization of Good Clinical Practice and registered as a clinical trial. The study protocol (Data Supplement) was approved by the institutional review

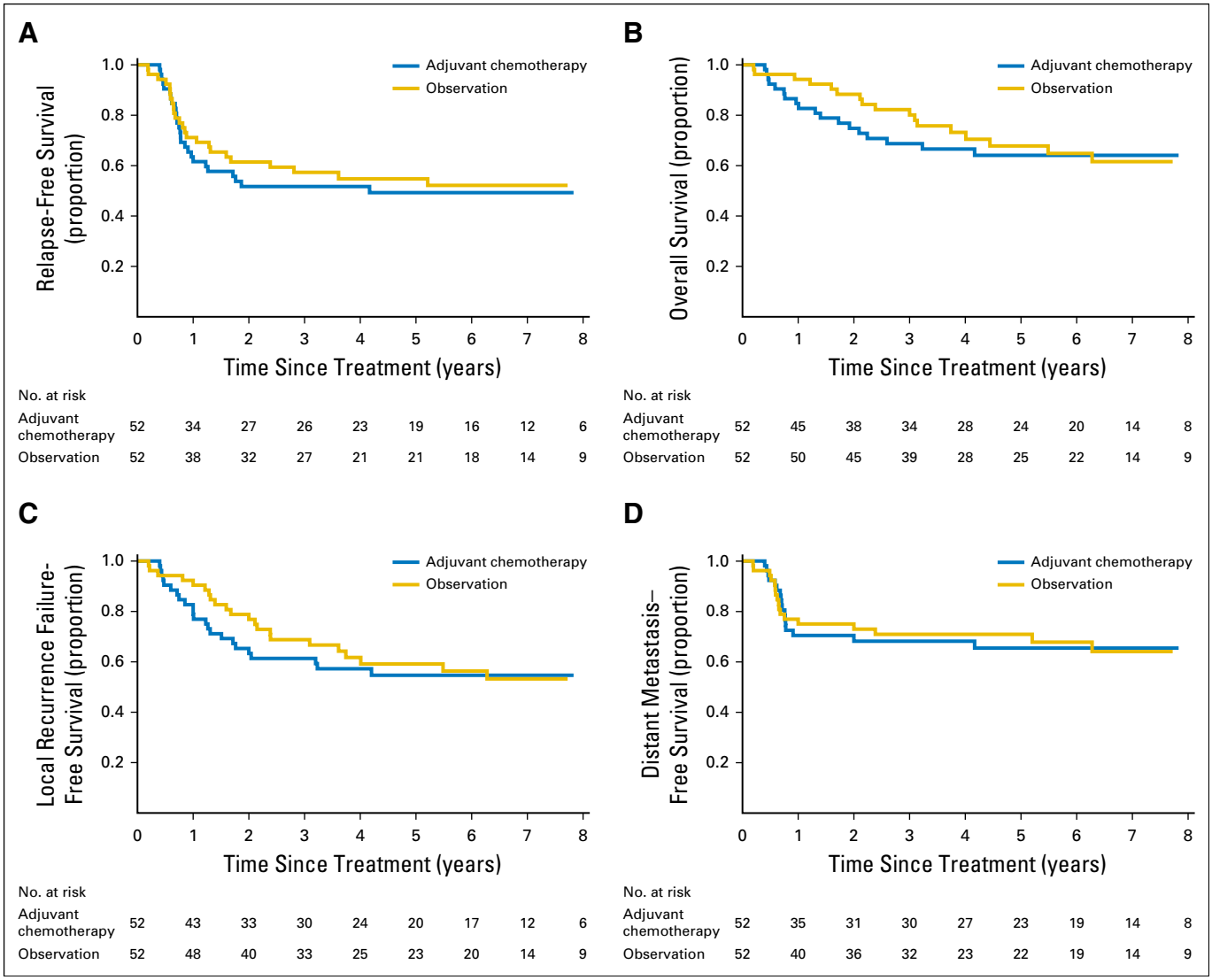


Fig 2. Kaplan-Meier estimates of survival among the study patients randomly allocated to adjuvant chemotherapy versus observation. (A) Relapse-free survival by treatment group. (B) Overall survival by treatment group. (C) Locoregional failure-free survival by treatment groups (D) Distant metastasis-free survival by treatment group.

board of each study center. All patients provided written informed consent before any study procedure.

Randomization and Masking

Patients who met all eligibility criteria after restaging were randomly assigned by telephone from the central office of the Comprehensive Cancer Trials Unit at The Chinese University of Hong Kong. They were randomly assigned in a 1:1 ratio, with stratification for the type of primary treatment (RT *v* CRT) and cancer stage (II/III *v* IV) to either adjuvant chemotherapy (arm 1) or clinical observation (arm 2). The masking of EBV DNA and positron emission tomography/computed tomography (PET/CT) results are described in the Appendix.

Procedure

Ten milliliters of venous blood were collected into EDTA tubes; processed within 6 hours to obtain plasma; and transported to the Department of Chemical Pathology, The Chinese University of Hong Kong. Plasma EBV DNA was analyzed by real-time polymerase chain reaction assay that targeted the *Bam*HI-W fragment of the EBV genome, as described previously.[13,20,21] On the basis of the analysis of known EBV DNA standards, our assay was able to detect consistently 20 EBV genomes per milliliter of plasma.[21] All samples were analyzed in duplicate. Amplification signals in any replicate were registered as a positive result, regardless of the level.

Patients randomly assigned to adjuvant chemotherapy would receive cisplatin 40 mg/m^2 intravenously and gemcitabine 1,000 mg/m^2 intravenously, with both administered on day 1 and day 8 every 3 weeks for a total of six cycles. Dose modification criteria were predefined in the study protocol (Appendix). Adverse events were graded according to Common Terminology Criteria for Adverse Events (version 3). Late toxicity of skin, subcutaneous tissue, and salivary glands were assessed by the Radiation Therapy Oncology Group and European Organisation for Research and Treatment of Cancer Late Radiation Morbidity Scoring Criteria.[22]

Outcomes

The primary end point was RFS, which was defined as the time from random assignment to the time of first recurrence of NPC (locoregional failure or distant metastasis) or death as a result of any cause, whichever occurred first, or censored at last follow-up. Secondary end points were OS, locoregional failure-free survival (LRFS), distant metastasis–free survival

(DMFS), toxicity of adjuvant chemotherapy, and correlation of plasma EBV DNA with clinical outcome (Appendix).

Statistical Analysis

Historically, RFS at 18 months is approximately 30% in patients with detectable post-RT plasma EBV DNA[16,17]; therefore, we estimated a magnitude of benefit from adjuvant chemotherapy to be 60% for the study treatment of major clinical value. With an estimated HR of 2 (or 0.5 if observation arm as reference), approximately 100 patients were required with a power of 0.8 and α of .05. At the closure of study accrual in July 2015 (when 104 patients were randomly assigned) and on the basis of the projected event rate of four per year, we planned to observe 48 events by the end of 2016, which can provide 80% power for a revised HR of 2.4 (or 0.42 if observation arm as reference). After a median follow-up of > 6 years, 98% of the events occurred in the first 3 years. The RFS curve was essentially flat after 3 years. The data and safety monitoring committee recommended to conduct the primary end point analysis. Kaplan-Meier method, log-rank test, and Cox proportional hazards regression modeling were used to analyze all time-to-event data (Appendix). We regarded $P < .05$ as significant. All statistical analyses were performed using SAS 9.3 software (SAS Institute, Cary, NC).

RESULTS

From September 2006 to July 2015, 789 patients with NPC who completed curative-intent RT or CRT consented for plasma EBV DNA screening (Fig 1); 573 patients (72.6%) had undetectable plasma EBV DNA, and 216 (27.4%) had detectable plasma EBV DNA (median, 61 copies/mL; interquartile range, 22.5 to 251 copies/mL). The baseline characteristics of the study population are listed in Table 1. Patients with detectable post-RT EBV DNA (cohort C in Table 1) had a higher T stage, N stage, and UICC overall stage than patients with undetectable plasma EBV DNA (cohort B in Table 1). Although RT technique was not a stratification factor in the randomization, intensity-modulated radiotherapy was adopted by all except one center during the study period. In one center where two-dimensional RT was still used in < 5% of patients before 2008, 11 patients recruited at that center received two-dimensional RT, including two who were subsequently randomly assigned (one to adjuvant chemotherapy and one to observation).

After work-up, 104 patients (13.2% of all screened) with detectable post-RT plasma EBV DNA were eligible and randomly assigned to adjuvant chemotherapy (n = 52) or observation (n = 52). The baseline clinical characteristics, including age, sex, Eastern Cooperative Oncology Group performance status, T stage, N stage, UICC overall stage, primary treatment modality, post-RT EBV DNA level, and baseline PET/CT positivity (blinded at random assignment), were evenly balanced in the two arms (cohort C1 *v* C2 in Table 1). The median follow-up was 6.6 years, and the relapse and death events in the study population are listed in Appendix Table A1 (online only). No significant difference was found in the 5-year RFS rate between adjuvant chemotherapy and observation (49.3% *v* 54.7%; HR for relapse or death, 1.09; 95% CI, 0.63 to 1.89; $P = .75$). Similarly, no significant difference was found in OS, LRFS, and DMFS between adjuvant chemotherapy and observation (Fig 2; Table 2). A sensitivity analysis that included patients who received at least one dose of adjuvant chemotherapy showed similar results (Appendix Table A2, online only). Forest plot analysis of RFS and OS (Figs 3A and 3B) in patient subgroups did not reveal heterogeneity in treatment effect except for female sex, which showed worse RFS in the adjuvant chemotherapy arm.

For patients randomly assigned to adjuvant chemotherapy, 84.6% started chemotherapy, and 50.0% completed six cycles (Appendix Table A3, online only). Three patients died within 90 days after the last dose of adjuvant chemotherapy. The cause of death was a result of a treatment-related complication in two (nasopharyngeal hemorrhage, hematemesis) and intercurrent illness in one. Other high-grade treatment-related toxicities were mainly hematologic (Appendix Table A4, online only). Late radiation toxicity was comparable in both arms, with the exception of subcutaneous toxicity, which was more common and severe in the adjuvant chemotherapy arm (Appendix Table A5, online only).

The absolute level of post-RT plasma EBV DNA correlated significantly with the risk of locoregional failure, distant metastasis,

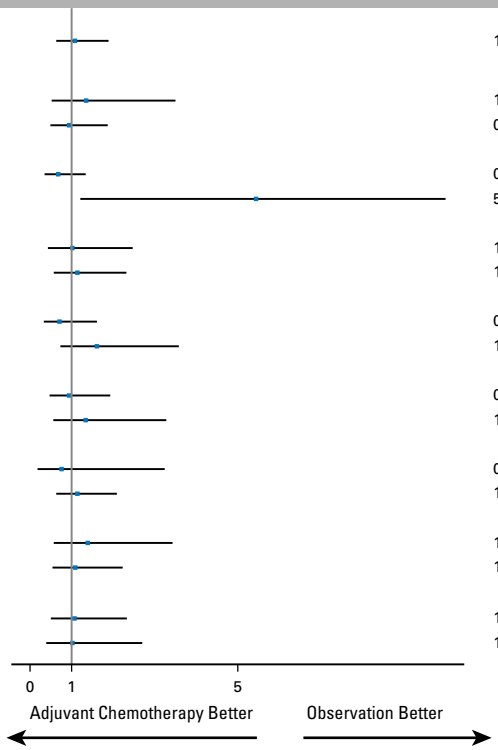
Table 2. Survival Outcome of Study Population

Outcome	Patients, %					Randomly Assigned Patients, %			
	A: All With Plasma EBV DNA	B: With Undetectable Plasma EBV DNA	C: With Detectable Plasma EBV DNA	B *v* C: HR (95% CI)	*P**	C1: Adjuvant Chemotherapy	C2: Observation	C1 *v* C2: HR (95% CI)	*P**
No. of patients	789	573	216			52	52		
RFS rate, %					< .001				.75
3-year	74.1	82.8	51.3	3.21 (2.50 to 4.11)		51.7	57.3	1.09 (0.63 to 1.89)	
5-year	68.4	77.1	45.4			49.3	54.7		
OS rate, %					< .001				.79
3-year	87.1	93.3	70.8	3.16 (2.36 to 4.22)		70.8	82.2	1.09 (0.56 to 2.11)	
5-year	79.8	87.3	60.2			64.0	67.8		
LRFS rate, %					< .001				.68
3-year	78.9	86.7	58.3	3.11 (2.40 to 4.03)		61.3	68.8	1.13 (0.63 to 2.02)	
5-year	72.3	80.3	51.5			54.6	59.1		
DMFS rate, %					< .001				.84
3-year	81.1	88.5	61.6	3.14 (2.39 to 4.12)		61.3	70.9	1.07 (0.58 to 1.97)	
5-year	75.1	83.1	54.2			58.9	63.8		

Abbreviations: DMFS, distant metastasis–free survival; EBV, Epstein-Barr virus; HR, hazard ratio; LRFS, locoregional failure-free survival; OS, overall survival; RFS, relapse-free survival.
*P value by log-rank test

A

Subgroup	Patients	Adjuvant chemotherapy	Observation	HR (95% CI)
Overall	104	26 of 52	25 of 52	1.09 (0.63 to 1.89)
Age				
≤ 50 years	44	9 of 21	8 of 23	1.36 (0.52 to 3.50)
> 50 years	60	17 of 31	17 of 29	0.95 (0.49 to 1.87)
Sex				
Male	72	14 of 33	23 of 39	0.69 (0.35 to 1.34)
Female	32	12 of 19	2 of 13	5.45 (1.21 to 24.5)
T stage				
T1-T2	53	10 of 26	10 of 27	1.03 (0.43 to 2.47)
T3-T4	51	16 of 26	15 of 25	1.15 (0.57 to 2.32)
N stage				
N0-N1	51	10 of 24	15 of 27	0.72 (0.33 to 1.61)
N2-N3	53	16 of 28	10 of 25	1.62 (0.73 to 3.58)
Overall stage				
II/III	68	15 of 33	16 of 35	0.95 (0.47 to 1.93)
IV	36	11 of 19	9 of 17	1.35 (0.56 to 3.28)
Primary treatment				
RT alone	16	3 of 7	5 of 9	0.77 (0.18 to 3.24)
CRT	88	23 of 45	20 of 43	1.15 (0.63 to 2.09)
EBV DNA. copies/mL				
1-49	58	12 of 31	8 of 27	1.40 (0.57 to 3.43)
≥ 50	46	14 of 21	17 of 25	1.10 (0.54 to 2.23)
PET/CT				
Negative	77	13 of 38	13 of 39	1.08 (0.50 to 2.33)
Positive	19	9 of 9	9 of 10	1.03 (0.39 to 2.70)



B

Subgroup	Patients	Adjuvant chemotherapy	Observation	HR (95% CI)
Overall	104	18 of 52	18 of 52	1.10 (0.56 to 2.10)
Age				
≤ 50 years	44	2 of 21	6 of 23	0.40 (0.07 to 1.80)
> 50 years	60	16 of 31	12 of 29	1.50 (0.70 to 3.20)
Sex				
Male	72	11 of 33	16 of 39	0.90 (0.41 to 1.90)
Female	32	7 of 19	2 of 13	2.59 (0.54 to 12.5)
T stage				
T1-T2	53	6 of 26	6 of 27	1.06 (0.34 to 3.30)
T3-T4	51	12 of 26	12 of 25	1.08 (0.48 to 2.40)
N stage				
N0-N1	51	7 of 24	11 of 27	0.79 (0.30 to 2.03)
N2-N3	53	11 of 28	7 of 25	1.54 (0.60 to 3.98)
Overall stage				
II/III	68	8 of 33	12 of 35	0.69 (0.28 to 1.70)
IV	36	10 of 19	6 of 17	1.95 (0.71 to 5.40)
Primary treatment				
RT alone	16	2 of 7	2 of 9	1.26 (0.18 to 9.04)
CRT	88	16 of 45	16 of 43	1.06 (0.53 to 2.10)
EBV DNA. copies/mL				
1-49	58	6 of 31	6 of 27	0.94 (0.30 to 2.90)
≥ 50	46	12 of 21	12 of 25	1.46 (0.60 to 3.30)
PET/CT				
Negative	77	7 of 38	8 of 39	0.91 (0.33 to 2.50)
Positive	19	7 of 9	8 of 10	1.28 (0.46 to 3.60)

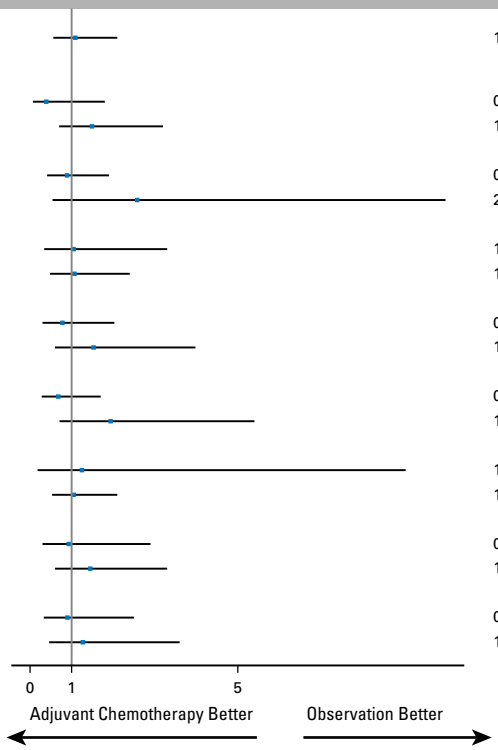


Fig 3. Forest plot analysis of (A) relapse-free survival and (B) overall survival in patient subgroups (adjuvant chemotherapy *v* observation). CRT, chemoradiation therapy; EBV, Epstein-Barr virus; HR, hazard ratio; PET/CT, positron emission tomography/computed tomography; RT, radiotherapy.

and death (Table 3; Fig 4). For each 10-log increase in plasma EBV DNA concentration, the HR was 1.96 (95% CI, 1.77 to 2.16) for locoregional failure, 2.14 (95% CI, 1.93 to 2.39) for distant metastasis, and 2.12 (95% CI, 1.89 to 2.37) for death. Post-RT plasma EBV DNA followed by UICC overall stage was the most significant prognostic factor in both univariable and multivariable analysis (Appendix Table A6, online only).

DISCUSSION

This study is the first biomarker-driven RCT in our knowledge to compare adjuvant chemotherapy versus observation in high-risk patients with NPC identified by detectable plasma EBV DNA after completion of RT and CRT. We used cisplatin and gemcitabine as the adjuvant chemotherapy, which has been shown to be more effective than cisplatin and fluorouracil in the metastatic setting.[19] The current results show that adjuvant cisplatin and gemcitabine chemotherapy do not improve RFS or OS in this biomarker (plasma EBV DNA)–selected high-risk group of patients with NPC.

We observed several deaths without documented progression in the study arms (Appendix Table A1). To avoid the confounding effect of death as a competing event, we adopted a conservative composite end point definition in the analysis of RFS, LRFS, and DMFS by including either failure or death, whichever occurred first (Appendix). In a separate analysis with death censored, the results were similar (5-year RFS, 56.3% *v* 54.7%; $P = .79$; HR for relapse, 0.92; 95% CI, 0.51 to 1.66).

This study has several limitations. The compliance to adjuvant cisplatin and gemcitabine was suboptimal because only 84.6% of patients randomly assigned to adjuvant chemotherapy actually started treatment, and 65.4%, 57.7%, and 50.0% completed four, five, and six cycles of adjuvant cisplatin and gemcitabine, respectively (Appendix Table A3). The reasons for early discontinuation were patient refusal (73%) or treatment-related toxicity (27%). In the phase III trial that compared cisplatin and gemcitabine with cisplatin and fluorouracil in the first-line setting of recurrent or metastatic NPC, 82.9%, 66.9%, and 58% of patients were able to complete four, five, and six cycles of cisplatin and gemcitabine versus 75.7%, 56.4%, and 58.0% for cisplatin and fluorouracil, respectively.[19] The lower compliance in the adjuvant setting than in the metastatic setting probably reflects a generally poor tolerance in the first 6 months after completion of RT and CRT. Another contributing factor could have been lower patient motivation to finish a regimen with significant toxicity but uncertain benefit in the adjuvant setting. Nevertheless, in a sensitivity analysis of survival outcome according to compliance of adjuvant chemotherapy, there was no suggestion of a different outcome in patients who received more than three cycles of adjuvant chemotherapy (Appendix Table A7, online only).

In the current study, the randomization was performed at a median of 78 days after completion of RT or CRT to accommodate the time to complete all prerandomization evaluations to assess patient disease status and eligibility criteria. As a result, of 216 patients with detectable post-RT plasma EBV DNA, 112 were excluded before random assignment (Fig 1). The reasons for exclusion were patient refusal (n = 39), presence of residual disease (n = 36) or distant metastasis (n = 25), inadequate renal (n = 6) or bone marrow function (n = 2), preexisting neuropathy (n = 2), poor general condition (n = 1), and diagnosis of second primary cancer (n = 1). We believe that the stringent prerandomization evaluation in the current study actually selected a more homogenous study population for adjuvant chemotherapy. Patients with clinically or radiologic-persistent locoregional disease, distant metastases, or inadequate organ function after completion of RT/CRT were excluded before random assignment. Therefore, the patients eventually randomly assigned had a much lower risk compared with the historical data.

We postulated that a time frame of 12 weeks after completing CRT would be necessary for patient recovery from treatment-related adverse effects but may be too late for adjuvant chemotherapy to be effective in minimal residual disease. In this study, the median time from the day of last RT to the day of first dose of adjuvant chemotherapy was 91 days (interquartile range, 81 to 99 days; Appendix Table A3). In comparison, the median time from last day of RT to first day of adjuvant chemotherapy was 33 days (interquartile range, 28 to 39 days) in another phase III study of adjuvant chemotherapy in NPC.[8] In a sensitivity analysis on survival outcome according to time to initiation of adjuvant chemotherapy (Appendix Table A8, online only), there was no indication for a better outcome if the adjuvant chemotherapy was started earlier (less than the median) rather than later (greater than the median).

Because the companion PET/CT study was not reported until the second PET/CT scan was completed at 6 months after randomization, the blinding of baseline PET/CT could introduce imbalance into the two study arms. However, the number of baseline PET/CT scans retrospectively reported as positive were well balanced in the two study arms (19.1% in the adjuvant

Table 3. Correlation of Post-RT Plasma EBV DNA Level With LRFS, DMFS, and OS

Post-RT Plasma EBV DNA Level (copies/mL)	No. of Patients (N = 789)	LRFS				DMFS				OS			
		No. (%)	5-Year Rate, %	HR (95% CI)	*P*	No. (%)	5-Year Rate, %	HR (95% CI)	*P*	No. (%)	5-Year Rate, %	HR (95% CI)	*P*
0	573	120 (20.9)	80.3	1.00	—	109 (19.0)	83.1	1.00	—	93 (16.2)	87.3	1.00	
1-49	100	31 (31.0)	71.2	1.58 (1.06 to 2.34)	.024	22 (22.0)	79.4	1.23 (0.78 to 1.95)	.37	20 (20.0)	82.5	1.28 (0.79 to 2.07)	.32
50-499	72	43 (59.7)	43.4	3.74 (2.64 to 5.30)	< .001	40 (55.6)	43.1	3.85 (2.68 to 5.54)	< .001	36 (50.0)	51.2	3.77 (2.57 to 5.55)	< .001
≥ 500	44	36 (81.8)	21.0	8.95 (6.14 to 13.05)	< .001	36 (81.8)	18.2	13.04 (8.88 to 19.14)	< .001	33 (75.0)	27.2	11.60 (7.76 to 17.36)	< .001

Abbreviations: DMFS, distant metastasis–free survival; EBV, Epstein-Barr virus; HR, hazard ratio; LRFS, locoregional failure-free survival; OS, overall survival; RT, radiotherapy.

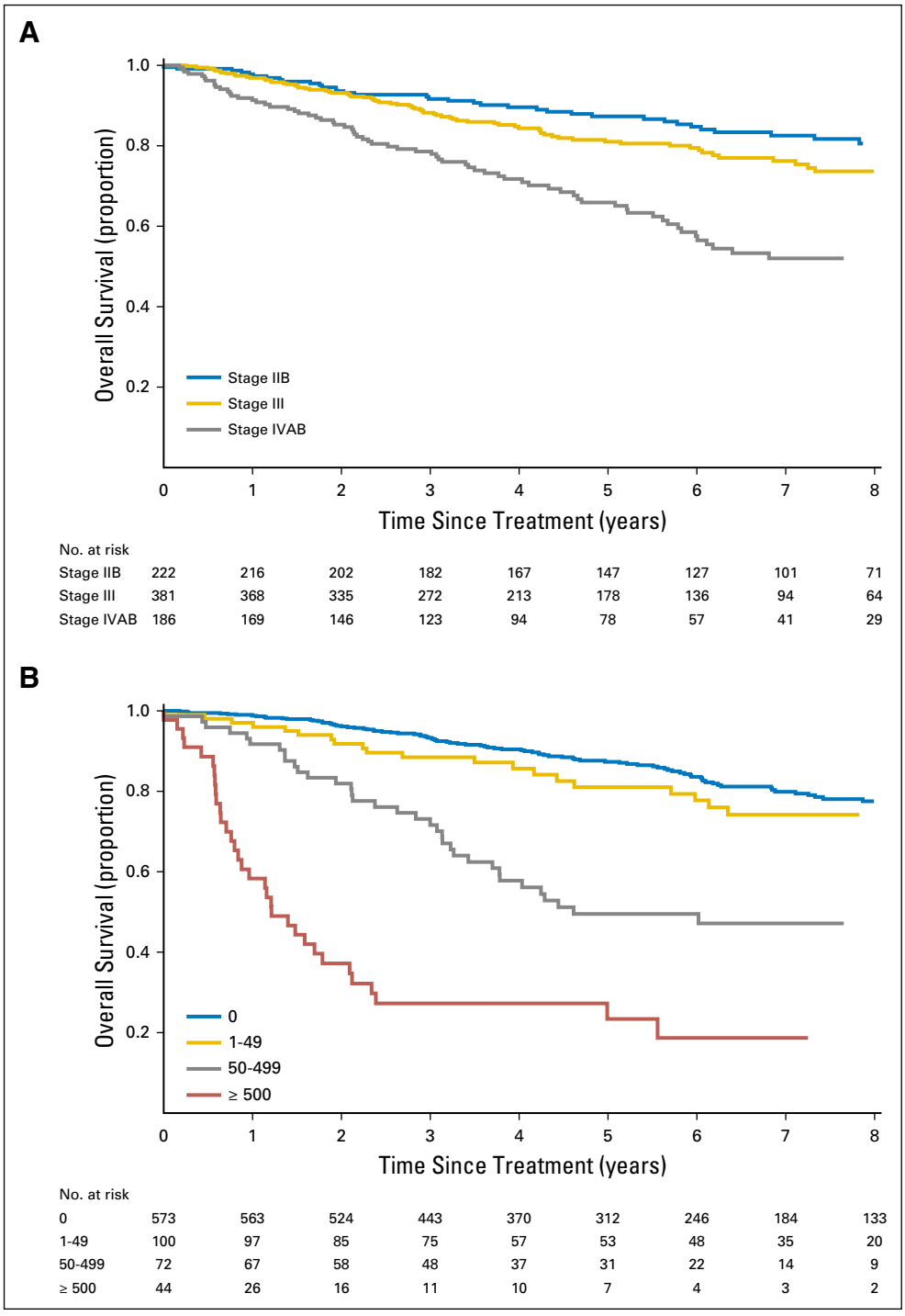


Fig 4. Prognostic group by (A) Union for International Cancer Control stage and (B) postradiotherapy plasma Epstein-Barr virus DNA level in the screening cohort (N = 789).

chemotherapy arm *v* 20.4% in the observation arm; Table 1). In a sensitivity analysis that excluded patients with positive baseline PET/CT scans, the survival outcome remained similar to that in the primary analysis (Appendix Table A9, online only).

The clinical utility and current state of polymerase chain reaction–based EBV DNA testing in NPC with recommendation for assay harmonization and validation have been recently discussed in a workshop meeting.[15,23] Currently, no consensus exists on the optimal cutoff for post-RT plasma EBV DNA levels for risk classification.[15] Previous studies have suggested that the quantification of a low level of plasma EBV DNA close to the detection limit of the assay system may be less accurate in patients with NPC.[24,25] In the current study, we report any detectable signal as a positive result to maximize the sensitivity for detecting residual disease.

In the current RCT, 85% of patients had received prior cisplatin. We hypothesized that in patients with NPC with detectable plasma EBV DNA after cisplatin and RT, the residual subclinical disease is more likely platinum resistant. Early administration of cisplatin-based chemotherapy in the adjuvant setting not only is

unable to eradicate the platinum-resistant clone but also adds toxicity, which further compromises the patient's ability to receive subsequent chemotherapy. Indeed, we observed that most patients in the adjuvant chemotherapy arm responded poorly to subsequent chemotherapy at relapse. Retrospective data have suggested that the increased risk of relapse and death in patients with elevated plasma EBV DNA after CRT could be reduced by metronomic adjuvant chemotherapy with oral tegafur-uracil for 12 months.[26] Recently, the use of immune checkpoint inhibitors has been demonstrated to improve progression-free survival in patients with stage III non–small-cell lung cancer after chemoradiation.[27] The NRG Oncology Cooperative Group initiated NRG-HN001 (ClinicalTrials.gov identifier: NCT02135042) that used post-RT plasma EBV DNA to divide patients with NPC into low-risk and high-risk groups, with the hypothesis that the low-risk group would not need adjuvant chemotherapy and that the high-risk group would benefit from more-aggressive non–cross-resistant paclitaxel and gemcitabine chemotherapy.[23]

Current clinical guidelines in NPC generally recommend that the treatment decision be based on the anatomic classification of UICC TNM staging.[1] The current study provides strong validation that post-RT plasma EBV DNA level is the major determinant of RFS and OS in patients with NPC at the end of RT or CRT. We propose to include post-RT EBV DNA level as a nonanatomic determinant of risk classification and treatment selection for adjuvant therapy in NPC. The optimal management of patients with NPC with detectable post-RT plasma EBV DNA remains undetermined. Patients should be encouraged to participate in clinical trials that investigate non–platinum-based systemic therapy or immune checkpoint inhibitors.[28,29] Until more evidence from RCTs becomes available, concurrent cisplatin and RT should remain the standard of care in endemic locally advanced NPC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Support

Supported by research funding from The Chinese University of Hong Kong and Hong Kong Hospital Authority. Gemcitabine was provided by Eli Lilly. The Partner State Key Laboratory of Oncology in South China at The Chinese University of Hong Kong was supported by funding from the Innovative Technology Commission. Any opinions, findings, conclusions, or recommendations expressed in this article (or by members of the project team) do not reflect the views of the Government of the Hong Kong Special Administrative Region, the Innovation and Technology Commission, or the Panel of Assessors for the Innovation and Technology Support Programme of the Innovation and Technology Fund.

Prior Presentation

Presented at the 2017 Annual Meeting of American Society of Clinical Oncology, Chicago, IL, June 2-6, 2017, and the European Society of Medical Oncology Asia 2017 Congress, Singapore, November 17-19, 2017.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Analysis of Plasma Epstein-Barr Virus DNA in Nasopharyngeal Cancer After Chemoradiation to Identify High-Risk Patients for Adjuvant Chemotherapy: A Randomized Controlled Trial

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Appendix

Study Design and Participants

The Hong Kong NPC Study Group 0502 trial is a prospective, open-label, multicenter, randomized controlled trial conducted at all six oncology centers in Hong Kong. The primary objective is to study the benefit of adjuvant chemotherapy with gemcitabine and cisplatin in patients with nasopharyngeal cancer (NPC) at higher risk of relapse defined by a detectable plasma Epstein-Barr virus (EBV) DNA level after completion of curative-intent radiotherapy (RT) or chemoradiation therapy (CRT).

Eligible patients were age $\geq$ 18 years with a histologic diagnosis of locoregionally advanced NPC of Union for International Cancer Control (6th edition) stage IIB, III, IVA, or IVB; no clinical evidence of persistent locoregional disease after completion of primary treatment; Eastern Collaborative Oncology Group performance status of 0 or 1; adequate bone marrow and organ function (defined as leukocytes $\geq$ 3,000/μL, absolute neutrophil count $\geq$ 1,500/μL; platelets $\geq$ 100,000/μL, total bilirubin $\leq$ 1.5 times the institutional upper limit of normal; transaminase $\leq$ 2.5 times the upper limit of normal; and creatinine clearance $\geq$ 50 mL/min as estimated by the Cockcroft-Gault equation. Exclusion criteria were hypercalcemia, second primary malignancy (except for in situ carcinoma of the cervix or adequately treated basal cell carcinoma of the skin), $>$ 12 weeks after completion of primary RT, receipt of prior adjuvant chemotherapy, peripheral or ototoxicity with a grade $>$ 2, serious active infection, pregnant or lactating female patients, and patients with reproductive potential who were not implementing adequate contraceptive measures.

Eligible patients were consented for plasma EBV DNA screening at 6 to 8 weeks post-RT. Those with undetectable plasma EBV DNA were followed up by standard surveillance. Those with detectable plasma EBV DNA were consented for work-up and random assignment to adjuvant chemotherapy versus clinical observation and surveillance. The protocol allowed primary RT to be delivered with either two- or three-dimensional techniques of $\geq$ 66 Gy in 33 fractions over 6.5 weeks (Lee et al: Int J Radiat Oncol Biol Phys 61:1107-1116, 2005). Concurrent cisplatin was administered per institutional practice either as a 100 mg/m^2 bolus on days 1, 22, and 43 of RT,[4] or 40 mg/m^2 weekly on days 1, 8, 15, 22, 29, 36, and 43 of RT.[3] Neoadjuvant chemotherapy was allowed. No patient received adjuvant chemotherapy, which was an exclusion criterion except for the patients randomly assigned to the adjuvant chemotherapy study arm.

Randomization and Masking

The randomization procedure was performed through telephone from the central office of the Comprehensive Cancer Trials Unit at The Chinese University of Hong Kong. A computer program was used to generate the allocation list. Randomization was done 1:1, with stratification for the type of primary treatment (RT *v* CRT) and cancer stage (II/III *v* IV). Patients fulfilling all eligibility criteria were randomly assigned to either adjuvant chemotherapy followed by clinical observation and surveillance (arm 1) or clinical observation and surveillance only (arm 2).

All patients in both study arms had plasma EBV DNA checked at 6 to 8 weeks after completion of primary RT and again at 6 to 8 months after random assignment. Patients assigned to arm 1 would have the baseline positron emission tomography/computed tomography (PET/CT) scan at 6 to 12 weeks after completion of primary RT and then proceed to adjuvant chemotherapy. Patients assigned to arm 2 would have the baseline PET/CT scan at 6 to 12 weeks after completion of primary RT; they would then proceed to clinical observation and surveillance. When the study was first conceived in 2005, the value of performing plasma EBV DNA and PET/CT scan in patients with NPC who had already completed standard conventional staging procedures (as discussed next under Procedure) after completion of primary RT was undefined (King et al: Br J Radiol 81:291-298, 2008) and is currently not a routine investigation in the clinical care of asymptomatic patients with NPC in the study centers (Au et al: Oral Oncol 77:16-21, 2018). In the current study, the post-RT plasma EBV DNA was only reported as negative (undetectable) or positive (detectable) at the time of random assignment. The result of the baseline PET/CT was blinded in both arms to patients and investigators at the time of random assignment. This blinding was part of the study design and was included in the patients' consent. When the patient had completed the second follow-up plasma EBV DNA and PET/CT scan at 6 to 8 months after random assignment, the result of both the baseline and the second follow-up PET/CT scan and the result of plasma EBV DNA would then be unblinded to both patient and investigator. Additional clinical management of the patient was at the discretion of the investigator or the attending clinician.

Procedure

All pretreatment screening procedures had to be completed within 28 days before the day of randomization, which included demographic data, medical history (including concomitant medication), disease history (including date of initial diagnosis, histology, and Union for International Cancer Control/American Joint Committee on Cancer stage classification of NPC), complete physical examination (including performance status, body weight, height, and vital signs), baseline laboratory tests (hematology, biochemistry, and hepatitis B surface antigen), ECG, chest radiograph, ultrasound liver or CT scan of abdomen, and

bone scan. All were the standard conventional staging procedures available at the study centers (unless the patient already had a staging PET or PET/CT scan as part of staging investigations [Au et al: Oral Oncol 77:16-21, 2018]).

Patients randomly assigned to adjuvant chemotherapy would receive cisplatin 40 mg/m^2 intravenously and gemcitabine 1,000 mg/m^2 intravenously, both administered on days 1 and 8 every 3 weeks for a total of six cycles. Carboplatin (area under the curve, 5) administered on day 1 only was used to replace cisplatin when significant (grade $\geq$ 2) peripheral or otoneurotoxicity existed. During adjuvant chemotherapy, laboratory hematology, biochemistry, and adverse events were evaluated on days 1 and 8 of each cycle. Adverse events were graded according to Common Terminology Criteria for Adverse Events (version 3). Chemotherapy was delayed by 1 week if the absolute neutrophil count was $< 1.5 \times 10^9$/L or platelet count was $< 75 \times 10^9$/L. Criteria of cisplatin dose modification for impaired renal function, neutropenic sepsis or grade 4 hematologic toxicity, and other nonhematologic toxicity were predefined in the study protocol. If adjuvant chemotherapy was delayed for $>$ 4 weeks, the patient would be discontinued from subsequent doses of chemotherapy but would continue to complete the follow-up schedule.

All patients in both study arms were followed every 3 months in the first and second years, every 4 months in the third year, every 6 months in the fourth and fifth years, and then every year thereafter. Nasopharynx examination (with nasopharyngoscopy), physical examination (including body weight and performance status), and documentation of the nodal status were performed at each scheduled visit. Assessment of late toxicity (defined as toxicity that occurred at least 3 months after the last day of RT) of the skin, subcutaneous tissue, and salivary glands was conducted at each visit using the Radiation Therapy Oncology Group and European Organisation for Research and Treatment of Cancer late radiation morbidity scoring criteria.[22]

Outcomes

The primary end point of the trial is relapse-free survival (RFS), which is defined as the time from random assignment to the time of first recurrence of NPC (locoregional or distant metastasis) or death as a result of any cause, whichever was observed first, or until date of last follow-up. Secondary end points were overall survival, locoregional control, distant metastasis–free survival, toxicity of adjuvant chemotherapy, and correlation of plasma EBV DNA and PET/CT scan with clinical course and outcome. Overall survival is defined as the duration from the date of random assignment to the date of death as a result of any cause or censored at the date of last follow-up. Local recurrence-free survival is defined as the duration from the date of random assignment to the date of first local recurrence or death as a result of any cause or censored at the date of last follow-up. For the purpose of this study, local recurrence also includes regional (neck) nodal recurrence. Distant metastasis–free survival is defined as the duration from the date of random assignment to the date of first distant metastasis or death as a result of any cause or censored at the date of last follow-up. In the analysis of the whole patient population that underwent EBV DNA screening, the origin of all end points was calculated since the date of consent for post-RT plasma EBV DNA test.

Sample Size and Statistical Analysis

When the current study was first conceptualized in 2005, we used the historical figure of approximately 30% for the RFS at 18 months in patients with detectable plasma EBV DNA post-RT.[16,17] The magnitude of benefit from adjuvant chemotherapy is estimated to be approximately 60% for the study treatment to be of major clinical value. With an estimated hazard ratio (HR) of 2 (or 0.5 if observation arm as reference), approximately 100 patients were required with a power of 0.8 and an α of .05.

In the phase III trial that compared cisplatin and gemcitabine with cisplatin and fluorouracil for six cycles in the first-line recurrent or metastatic setting, the HR for RFS was 0.55 (95% CI, 0.44 to 0.68).[19] Because the current study incorporated a follow-up PET/CT scan at 6 months after randomization, we estimated that we would be able to detect a much higher event rate in the observation arm on the basis of our previous historical data. Therefore, we anticipated that in a high-risk group defined by detectable plasma EBV DNA (a group with a very high relapse rate within 18 months) and as receiving a more active regimen of gemcitabine and cisplatin for six cycles, we could reasonably achieve an HR of 0.42 to 0.50 for RFS when the more active chemotherapy was compared with observation alone.

All analyses were performed by intention-to-treat principle except for toxicity of adjuvant chemotherapy, which was analyzed in the safety population (defined as patients who received at least one dose of chemotherapy). We performed a post hoc sensitivity analysis on patient groups of special interest: patients who received at least one dose of adjuvant chemotherapy, patients who complied with adjuvant chemotherapy, timing of initiation of adjuvant chemotherapy, and exclusion of patients with positive baseline PET/CT results. The linearity and proportional hazard assumptions were confirmed by model checking using plots of log-survival versus time, log-log curve, and martingale residuals (Altman et al: BMC Med 10:51, 2012). A Kaplan-Meier survival curve was used to analyze all time-to-event data. A log-rank test was used to assess the difference in survival between treatment groups. We regarded $P < .05$ as significant. All statistical analyses were performed using SAS 9.3 software (SAS Institute, Cary, NC).

Table A1. Pattern of Failure and Cause of Death in Study Population

Treatment Failure	Patients, No. (%)			Randomly Assigned Patients, No. (%)	
	A: All With Plasma EBV DNA	B: With Undetectable Plasma EBV DNA	C: With Detectable Plasma EBV DNA	C1: Adjuvant Chemotherapy	C2: Observation
No. of patients	789	573	216	52	52
Relapse	221 (28.0)	114 (19.9)	107 (49.5)	21 (40.4)	24 (46.2)
Site of progression					
Local	87 (11.0)	55 (9.6)	32 (14.8)	7 (13.5)	6 (11.5)
Regional (neck lymph node)	41 (5.2)	21 (3.7)	20 (9.3)	4 (7.7)	4 (7.7)
Locoregional	114 (14.4)	64 (11.2)	47 (21.8)	11 (21.2)	9 (17.3)
Distant metastasis	142 (18.0)	67 (11.7)	75 (34.7)	12 (23.1)	17 (32.7)
Death	182 (23.1)	93 (16.2)	89 (41.2)	18 (34.6)	18 (34.6)
Death without progression	31 (3.9)	20 (3.5)	11 (5.1)	5 (9.6)	1 (1.9)
Cause of death					
NPC progression	141 (17.9)	65 (11.3)	76 (35.2)	12 (23.1)	16 (30.8)
Second primary cancer*	8 (1.0)	7 (1.2)	1 (0.5)	1 (1.9)	0 (0.0)
Treatment-related complication	6 (0.8)	3 (0.5)	3 (1.4)	2 (3.8)	1 (1.9)
Intercurrent illness	24 (3.0)	17 (3.0)	7 (3.2)	1 (1.9)	1 (1.9)
Unknown cause	3 (0.4)	1 (0.2)	2 (0.9)	2 (3.8)	0 (0.0)

Abbreviations: EBV, Epstein-Barr virus; NPC, nasopharyngeal cancer.
*Site of second primary cancer: colorectal (n = 2), hepatobiliary (n = 2), breast (n = 1), head and neck (n = 1), lung (n = 1), and stomach (n = 1).

Table A2. Sensitivity Analysis

Outcome	C1: Received at Least One Dose of Adjuvant Chemotherapy	C2: Observation	C1 *v* C2: HR (95% CI)	*P**
No. of patients	44	52		
RFS rate, %			1.01 (0.57 to 1.81)	.96
3-year	54.3	57.3		
5-year	51.4	54.7		
OS rate, %			1.08 (0.54 to 2.14)	.84
3-year	70.0	82.2		
5-year	64.6	67.8		
LRFS rate, %			1.09 (0.59 to 2.00)	.79
3-year	61.1	68.8		
5-year	55.7	59.1		
DMFS rate, %			1.00 (0.52 to 1.91)	.99
3-year	63.3	70.9		
5-year	60.0	63.8		

NOTE. Patients received at least one dose of chemotherapy versus observation. Patients who refused adjuvant chemotherapy after random assignment were excluded (n = 8).
Abbreviations: DMFS, distance metastasis–free survival; HR, hazard ratio; LRFS, locoregional failure-free survival; OS, overall survival; RFS, relapse-free survival.
**P* value by log-rank test.

Table A3. Timing of and Patient Compliance With Adjuvant Chemotherapy

Variable	Adjuvant Chemotherapy Group, No. (%)
No. of patients randomly assigned	52
Completed cycles	
1	44 (84.6)
2	39 (75.0)
3	36 (69.2)
4	34 (65.4)
5	30 (57.7)
6	26 (50.0)
Reason for discontinuation, No.	
Progressive disease	0
Patient refusal	19
Adverse event	5
Bone marrow toxicity	1
Death during treatment	1
Dose modification	27 (51.9)
Cisplatin only	11
Gemcitabine only	8
Gemcitabine and cisplatin	7
Gemcitabine and carboplatin	1
Reason for modification	
Hematologic toxicity, No.	27
Changed from cisplatin to carboplatin	2 (3.8)
Dose delay	38 (73.1)
Gemcitabine only	6
Gemcitabine and cisplatin	31
Gemcitabine and carboplatin	1
Reason for delay	
Hematologic toxicity, No.	38
Median delivered dose intensity, % (IQR)	
Cisplatin	93.2 (32.8-99.7)
Gemcitabine	81.0 (67.8-83.7)
Median time from last day of RT to date of plasma EBV DNA screening, days (IQR)	45 (40-48)
Median time from date of plasma EBV DNA screening to date of random assignment, days (IQR)	35 (30-41.5)
Median time from last day of RT to date of random assignment, days (IQR)	78 (75-84)
Median time from last day of RT to first dose of adjuvant chemotherapy, days (IQR)	91 (81-99)
Median time from date of random assignment to first dose of adjuvant chemotherapy, days (IQR)	11 (7.5-19)

Abbreviations: EBV, Epstein-Barr virus; IQR, interquartile range; RT, radiotherapy.

Table A4. Treatment-Related Adverse Events Graded by CTCAE During Adjuvant Chemotherapy in Patients Randomly Assigned to Adjuvant Chemotherapy and Who Received at Least One Dose

Adverse Event	All Grades, No. (%)	Grade 3, No. (%)	Grade 4, No. (%)
Hematologic			
Neutropenia	38 (86)	24 (55)	11 (25)
Thrombocytopenia	19 (43)	3 (7)	0
Anemia	17 (39)	15 (34)	0
Leukopenia	6 (14)	3 (7)	2 (5)
Febrile neutropenia	1 (2)	1 (2)	0
Nonhematologic*			
Nausea	25 (57)	0	0
Fatigue	24 (55)	1 (2)	0
Anorexia	18 (41)	0	0
Pain	17 (39)	0	0
Vomiting	14 (32)	0	0
Constipation	13 (30)	0	0
Neuropathy: sensory	13 (30)	0	0
Infection	7 (16)	1 (2)	0
Mucositis	7 (16)	0	1 (2)
Rash	7 (16)	0	0
Renal	7 (16)	0	0
Fever	6 (14)	0	0
Injection site reaction	6 (14)	0	0
Electrolyte imbalance	5 (11)	4 (9)	1 (2)

NOTE. Safety population (n = 44).
Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events (version 3.0).
*Nonhematologic adverse events reported in ≥ 10% of patients. One patient died as a result of grade 5 hematemesis.

Table A5. Late Radiation Toxicity by RTOG/EORTC Radiation Morbidity Scoring Criteria

	Randomly Assigned to Adjuvant Chemotherapy (n = 52), No.			Randomly Assigned to Observation (n = 52), No.			
Site	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	*P**
Esophagus	26	2	1	22	2	3	.250
Eye	0	0	0	1	0	0	NA
Heart	0	0	0	0	0	0	NA
Larynx	0	0	0	1	0	0	NA
Mucous membrane†	13	17	0	16	19	0	NA
Salivary glands	4	20	12	8	21	13	.820
Skin	21	9	1	30	5	0	.500
Spinal cord	0	0	0	1	0	0	NA
Subcutaneous tissue‡	9	12	9	23	9	2	.022

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; NA, not applicable; RTOG, Radiation Therapy Oncology Group.
**P* value calculated for the comparison of the difference in the incidence of grade 3 toxicities between two groups.
†Mucous membrane toxicity refers to graded changes from dryness to atrophy, telangiectasia, and ulceration.
‡Subcutaneous tissue toxicity refers to graded changes from loss of subcutaneous fat/induration to fibrosis, contracture, and necrosis.

Table A6. Prognostic Factors for Relapse-Free Survival and Overall Survival in the Screening Cohort (N = 789)

Covariate	Relapse-Free Survival		Overall Survival	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Univariable analysis				
Age				
> 50 *v* ≤ 50	1.11 (0.87 to 1.43)	.400	1.46 (1.09 to 1.97)	.010
Sex				
Male *v* female	1.15 (0.85 to 1.54)	.360	1.45 (1.00 to 2.08)	.050
UICC T stage				
T1/2 *v* T3/4	1.54 (1.19 to 1.98)	.001	1.84 (1.36 to 2.49)	< .001
UICC N stage	1.48 (1.15 to 1.90)	.002	1.84 (1.37 to 2.48)	< .001
N0/1 *v* N2/3				
UICC overall stage				
II/III *v* IV	2.20 (1.70 to 2.85)	< .001	2.49 (1.85 to 3.36)	< .001
Treatment modality				
RT *v* CRT	1.40 (1.00 to 1.97)	.050	1.55 (1.04 to 2.31)	.030
Post-RT EBV DNA				
0 *v* > 0 copies/mL	3.21 (2.50 to 4.11)	< .001	3.16 (2.36 to 4.22)	< .001
Multivariable analysis*				
Age				
> 50 *v* ≤ 50	1.07 (0.83 to 1.37)	.620	1.42 (1.05 to 1.92)	.020
Sex				
Male *v* female	1.24 (0.92 to 1.67)	.150	1.53 (1.05 to 2.21)	.030
UICC T stage				
T1/2 *v* T3/4	1.32 (0.99 to 1.75)	.060	1.47 (1.05 to 2.06)	.030
UICC N stage				
N0/1 *v* N2/3	1.15 (0.88 to 1.51)	.310	1.45 (1.05 to 2.00)	.030
UICC overall stage				
II/III *v* IV	1.51 (1.13 to 2.02)	.010	1.59 (1.13 to 2.23)	.010
Treatment modality				
RT *v* CRT	0.94 (0.64 to 1.39)	.760	0.91 (0.57 to 1.43)	.670
Post-RT EBV DNA				
0 *v* > 0 copies/mL	2.97 (2.29 to 3.84)	< .001	2.90 (2.15 to 3.92)	< .001

Abbreviations: CRT, chemoradiation therapy; EBV, Epstein-Barr virus; HR, hazard ratio; RT, radiotherapy; UICC, Union for International Cancer Control.
*Estimates for each covariate in multivariable analysis were adjusted for all other covariates listed.

Table A7. Sensitivity Analysis: Survival Outcome According to Compliance With Adjuvant Chemotherapy

Outcome	Adjuvant Chemotherapy Arm: All ITT	D3: Completed Three or Fewer Cycles of Adjuvant Chemotherapy	D4: Completed More Than Three Cycles of Adjuvant Chemotherapy	D4 *v* D3: HR (95% CI)	*P**
No. of patients	52	18	34		
RFS rate, %				1.63 (0.69 to 3.88)	.26
3-year	51.7	61.1	46.6		
5-year	49.3	61.1	43.0		
OS rate, %				2.24 (0.74 to 6.82)	.14
3-year	70.8	82.6	64.5		
5-year	64.0	76.7	57.6		
LRFS rate, %				1.81 (0.71 to 4.61)	.20
3-year	61.3	72.0	55.5		
5-year	54.6	66.7	48.5		
DMFS rate, %				1.89 (0.69 to 5.16)	.21
3-year	61.3	72.2	55.6		
5-year	58.9	72.2	52.1		

Abbreviations: DMFS, distant metastasis–free survival; HR, hazard ratio; ITT, intention to treat; LRFS, locoregional failure-free survival; OS, overall survival; RFS, relapse-free survival.
**P* value by log-rank test.

Table A8. Sensitivity Analysis: Survival Outcome According to Time to Initiation of Adjuvant Chemotherapy

Outcome	Received at Least One Dose of Adjuvant Chemotherapy	T1: Time to Initiate Adjuvant Chemotherapy Less Than the Median	T2: Time to Initiate Adjuvant Chemotherapy at or Greater Than the Median	T2 *v* T1: HR (95% CI)	*P**
No. of patients	44	20	24		
RFS rate, %				0.47 (0.20 to 1.13)	.08
3-year	54.3	38.9	66.7		
5-year	51.4	38.9	61.5		
OS rate, %				0.43 (0.15 to 1.21)	.10
3-year	70.0	53.2	83.3		
5-year	64.6	53.2	73.7		
LRFS rate, %				0.47 (0.19 to 1.18)	.10
3-year	61.1	43.6	75.0		
5-year	55.7	43.6	65.2		
DMFS rate, %				0.46 (0.17 to 1.20)	.10
3-year	63.3	48.9	75.0		
5-year	60.4	48.9	75.0		

NOTE. The median time from the last dose of radiation to the first dose of adjuvant chemotherapy was 91 days (interquartile range, 81 to 99 days).
Abbreviations: DMFS, distant metastasis–free survival; HR, hazard ratio; LRFS, locoregional failure-free survival; OS, overall survival; RFS, relapse-free survival.
**P* value by log-rank test.

Table A9. Sensitivity Analysis: Exclusion of Patients With Positive Baseline PET/CT Scan

Outcome	C1: Adjuvant Chemotherapy*	C2: Observation*	C1 *v* C2: HR (95% CI)	*P*†
No. of patients	38	39		
RFS rate, %			1.08 (0.50 to 2.33)	.85
3-year	68.1	73.9		
5-year	64.9	70.6		
OS rate, %			0.91 (0.33 to 2.50)	.85
3-year	86.4	91.8		
5-year	80.3	81.5		
LRFS rate, %			1.02 (0.45 to 2.30)	.97
3-year	76.1	81.6		
5-year	70.0	72.1		
DMFS rate, %			1.03 (0.41 to 2.60)	.95
3-year	78.6	84.2		
5-year	75.3	80.6		

Abbreviations: DMFS, distant metastasis–free survival; HR, hazard ratio; LRFS, locoregional failure-free survival; OS, overall survival; PET/CT, positron emission tomography/computed tomography; RFS, relapse-free survival.
*Patients whose baseline PET/CT scan showed positive findings (nine in adjuvant chemotherapy arm, 10 in observation arm) were excluded. Eight patients who did not complete a second PET/CT scan study (five in adjuvant chemotherapy arm, three in observation arm) also were excluded.
†*P* value by log-rank test.