The Clinical Utility of Plasma Epstein–Barr Virus DNA Assays in Nasopharyngeal Carcinoma: The Dawn of a New Era?

A Systematic Review and Meta-Analysis of 7836 Cases

Wenna Zhang, MD, Yupei Chen, MD, Lei Chen, MD, Rui Guo, MD, Guanqun Zhou, MD, Linglong Tang, MD, Yanping Mao, MD, Wenfei Li, MD, Xu Liu, MD, Xiaojing Du, MD, Ying Sun, MD, and Jun Ma, MD

Abstract: In this study, we assessed the potential of plasma Epstein– Barr virus (EBV) DNA assays to predict clinical outcomes in a large sample of nasopharyngeal carcinoma (NPC) patients and proposed a risk stratification model based on standardized EBV DNA load monitoring.

We conducted a meta-analysis of 14 prospective and retrospective comparative studies (n = 7 836 patients) to evaluate the correlation between pretreatment plasma EBV DNA (pre-DNA), midtreatment plasma EBV DNA (mid-DNA), posttreatment plasma EBV DNA (post-DNA), the half-life value of plasma EBV DNA clearance rate ($t_{1/2}$), and clinical outcomes. Our primary endpoint was overall survival (OS). Our secondary endpoints were progression-free survival (PFS), distant-metastasis-free survival (DMFS), and local-regional-failure-free survival (LRFS).

High pre-DNA, detectable mid-DNA, detectable post-DNA, and slow EBV DNA clearance rates were all significantly associated with poorer OS, with hazard radios (HRs) equal to 2.81, 3.29, 4.26, and 3.58, respectively. Pre-DNA, mid-DNA, and post-DNA had the same effects on PFS, DMFS, and LRFS.

Plasma EBV DNA assays are highly prognostic of long-term survival and distant metastasis in NPC patients. Based on the results of this metaanalysis, we propose a 4-grade systematic risk stratification model. Given the inherent limitations of the included studies, future well-designed randomized clinical trials are required to confirm to the findings of this analysis and to contribute to the development of individualized treatment strategies for NPC patients.

(Medicine 94(20):e845)

Abbreviations: AC = adjuvant chemotherapy, CCRT = concurrent chemoradiotherapy, CI = confidence interval, DFS = disease-free

Editor: Joshua Meyer.

WZ, YC, and LC contributed equally to this study.

This work was supported by grants from the Key Laboratory Construction Project of Guangzhou City, China (No. 121800085), the Health & Medical Collaborative Innovation Project of Guangzhou City, China (201400000001), and the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period (2014BAI09B10).

The authors have no conflicts of interest to disclose.

DOI: 10.1097/MD.00000000000845

survival, DMFS = distant-metastasis-free survival, EBNA-1 = EBV nuclear antigen 1, EBV = Epstein–Barr virus, HR = hazard radio, LRFS = local-regional-failure-free survival, mid-DNA = midtreatment plasma EBV DNA, NCCN = National Comprehensive Cancer Network, NPC = nasopharyngeal carcinoma, O-E = observed minus expected number of deaths, OS = overall survival, PCR = Polymerase chain reaction, PFS = progression-free survival, post-DNA = post-treatment plasma EBV-DNA, pre-DNA = pretreatment plasma EBV DNA, PRISMA-P = preferred reporting items for systematic review and meta-analysis protocols, RCT = randomized clinical trials, $t_{1/2}$ = the half-life value of plasma EBV DNA clearance rate, TNM staging = tumor-node-metastasis staging.

INTRODUCTION

The incidence of nasopharyngeal carcinoma (NPC) has remained consistently high in endemic regions (ie, Southern China).¹ Among men ages 20 to 44 years, NPC is the most prevalent form of cancer, comprising 19% of the overall cancer incidence in Hong Kong.²

According to the National Comprehensive Cancer Network (NCCN) guidelines,³ radiotherapy is fundamental to the treatment of NPC combined with different chemotherapies according to staging (ie, concurrent chemoradiotherapy [CCRT] for locoregionally advanced NPC). With the improved local control resulting from more precise imaging and radiotherapy, distant metastases have become the main cause of the failure of this mode of treatment;⁴ despite the use of CCRT, patients with locoregionally advanced NPC will still have a poor prognosis.⁵ Furthermore, until further data emerge, the generalized treatment strategies currently in use cannot be diversified to meet the need for individualized treatment.

Circulating Epstein-Barr virus (EBV) DNA concentrations correlate positively with disease stage as well as exhibiting prognostic importance in NPC.6 Based on the great variety of published studies, EBV DNA concentrations and plasma EBV DNA clearance rates have been identified as emerging biomarkers for monitoring survival,⁷⁻¹¹ although the mechanism remains unclear and no risk classification has been effectively demonstrated. Thus, we hypothesized that plasma EBV DNA assays can be applied clinically to the development of a systematic risk stratification model to monitor disease, responses to treatment, and outcomes. Currently, the published data are limited to those obtained in small-scale series studies; therefore, we conducted this meta-analysis in a largescale population ($n = 7\,836$ patients) to test our hypothesis with the aim of standardizing EBV DNA load monitoring based on a proposed 4-grade systematic risk stratification model. Such a

Received: February 27, 2015; revised and accepted: April 10, 2015.

State Key Laboratory of Oncology in South China, Department of Radiation Oncology, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, People's Republic of China.

Correspondence: Jun Ma, State Key Laboratory of Oncology in South China, Department of Radiation Oncology, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou 510060, People's Republic of China (e-mail: majun2@mail.sysu.edu.cn).

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

ISSN: 0025-7974

model could be used to design more biomarker-integrated clinical trials to optimize individualized treatment strategies.

MATERIALS AND METHODS

This meta-analysis was performed in accordance with preferred reporting items for systematic review and metaanalysis protocols (PRISMA-P) 2015 statement.¹²

Literature Search and Selection of Studies

Initially, we identified all studies focusing on plasma EBV DNA in NPC patients, regardless of publication language. Sources included PubMed, Web of Science and the Cochrane library (last search update January 2015). The search strategy was based on combinations of "nasopharyngeal carcinoma/ cancer/neoplasm" and "EBV DNA/Epstein-Barr virus DNA/ Epstein-Barr viral DNA/EBV deoxyribonucleic acid/Epstein-Barr virus deoxyribonucleic acid/Epstein-Barr viral deoxyribonucleic acid" in [Title/Abstract]. References of retrieved articles were also screened to broaden the search. Lin et al.¹³ used detectable EBV nuclear antigen 1 (EBNA-1) as the marker of positive EBV DNA; this study was also included in our metaanalysis. All analyses were based on previous published studies,thus no ethical approval or patient consent were required.

Inclusion Criteria and Exclusion Criteria

We included all available prospective and retrospective comparative studies (cohort or case-control studies) that compared different clinical outcomes of treatment with high pretreatment plasma EBV DNA (pre-DNA)/ mid-treatment plasma EBV DNA (mid-DNA)/ post-treatment plasma EBV-DNA (post-DNA) versus low pre-DNA/ mid-DNA/ post-DNA or different EBV DNA clearance rates regardless of stage or the population under investigation.

Studies for which it was impossible to extract at least one of the quantitative outcomes mentioned in the next section of this report were excluded. Responding letters, comments, review articles, case reports and experimental animal studies were also excluded. In cases of multiple reports describing the same population, the most recent or most complete report was selected. To ensure better comparison of the outcomes, studies with a median follow-up time of less than 24 months^{14,15} or for which basic information was not available¹⁶ were also excluded (Supplementary Fig. S1).

Data Extraction and Outcomes

Two investigators (WNZ and YPC) extracted the data from eligible studies independently and reached a consensus for all items. Data on characteristics of studies and patients, measurements, and results were extracted. We also recorded the first author, year of publication, country of origin, number of patients analyzed, median follow-up time, tumor-node-metastasis (TNM) staging, pre-DNA and post-DNA cut-off values, and the inclusion period for each study.

Cut-off values for pre-DNA varied among studies; 4000 copies/ml and 1500 copies/ml were the most commonly used values. No attempt at repeated analysis with alternative cut-offs was made. Wang et al.¹¹ detected an association between different pre-DNA values and 2-year overall survival (OS) based on the same sample, which found only 50 000 copies/ml was positive [HR 0.26, 95% Confidence Interval (CI) 0.02–0.51, P = 0.0055]. However, there were only 34 patients in total in this study; therefore, the result is debatable.

The post-DNA cut-off value was defined as 0 copies/ml in five of the included studies.^{8,10,17-19} To investigate the effects of different cut-off values, we also conducted subgroup analysis of long-term survival.

Our primary endpoint was OS, which was defined as the time from diagnosis to death or the last reported date. Our secondary endpoints were progression-free survival (PFS), distant-metastasis-free survival (DMFS) and local-regionalfailure-free survival (LRFS), which were defined as the time from diagnosis to the date of progression, distant metastasis or local regional recurrence respectively. We also defined diseasefree survival (DFS) as the time from diagnosis to the date of any event or when censored at the last report date. If there was no PFS but DFS was mentioned in individual studies, the DFS was included in LRFS.

Quality Assessment and Statistical Analysis

Studies were provided a level of evidence based on the criteria of the Centre for Evidence-based Medicine in Oxford, UK.²⁰ The quality of all the nonrandomized studies was assessed by the modified Newcastle-Ottawa scale,²¹ including patient selection, comparability of the study groups, and assessment of outcomes. Scores ranked from 0-9 (allocated as stars) and studies achieving six stars or more were considered of high quality.

The meta-analysis was performed using Review Manager 5.3.5 (Cochrane Collaboration, Oxford, UK) and STATA 12.0 (Stata Corp, College Station, TX) for testing publication bias. Results regarding the survival endpoints were expressed as hazard radios (HRs), which is the only summary statistic allowing for both censoring and time to an event. HR and its 95% confidence intervals were used directly if available in the individual study, or extraction of summary statistics was performed according to the methods detailed by Parmar et al.²² The observed minus the expected number of deaths (O-E) and its variance and 2-year events if not available were then calculated for each trial using the same method. *P*-values < 0.05 were defined as statistically significant.

The fixed effect model (Mantel–Haenszel), which assumes that differences between the results of various studies are due to chance, was used in our meta-analysis. Heterogeneity across studies was evaluated by the Chi² (χ^2) test and the I^2 statistic in combination with a forest plot. Statistically significant heterogeneity was defined as a $\chi^2 P$ -value < 0.1. Subgroup analysis was performed in instances of heterogeneity across studies. Subgroup analysis were also performed to compare the differences based on population, inclusion stage and cut-off values. Sensitivity analysis was performed to examine the effect of variations in study quality. Potential publication bias was examined using Egger's test. *P*-values < 0.05 were defined as statistically significant.

RESULTS

Eligible Studies

A total of 359 references were retrieved using the initial search algorithm, of which 14 studies were finally included (n = 7 836 patients). Characteristics of the 14 eligible studies are listed in Table 1; no randomized controlled trial (RCT) was available for our research. There was variation among the studies in terms of the level of evidence, study design and quality score. The included studies comprised eight prospective studies^{7,8,11,13,18,19,23,24} and six retrospective studies.^{9,10,17,25–27}

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

	-					Cut-of	f value*	E		
Study	Level of evidence	Design	Z	Inclusion period	1111 Stage (1997AJCC)	Pre-DNA	Post-DNA	L reatment strategy	Median Tollow-up (months)	Quality score
Lin, J. C. 2001 Tw	2c	Р	124	1996.4-1998.11	I-IV,M0	0	/	RT+/-neoCT+/-conCT $\pm/-\Delta dinvant CT$	38.0(24-56)	******
Chan. A.T. 2002 HK	2b	Ч	170	1997.9 - 1999.10	I-IV.M0	4000	500	RT+/-conCT	41.5(9-60)	*****
Leung, S. F. 2006 HK	3b	R	155 ^a	cohort A: 1993-1994	I/II ^c	4000	_	RT+/-conCT	5.8 years	****
ò				cohort B: 1997-2000					•	
Twu, C. W. 2007 Tw	2c	Р	114	NA	I-IV,M0	1500	0	neoCT+/-RT	46.0(22 - 67)	******
Lin, J. C. 2007 Tw	3b	R	152	1994.5 - 2001.5	I-IV,M0	1500	0	CCRT	78.0(48-126)	******
Wang, W. Y. 2010 Tw	2c	Ч	34	2005.3 - 2008.5	R/M	1500	/	CT+/-RT	30.0(18-50)	******
Hou, X. 2011 GZ	4	R	69	2002.5 - 2003.12	I-IV,M0	/	0	RT+/-neoCT+/-conCT	70.0(13 - 79)	*****
Hsu, C. L. 2012 Tw	2c	Ч	73	2007.1 - 2010.2	R/M	5000	/	CT+/-RT	$31.0(20-40)^{d}$	*****
Wang, W. Y. 2013 Tw	2c	Р	210	NA	IIb-IV,M0	1500	0	neoCT+RT	> 6 years	******
Li, S. W. 2013 GZ	2c	Р	210	2003.1 - 2004.12	III/IV,M0	median	/	RT+/-neoCT+/-conCT	73.0(10 - 104)	*****
Wei, W. 2014 GZ	3b	R	155 ^b	2006.7 - 2009.6	I-IV,M0	1500	/	CCRT	60.1(9-80)	******
Leung, S. F. 2014 HK	2c	Р	107	2004.6 - 2006.4	IIb-IV,M0	4000	0	CRT/RT	73.0(4.9 - 89.6)	****
Chen, W. H. 2014 GZ	3b	R	717	2008.1 - 2011.11	I-IV,M0	4000	/	RT+/-neoCT+/-conCT	31.0(24 - 42)	******
Tang, L. Q. 2014 GZ	4	R	6277	2007.1 - 2009.12	I-IV,M0	4000	/	RT+/-neoCT+/-conCT	#	******
				2011.1-2011.12						
1997 AJCC TNM stag GZ = Guangzhou, HK = Fw - Taiwan	e = 1997 Arr Hong Kong,	nerican Joi NA=no	int Com t availat	mittee on Cancer tumor-n ble, neoCT = neo-adjuvan	node-metastasis t chemotherapy.	staging syste , P=prospec	m, conCT = c tive design, F	concurrent chemotherapy, CT = V/M = relapse or metastasis, R	chemotherapy, EBV = = retrospective desig	= Epstein-Barr virus, t, RT = radiotherapy,
* Copies/ml. # training	; set: 51.0 (4	40-62), vi	alidation	t set: 50.0 (40-62), valida	tion set: 29.0 (2	(6-32).				

^a Follow-up data available in these cases. ^b Pretreatment level of EBV DNA was available for only 155 patients in the total dataset of 214. ^c Data included were for stage/II. ^d For surviving patients only.

With the exception of two studies,^{11,23} all were conducted in newly diagnosed non-metastatic NPC patients. The numbers of 2-year events in each study concerning different clinical outcomes are also shown in each figure below.

Regarding the relationship between EBV DNA and clinical outcomes, 13 studies reported pre-DNA^{7-11,13,18,19,23-27} and six reported post-DNA.^{7,8,10,17-19} Two studies (n = 104 patients) provided OS data for the EBV DNA clearance rate,^{11,23} in which the half-life time of EBV DNA clearance rates $(t_{1/2})$ were comparable for three cut-off values ($t_{1/2} = 4$ days, 5 days and 7 days). In comparisons of the clinical outcomes based on pre-DNA, the data for OS, PFS, DMFS, and LRFS were available for 13 studies (n = 8443 patients), six studies (n = 7526 patients), six studies (n = 7600 patients) and six studies (n = 917 patients), respectively. Studies of post-DNA and clinical outcomes were relatively few; OS, PFS, DMFS, and LRFS data were available in only six studies (n = 822 patients), two studies (n = 277 patients), two studies (n = 176 patients) and four studies (n = 583 patients), respectively. Only one study⁸ reported the clinical application of mid-DNA; therefore, this parameter was not analyzed. A summary of the meta-analysis results are shown in Table 2.

Data synthesis

Pre-DNA and Clinical Outcomes

TABLE 2. Summary of Meta-Analysis Results

High levels of pre-DNA were associated with a poorer prognosis in terms of the risk of death, recurrence and metastasis (Figure 1). Analysis of pooled data from 13 studies showed that mortality was almost 3-fold higher in high pre-DNA patients (OS, HR 2.81, 95% CI 2.44–3.24, P < 0.00001). There was significant between-study heterogeneity (I^2 48%, P = 0.03), which we subsequently evaluated in subgroup analyses. Similar results were obtained when studies with a

median follow-up period of less than 3 years^{23,25} were excluded from the analysis (OS, HR 2.80; I^2 39%, P = 0.10). The risks for progression and local-regional failure according to pre-DNA levels were 2.74 and 2.02, respectively. However, the risk of distant metastasis was almost 4-fold higher in high pre-DNA patients (DMFS, HR 3.89, 95% CI 3.39–4.47, P < 0.00001).

Mid-DNA, Post-DNA and Clinical Outcomes

Detectable post-DNA showed an even stronger association with a poorer prognosis than the risk associated with high pre-DNA, especially for DMFS (HR 7.54, 95% CI 3.39–16.77, P < 0.00001) and LRFS (HR 7.51, 95% CI 5.11–11.02, P < 0.00001) (Supplementary Fig. S2). The risk of mortality was 4.26-fold higher in detectable post-DNA patients (OS, HR 4.26, 95% CI 3.26–5.57, P < 0.00001). Again, between-study heterogeneity was detected (I^2 47%, P = 0.09), the source of which was subsequently evaluated in subgroup analyses. Similarly, the HR was 5.21 (PFS, 95% CI 3.29–8.27, P < 0.00001) for progression in detectable post-DNA NPC patients compared with undetectable post-DNA NPC patients.

Although the studies reporting mid-DNA concentrations were limited, we have shown the results of a single study in Table 2 because of the remarkable HR value for DMFS (HR 12.02, 95% CI 2.78–51.93, P < 0.00001).

EBV DNA Clearance Rate and Clinical Outcomes

Plasma EBV DNA clearance rates within the first month of treatment were sufficiently predictive of the clinical outcome to allow timely changes in the treatment regimen. Patients with a short $t_{1/2}$ had significantly higher OS than those with a long $t_{1/2}$ (Supplementary Fig. S3). Taking the $t_{1/2}$ value as 7 days, the HR of $t_{1/2} > 7$ vs. ≤ 7 was 3.58 (95% CI 2.07–6.20, P < 0.00001).

	Studios	Hazand radio	Z-value	P-value	5	Study he	eterogeneit	y
Outcomes	No.	95%CI			Chi^2 (χ^2)	df	$I^2, \%$	P value [*]
Pre-DNA								
OS	13	2.81, 2.44-3.24	14.31	< 0.00001	23.03	12	48%	0.03
PFS	6	2.74, 2.37-3.18	13.33	< 0.00001	8.21	5	39%	0.15
DMFS	6	3.89, 3.39-4.47	19.27	< 0.00001	5.90	5	15%	0.32
LRFS	6	2.02, 1.52-2.70	4.82	< 0.00001	2.39	5	0%	0.79
Post-DNA								
OS	6	4.26, 3.26-5.57	10.58	< 0.00001	9.46	5	47%	0.09
PFS	2	5.21, 3.29-8.27	7.01	< 0.00001	0.00	1	0%	0.95
DMFS	2	7.54, 3.39-16.77	4.95	< 0.00001	1.35	1	26%	0.24
LRFS	4	7.51, 5.11-11.02	10.30	< 0.00001	4.72	3	36%	0.19
Mid-DNA								
OS	1	3.29, 1.37-7.89	2.67	0.0077				
PFS	1	4.05, 1.89-8.67	3.60	0.0003				
DMFS	1	12.02, 2.78-51.93	3.33	0.0009				
LRFS	1	2.05, 0.79-5.31	1.48	0.1378				
EBV DNA clearance ra	ate							
$T_{1/2} > 4$ vs. $\leq 4, os$	2	3.13, 1.76-5.57	3.90	< 0.0001	0.24	1	0%	0.62
$T_{1/2} > 5$ vs. <5,os	2	2.66, 1.55-4.55	3.56	0.0004	0.19	1	0%	0.66
$T_{1/2} > 7$ vs. ≤ 7 , os	2	3.58, 2.07-6.20	4.56	< 0.00001	0.39	1	0%	0.53

EBV = Epstein-Barr virus.

P < 0.1 for between-study heterogeneity and statistically significant heterogeneity are shown in bold.

A	High pre	e-DNA	Low pre	-DNA				Hazard Ratio	Hazard Ratio
Study or Subgroup	Events	Total	Events	Total	O-E	Variance	Weight	Exp[(O-E) / V], Fixed, 95% CI Year	Exp[(O-E) / V], Fixed, 95% Cl
Lin, J. C.2001	22	88	1	36	3.39	1.81	0.9%	6.51 [1.52, 27.93] 2001	
Chan.A.T.2002	33	70	30	66	20.45	26.87	14.0%	2.14 [1.47, 3.12] 2002	
Leung, S. F.2006	13	47	7	108	4.92	3.43	1.8%	4.20 [1.46, 12.09] 2006	
Twu, C. W.2007	13	51	1	63	5.02	2.27	1.2%	9.13 [2.49, 33.53] 2007	(x <u>, x</u>)
Lin. J. C.2007	4	36	0	85	6.76	6.57	3.4%	2.80 [1.30, 6.01] 2007	
Wang, W. Y.2010	12	25	4	9	-1.45	3.14	1.6%	0.63 [0.21, 1.90] 2010	
Hsu, C. L.2012	27	28	17	45	6.4	4.8	2.5%	3.79 [1.55, 9.28] 2012	
Li, S. W.2013	48	103	26	107	43.44	34.58	18.1%	3.51 [2.52, 4.90] 2013	
Wang, W. Y.2013	25	103	3	107	10.15	17.95	9.4%	1.76 [1.11, 2.80] 2013	
Leung, S. F.2014	0	93	0	14	3.05	6.01	3.1%	1.66 [0.75, 3.69] 2014	
Chen. W. H.2014	73	306	94	411	23.62	18.85	9.8%	3.50 [2.23, 5.50] 2014	
Tang, L. Q.2014	229	2748	74	3539	64.59	57.92	30.2%	3.05 [2.36, 3.95] 2014	-8-
Wei, W.2014	6	47	4	108	7.77	7.37	3.8%	2.87 [1.39, 5.91] 2014	· · · · · ·
									8
Total (95% CI)		3745		4698			100.0%	2.81 [2.44, 3.24]	•
Total events	505		261						
Heterogeneity: Chi ² =	23.03. df =	12 (P =	0.03): 12 =	48%					
Test for overall effect:	Z = 14.31 (P < 0.00	001)						0.05 0.2 1 5 20
			-						Favours [High pre-DNA] Favours [Low pre-DNA]
в	High pre	DNA	Low pro	DNA				Harard Patio	Hazard Patio
Study or Subaroup	Evente	Total	Evente	Total	0.5	Variance	Weight	Evol(O_E) / VI Eived 95% CI Ver	Exp[()_E) / V] Eixed 95% Cl
Lin L C 2001	20	00	2	26	2.02	1 0/	1 1 10/	P 02 [1 90 24 00] 2001	
Chap & T 2002	25	70	24	66	10.00	27 4	15 59/	1 00 [1 27 2 00] 2001	
Tana L 0 2014	621	2749	217	2520	107.05	05.05	EE 00/	2 09 [2 52 2 76] 2002	-
Tang, L. Q.2014	14	2/40	12	109	107.90	15.00	0 00%	1 09 [1 20 2 20] 2014	
Vvel, vv.2014	14	4/	13	100	10.32	15.00	0.0%	1.90 [1.20, 3.29] 2014	
Chap W U 2014	111	306	106	411	1.1	0.0	4.9%	2.20 [1.17, 4.45] 2014	
Glien, W. H.2014		300	100	411	20.20	20.90	14.976	2.97 [2.02, 4.30] 2014	
Total (95% CI)		3352		4174			100.0%	2.74 [2.37, 3.18]	•
Total events	820		372						
Heterogeneity: Chi2 =	8.21, df = 5	5 (P = 0.1	(5); ² = 39	9%					
Test for overall effect:	Z = 13.33 (P < 0.00	001)						Eavours [Hinh pre-DNA1 Eavours [Low pre-DNA1
									i arous (right pre shell i arous (aon pre shell
с	High pre	-DNA	Low pre	-DNA				Hazard Ratio	Hazard Ratio
Study or Subgroup	Events	Total	Events	Total	0-E	Variance	Weight	Exp[(O-E) / V], Fixed, 95% CI Year	Exp[(O-E) / V], Fixed, 95% CI
Lin .L C 2001	28	88	1	36	2 53	0.96	0.5%	13 95 [1 89 103 11] 2001	· · · · · · · · · · · · · · · · · · ·
Li, S. W.2013	40	103	24	107	167.75	113.72	56.6%	4.37 [3.64. 5.25] 2013	
Chen. W. H.2014	111	306	109	411	22.05	16.89	8.4%	3.69 [2.29, 5.94] 2014	
Leung, S. F.2014	0	93	0	14	5.63	4.91	2.4%	3.15[1.30, 7.62] 2014	
Wei W 2014	14	47	11	108	6.29	6.15	3 1%	2 78 [1 26 6 13] 2014	
Tang I Q 2014	509	2748	152	3539	68.94	58.34	29.0%	3 26 12 52 4 211 2014	+
		-							
Total (95% CI)		3385		4215			100.0%	3.89 [3.39, 4.47]	•
Total events	702		297						
Heterogeneity: Chi ² =	5.90, df = 5	5 (P = 0.3)	32); I ² = 15	5%					0.01 0.1 1 10 100
Test for overall effect:	Z = 19.27 ((P < 0.00	001)						Favours [High pre-DNA] Favours [Low pre-DNA]
D	High pre	DNA	Low pre	-DNA				Hazard Ratio	Hazard Ratio
Study or Subgroup	Events	Total	Events	Total	0-E	Variance	Weight	Exp[(O-E) / V], Fixed, 95% Cl Year	Exp[(O-E) / V], Fixed, 95% Cl
Lin, J. C.2007	5	36	6	85	1.01	3.69	7.9%	1.31 [0.47, 3.65] 2007	· · · · ·
Twu, C. W.2007	17	51	11	63	5.41	5.49	11.8%	2.68 [1.16, 6.18] 2007	
Li, S. W.2013	26	103	14	107	13.5	16.1	34.5%	2.31 [1.42, 3.77] 2013	
Wang, W. Y.2013	34	103	11	107	9.5	14.22	30.4%	1.95 [1.16, 3.28] 2013	
Leung, S. F.2014	0	93	0	14	0.9	4.02	8.6%	1.25 [0.47, 3.32] 2014	· · · · ·
Wei, W.2014	3	47	4	108	2.59	3.18	6.8%	2.26 [0.75, 6.78] 2014	
Total (95% CI)		433		484			100.0%	2.02 [1.52, 2.70]	
Total events	85		46						
Heterogeneity: Chi ² =	2.39, df = 5	6 (P = 0.7	79); l ² = 0%	6					02 05 1 2 5
Test for overall effect:	Z = 4.82 (F	< 0.000	01)						C.2 U.0 I 2 5 Eavoure [High pre_DNA] Eavoure [Low pre_DNA]
									r avours [right pre-brand r avours [cow pre-brand]

FIGURE 1. Meta-analysis of pre-DNA associated clinical outcomes, for (A) overall survival (OS), (B) progression-free survival (PFS), (C) distant-metastasis-free survival (DMFS), (D) local-regional-failure-free survival (LRFS). Note: Events for Leung, 2014 were unavailable.

Subgroup Analysis

Pre-DNA Associated OS, Subdivided by Population, Cutoff Value and Inclusion Stage

There were no significant differences in the results of this subgroup analysis compared with those of the original analysis, except in terms of the stage of patients included in each study (inclusion stage) (Figure 2). Pre-DNA was not predictable in relapse or metastasis patients before treatment [2 studies (n = 54 patients), HR 1.87, 95% CI 0.93–3.74, P = 0.08].

Post-DNA Associated OS, Subdivided by Population and Cutoff Value

There were no significant differences in the results of this subgroup analysis compared with those of the original analysis (Supplementary Fig. S4). However, regarding the subgroup analysis based on post-DNA cut-off value, five studies^{8,10,17–19} including 549 patients indicated that 0 copies/ml was a better prognosticator than 500 copies/ml (HR 5.81 compared with 2.82, P = 0.009).

Sensitivity Analysis and Publication Bias

Nine studies achieving six or more stars on the modified Newcastle-Ottawa scale (Supplementary Table S1) were included in sensitivity analysis (Table 3). There was no change in the significance of any of the outcomes except for the inability to perform post-DNA meta-analysis of PFS and DMFS for a few studies. The degree of between-study heterogeneity decreased slightly for OS data of post-DNA, but not for OS data of pre-DNA.

As shown in our data synthesis report, there was significant between-study heterogeneity in the OS analysis of both pre-DNA and post-DNA. According to the results of subgroup analysis, population variation was associated with pre-DNA predicted OS (P = 0.05) and different cut-off values were the key factors for post-DNA (P = 0.009). An Egger's publication bias plot of 13 studies^{7-11,13,18,19,23-27} that reported pre-DNA associated OS included in this meta-analysis was constructed (Figure 3), which indicated no obvious publication bias (P = 0.879).

	High pre-	DNA	Low pre-	DNA	0.5	Variance	Walaht	Hazard Ratio	Hazard Ratio
1.1.1 Taiwan	Events	Iotal	Events	Iotai	0-E	variance	weight	Exp[(O-E) / V], Fixed, 95% Ci Year	Exp[(0-E) / V, Fixed, 95% CI
Lin, J. C. 2001	22	88	1	36	3.39	1.81	0.9%	6.51 [1.52, 27.93] 2001	
Twu, C. W. 2007	13	51	1	63	5.02	2.27	1.2%	9.13 [2.49, 33.53] 2007	
Wang, W. Y. 2010	12	25	4	9	-1.45	3.14	1.6%	0.63 [0.21, 1.90] 2010	
Hsu, C. L. 2012 Wang, W. Y. 2013	27	28	17	45	6.4	4.8	2.5%	3.79 [1.55, 9.28] 2012 1.76 [1.11, 2.80] 2013	·
Subtotal (95% CI)	20	331		345	10.10	11.00	19.1%	2.29 [1.66, 3.17]	•
Total events Heterogeneity: Chi2 =	103 14 27 df = F	5/P=0	26 01): 1 ² = 6	5%					
Test for overall effect:	Z = 5.01 (P	< 0.000	01)						
1.1.2 Hong Kong									
Chan, A.T. 2002	33	70	30	66	20.45	26.87	14.0%	2.14 [1.47, 3.12] 2002	
Leung, S. F. 2006	13	47	7	108	4.92	3.43	1.8%	4.20 [1.46, 12.09] 2006	
Subtotal (95% CI)	ă	210		188	0.00	0.01	19.0%	2.19 [1.58, 3.03]	•
Total events	46	(P = 0.2	37						
Test for overall effect:	Z = 4.72 (P	< 0.000	01)						
1.1.3 Guangzhou									
Li, S. W. 2013	48	103	26	107	43.44	34.58	18.1%	3.51 [2.52, 4.90] 2013	
Chen, W. H. 2014	73	306	94	411	23.62	18.85	9.8%	3.50 [2.23, 5.50] 2014	
Tang, L. Q. 2014	229	2748	74	3539	64.59	57.92	30.2%	3.05 [2.36, 3.95] 2014	
Subtotal (95% CI)		3204		4165			62.0%	3.24 [2.70, 3.87]	•
Total events Heterogeneity: Chi ² =	356 0.66. df = 3	(P = 0.8	198 (8): 1 ² = 0%						
Test for overail effect.	Z = 12.80 (F	P < 0.00	001)						
Total (95% CI)		3745		4698			100.0%	2 81 [2 44 3 24]	
Total events	505	0140	261	4000			100.074	2.01 [2.44, 0.24]	· · · · · · · · · · · · · · · · · · ·
Heterogeneity: Chi ^z =	23.03, df = 1	12 (P = 0	0.03); (2 = -	48%					0.05 0.2 1 5 20
Test for subaroup diffe	Z = 14.31 (F erences: Chi	² = 6.18	. df = 2 (P	= 0.05).	l² = 67.	6%			Favours [High pre-DNA] Favours [Low pre-DNA]
	Winh ere	DNA	Lowers	DNA				Harord Patio	Harand Ratio
Study or Subgroup	Events	Total	Events	Total	O-E	Variance	Weight	Exp[(O-E) / V], Fixed, 95% Cl Year	Exp[(O-E) / V]. Fixed, 95% Cl
1.2.1 Cut-off value =	4000 copies	s/ml	-	-	20.45		14.00	0 14 14 17 0 101 0000	
Leung, S. F. 2002	33	47	30	108	4.92	20.87	14.0%	2.14 [1.47, 3.12] 2002 4.20 [1.46, 12.09] 2006	
Chen, W. H. 2014	73	306	94	411	23.62	18.85	9.8%	3.50 [2.23, 5.50] 2014	
Tang, L. Q. 2014	229	2748	74	3539	64.59	57.92	30.2%	3.05 [2.36, 3.95] 2014	
Subtotal (95% CI)	0	3264	0	4138	5.05	0.01	59.0%	2.80 [2.33, 3.37]	•
Total events	348		205						
Test for overall effect:	5.50, df = 4 Z = 10.97 (F	(P = 0.2 P < 0.00	(4); 1* = 27 (001)	%					
1.2.2 Gut-off value = Lin. J. C. 2007	1500 copies 4	s/ml 36	0	85	6.76	6.57	3.4%	2 80 [1 30 6 01] 2007	
Twu, C. W. 2007	13	51	1	63	5.02	2.27	1.2%	9.13 [2.49, 33.53] 2007	· · · · · · · · ·
Wang, W. Y. 2010	12	25	4	9	-1.45	3.14	1.6%	0.63 [0.21, 1.90] 2010	
Wei, W. 2014	25	47	3	107	7.77	7.37	3.8%	2.87 [1.39, 5.91] 2014	
Subtotal (95% CI)		262	223	372			19.5%	2.13 [1.55, 2.94]	•
Heterogeneity: Chi ² =	60 11.26, df = 4	(P=0.	12 02): 1 ² = 6	4%					
Test for overall effect:	Z = 4.63 (P	< 0.000	01)						
1.2.3 Other cut-off va									
	alues								
Lin, J. C. 2001	alues 22	88	1	36	3.39	1.81	0.9%	6.51 [1.52, 27.93] 2001	· · · · · · · · · · · · · · · · · · ·
Lin, J. C. 2001 Hsu, C. L. 2012	alues 22 27 48	88 28 103	1 17 26	36 45 107	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18 1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI)	22 27 48	88 28 103 219	1 17 26	36 45 107 188	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.94]	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% Cl) Total events Heterogeneity: Ch ² =	22 27 48 97 0.65 cff = 2	88 28 103 219	1 17 26 44	36 45 107 188	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.94]	•
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI) Total events Heterogeneity: Chi ^a = Test for overall effect:	22 27 48 97 0.66, df = 2 Z = 8.29 (P	88 28 103 219 (P = 0.7 < 0.000	1 17 26 44 (2); l ² = 0% 01)	36 45 107 188	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.94]	•
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI) Total events Heterogeneity: Chi ^a = Test for overall effect: Total (95% CI)	22 27 48 97 0.66, df = 2 Z = 8.29 (P	88 28 103 219 (P = 0.7 < 0.000 3745	1 17 26 44 2); I ² = 0% 01)	36 45 107 188 4698	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.34] 2.81 [2.44, 3.24]	•
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% Cl) Total events Heterogeneity: Ch) ^a = Test for overall effect: Total (95% Cl) Total events	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505	88 28 103 219 (P = 0.7 < 0.000 3745	1 17 26 44 2); ² = 0% 01) 261	36 45 107 188 4698	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24]	•
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI) Total events Heterogeneity: Chi ^a = Test for overall effect: Total (95% CI) Total events Heterogeneity: Chi ^a =	22 27 48 97 0.66, df = 2 Z = 8,29 (P 505 23.03, df = 1 Z = 14.31 (F	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = 0 P < 0.00	1 17 26 44 (2); ² = 0% 01) 261 0.03); ² = 0 001)	36 45 107 188 4698 48%	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24]	→ → → → → → → → →
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI) Total events Heterogeneity: Chi ^a = Test for overall effect: Total (95% CI) Total events Heterogeneity: Chi ^a = Test for overall effect. Test for subarouo diffe	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F erences: Chi	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = (P < 0.00 z = 5.60	1 17 26 44 2); I ² = 0% 01) 261 0.03); I ² = - 001) . df = 2 (P	36 45 107 188 4698 48% = 0.06).	3.39 6.4 43.44	1.81 4.8 34.58 3%	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.61 [2.44, 3.24]	0.05 0.2 1 5 20 Favours [thigh pre-DNA] Favours [Low pre-DNA]
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5% CI) Total events Heterogeneity: Ch ² = Test for overall effect Total (\$5% CI) Total events Heterogeneity: Ch ² Test for subarous diffe	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F erences: Chi High pre-	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = 0 > < 0.00 2 = 5.60 DNA	1 17 26 44 2); l ² = 0% 01) 261 0.03); l ² = . 001) . df = 2 (P Low pre-	36 45 107 188 4698 48% = 0.06).	3.39 6.4 43.44	1.81 4.8 34.58	0.9% 2.5% 18.1% 21.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.61 [2.44, 3.24] Hazard Ratio	0.05 0.2 1 5 20 Favours [High pre-DNA] Favours [Low pre-DNA] Hazard Ratio
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5% CI) Total events Heterogeneity: Ch ² = Test for overall effect: Total events Heterogeneity: Ch ² = Test for overall effect rest for subarouro diff Study or Subarouro	22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F rences: Chi High pre- Events asis	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = 0 P < 0.00 2 = 5.60 -DNA Total	1 17 26 2); ² = 0% 01) 261 0.03); ² = 0% 001) . df = 2 (P Low pre- Events	36 45 107 188 4698 48% = 0.06). DNA Total	3.39 6.4 43.44 J ² = 64. O-E	1.81 4.8 34.58 3% Variance	0.9% 2.5% 18.1% 21.5% 100.0% Weight	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.52, 4.90] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(O-E1) VI, Fixed, 35% CI Year	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5%, CI) Total events Heterogeneity: Chi ² = Test for overall effect Total (\$5%, CI) Total events Heterogeneity: Chi ² = Test for overall effect Test for subsroup 1.3.1 Relapse/fileas	22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 52 = 14.31 (F erences: Chi High pre- Events tasis 12	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = (P < 0.00 ² = 5.60 -DNA Total 25	1 17 26 44 (2); ² = 0% 01) 261 0.03); ² = - 001) . df = 2 (P Low pre- <u>Events</u> 4	36 45 107 188 4698 48% = 0.06). -DNA Total 9	3.39 6.4 43.44 ² = 64. <u>0-E</u> -1.45	1.81 4.8 34.58 3% <u>Variance</u> 3.14	0.9% 2.5% 18.1% 21.5% 100.0% Weight	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.51 [2.52, 4.69] 2013 3.64 [2.58, 4.54] 2.81 [2.44, 3.24] Hazard Ratio Exp[(C-E) / VJ. Fixed, 95% Cl Year 0.65 [0.21, 1.90] 2010	0.05 0.2 1 5 20 Favours [High pre-DNA] Hazard Ratio Exp[(0-E)/ V], Fixed, \$5% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li S. W. 2013 Subtotal (95% CI) Total events Heterogeneity. Ch ² = Test for overall effect Total (95% CI) Total events Heterogeneity. Ch ² = Test for subarroup diff Study or Subarroup 1.3.1 Relapse/Measure Mang, W. 2. 2010 Hsu, C. L. 2012 Subtotal (95% CI)	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F erences: Chi High pre- Events tasis 12 27	88 28 103 219 (P = 0.7 < 0.000 3745 212 (P = (P < 0.000 3745 212 (P = (P < 0.000 2 = 5.60 DNA Total 25 28 53	1 17 26 44 2); ² = 0% 01) 261 0.03); ² = - 001) . df = 2 (P Low pre- Events 4 17	36 45 107 188 4698 48% = 0.06), DNA Total 9 45 54	3.39 6.4 43.44 ² = 64. <u>0-E</u> 6.4	1.81 4.8 34.58 3% <u>Variance</u> 3.14 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 282, 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[IO-E]/VL Fixed, 95% C1 Year 0.63 [0.21, 1.50] 2010 3.79 [1.55, 2.82] 2012 1.87 [0.53, 3.74]	0.05 0.2 1 5 20 Favours [High pre-DNA] Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) //V, Fixed, 95%, Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtoal (\$5% CI) Total events Heterogeneity: Ch ² = Total (\$5% CI) Total events Heterogeneity: Ch ² Test for svall effect Test for svall effect Test for subaroup diff <u>Study or Subaroup</u> 1.3.1 Relapse/Metasi Wang W. 2. 2012 Subtoal (\$5% CI) Total events	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F erences: Chi High pre- Events tasis 12 27 39	88 28 103 219 (P = 0.7 < 0.000 3745 12 (P = (2 < 0.00 2 < 0.00 2 < 0.00 2 < 0.00 DNA Total 25 28 53	1 17 26 44 2); ² = 0% 01) 261 0.03); ² = - 001) . df = 2 (P Low pre- Events 4 17 21	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54	3.39 6.4 43.44] ² = 64. -1.45 6.4	1.81 4.8 34.58 3% <u>Variance</u> 3.14 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.25] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E) / V], Fixed, 35% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 9.28] 2012 1.87 [0.58, 3.74]	0.05 0.2 1 5 20 Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / VJ. Fixed, 95% Cl
Lin, J.C. 2001 Hsu, C.L. 2012 Li, S.W. 2013 Subtolai (\$5%, CI) Total events Heterogeneity, Chi ² = Test for overall effect. Test for subaroup diff. <u>Study or Subaroup</u> 1.3.1 Relapse/Metast Wang, W. 7. 2010 Heterogeneity, Chi ² = Total (\$5%, CI) Total events Heterogeneity, Chi ² = Total events Usage, C.L. 2012 Subtoal (\$5%, CI) Total events Heterogeneity, Chi ² = Test for overall affect.	22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F rences: Chi High pre- Events iasis 12 27 39 6.12, df = 1 Z = 1.76 (<i>J</i>)	88 28 103 219 (P = 0.7 3745 12 (P = (0 < 0.000 ² = 5.60 DNA 25 28 53 (P = 0.0 8 20 20 20 20 20 20 20 20 20 20 20 20 20	1 17 26 (2); ² = 0% (01) 261 0.03); ² = . (001) . df = 2 (P Low pre- Events 4 17 (1); ² = 84	36 45 107 188 4698 48% = 0.06), DNA Total 9 45 54	3.39 6.4 43.44 ^{[7} = 64. -1.45 6.4	1.81 4.8 34.58 3% <u>Variance</u> 3.14 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.24, 400] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E)/V], Fuxed, 95% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.28] 2012 1.87 [0.53, 3.74]	0.05 0.2 1 5 20 Favous [high pre-DNA] Favours [Low pre-DNA] Hazard Ratio Exp[(0-5) / V), Fixed, 95%, Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (9% C)) Total events Heterogeneity: Ch ² = Test for overail effect Test for overail effect Heterogeneity: Ch ² = Test for overail effect 7 Subtoroup 5 Nutl or Subtoroup 6 Nutl or Subtoroup 6 Nutl or Subtoroup 6 Nutl or Subtoroup 6 Nutl or Subtoroup 7 Total events Heterogeneity: Ch ² = Test for overail effect	22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F rences: Chi High pre- Events iasis 12 27 39 6.12, df = 1 Z = 1.76 (P	88 28 103 219 (P = 0.7 < 0.000 3745 219 (P = 0.000 2 < 0.000 2 = 5.60 DNA Total 25 28 53 (P = 0.08)	1 17 26 44 (2); l ² = 0% 01) 261 0.03); l ² = . 001) . df = 2 (P Low pre- Events 4 17 21 17; l ² = 84	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54	3.39 6.4 43.44 ² = 64. O-E -1.45 6.4	1.81 4.8 34.58 3% <u>Variance</u> 3.14 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 228] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.61 [2.44, 3.24] Hazard Ratio Exp[(C-E) / V]. Fixed. 35% Cl Year 0.63 [0:21, 1.90] 2010 3.79 [15.5, 228] 2012 1.87 [0.53, 3.74]	0.05 0.2 1 5 20 Favours [Lingh pre-DNA] Hazard Ratio Exp[(0-E) / V), Fixed, 95% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subbtal (\$5%, CI) Total events Heterogeneity: Ch ² = Test for overall effect Test for overall effect Test for overall effect Test for overall effect Subtoral (\$5%, CI) Total events Heterogeneity: Ch ² = Test for overall effect 3.3.1 Religner/Metas Subtoral (\$5%, CI) Total events Heterogeneity: Ch ² = Test for overall effect 1.3.2 Stape HV, Mo	Alues 22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F rerences: Chi High pre- Events 12 27 39 6.12, df = 1 Z = 1.76 (P 22 22 22 22 22 22 22 22 22 2	88 28 103 219 (P = 0.7 3745 2 0.000 3745 2 0.000 2 < 0.000 2 < 0.000 2 = 5.60 DNA 25 28 53 (P = 0.08) (P = 0.08)	1 17 26 44 21; ² = 0% 001) 001) 261 2001) df = 2 (P Low pre- Events 17 21 17; ² = 84	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54 %	3.39 6.4 43.44 F = 64. -1.45 6.4	1.81 4.8 34.58 3% <u>Variance</u> 3.14 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.28, 2012 3.51 [2.52, 4.69] 2013 3.64 [2.58, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(C-E) / VJ. Fixed, 35% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.28] 2012 1.87 [0.53, 3.74]	0.05 0.2 1 5 20 Favours [High pre-DNA] Favours [Low pre-DNA] Hazard Ratio Exp[(0-E)/V]. Fixed, 95% Cl
Lin. J.C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Test for overall effect Test for subaroup diff Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for subaroup diff Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect 1.3.2 Stage HV, MO Lin. J. C. 2001 Chan, A.T. 2002	Alues 22 27 48 97 0.66, df = 2: Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F prences: Chi High pre- Events 12 27 39 6.12, df = 1 Z = 1.76 (P 22 33	88 28 103 219 (P = 0.7 3745 20,000 3745 20,000 2 < 0.000 2 < 0.0000 2 < 0.00000 2 < 0.0000 2 < 0.0000 2 < 0.0000 2 < 0.00000 2 < 0.00000 2 < 0.0000000 2 < 0.0000000000000000000000000000000000	1 17 26 44 (2): P = 0% 01) 261 0001	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54 %	3.39 6.4 43.44 F = 64. -1.45 6.4 3.39 20.45	1.81 4.8 34.58 3% Variance 3.14 4.8 1.81 26.87	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.82] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E]) VI, Fixed, 95% CI Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.08] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.12] 2002	0.05 0.2 1 5 20 Favours [Low pre-DNA] Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / V, Fixed, 95% Cl
Lin, J.C. 2001 Hou, C.L. 2012 Li, S.W. 2013 Subtotal (\$5% CI) Total events Heterogeneity: Ch ² = Test for overall effect Test for subarous diff Study of Subgroup 1.3.1 Relapse/Metasi Subtotal (\$5% CI) Total events Heterogeneity: Ch ² = Subtotal (\$5% CI) Total events Heterogeneity: Ch ² Test for subarous diff Chal events Heterogeneity: Ch ² Test for subarous diffect 1.3.2 Stage HV, M0 Lin, J. C. 2001 Chan, A.T. 2002 Leung, S. F. 2006	alues 22 27 48 97 0.66, df = 1 27 2 = 8.29 (P 505 23.03, df = 1 2 = 14.31 (F arences: Chi High pre- Events 12 27 39 6.12, df = 1 Z = 1.76 (P 22 33 13 13	88 28 103 219 (P = 0.7 < 0.000 3745 2 (P = 0. - < 0.000 2 < 0.000 2 < 5.60 DNA Total 25 28 53 (P = 0.08) (P = 0.08) 88 70 47 7	1 17 26 44 (2): P = 0% 01) 2611 0001) (00): P =. Events 4 17 21 17): P = 84 1 30 7 7	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54 %	3.39 6.4 43.44 F = 64. -1.45 6.4 3.39 20.45 4.92	1.81 4.8 34.58 3% Variance 4.8 1.81 1.81 26.87 3.43 4.8	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.23] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E) / VI, Fixed, 35% Cl Year 0.53 [0.21, 1.90] 2010 3.79 [1.55, 9.28] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.72] 2002 4.20 [1.46, 12.09] 2006 9.99 [1.46, 12.09] 2006	0.05 0.2 1 5 20 Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / VJ. Fixed, 95% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (9% C) Total events Heterogeneity, Ch ² = Test for overall effect Test for overall effect Heterogeneity: Ch ² = Test for overall effect 7 at for overall effect 7 at for overall effect 7 at for overall effect 1 at Suborou off 8 tudy or Suboroup 1 at Relapse/Metast Wang, W. 2010 Total events 1 at Stapse/Metast Wang, W. 2010 Total events 1 at Stapse/Hythol 1 at J. C. 2001 Chan, AT, 2002 Lin, J. C. 2001 Chan, AT, 2002	alues 22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 Z = 14.31 (F perences: Chi High pre- Events 12 27 39 6.12, df = 1 Z = 1.76 (P 22 33 13 4 13	88 28 103 219 (P = 0.7 < 0.000 3745 2 0.000 2 < 0.000 2 < 0.000 2 < 5.60 DNA Total 25 28 53 (P = 0.08) 88 70 47 36 51	1 17 26 44 (2); P = 0% 001) 261 2001) (d = 2 (P) Low pre- Events 4 17 21 10; P = 84 17 21 11; P = 84 10 7 0 1 10 10 10 10 10 10 10 10	36 45 107 188 4698 48% = 0.061) DNA Total 9 45 54 %	3.39 6.4 43.44 F = 64. -1.45 6.4 3.39 20.45 4.92 6.76 5.02	1.81 4.8 34.58 3% Variance 3.14 4.8 1.81 26.87 3.43 6.57 2.27	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.25] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E)/V], Fixed, 95% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.29] 2011 2.14 [1.47, 312] 2002 4.20 [1.40, 7.20] 2001 2.14 [1.47, 312] 2002 4.20 [1.40, 2.20] 2001 2.167 [0.53, 3.74]	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$9%, CI) Total events Heterogeneity: Chi ² = Test for overail effect Test for overail effect Heterogeneity: Chi ² = Test for overail effect Test for Subtotal (35%, CI) Total events Heterogeneity: Chi ² = Test for overail effect Una, J. C. 2010 Chai, A. T. 2002 Chai, A. T. 2002 Chai, A. T. 2005 Lin, J. C. 2001 Chai, A. T. 2005 Lin, J. C. 2007 Twu, C. W. 2007 Wang, W. 2013	alues 22 27 48 9 0.66, df = 2 Z = 8.29 (P 505 23.03, df = 1 2 = 1.43 : (R High pre- High pre- High pre- High pre- Z = 1.76 (P 22 33 33 4 4 32 52	88 28 219 (P = 0.7 < 0.000 3745 2 < 0.000 2 < 0.000 2 = 5.60 DNA Total 25 25 25 33 (P = 0.0 PNA 70 47 36 51 103	1 17 26 44 (1): P = 0% 001) 261 2003); P =. 0001) . df = 2 (P Low pre- Events 4 17 21 11); P = 84 17 21 13 00 7 0 1 3	36 45 107 188 4698 48% = 0.061) DNA Total 9 45 54 % 36 66 108 85 53 107	3.39 6.4 43.44 43.44 -1.45 6.4 3.39 20.45 4.92 6.76 6.2 10.15	1.81 4.8 34.58 3% Variance 1.81 4.8 3.43 6.57 2.27 7.7.95	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1% 0.9% 4.1% 9.4% 9.4%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.28, 2012 3.64 [2.58, 4.54] 2.81 [2.44, 3.24] Hazard Ratio Exp[(C-E) / VJ. Fixed, 95% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.28] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 312] 2002 4.20 [1.40, 212] 2002 1.97 [1.50, 3.007] 1.17 [1.11, 200] 2010 3.11 [1.12, 2002]	0.05 0.2 1 5 20 Favours [Ligh pre-DNA] Favours [Low pre-DNA] Hazard Ratio Exp[(0-E)/V), Fixed, 95% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Test for overall effect Test for overall effect Test for overall effect Test for subcround diff. Bludy or Subcroup, Ch ² = Test for overall effect Test for subcround diff. Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect 1.3.2 Stage I-IV , MO Lin, J. C. 2001 Total, Z. 2007 Twu, C. W. 2007 Twu, C. W. 2007 Twu, C. W. 2007 Twu, C. W. 2007	alues 22 27 48 97 505 23.03, df = 1 22 23.03, df = 2 23.03, df = 1 24.03 27 14.04 14.04 14.05 17 12.27 39 6.12, df = 1 1.76 (P 23.33 13 13.32 25 48 n	88 28 219 (P = 0.7 < 0.000 3745 2 < 0.000 2 < 0.000 2 = 5.60 DNA Total (P = 0.0 25 28 53 (P = 0.008) 88 70 47 36 51 103 103	1 1 17 26 44 44 26 1 261 201) 261 2001 1 261 2001 4 17 21 12 1 30 0 7 0 1 1 1 1 1 1 1 1 1 1 1 1 1	36 45 107 188 4698 48% = 0.06). DNA Total 9 9 5 4 54 %	3.39 6.4 43.44 7 = 64 -1.45 6.4 3.39 20.45 4.92 6.76 5.02 10.15 43.44	1.81 4.8 34.58 3% 1.81 26.67 3.34 4.8 6.57 2.277 2.277 2.277 3.438 6.57 3.458 6.57 2.277 2.277 2.277 3.458 4.58 3.458 3.458 3.458 3.458 3.458 3.58 3.58 3.58 3.58 3.58 3.58 3.58 3.	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.26] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.58, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[IO-E] / VL Fixed, 95% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.28] 2012 1.67 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.12] 2002 4.20 [1.46, 12:09] 2006 2.80 [1.03, 0.61] 2007 5.13 [2.44, 3.35] 2007 5.13 [2.44, 3.26] 2006 2.80 [1.12, 20] 2006 2.80 [1.12, 20] 2006 0.80 [1.12, 20] 2016 3.51 [2.52, 4.00] 2013 3.51 [2.52, 4.00] 2014 3.51 [2.52, 4.00] 2015 3.51 [2.52, 4.50] 2015	ODS 0.2 1 5 20 Favours [Low pre-DNA] Hazard Ratio Exp[O-E]/V, Fixed, 95% CI
Lin. J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (§5%, CI) Total events Heterogeneity, Chi ² = Test for overall effect Test for overall effect Test for subarou diff Subtotal (§5%, CI) Total events Heterogeneity, Chi ² = Test for subarou diff Subtotal (§5%, CI) Total events Heterogeneity, Chi ² = Test for subarou diff 1.3.1 Relapse/Metasi Subtotal (§5%, CI) Total events Heterogeneity, Chi ² = Chan, A.T. 2002 Leung, S. F. 2004 Lin. J. C. 2007 Twu, C. W. 2007 Wang, W. Y. 2013 Leung, S. F. 2014 Le, W. 2013 Leung, S. F. 2014 Len, W. 1. 2014	lues 22 27 48 97 97 505 523.03, df = 1 505 523.03, df = 1 2 23.03, df = 1 2 23.03, df = 1 2 23.03, df = 1 2 2.13, df = 1 2 2.2 2.3 3 13 13 2.5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	88 28 103 219 (P = 0.7, < 0.000 3745 22 (P = 0.00 2 = 5.60 DNA Total 25 28 53 (P = 0.0, 0) 25 28 53 (P = 0.0, 0) 00 47 36 65 11 103 103 306	1 17 26 44 44 26 10 10 26 10 10 10 10 10 10 10 10 10 10	36 45 107 188 4698 48% = 0.06). DNA Total 9 9 5 54 % %	3.39 6.4 43.44 7 = 64 -1.45 6.4 3.39 20.45 4.92 6.76 6.4 3.39 20.45 4.92 6.76 23.62	1.81 4.8 34.58 3% Variance 1.81 26.87 3.43 3.53 5.53 4.58 5.34 5.85 5.34 5.34 5.34 5.34 5.34 5.34 5.34 5.3	0.9% 2.5% 100.0% 21.5% 100.0% 0.9% 14.0% 2.5% 4.1% 0.9% 14.8% 3.4% 1.8% 3.4% 18.1% 3.1%	6.51 [1.52, 27,93] 2001 3.79 [1.55, 2.26] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Exp[(0-5]) VI, Fixed, 95% CI Year 0.63 [0,21, 1.90] 2010 3.79 [1.55, 9.28] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27,93] 2001 2.14 [1.47, 3.12] 2002 4.20 [1.46, 12:09] 2060 2.80 [1.03, 6.01] 2010 3.35 [1.22, 4.90] 2013 3.35 [1.24, 3.353] 2007 1.76 [1.11, 2.80] 2013 3.35 [1.24, 3.353] 2007 1.76 [1.11, 2.80] 2013 3.35 [1.24, 3.353] 2007	0.05 0.2 1 5 20 Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / V, Fixed, 95% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (9% C)] Total events Heterogeneity: Ch ² = Test for overall effect Test for overall effect Heterogeneity: Ch ² = Test for overall effect Test for overall effect (1.3.1 Relapse/Meast Wang, W. 2010 Heterogeneity: Ch ² = Test for overall effect (1.3.2 Relapse/Meast Wang, W. 2010 Chai, A.T. 2002 Chai, A.T. 2003 Chai, A.T. 2003 Chai, A.T. 2003 Chai, A.T. 2003 Chai, A.T. 2003 Chai, A.T. 2003 Lin, J. C. 2001 Chai, A.T. 2003 Lin, J. C. 2001 Chai, A.T. 2002 Lin, J. C. 2001 Chai, M.T. 2003 Lin, J. C. 2001 Chai, M.T. 2002 Lin, J. C. 2001 Chai, M.T. 2002 Lin, J. C. 2001 Chai, M.T. 2003 Lin, J. C. 2001 Chai, M.T. 2003 Lin, J. C. 2001 Chai, M.T. 2003 Lin, J. C. 2001 Chai, M.T. 2004 Lin, J. C. 2001 Chai, M.T. 2002 Lin, J. C. 2001 Chai, M.T. 2004 Lin, J. C. 2007 Wang, W.Y. 2014 Lin, J. C. 2007 Wang, W.Y. 2014 King, C. 2007 King, C. 2007 K	22 27 48 97 0.66, df = 2 Z = 8.29 (P 505 505 505 505 505 505 505 50	88 28 103 219 (P = 0.7, < 0.000 3745 22 (P = 0.00) 2 = 5.60 DNA Total 25 28 53 (P = 0.0, 0) 25 28 53 (P = 0.0, 0) 25 28 53 (P = 0.0, 0) 25 28 53 30 6 47 36 6 51 103 306 47 306 51 103 306 47 27 27 27 27 27 27 27 27 27 27 27 27 27	1 17 26 44 (2); P = 00 00.03); P = (3); P = (4); P = 00 (3); P = (4); P = 00 (4); P = (4); P = 00 (4); P = 000 (4); P = 00 (4); P = 000(4); P = 000(4)	36 45 107 188 4698 4698 48% 9 45 54 7 000 7 45 54 % %	3.39 6.4 43.44 43.44 -1.45 6.4 -1.45 6.4 3.39 20.45 4.92 6.76 5.02 10.15 4.345 23.62 7.77	1.81 4.8 34.58 3% Variance 3.14 4.8 1.81 4.8 3.43 6.57 7.7.95 5.667 7.7.95 6.015 7.7.75 7.7.75 7.777	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1% 0.9% 4.1% 1.8% 9.4% 3.4% 1.2% 9.8% 3.8%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.25] 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E]/V], Fixed, 35% CI Year 0.53 [0.21, 1.50] 2010 3.79 [1.55, 2.28] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.12] 2002 4.20 [1.40, 12.09] 2006 2.80 [1.30, 601] 2007 1.76 [1.11, 2.00] 2013 3.55 [2.24, 4.00] 2013 3.56 [2.75, 3.69] 2014 3.56 [2.75, 5.92] 2014	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subbotal (\$9%, CI) Total events Heterogeneity: Chi ² = Test for overall effect Test for overall effect Test for overall effect Test for overall effect Subbotal (39%, CI) 13.1 RelapserMetas Subbotal (39%, CI) Total events Heterogeneity: Chi ² = Test for overall effect 1.3.2 StapserMetas Subbotal (39%, CI) Total events Heterogeneity: Chi ² = Test for overall effect 1.3.2 StapserMetas Uni, J. C. 2001 Chan, A.T. 2002 Leang, S.F. 2004 Chan, A.T. 2002 Leang, S.F. 2014 Chen, W. 2013 Lie, S.W. 2014 Chen, W. 2014 Chen, W. H. 2014 Chen, W. H. 2014 Chen, Q. 2014 Chen, Q. 2014	alues 22 27 48 97 97 0.66, df = 2 Z = 3.29 (P 505 23.03, df = 2 Z = 3.29 (P Events 22.03, df = 1 2 22.03, df = 1 2 2.03, df = 1 2.03, df = 1	88 28 103 219 (P = 0.7 < 0.000 3745 2 < 0.000 2 < 0.000 2 = 5.60 Total 2 (P = 0.0 - < 0.000 DNA Total 2 (P = 0.0 - < 0.000 DNA 25 28 53 (P = 0.0 - < 0.000 DNA 25 28 53 (P = 0.0 - < 0.000 DNA 25 28 53 30 (P = 0.0 - < 0.000 DNA 25 28 53 30 20 20 20 20 20 20 20 20 20 20 20 20 20	1 17 26 261 2001) 261 2001) .df = 2 (P) Low pre- Events- 4 17 21 1 30 0 7 0 1 3 26 4 4 7 4 7 9 9 4 4 7 9 9 9 9 9 9 9 9 9 9 9 9 9	36 45 107 188 4698 48% = 0.06). -DNA Total 9 45 54 % % % %	3.39 6.4 43.44 43.44 -1.45 6.4 -1.45 6.4 3.39 20.45 4.92 6.76 5.02 10.15 43.04 3.062 7.77 64.59	1.81 4.8 3.4.58 3% Variance 3.14 4.8 1.81 2.8.7 7 1.7.95 3.4.58 5.01 1.8.57 3.4.58 5.01 1.8.57 7.7.57.92	0.9% 2.5% 18.1% 21.5% 100.0% 14.0% 1.8% 2.5% 4.1% 0.9% 1.4% 1.2% 9.4% 1.8% 9.4% 1.8% 3.3% 3.3% 3.3%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 9.28] 2012 3.64 [2.58, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(C-E) / VJ. Fixed, 35% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 9.28] 2012 1.67 [0.53, 3.74] 6.51 [1.52, 27.95] 2001 2.14 [1.47, 312] 2002 4.20 [1.46, 1.20] 2006 2.80 [1.30, 8.01] 2007 6.15 [2.52, 4.90] 2011 3.51 [2.52, 4.90] 2013 3.51 [2.52, 4.90] 2014 3.50 [2.33, 5.90] 2014 2.87 [3.55, 50] 2014 2.86 [3.50, 2014] 2.85 [3.50] 2.85	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Test for overall effect Test for overall effect Test for overall effect Test for subsroug 3.1.1 Relapse/fitetas Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect 1.3.2 Statege HJ, Mo Lin, J. C. 2017 Total events Heterogeneity, Ch ² = Test for overall effect 1.3.2 Statege HJ, Mo Lin, J. C. 2017 Twu, C. W. 2007 Twu, C. W. 2007 Twu, C. W. 2014 Leung, S. F. 2016 Chan, A.T. 2002 Leung, S. F. 2014 Chen, W. 2014 Leung, S. F. 2014 Chen, W. H. 2014 Chen, W. 2014 Che	alues 22 27 48 97 0.66, df = 2 2 = 8.29 (P 505 505 505 505 505 505 505 505 505 50	88 28 103 219 (P = 0.7 < 0.000 3745 2 < 0.000 2 < 0.000 2 < 0.000 DNA Total 2 2 5 2 8 53 (P = 0.0 88 70 0 47 36 51 103 93 306 47 2748	1 17 26 42); I* = 0% 001) 2611 2031; I* = 2 1003; I* = - I = 2 17 21 1 30 0 7 0 1 3 26 0 4 4 7 4 21 21 1 30 0 7 20 1 21 21 21 21 21 21 21 21 21	36 4598 4698 48% = 0.06). DNA Total 9 9 5 4 % % 36 66 66 66 66 66 66 66 66 66 66 66 66	3.39 6.4 43.44 43.44 F = 64. -1.45 6.45 6.45 6.45 7.777 6.45 6.45 7.777 6.45 7.777 6.45 7.777 6.45 7.7777 6.45 7.7777 6.45 7.77777 6.45 7.7777777777777777777777777777777777	1.81 4.8 34.58 3% Variance 1.81 2.677 3.43 6.57 5.34.58 6.01 1.855 5.7.92	0.9% 2.5% 18.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1% 0.9% 4.1% 9.4% 18.1% 9.4% 18.1% 9.4% 3.0% 9.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.82, 2012 3.54 [2.54, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[IC=E] VL Fixed, 35% CI Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.82] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.12] 2002 4.20 [1.44, 1.20] 2016 2.80 [1.24, 3.35] 2007 1.176 [1.12, 20] 2016 3.51 [2.24, 4.90] 2016 3.51 [2.24, 9.00] 2016 3.55 [2.24, 9.00] 201	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Test for overall effect Test for overall effect Test for subscrud dff Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Heterogeneity, Ch ² = Test for overall effect 1.3.2 Stage HV, M0 Lin, J. C. 2001 Total events Heterogeneity, Ch ² = Test for overall effect 1.3.2 Stage HV, M0 Lin, J. C. 2001 Twu, C. W. 2014 Lin, J. C. 2014 Subtotal (\$5%, CI) Total events Heterogeneity, Ch ² = Test for overall effect Total events Heterogeneity, Ch ² = Test for overall effect Heterogeneity, Ch ² = Test for overall effect	alues 22 27 8 97 0.66, df = 2 2 = 8.29 (P 505 523.03, df = 2 2 = 1.431 (R 12, 21, 431 (R 13, 431 (R 13, 431 (R), 4	88 28 219 (P = 0.7 3745 22 (P = (- 0.000 25 - 0.000 25 - 2.00 25 - 2.00 25 - 2.00 25 - 2.00 25 - 2.00 20 - 0.00 25 - 2.00 25 - 2.00 20 - 0.00 25 - 2.00 25 - 2.00 27 - 2.00 20 - 2.000 20 - 2.00 20	1 1 1 1 2 4 2 2 2 2 2 2 2 1 3 0 0 1 1 2 2 1 3 0 2 2 1 3 0 2 1 2 1 2 1 2 2 1 2 2 1 2 2 2 3 2 2 3 2 2 1 2 3 2 2 3 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 3 2 3 3 2 3 2 3 2 3 2 3 3 2 3 3 2 4 4 3 3 2 3 2 3 3 3 2 4 4 3 3 3 2 4 4 3 3 3 3 3 3 3 2 4 4 3 3 3 3 3 3 3 3 3 3 3 3 3	36 4598 4698 48% = 0.06). DNA Total 9 9 5 5 4 % % 36 66 66 66 66 66 66 66 66 66 66 66 66	3.39 6.4 43.44 43.44 F = 64. -1.45 6.45 -1.45 6.45 -1.45 6.45 -1.45 6.45 -1.45 6.45 6.45 6.45 6.45 6.45 6.45 6.45 6	1,81 4,8 34,58 3% Variance 3,14 4,8 3,14 4,8 4,8 4,8 4,8 4,8 4,8 4,8 4,8 4,8 4,	0.9% 2.5% 18.1% 21.5% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0% 10.0% 2.5% 4.1% 14.0	6.51 [1.52, 27,93] 2001 3.79 [1.55, 226, 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E]) VI, Fixed, 95% CI Year 0.63 [0,21, 1.90] 2010 3.79 [1.55, 2.08] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27,93] 2001 2.14 [1.47, 3.12] 2002 4.20 [1.46, 1.209] 2006 2.80 [1.03, 0.61] 2077 1.76 [1.12, 20] 2016 2.14 [1.47, 3.12] 2002 4.20 [1.46, 1.209] 2016 3.51 [2.52, 4.50] 2014 3.51 [2.52, 4.50] 2014 3.52 [2.53, 3.50] 2014 2.86 [2.48, 3.31]	ODS 0.2 Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / V, Fixed, 95% CI T
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (S% C)] Total events Heterogeneity, Chi ² = Test for overall effect. Total (S% C)] Total events Heterogeneity, Chi ² = Test for overall effect. Test for overall effect. Test for overall effect. 13.1 RelpaperMetasi Wang, W. 2.010 Hsu, C. L. 2012 Subtotal (S% C)] Total events Heterogeneity, Chi ² = Test for overall effect. 13.2 Stape-Metasi Heterogeneity, Chi ² = Test for overall effect. J. 2.2 C001 Chan, A.T. 2002 Lin, J. C. 2001 Chan, A.T. 2002 Lin, S. W. 2013 Leung, S. F. 2014 Leung, S. F. 2014 Chen, W.H. 2014 Subtotal (S% C)] Total events Heterogeneity, Chi ² = Test for overall effect.	alues 22 27 0 66, df = 2 27 - 505 505 505 505 505 505 505 505	88 28 219 (P = 0.7 < 0.000 3745 22 (P = 0 < 0.000 25 < 0.000 DNA Total 25 28 53 (P = 0.0 25 28 53 (P = 0.08) 25 28 53 (P = 0.08) 25 28 53 (P = 0.08) 26 51 27 27 88 87 0 47 7 2748 3692 20 (P = 0.7 2748 3692 20 27 27 48 3692 20 27 27 48 3692 20 27 27 48 3692 20 27 48 27 27 48 27 27 48 27 27 48 27 27 48 27 27 27 48 27 27 48 27 27 27 48 27 27 27 48 27 27 27 27 27 27 27 27 27 27 27 27 27	1 1 17 26 24 21; I ² = 0% 001) 2261 2001; I ² = 1 4 17 121 121 130 7 0 14 130 7 0 14 130 26 14 15 15 16 16 16 16 16 16 16 16 16 16	36 45 107 188 4698 48% = 0.06). DNA Total 9 45 54 % % 36 66 66 66 66 66 66 63 107 107 14 108 3539 4644 35%	3.39 6.4 43.44 43.44 1 ⁷ = 64. -1.45 6.4 920.45 4.92 20.45 5.02 5.02 5.02 5.02 5.02 5.02 5.02 5.0	1.81 4.8 34.58 3% Variance 3.14 4.8 3.3 4.8 3.7 7 7.75 5.7.92	0.9% 2.5% 18.1% 21.5% 100.0% 1.6% 2.5% 4.1% 0.9% 14.0% 1.2% 9.4% 1.2% 9.4% 18.1% 9.4% 18.1% 9.8% 9.8% 9.8% 9.5%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 223, 2012 3.51 [2.52, 4.60] 2013 3.64 [2.68, 4.54] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-5]) VI. Fixed, 95% CI Year 0.63 [0,21, 1:90] 2010 3.79 [1.55, 928] 2012 1.87 [0.53, 3.74] 6.51 [1.12, 22, 409] 2013 3.56 [2.24, 403, 202] 1.76 [1.11, 2.60] 2016 2.80 [1.03, 5.01] 1.66 [0.75, 3.69] 2014 3.56 [2.24, 403, 202] 1.66 [0.75, 3.69] 2014 3.56 [2.24, 403, 202] 1.67 [1.39, 5.91] 2014 3.56 [2.24, 403, 202] 1.67 [1.39, 5.91] 2014 3.56 [2.24, 403, 202] 1.67 [1.39, 5.91] 2014 3.56 [2.24, 503, 2014 2.86 [2.48, 3.31]	0.05 0.2 1 5 20 Favours [Low pre-DNA] Hazard Ratio Exp[(0-E) / V. Fixed, 95% Cl
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subtotal (95% CI) Total events Heterogeneity: Ch ² = Test for overall effect Test for overall effect Test for overall effect Heterogeneity: Ch ² = Subtotal (95% CI) Total events Heterogeneity: Ch ² = Test for overall effect 3.3 Relapse/Metas Wang, W. 2010 Chai, A. 2017 Subtotal (95% CI) Total events Heterogeneity: Ch ² = Test for overall effect 1.3.2 Stapse HV, Mo Chai, J. C. 2001 Chai, A.T. 2002 Lin, J. C. 2001 Chai, S.W. 2013 Lisung, S. F. 2014 Chen, W. H. 2014 Vang, W. 2014 Tang, L. O. 2014 Total events Heterogeneity: Ch ² = Test for overall effect Total (95% CI)	alues 22 27 48 97 0.66, df = 2 2 = 8.29 (P 505 505 505 505 512 2 = 8.29 (P High pre- Events 12 27 39 6.12, df = 1 22 33 13 4 4 13 25 48 6 22 33 13 13 25 55 55 55 52 52 52 52 52 52	88 28 219 (P = 0,7 < 0,000 3745 2 (2 (P = (0 < 0,000 = 5,60 2 (5,60 2 (P = 0,00 2 (5,60 2 (P = 0,00) 2 (P =	1 1 1 26 1 26 1 26 1 26 1 26 1 26 1 30 7 21 1 30 7 0 1 3 26 4 4 7 21 1 30 7 0 1 1 21 21 21 21 21 21 21 21	366 4598 4698 48% = 0.06). JONA Total 9 9 455 54 % % 366 108 855 33 107 107 107 107 107 107 14 4598 3539 4654 46598	3.39 6.4 43.44 43.44 F = 64. -1.45 6.4 920.45 4.92 20.45 4.92 20.45 5.02 10.15 4.92 23.62 23.62 23.62 23.65 9	1.81 4.8 34.58 3% Variance 2.8 67 3.43 6.57 7.7 7.55 8.60 1.8 8.60 7.37 7.57.92	0.9% 2.5% 18.1% 116.1% 21.5% 100.0% Weight 1.6% 2.5% 4.1% 1.6% 3.4% 1.2% 9.4% 1.2% 9.4% 1.2% 9.9% 9.9% 9.5% 5.9%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 2.23, 2012 3.51 [2.52, 4.60] 2013 3.54 [2.68, 4.54] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E] / V]. Fixed, 35% CI Year 0.83 [0.21, 1.50] 2010 0.379 [1.55, 9.28] 2012 1.87 [0.53, 3.74] 6.51 [1.52, 27.93] 2001 2.14 [1.47, 3.12] 2020 4.20 [1.46, 12.09] 2006 2.80 [1.30, 601] 2007 1.76 [1.11, 2.60] 2006 2.80 [1.30, 601] 2007 1.76 [1.11, 2.60] 2013 3.55 [2.24, 402] 2013 1.66 [0.75, 3.69] 2014 3.59 [2.54, 3.55] 2014 2.87 [2.54, 3.35]	
Lin, J. C. 2001 Hsu, C. L. 2012 Li, S. W. 2013 Subbotal (\$9%, CI) Total events Heterogeneity: Chi ² = Test for overall effect Test for overall effect Test for overall effect Test for overall effect Subbotal (39%, CI) 13.1 Relapse/Meass Wang, W. 2.010 Hsu, C. L. 2012 Subbotal (39%, CI) Total events Heterogeneity: Chi ² = Test for overall effect 1.3.2 Stage HJ, Mo Chan, A.T. 2002 Chan, A.T. 2002 Chan, A.T. 2002 Chan, A.T. 2001 Chan, A.T. 2001 Chan, A.T. 2001 Chan, A.T. 2002 Lin, J. C. 2001 Twu, C. W. 2013 Lueng, S.F. 2014 Chen, W. 2014 Chen, W. 2014 Chen, W. 2014 Chen, W. 2014 Chen, W. H. 2014 Chen, W. H. 2014 Chen, W.H. 2014 Chen	alues 22 27 48 97 0 66, df = 2 2 = 8.29 (P 505 505 505 505 505 505 12 27 39 12 27 31 13 4 12 27 31 13 4 13 22 22 33 13 4 13 12 27 22 22 22 22 22 22 22 22 2	88 28 219 (P = 0,7 < 0,000 3745 2 < 0,000 2 = 5,60 2 < 0,000 2 = 5,60 2 < 0,000 2 = 5,60 2 < 0,000 2 = 5,60 2 = 5,60 2 = 0,00 2 = 5,60 2 = 0,000 2 = 5,60 2 = 0,000 2 = 0,0000 2 = 0,000 2 = 0,0000 2 = 0,00000 2 = 0,00000 2 = 0,00000 2 = 0,000000 2 = 0,0000000000000000000000000000000000	1 1 1 1 2 4 4 4 4 2 2 1 2 6 1 3 0 0 1 1 3 2 6 1 2 1 1 3 0 0 1 1 1 1 1 2 1 1 2 2 1 2 2 1 2 1 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2	366 453 107 188 4698 48% 9 9 455 54 % 366 108 85 54 % 366 108 85 53 107 107 107 107 14 4548 3539 4654 4658	3.39 6.4 43.44 43.44 -1.45 6.4 -1.45 6.4 3.39 20.45 4.92 6.76 5.02 10.15 43.05 23.62 23.62 23.65 9	1.81 4.8 34.58 3% Variance 1.81 28.87 3.14 4.8 6.87 17.95 34.58 6.01 18.85 7.77 57.92	0.9% 2.5% 101.1% 110.1% 100.0% 4.1% 1.6% 2.5% 4.1% 14.0% 1.6% 3.4% 14.0% 1.6% 3.4% 3.4% 3.4% 3.9% 9.9%	6.51 [1.52, 27.93] 2001 3.79 [1.55, 223, 2012 3.64 [2.54, 4.94] 2.81 [2.44, 3.24] Hazard Ratio Exp[(0-E]) / U, Fixed, 35% Cl Year 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.23] 2012 1.87 [0.53, 3.74] 0.63 [0.21, 1.90] 2010 3.79 [1.55, 2.23] 2012 2.14 [1.47, 3.12] 2002 4.20 [1.4, 1.209] 2016 2.16 [1.52, 27.93] 2011 2.14 [1.47, 3.12] 2002 4.20 [1.4, 1.209] 2016 3.51 [2.24, 4.0] 2017 1.76 [1.17, 3.0] 2017 1.76 [1.17, 3.0] 2017 1.75 [.55, 3.0] 214 3.50 [2.35, 5.9] 214 3.55 [2.3, 3.95] 2014 2.86 [2.48, 3.31] 2.81 [2.44, 3.24]	

FIGURE 2. Subgroup analysis of pre-DNA associated overall survival (OS), subdivided by population, cut-off value and inclusion stage. Note: Events for Leung, 2014 were unavailable.

	Studios	Hazand nadia			S	tudy he	eterogenei	ty
Outcomes	No.	95%CI	Z-value	P-value	$\mathrm{Chi}^2~(\chi^2)$	df	$I^2, \%$	P value [*]
Pre-DNA								
OS	9	2.85, 2.38-3.40	11.49	< 0.00001	17.09	8	53%	0.03
PFS	4	2.95, 2.50-3.49	12.76	< 0.00001	4.39	3	32%	0.22
DMFS	4	2.01, 1.37-2.93	11.00	< 0.00001	2.37	3	0%	0.50
LRFS	4	2.02, 1.52-2.70	3.59	0.0003	1.17	3	0%	0.76
Post-DNA								
OS	3	5.62, 3.73-8.48	8.24	< 0.00001	2.54	2	21%	0.28
PFS	0	-	-	-	-	-	-	-
DMFS	0	-	-	-	-	-	-	-
LRFS	3	8.55, 5.66-12.92	10.19	< 0.00001	1.91	2	0%	0.39
Mid-DNA								
OS	1	3.29, 1.37-7.89	2.67	0.0077				
PFS	1	4.05, 1.89-8.67	3.60	0.0003				
DMFS	1	12.02, 2.78-51.93	3.33	0.0009				
LRFS	1	2.05, 0.79-5.31	1.48	0.1378				
EBV DNA clearance rate	2							
$T_{1/2} > 4$ vs. $\leq 4, os$	2	3.13, 1.76-5.57	3.90	< 0.0001	0.24	1	0%	0.62
$T_{1/2} > 5$ vs. $\leq 5, os$	2	2.66, 1.55-4.55	3.56	0.0004	0.19	1	0%	0.66
$T_{1/2} > 7$ vs. ≤ 7 ,os	2	3.58, 2.07-6.20	4.56	< 0.00001	0.39	1	0%	0.53

TABLE 3. Summary of Sensitivity Analysis Results Excluding Studies Achieving <6 stars on the Modified Newcastle-Ottawa Scale

EBV = Epstein-Barr virus.

*P < 0.1 for between-study heterogeneity and statistically significant heterogeneity are shown in bold.

DISCUSSION

This meta-analysis of eight prospective studies and six retrospective studies including 7 836 patients showed that pre-DNA, mid-DNA, post-DNA and EBV DNA clearance rate were all strongly associated with cancer-specific outcomes (OS, PFS, DMFS and LRFS) in NPC patients. However, no RCTs were available for inclusion in this analysis. Because of the number and quality of studies included, our results should be interpreted with caution and further clinical trials are required to validate our conclusions. Nevertheless, we propose a 4-grade systematic risk stratification model for NPC to allow adjustment of clinical treatment based on the current NCCN guidelines for Head and Neck cancers,³ which represents a new attempt at the application of biomarkers.



FIGURE 3. Egger's publication bias plot of 13 studies that reported the pre-DNA associated OS.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

NPC is highly radiosensitive and CCRT with or without adjuvant chemotherapy (AC) has been the mainstay treatment for patients with loco-regionally advanced stages II–IVB disease.⁴ However, this type of AC (cisplatin + 5-flurouracil) is controversial due to its poor compliance and relatively low failure-free survival.²⁸ A recent meta-analysis²⁹ including relevant RCTs also showed no significant improvement in survival following CCRT + AC compared with CCRT alone. In our opinion, CCRT alone was recommended as the main treatment strategy for loco-regionally advanced NPC patients in the following prognostic groupings, which would have important implications for the selection of appropriate treatment strategies in this model. Given the limited published data of combined treatment strategies that included with EBV DNA, the selection of changes in the treatment decision was a primary suggestion.

Leung et al.⁹ found that pre-DNA was an independent prognostic factor secondary to 1997 AJCC/UICC staging in NPC. Their results indicated that the 5-year survival rates for the low and high pre-DNA groups in patients with stage II disease were 90% and 63%, respectively, with the former being similar to the survival in stage I disease (92%, 95% CI 83-100%) and the latter worse than that in stage III disease (73%, 95%CI 64-82%). Leung et al.9 did not found significant differences in survival between the high and low pre-DNA groups of patients with stage III and IV disease, respectively; however, they proposed that the majority of low-risk stage II patients should be treated with radiotherapy alone to avoid non-contributive therapy. In our meta-analysis of 13 related studies, similar results were obtained. In subgroup analysis, we found that pre-DNA was not predictive of OS in relapse or metastasis patients before treatment (HR 1.87, 95% CI 0.93-3.74, P = 0.08). The pre-DNA result was applicable only in 1997 AJCC/UICC stage I-IV disease patients without metastasis and a pre-DNA cut-off value of 4 000 copies/ml was recommended (HR 2.80, 95% CI 2.33– 3.37, P < 0.00001). A recent study³⁰ has successfully realized the international harmonization and standardization of detectable EBV DNA through the use of common calibrators (all prepared in Hong Kong) and Polymerase Chain Reaction (PCR) master mix (Roche master mix). Reliable and comparable cut-off values across centers were available; thus, the following primary risk stratification was proposed:

- (1) Stage I–II patients with pre-DNA < 4 000 copies/ml can be regarded as modified stage I disease.
- (2) Stage I patients with pre-DNA \geq 4 000 copies/ml can be regarded as modified stage II disease.
- (3) Stage II patients with pre-DNA \geq 4 000 copies/ml can be regarded as modified stage III disease.

Based on the primary risk stratification, patients with high pre-DNA levels could be treated with aggressive strategies, such as neo-adjuvant chemotherapy + CCRT with or without AC, while the low group could be spared non-contributive therapy and be treated with radical radiotherapy alone.

Studies of mid-DNA on survival were limited. To date, only one study from Hong Kong⁸ with a median follow-up time of 73 months indicated that detectable mid-DNA (measured at completion of 4 weeks of CCRT/radiotherapy) encompassed approximately three-quarters of all failures, while the rest were associated with detectable post-DNA. They also found that patients with detectable mid-DNA and then undetectable post-DNA still had a poorer prognosis than those with undetectable mid-DNA. Their data showed no significant difference in outcomes based on tumor stage stratification. Mid-DNA was found to be a strong prognosticator of distant metastasis (DMFS, HR 12.02, 95% CI 2.78–51.93, P = 0.0009), which might be important in current treatment status monitoring procedures. Thus, the secondary risk stratification was proposed as a suggestion based on the study described above:

- (1) Stage I–II patients with undetectable mid-DNA might be regarded as having modified stage I disease.
- (2) Stage III-IV patients with undetectable mid-DNA might be regarded as having modified stage II disease.
- (3) Stage I patients with detectable mid-DNA might be regarded as having modified stage II disease.
- (4) Stage II–IV patients with detectable mid-DNA might be regarded as being at high risk of relapse or distant metastasis.

Based on the secondary risk stratification, patients with a high risk stratification could be treated with aggressive strategies, such as CCRT + AC with targeted therapy also recommended if necessary. Patients with a low risk stratification could be treated with moderate strategies, such as radical radiotherapy or CCRT alone.

Large reductions in plasma EBV DNA levels were observed with a 49% detectable rate of mid-DNA and a 16% detectable rate of post-DNA.⁸ Post-radiotherapy biopsies carried out by some investigators³¹ revealed that patients with detectable post-DNA had either incomplete regression of the tumor or had developed distant metastasis. In total, we included six studies^{7,8,10,17–19} of the clinical utility of post-DNA. The prognostic value of post-DNA was first observed in a study involving 170 patients conducted by Chan et al.⁷ in 2002. The study also indicated that post-DNA less than 500 copies/ml indicated an excellent prognosis (2-year survival > 80%) and

that such patients might be spared adjuvant therapy. Lin et al.¹⁰ suggested that more chemotherapy should be offered to patients with persistently detectable post-DNA after CCRT, which is consistent with the results of our meta-analysis. Through subgroup analysis based on cut-off values, we found a statistically significant difference (0 copies/ml cut-off, HR 5.81 vs. 500 copies/ml, HR 2.82; P = 0.009). Based on these results, we proposed the following tertiary risk stratification:

- (1) Patients with post-DNA = 0 copies/ml might be spared adjuvant therapy;
- (2) Patients with post-DNA > 0 copies/ml could undertake AC or other treatments if necessary.

Some investigators^{11,23,32,33} collected three to five blood samples during the treatment and calculated the EBV clearance rate. In an investigation of the kinetics of plasma EBV DNA, Lo et al.³² found an initial rise in the first week of radiotherapy, which might be accounted for treatment-induced cancer cell death rather than due to a change in the clearance rate. A median $t_{1/2}$ of 3.8 days (interquartile range, 2.4–4.4 days) was also determined in this study. To et al.³³ observed that the median $t_{1/2}$ after surgical resection of NPC was 139 minutes, which demonstrated that the plasma EBV DNA concentration correlates with tumor burden. In addition to the biological features, the prognostic implications of EBV DNA clearance rate were further investigated. Wang et al.¹¹ found that the EBV DNA clearance rate was better than pre-DNA (before salvage treatment) in predicting OS. Both Wang et al.¹¹ and Hsu et al.²³ found that a pre-DNA of 5000 copies/ml combined with a plasma EBV DNA clearance rate of less than 7 days had better OS than other groups. We performed the meta-analysis of the two studies based on different half-life values and recommended a t_{1/2} of 7 days (OS, HR 3.58, 95% CI 2.07–6.20, P < 0.00001) to evaluate tumor response and OS. However, there were two limitations. Firstly, this parameter could only be applied to patients with relapse or metastasis before salvage treatment. Secondly, the pooled sample was still too small for the results to be convincing. Nevertheless, the EBV DNA clearance rate was more objective and sensitive than the current conditions for effect evaluation, which depends mainly on the morphology observed in radiological imaging after full-term chemotherapy. Early changes in the chemotherapy regimen may be considered timely. We then proposed the quaternary risk stratification of patients with recurrent disease or metastasis with assessable pre-DNA (before salvage treatment) and calculated the $t_{1/2}$ (during salvage treatment) as follows:

- (1) Patients with pre-DNA < 5000 copies/ml and $t_{1/2} \le 7$ days should continue with the current treatment strategies;
- (2) Patients not in the former group should be changed to other treatment strategies, such as altered chemotherapy or targeted therapy.

A point worth emphasizing is that the former three grades of our 4-grade systematic risk stratification model for individualized therapy are applicable only to patients without relapse or metastasis at diagnosis. The four grades should also be applied sequentially. However, this model should not be used outside the context of well-designed clinical trials. Although the first biomarker-integrated multicenter RCT (Clinical Trials.gov Identifier: NCT02135042) began in 2014, further validation clinical trials are required.

The present meta-analysis has some limitations that must be taken into account in the interpretation of our results. The main limitation is that all the included studies were nonrandomized clinical trials with relatively small sample sizes; the study by Wang et al.¹¹ was performed in only 34 patients. Moreover, publication and reporting bias could not be avoided because our analysis was based on data extracted from published reports rather than individual patient data, without which we were unable to include all the endpoint data and basic information for each study. For example, PFS data were not available in the study by Twu et al.¹⁸ Thus, access to individual patient data as well as unreported data would allow a more balanced evaluation of the endpoints included in this meta-analysis. The original meta-analysis was based on the assumption that differences between the results of various studies were due to chance. However, as shown in our description of the results, there was significant between-study heterogeneity in the OS analysis of both pre-DNA and post-DNA. In addition, there was variation in the quality of the included studies and subgroup analysis yielded some difference in the results. For instance, variation in the population was associated with pre-DNA prediction of OS and different cut-off values were the key factor for post-DNA. To balance the risk of bias, the data were extracted by two independent investigators. We also classified the level of evidence and scored the quality of the studies. Future systematic analysis should include the data obtained in the RCTs currently underway.

Nevertheless, meta-analysis of the application of EBV DNA as a biomarker was conducted at an appropriate time, when sufficient data was available to for evaluation by this method. We applied multiple strategies to minimize the heterogeneity, including study identification, strict inclusion criteria, and methodological quality evaluation of the eligible studies, subgroup analysis, sensitivity analysis and Egger's test to control for potential bias. Hence, the results of our analysis represent the most current systematic information available and furthermore, we have proposed a completely new risk stratification model that can be used to design RCTs in this area.

In conclusion, this meta-analysis indicates that pre-DNA, mid-DNA, post-DNA and EBV DNA clearance rate are all prognostic factors for different clinical outcomes in NPC patients and that different assays of this biomarker are applicable in clinical practice. Based on these data, we propose a new 4-grade systematic risk stratification model, which is also complementary to the current 1997 AJCC/UICC staging system. Given the inherent limitations of the included studies, future well-designed RCTs and validation trials are awaited to confirm and update the findings of this analysis and further the development of individualized strategies for the treatment of NPC.

ACKNOWLEDGEMENTS

We received writing assistance from native English speakers. We thank the anonymous reviewers for their insightful comments and great efforts to improve this manuscript. This manuscript has not been published in whole or in part, nor is it being considered for publication elsewhere. We also declare no potential conflicts of interest.

REFERENCES

 Wei WI, Sham JST. Nasopharyngeal carcinoma. Lancet. 2005;365:2041–2054.

- Le QT, Jones CD, Yau TK, et al. A comparison study of different PCR assays in measuring circulating plasma epstein-barr virus DNA levels in patients with nasopharyngeal carcinoma. *Clin Cancer Res.* 2005;11:5700–5707.
- Pfister DG, Ang KK, Brizel DM, et al. Head and neck cancers, version 2. 2013. Featured updates to the NCCN guidelines. *JNCCN*. 2013;11:917–923.
- Chan AT. Nasopharyngeal carcinoma. Ann Oncol. 2010;21 (suppl 7):vii308–312..
- Cheng SH, Yen KL, Jian JJ, et al. Examining prognostic factors and patterns of failure in nasopharyngeal carcinoma following concomitant radiotherapy and chemotherapy: impact on future clinical trials. *Int J Radiation Oncol Biol Phys.* 2001;50:717–726.
- Lo YM. Quantitative analysis of Epstein-Barr virus DNA in plasma and serum: applications to tumor detection and monitoring. *Ann New York Acad Sci.* 2001;945:68–72.
- 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.
- 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.
- 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.
- Lin JC, Wang WY, Liang WM, et al. Long-term prognostic effects of plasma epstein-barr virus DNA by minor groove binder-probe real-time quantitative PCR on nasopharyngeal carcinoma patients receiving concurrent chemoradiotherapy. *Int J Radiat Oncol Biol Phys.* 2007;68:1342–1348.
- Wang WY, Twu CW, Chen HH, et al. Plasma EBV DNA clearance rate as a novel prognostic marker for metastatic/recurrent nasopharyngeal carcinoma. *Clin Cancer Res.* 2010;16:1016–1024.
- Chan KCA, Lo YMD. Circulating EBV DNA as a tumor marker for nasopharyngeal carcinoma. *Semin Cancer Biol.* 2002;12:489–496.
- Lin JC, Chen KY, Wang WY, et al. Detection of Epstein-Barr virus DNA in the peripheral-blood cells of patients with nasopharyngeal carcinoma: relationship to distant metastasis and survival. *J Clin Oncol.* 2001;19:2607–2615.
- An X, Wang FH, Ding PR, et al. Plasma Epstein-Barr virus DNA level strongly predicts survival in metastatic/recurrent nasopharyngeal carcinoma treated with palliative chemotherapy. *Cancer*. 2011;117:3750–3757.
- Chan SC, Hsu CL, Yen TC, et al. The role of 18F-FDG PET/CT metabolic tumour volume in predicting survival in patients with metastatic nasopharyngeal carcinoma. *Oral Oncol.* 2013;49:71–78.
- Chai SJ, Pua KC, Saleh A, et al. Clinical significance of plasma Epstein-Barr Virus DNA loads in a large cohort of Malaysian patients with nasopharyngeal carcinoma. *J Clin Virol.* 2012;55:34– 39.
- Hou X, Zhao C, Guo Y, et al. Different clinical significance of preand post-treatment plasma Epstein-Barr virus DNA load in nasopharyngeal carcinoma treated with radiotherapy. *Clin Oncol.* 2011;23:128–133.
- Twu CW, Wang WY, Liang WM, et al. Comparison of the prognostic impact of serum anti-EBV antibody and plasma EBV DNA assays in nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys.* 2007;67:130–137.

- 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.
- Wang WY, Twu CW, Lin WY, et al. Plasma Epstein-Barr virus DNA screening followed by (1)(8)F-fluoro-2-deoxy-D-glucose positron emission tomography in detecting posttreatment failures of nasopharyngeal carcinoma. *Cancer.* 2011;117:4452–4459.
- Mutirangura A, Pornthanakasem W, Theamboonlers A, et al. Epstein-Barr viral DNA in serum of patients with nasopharyngeal carcinoma. *Clin Cancer Res.* 1998;4:665–669.
- Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med.* 1998;17:2815–2834.
- Hsu CL, Chang KP, Lin CY, et al. Plasma Epstein-Barr virus DNA concentration and clearance rate as novel prognostic factors for metastatic nasopharyngeal carcinoma. *Head Neck.* 2012;34:1064– 1070.
- 24. Li SW, Wang H, Xiang YQ, et al. Prospective study of prognostic value of Raf kinase inhibitory protein and pretreatment plasma Epstein-Barr virus DNA for distant metastasis in locoregionally advanced nasopharyngeal carcinoma. *Head Neck.* 2013;35:579–591.
- Chen WH, Tang LQ, Wang FW, et al. Elevated levels of plasma Ddimer predict a worse outcome in patients with nasopharyngeal carcinoma. *BMC Cancer.* 2014;14:583.
- 26. Tang LQ, Li CF, Chen QY, et al. High-sensitivity C-reactive protein complements plasma Epstein-Barr virus deoxyribonucleic acid prognostication in nasopharyngeal carcinoma: a large-scale retrospective and prospective cohort Study. *Int J Radiat Oncol Biol Phys.* 2014.

- 27. Wei W, Huang Z, Li S, et al. Pretreatment Epstein-Barr virus DNA load and cumulative cisplatin dose intensity affect long-term out-come of nasopharyngeal carcinoma treated with concurrent chemotherapy: experience of an institute in an endemic area. *Oncol Res Treat.* 2014;37:88–95.
- Chen L, Hu C-S, Chen X-Z, et al. Concurrent chemoradiotherapy plus adjuvant chemotherapy versus concurrent chemoradiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma: a phase 3 multicentre randomised controlled trial. *Lancet Oncol.* 2012;13:163–171.
- 29. Chen YP, Wang ZX, Chen L, et al. A Bayesian network metaanalysis comparing concurrent chemoradiotherapy followed by adjuvant chemotherapy, concurrent chemoradiotherapy alone and radiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma. Ann Oncol. 2015;26:205–211.
- 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.
- 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 Res.* 1999;59:1188–1191.
- Lo YM, Leung SF, Chan LY, et al. Kinetics of plasma Epstein-Barr virus DNA during radiation therapy for nasopharyngeal carcinoma. *Cancer Res.* 2000;60:2351–2355.
- To EW, Chan KC, Leung SF, et al. Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. *Clin Cancer Res.* 2003;9:3254–3259.