

Research Paper

# Prognostic Values of *CCNE1* Amplification and Overexpression in Cancer Patients: A Systematic Review and Meta-analysis

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## Abstract

A number of studies revealed that *CCNE1* copy number amplification and overexpression (on mRNA or protein expression level) were associated with prognosis of diverse cancers, however, the results were inconsistent among studies. So we conducted this systematic review and meta-analysis to investigate the prognostic values of *CCNE1* amplification and overexpression in cancer patients. PubMed, Cochrane library, Embase, CNKI and WanFang database (last update by February 15, 2018) were searched for literatures. A total of 20 studies were included and 5 survival assessment parameters were measured in this study, which included overall survival (OS), progression free survival (PFS), recurrence free survival (RFS), cancer specific survival (CSS) and distant metastasis free survival (DMFS). Pooled analyses showed that *CCNE1* amplification might predict poor OS (HR=1.59, 95% CI: 1.05-2.40,  $p=0.027$ ) rather than PFS (HR=1.49, 95% CI: 0.83-2.67,  $p=0.177$ ) and RFS (HR=0.982, 95% CI: 0.2376-4.059,  $p=0.9801$ ) in various cancers; *CCNE1* overexpression significantly correlated with poor OS (HR=1.52, 95% CI: 1.05-2.20,  $p=0.027$ ), PFS (HR=1.20, 95% CI: 1.07-1.34,  $p=0.001$ ) and DMFS (HR=1.62, 95% CI: 1.09-2.40,  $p=0.017$ ) rather than RFS (HR=1.68, 95% CI: 0.81-3.50,  $p=0.164$ ) and CSS (HR=1.54, 95% CI: 0.74-3.18,  $p=0.246$ ). On the whole, these results indicated *CCNE1* amplification and overexpression were associated with poor survival of patients with cancer, suggesting that *CCNE1* might be an effective prognostic signature for cancer patients.

Key words: cancer, *CCNE1*, prognosis, systematic review, meta-analysis

## Introduction

Cancer is becoming one of the main causes of death and is a hard challenge to the world [1]. Early diagnosis and prognosis prediction are helpful for clinical treatment of cancers [2], but traditional detection methods such as biopsy and imaging techniques still have their limitations. Therefore, it is urgently needed to excavate new molecular

signatures for the early and precise prognosis prediction of cancers.

Both gene copy number variations and mRNA expression levels (or protein expression levels) in cancer cells could be used as signatures for prognosis and response to therapies. For example, *HER2* amplification was an efficient predictor of overall

survival in breast cancer patients [3]. Amplification, genetic rearrangement and overexpression of cell cycle cyclins genes had been documented in almost all human cancer types and contributed to development and progression of cancers [4]. Cyclin E1, encoded by the *CCNE1*, drives the transition from G1 to S phase by binding and activation of cyclin dependent kinase 2 (CDK2), resulting in the initiation of DNA synthesis [5]. Up to now, an increasing number of studies had reported the relations between *CCNE1* and prognosis in diverse cancers, but still generated discrepant conclusions. For instance, overexpression and copy number amplification of *CCNE1* were related to poor survival in some kinds of malignant tumors such as bladder cancer [6], breast cancer [7], ovarian cancer [8] and so on, while some opposite results existed [9, 10]. Therefore, the prognostic value of *CCNE1* in cancer patients remained uncertain.

It's worth noting that, in some literatures, Cyclin E1 and Cyclin E2 (encoded by *CCNE2*) were collectively referred to as Cyclin E [11-13], while Cyclin E1 was formerly called as Cyclin E in some studies [14]. Furthermore, Cyclin E2 was also found to be associated with prognosis in several kinds of cancer [15, 16]. Therefore, research results derived from articles about Cyclin E without definite indication of Cyclin E1 (or *CCNE1*) in the full text were not explicit, because we couldn't determine whether these conclusions were the separate effect of Cyclin E1 or the combined effect of Cyclin E1 and Cyclin E2. So in this study, we conducted a rigorous systematic review and meta-analysis for the prognostic values of *CCNE1* amplification and overexpression rather than Cyclin E.

## Materials and Methods

### Literature search

Comprehensive literature search of PubMed, Cochrane library, Embase, CNKI database and WanFang database was performed for articles published prior to February 15, 2018 by using Medical Subject Headings (MeSH) combined with keywords as follows: "cyclin E1, human", "*CCNE1*", "Cyclin E1", "*CCNE*", "Cyclin Es", "Cyclin Et", "G1/S-Specific Cyclin-E1", "Prognosis", "Survival", "Prognoses", "Factor, Prognostic", "Prognostic Factor", "Carcinoma", "Neoplasms", "Neoplasia", "Neoplasm", "Tumor", "Cancer", "Malignancy", "Benign Neoplasm", "Neoplasm, Benign", "Malignant Epithelial Neoplasm", "Epithelioma", "Carcinomatosis". Additionally, we conducted the manual search of the references in all included articles to identify potential missing studies that were not found during the preliminary literature searches.

Inclusion criteria for the included studies: (1) all articles should be published either in English or Chinese language in the form of full article; (2) observational studies (case-control or cohort studies) that investigated patients with any type of cancer; (3) articles investigating the correlations between *CCNE1* expression levels (protein expression level or mRNA expression level) and the prognosis in cancer patients or the correlations of *CCNE1* amplification and the prognosis in cancer patients; (4) Hazard ratio (HR) and 95% confidence interval (95% CI) of survival assessment parameters were reported directly, or there was sufficient original data for estimating them in article; (5) when the patient cohorts overlapped among different publications, only the largest or the most recent one was included. Exclusion criteria were as follow: (1) studies without usable or sufficient data; (2) case reports, reviews, letters, comments, conference abstracts and expert opinions; (3) article about Cyclin E in which there was no definite indication of Cyclin E1 (*CCNE1*); (4) researches about a set of genes rather than *CCNE1* alone; (5) results from overlapping patients cohorts; (6) articles based on external datasets. Screening of eligible studies was independently performed by four investigators (HY.Z, JL.W, LS.L, HL.Y), disagreements were resolved by consensus.

### Data extraction and quality assessment

Four investigators (HY.Z, JL.W, LS.L, and HL.Y) independently extracted the available data from all included studies. The extracted data was crosschecked and any discrepancy was resolved by discussion. The following information was extracted from each included study: first author name, publication year, study design, cancer type, inclusion period, case and control numbers, research country, region, method for detecting *CCNE1* amplification, method for detecting *CCNE1* expression level, cut off value, research center, duration of follow-up, HR and 95% CI. If the article only reported Kaplan-Meier curve (K-M curve) without providing HR and 95% CI directly, appropriate data was extracted from the survival curves by using Engauge Digitizer 4.1 software and the logHR and selogHR were calculated according to Tierney et al [17]. This method was conducted by three investigators to reduce imprecision. For studies that reported both the univariate and multivariate analysis results, the latter was extracted. Survival assessment parameters with HR and 95% CI available in these included studies were selected in our research, including overall survival (OS), progression free survival (PFS), cancer specific survival (CSS), recurrence free survival (RFS) and distant metastasis free survival (DMFS). The

Newcastle-Ottawa Scale (NOS) [18] was used to assess the quality of each included study. The NOS score ranged from 0 to 9, and a study with score  $>7$  was considered as high quality. Three investigators (HY.Z, JL.W, LS.L) independently scored each study, any discrepancy was resolved by discussion.

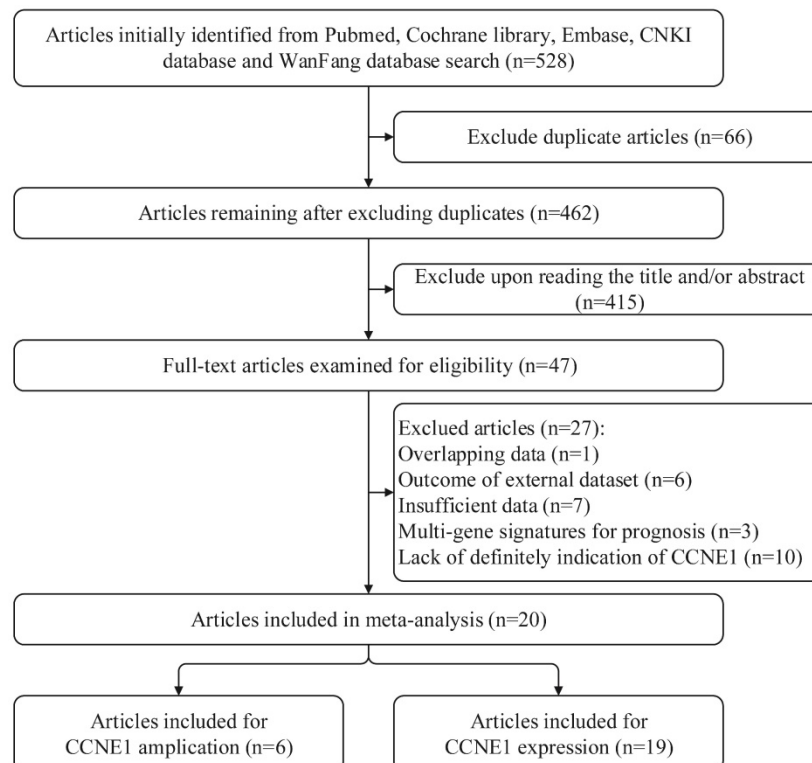
### Statistical Analysis

We used HR with corresponding 95% CI to calculate pooled data. By convention,  $HR > 1$  indicated poor prognosis for the *CCNE1* amplification or overexpression group. The heterogeneity among studies was determined by Q test and  $I^2$  value [19]. When  $p < 0.05$  for the Q test or  $I^2 > 50\%$ , heterogeneity was supposed to be significant, and then the random effect model was applied. On the contrary, fixed effect model was employed ( $p \geq 0.05$  or  $I^2 \leq 50\%$ ). Publication bias was evaluated by Egger's test [20]. And when the number of included studies was  $< 10$ , publication bias analysis was not conducted. Subgroup analysis was performed to find out factors contributing to heterogeneity. One-way sensitivity analysis was performed to assess the stability of results, but when the number of included studies was  $< 3$ , it was not conducted.  $P$  values in all analyses were two sides, and  $p < 0.05$  was considered statistically significant. All statistical analyses in this meta-analysis were conducted with STATA 12.0 (Stata Corporation, College Station, TX, USA).

## Results

### Identification of relevant studies

By applying the described searching strategy, we initially collected 528 publications. After exclusion of duplicates and irrelevant studies by reviewing the titles and abstracts, 47 articles remained for further evaluation. We reviewed all of the 47 publications by reading full manuscripts, and excluded 27 articles as follows: 7 [21-27] articles lacking sufficient information, 6 [28-33] articles based on external datasets, 1 [34] article with overlapping data, 3 [35-37] articles reporting gene sets including *CCNE1* rather than *CCNE1* as a single gene signature for prognosis and 10 [38-47] articles about Cyclin E in which there were no explicit indication of *CCNE1*. Finally, 20 studies were qualified and included in our research. Among them, 1 article only exploring the predictive roles of *CCNE1* copy number amplification in cancer prognosis [48], 14 articles were only about *CCNE1* expression levels (on mRNA or protein expression level) [6, 7, 10, 49-59], and 5 articles examined both *CCNE1* amplification and expression levels [8, 9, 60-62]. The detailed procedure of literature retrieval is revealed in Figure 1. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram to design, analyze, and report this meta-analysis [63].



**Figure 1.** Flow chart for searching databases and selecting eligible studies.

**Table 1A.** Characteristics of the included 6 studies that researching on the predictive value of *CCNE1* amplification for cancers prognosis

Author (Year)	Inclusion period	Country	Research center	Tumor Type	Case /Control	Median/Range of follow-up(Mo)	Detection method	Outcome measures	Amplification percentage	Variance analysis	HR source	Region
Ayhan A(2016)	1995-2013	Multi-country <sup>a</sup>	multicenter	ovarian cancer	13/70	NR	FISH	OS	15.66	Univariate	Reported	Mixed
Nakayama K(2015)	1998-2010	Japan	single	endometrial carcinomas	9/99	52(5-139)	FISH	OS PFS	8.33	Multivariate	Reported	Asia
Pils D(2014)	2005-2008	Multi-country <sup>b</sup>	multicenter	ovarian cancer	total: 167	49(1-69)	copy number PCR	OS PFS	25	Univariate	Reported	Europe
TCGA(2011)	NR	USA	multicenter	ovarian cancer	106/383	30(1-179)	microarray	OS	21.68	Univariate	K-M curve	North America
Nakayama N(2010)	NR	Japan	single	ovarian cancer	18/70	NR	FISH	OS PFS	20.45	Multivariate	Reported	Asia
Luhtala S(2016)	2003-2007	Finland	single	breast cancer	15/170	64.8	CISH	RFS	8.11	Univariate	Reported	Europe

a: Japan, USA. b: Germany, Belgium, Austria. c: total: the study didn't provide detail number of control and case group.

## Study characteristics

The main characteristics of the included 6 studies that explored the prognostic value of *CCNE1* amplification in cancers are presented in Table 1A. 1120 patients were enrolled in these researches totally, and the cohort sizes ranged from 83 to 489 cases with the mean of 186. There were 4 articles on ovarian cancer, 1 literature about endometrial carcinomas and 1 research on breast cancer. For detecting the amplification of *CCNE1*, fluorescence in situ hybridization (FISH) was applied in 3 studies, copy number polymerase chain reaction (PCR) in 1 study, microarray in 1 study and chromogenic in situ hybridization (CISH) in 1 study. *CCNE1* amplification proportion varied from 8.33% to 25%, with the mean percentage of 16.54%. HR and 95% CI were directly reported in 5 studies and extracted from K-M curves in 1 study. All studies assessed *CCNE1* amplification in tumor tissue.

The main characteristics of the 19 included studies that researched on the predictive roles of *CCNE1* expression level in cancers prognosis are shown in Table 1B. The total number of patients included in these articles was 4592, and the sample size ranged from 60 to 857 with the mean value of 242. There were 8 articles on breast cancer, 5 literatures about ovarian cancer, 3 researches on bladder cancer, 1 study about endometrial carcinomas, 1 article about esophageal adenocarcinoma and 1 research on upper tract urothelial carcinoma. For detecting expression levels of *CCNE1*, immunochemistry (IHC) was applied to evaluate *CCNE1* protein expression level in 12 studies, real-time quantitative polymerase chain reaction (RT-qPCR) was performed to detect *CCNE1* mRNA expression level in 6 studies, and microarray was conducted to detect *CCNE1* mRNA expression level in 1 study. 3 articles treated *CCNE1* expression level as continuous type variable, so there were no specific cut-off values in them [9, 56, 57]. In the remaining 16 articles, *CCNE1* expression level was all

classified as high and low group, but the cut-off definitions were various. HR and 95% CI were directly reported in 15 studies and extracted from K-M curves in 4 studies. All studies assessed *CCNE1* expression levels in tumor tissue.

As shown in Table S1, global quality assessment scores of the 20 included studies in this study were generally high with 14 (70%) articles being more than 7 points, ranged from 6 to 8.

## Survival analysis for *CCNE1* amplification carriers with cancers

**OS analysis:** 5 studies with totally 935 patients investigated the association between *CCNE1* amplification and OS. A random effect model was used to estimate the pooled HR and 95% CI, due to a significant heterogeneity among these 5 studies ( $I^2=75%$ ,  $p=0.003$ ). As shown in Figure 2A, the results demonstrated that *CCNE1* amplification carriers were associated with worse OS than non-carriers, with a pooled HR of 1.59 (95% CI: 1.05-2.40,  $p=0.027$ ). The results of subgroup analyses were presented in Table S2A. It revealed that analysis method, detection method, region, and research center might contribute to the heterogeneity. It was noteworthy that, of these 5 included studies, 4 studies focused on ovarian cancer, but *CCNE1* amplification had no significant relationship with OS in ovarian cancer patients (HR=1.46, 95% CI: 0.98-2.18,  $p=0.064$ ). In studies adopting multivariate analysis rather than univariate analysis, *CCNE1* amplification carriers had statistically significantly worse OS (multivariate analysis: HR=3.45, 95% CI: 1.76-6.77,  $p<0.001$ ; univariate analysis: HR=1.22, 95% CI: 0.90-1.65,  $p=0.193$ ).

**PFS analysis:** 3 studies of 363 patients assessed the predictive value of *CCNE1* amplification for PFS. Significant heterogeneity was found across these included studies ( $I^2=62.3%$ ,  $p=0.07$ ), so the pooled HR was calculated based upon a random effects model. Meta-analysis results demonstrated no significant



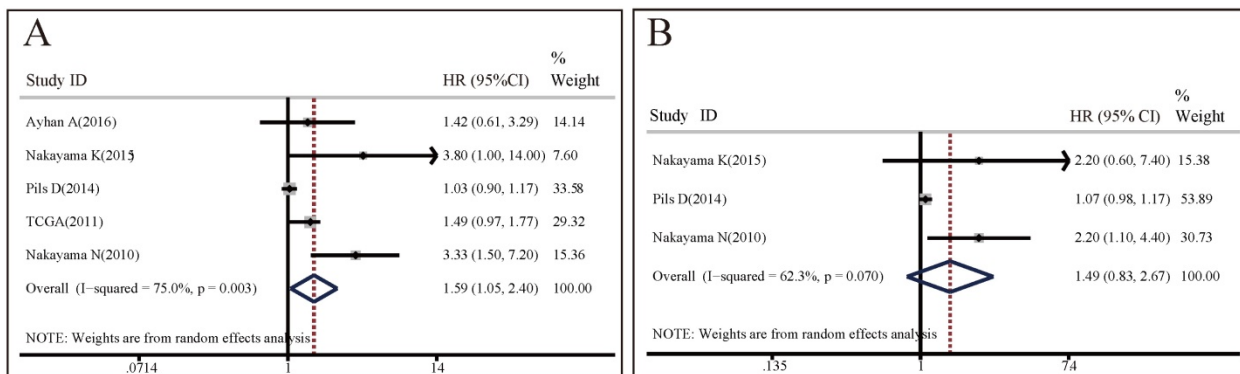
association between *CCNE1* amplification and PFS (HR=1.49, 95% CI: 0.83-2.67,  $p=0.177$ ) (Figure 2B). As shown in Table S2B, the results of subgroup analyses revealed that analysis method, detection method, region, and research center might contribute to the heterogeneity. We also found that there was no

statistical difference in PFS between *CCNE1* amplification carriers and non-carriers, regardless of tumor type or study quality. However, it was worth noting that, in studies adopting multivariate analysis, *CCNE1* amplification carriers showed significantly worse PFS (HR=2.20, 95% CI: 1.20-4.04,  $p=0.011$ ).

**Table 1B.** Characteristics of the included 19 studies that researching on the predictive value of *CCNE1* overexpression for cancers prognosis.

Author (Year)	Inclusion period	Country	Research center	Tumor Type	Case/Control	Median/Range of follow-up(Mo)	Detection method	Cut-off	Outcome measures	High expression percentage	Variance analysis	HR source	Region
Ayhan A(2016)	1995-2013	Muti-country <sup>a</sup>	multicenter	ovarian cancer	20/63	NR	IHC	80% <sup>b</sup>	OS	24.1	Multivariate	Reported	Mixed
Nakayama K(2015)	1998-2010	Japan	single	EC	54/54	52(5-139)	IHC	median <sup>c</sup>	OS PFS	50	Univariate	Reported	Asia
Zhou Z(2014)	2002-2008	USA	single	EAC	total <sup>d</sup> 112	39(0.3-142)	IHC	10% <sup>e</sup>	OS	NR	Univariate	K-M curve	North America
Wu S(2014)	NR	China	single	UTUC	59/44	39(1-98)	IHC	5 <sup>f</sup>	OS	57.28	Multivariate	Reported	Asia
Nakayama N(2010)	NR	Japan	single	ovarian cancer	44/44	NR	IHC	median	OS PFS	50	Univariate	Reported	Asia
Farley J(2003)	NR	USA	multicenter	ovarian cancer	62/77	NR	IHC	means <sup>g</sup>	OS	44.6	Multivariate	Reported	North America
Shariat SF(2006)	1987-2002	USA	single	bladder cancer	125/99	NR	IHC	30% <sup>h</sup>	CSS RFS	55.31	Univariate	K-M curve	North America
Chappuis PO(2005)	1980-1995	Canada	single	breast cancer	67/186	96	IHC	50% <sup>i</sup>	CSS	26.48	Univariate	K-M curve	North America
Lotan Y(2013)	2002-2012	USA	single	bladder cancer	183/216	NR	IHC	30% <sup>j</sup>	CSS RFS	84.72	Univariate	Reported	North America
Lundgren C(2015)	1993-2004	Sweden	multicenter	breast cancer	186/186	NR	IHC	NR	CSS DMFS	48.45	Univariate	Reported	Europe
Luhtala S(2016)	2003-2007	Finland	single	breast cancer	74/128	64.8	IHC	50% <sup>k</sup>	RFS	36.63	Univariate	Reported	Europe
Matsushita R(2015)	2003-2013	Japan	single	bladder cancer	30/30	NR	RT-qPCR	median	OS	50	Univariate	K-M curve	Asia
Pils D(2014)	2005-2008	Muti-country <sup>l</sup>	multicenter	ovarian cancer	total <sup>d</sup> 167	49(1-69)	RT-qPCR	none	OS PFS	none	OS:Multivariate PFS:Univariate	Reported	Europe
Marchini S(2008)	1992-2003	Italy	single	ovarian cancer	OS:9/59; PFS:10/57	NR	RT-qPCR	OS(0.124); PFS(0.34)	OS PFS	OS:13.23 PFS:14.93	Multivariate	Reported	Europe
Sieuwerts AM(2006)	1979-1995	Netherland	multicenter	breast cancer	317/318	95(11-202)	RT-qPCR	median	OS DMFS	49.92	Multivariate	Reported	Europe
Jansen MP(2011)	1981-1996	Netherland	single	breast cancer	total <sup>d</sup> 226	89(10-165)	RT-qPCR	none	PFS	none	Multivariate	Reported	Europe
Kreike B(2010)	1984-1995	Netherland	single	breast cancer	total <sup>d</sup> 291	80.4(0.6-237.9)	microarray	none	RFS	none	Univariate	Reported	Europe
Desmedt C(2006)	NR	UK	single	breast cancer	total <sup>d</sup> 205	97.32	RT-qPCR	median	RFS	50	Univariate	Reported	Europe
Fredholm H(2017)	1992-2005	Swedish	multicenter	breast cancer	435/422	115.2	IHC	10% <sup>m</sup>	DMFS	50.76	Univariate	Reported	Europe

a: Japan, USA. b: diffuse and intense immunoreactivity in 80% of tumor cells were considered highly expressed. c: median value. d: total: the study didn't provide detail number of control and case group. e: 10% or more of cells stained with a moderate to strong intensity was considered highly expressed. f: staining index score = 5. g: mean value. h: *CCNE1* immunoreactivity was considered high when samples demonstrated more than 30% nuclear reactivity. i: *CCNE1* immunoreactivity was considered high when samples demonstrated more than 50% nuclear reactivity. j: *CCNE1* immunoreactivity was considered high when samples demonstrated more than 30% nuclear reactivity. k: *CCNE1* immunoreactivity was considered high when samples demonstrated more than 50% nuclear reactivity. l: Germany, Belgium, Austria. m: *CCNE1* immunoreactivity was considered high when samples demonstrated more than 10% nuclear reactivity.



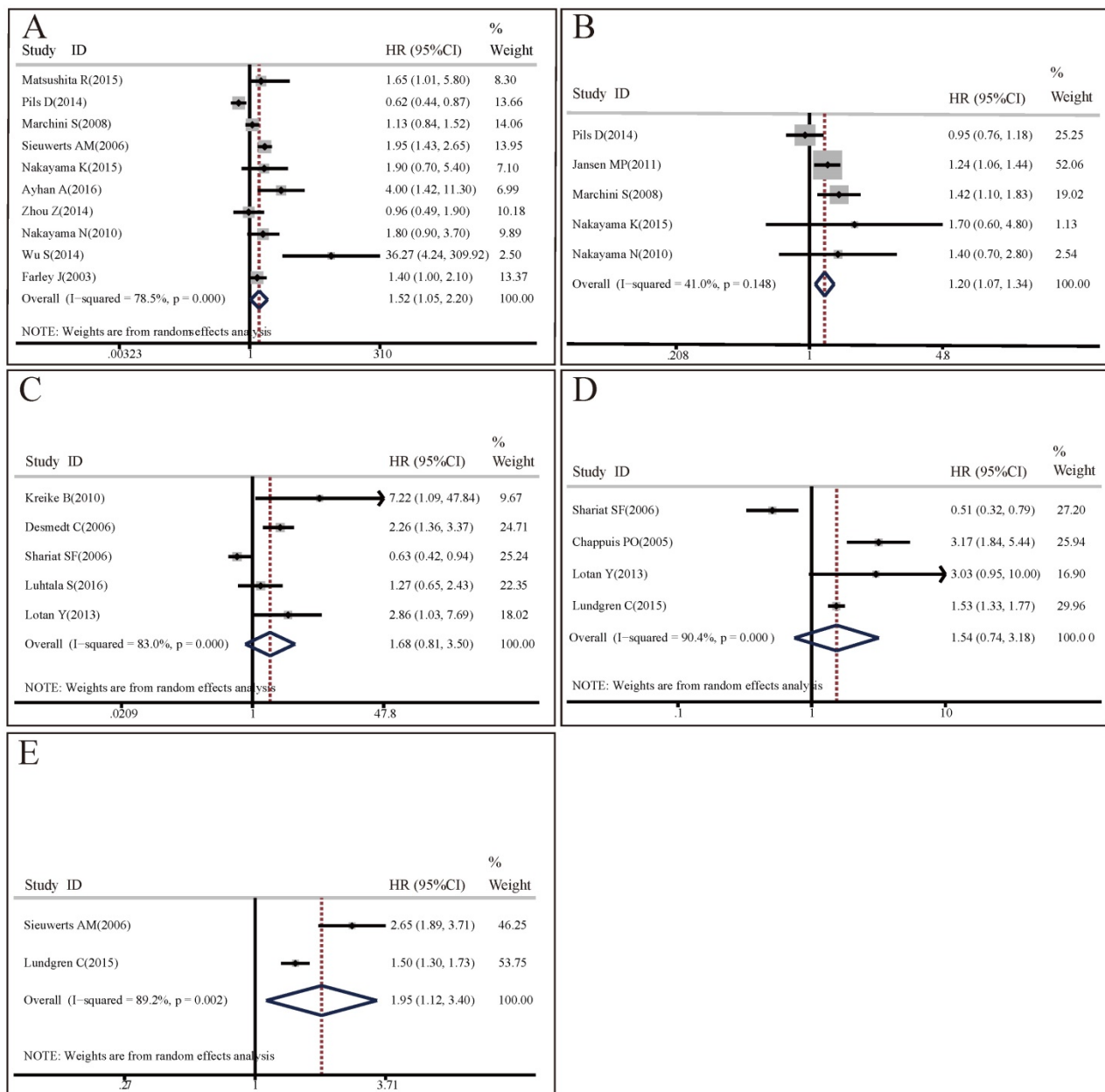
**Figure 2.** Forest plots of associations between *CCNE1* amplification and survival in cancer patients. **(A)** Effect of *CCNE1* amplification on overall survival. **(B)** Effect of *CCNE1* amplification on overall survival progression free survival.

**RFS analysis:** only 1 study enrolling 185 patients reported the predictive values of *CCNE1* amplification for RFS in breast cancer, with the HR of 0.982 (95% CI: 0.2376-4.059,  $p=0.9801$ ), indicating no significant relationship between them in breast cancer patients.

**Survival analysis for *CCNE1* overexpression carriers with cancers**

**OS analysis:** 10 studies with 1562 patients researched on the association between *CCNE1* expression levels and OS. The random effect model was applied to estimate the pooled HR with corresponding 95% CI. As shown in Figure 3A, there

was significant heterogeneity among these included studies ( $I^2=78.5%$ ,  $p<0.001$ ), meta-analysis results demonstrated that there was significant difference in OS between high *CCNE1* expression level group and low *CCNE1* expression level group. *CCNE1* overexpression carriers were associated with worse OS than non-carriers, with a pooled HR of 1.52 (95% CI: 1.05-2.20,  $p=0.027$ ). Subgroup analyses revealed that *CCNE1* overexpression carriers had statistically significantly shorter OS compared to non-carriers in studies as followed: on breast cancer or upper tract urothelial carcinoma; detecting by IHC method; about protein expression level; conducted in Asia or mixed



**Figure 3.** Forest plots of associations between *CCNE1* overexpression and survival in cancer patients. **(A)** Effect of *CCNE1* overexpression on overall survival. **(B)** Effect of *CCNE1* overexpression on progression free survival. **(C)** Effect of *CCNE1* overexpression on recurrence free survival. **(D)** Effect of *CCNE1* overexpression on cancer specific survival. **(E)** Effect of *CCNE1* overexpression on distant metastasis free survival.

region; with HR and 95% CI reported directly or with high quality. Noteworthy, of these 10 included studies, 5 studies were about ovarian cancer, but *CCNE1* overexpression had no significant relationship with OS in ovarian cancer patients (HR=1.28, 95% CI: 0.81-2.03,  $p=0.283$ ) (Table S3A).

**PFS analysis:** Since there was no significant heterogeneity across 5 included studies involving 666 patients ( $I^2=41%$ ,  $p=0.148$ ), the fixed effect model was applied. Compared with low *CCNE1* expression group, *CCNE1* overexpression group had a significantly worse PFS (HR=1.20, 95% CI: 1.07-1.34,  $p=0.001$ ) (Figure 3B). The results of subgroup analyses were presented in Table S3B. It revealed that analysis method might contribute to the heterogeneity. In studies adopting multivariate analysis, *CCNE1* overexpression predicted statistically significantly poorer PFS (multivariate analysis: HR=1.29, 95% CI: 1.13-1.47,  $p<0.001$ ; univariate analysis: HR=1.01, 95% CI: 0.82-1.24,  $p=0.958$ ). However, of these 5 included studies, 3 studies were about ovarian cancer, but *CCNE1* overexpression didn't show prognostic prediction ability for PFS in ovarian cancer patients (HR=1.14, 95% CI: 0.97-1.34,  $p=0.109$ ).

**RFS analysis:** 5 studies encompassing 1140 patients investigated the predictive values of *CCNE1* overexpression for RFS in cancers. As the result of meta-analysis exhibited obvious heterogeneity ( $I^2=83.0%$ ,  $p<0.001$ ), the random effect model was used. As shown in Figure 3C, meta-analysis results revealed no association between *CCNE1* overexpression and RFS, with a pooled HR of 1.68 (95% CI: 0.81-3.50,  $p=0.164$ ). Subgroup analyses results showed that the source of HR might contribute to the heterogeneity. It was notable that, of these 5 included studies, there were 4 studies with HR and 95% CI from literatures reported directly, and *CCNE1*

overexpression were associated with poorer RFS (HR=2.1, 95% CI: 1.31-3.36,  $p=0.002$ ) in them (Table S3C).

**CSS analysis:** 4 studies enrolling 881 patients assessed the association between *CCNE1* expression levels and CSS. The  $p$  and  $I^2$  values of heterogeneity test were  $<0.001$  and 90.4% respectively. After using random effect model, the pooled HR was 1.54 (95% CI: 0.74-3.18,  $p=0.246$ ), indicating *CCNE1* expression level didn't have significant correlation with CSS in cancer patients (Figure 3D). The results of subgroup analyses were presented in Table S3D.

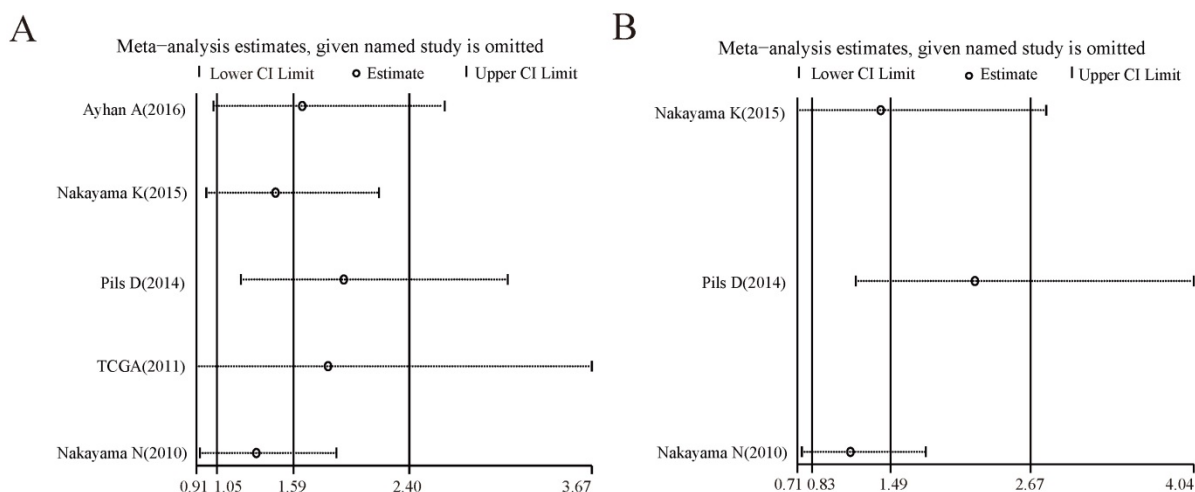
**DMFS analysis:** A random effect model was used to estimate the pooled HR with corresponding 95% CI, due to a significant heterogeneity across 3 included studies with 1678 patients ( $I^2=86.6%$ ,  $p=0.001$ ). As shown in Figure 3E, overexpression of *CCNE1* might predict poorer DMFS in cancer patients, with a pooled HR of 1.62 (95% CI: 1.09-2.40,  $p=0.017$ ). The results of subgroup analyses were presented in Table S3E.

### Sensitivity analysis

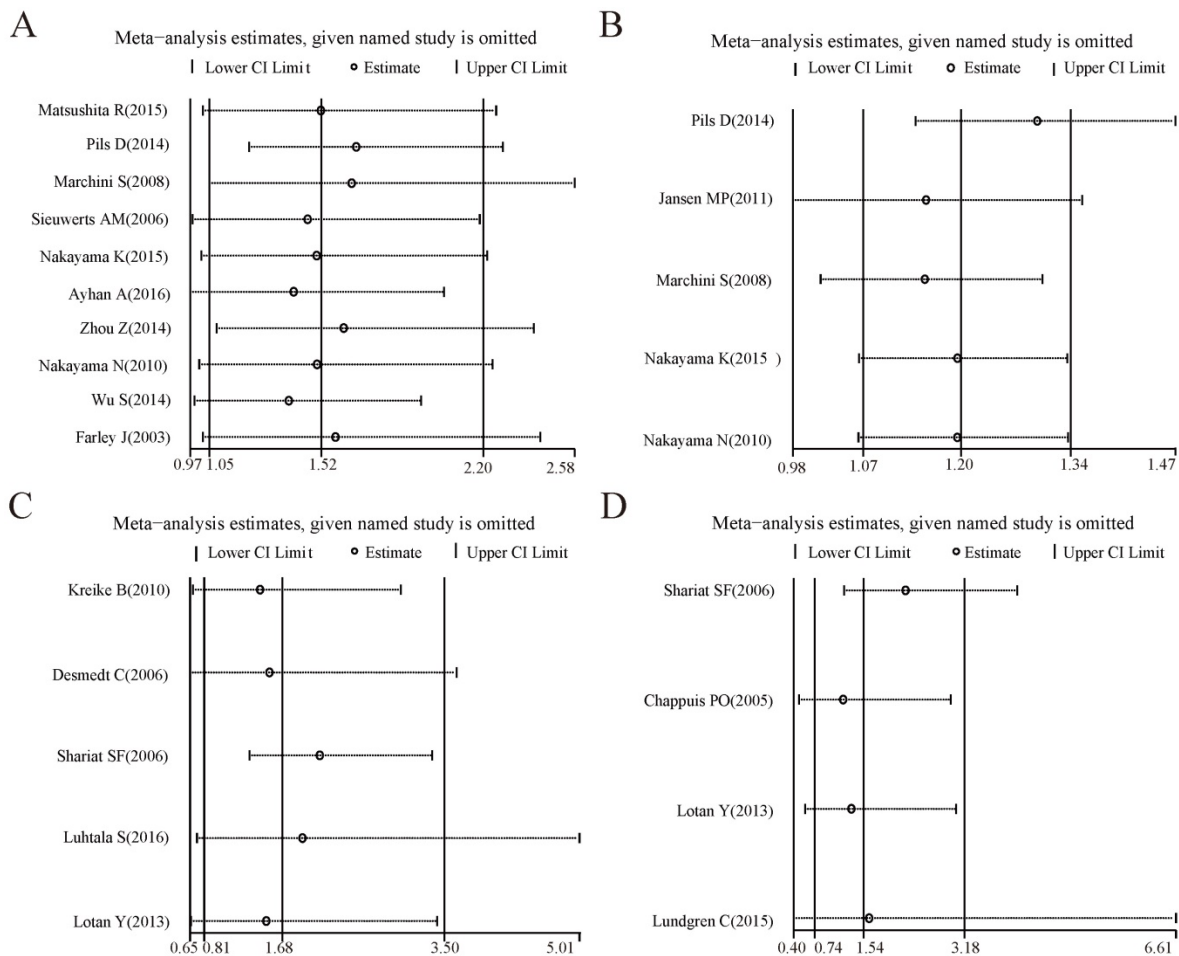
For the analysis groups about prognostic values of *CCNE1* amplification on OS and PFS or about prognostic values of *CCNE1* overexpression on OS, PFS, CSS, RFS and DMFS in cancer patients, sensitivity analyses were all performed by removing each study in turn from the pooled analysis. As showed in Figure 4-5, removing any of the included studies had no significant influence on the overall results of these groups mentioned above.

### Publication bias

We used funnel plot and Egger's tests to evaluate the publication bias of included studies researching on the association between *CCNE1* overexpression

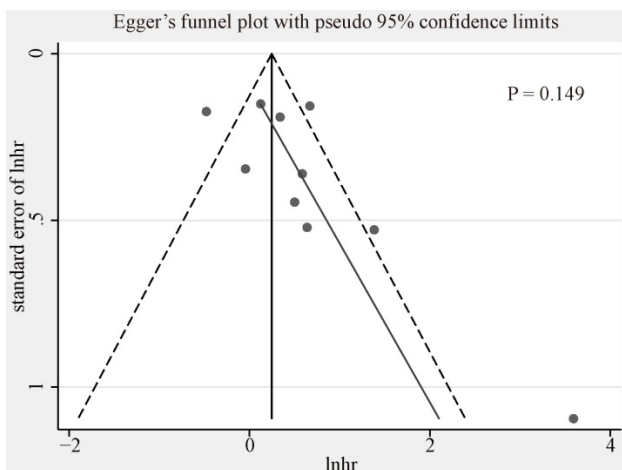


**Figure 4.** Forest plots of sensitivity analysis for the included studies on *CCNE1* amplification. Pooled hazard ratios and 95% confidence intervals by omitting each study. (A) For overall survival group. (B) For progression free survival group.



**Figure 5.** Forest plots of sensitivity analysis for the included studies on *CCNE1* overexpression. Pooled hazard ratios and 95% confidence intervals by omitting each study. **(A)** For overall survival group. **(B)** For progression free survival group. **(C)** For recurrence free survival group. **(D)** For cancer specific survival group. **(E)** For distant metastasis free survival group.

and OS (Figure 5, Egger’s test,  $p=0.149$ ). No evidence of significant publication bias was found. We did not analyze the publication bias of meta-analyses for the other groups mentioned above as the quantity of included studies was too small (<10) to make a valid statistical test.



**Figure 6.** Egger’s funnel plot for publication bias among the included studies exploring prognostic values of *CCNE1* overexpression for overall survival.

## Discussion

In cancer development, deregulation of cell cycle regulators was considered to be a prerequisite. *CCNE1* located on the chromosomal band 19q12, was first isolated in 1991 [64]. Cyclin E1 functioning as a regulatory subunit of CDK2, played significant roles in chromosomal instability and apoptosis in cancer cells [5]. In addition, researchers recently discovered that Cyclin E1 also had kinase-independent functions in cell-cycle progression [13]. Overexpression and amplification of *CCNE1* were reported to be associated with poor prognosis in a variety of cancers such as bladder cancer [6], ovarian cancer [8] and so on, while some opposite results existed [9, 10]. Even in subtypes of a single type cancer, *CCNE1* had different prognosis roles. For instance, *CCNE1* overexpression was related to a significantly increased risk of death and relapse in basal-like and triple receptor-negative breast cancers, while there was a lack of such association in hormone receptor-positive and luminal breast cancers [65]. So the prognostic value of *CCNE1* in cancer patients remained unclear.



*CCNE2* was located on the chromosomal band 8q22.1 [66]. Although Cyclin E1 and E2 were often coordinately regulated, and exhibited high homology within their important functional regions (47% between the overall sequences and 70% identity between the cyclin box) [67], which appeared to be functionally redundant, recently, more and more evidence suggested that Cyclin E1 and E2 were frequently expressed independently of one another in cancers, which had unique relationship with cancer prognosis. For example, it had been reported that Cyclin E2 overexpression was an independent and better prognostic signature for HER2 and luminal B breast cancer, when compared with Cyclin E1 [12]. Besides, both *CCNE2* overexpression and amplification were related to endocrine therapy resistance in breast cancer [12, 68]. However, in many studies, Cyclin E1 and Cyclin E2 were collectively referred to as Cyclin E, while in other articles, Cyclin E1 was called as Cyclin E. This inconsistency led us to conduct this meta-analysis for the prognosis value of Cyclin E1 in cancers rather than Cyclin E. We excluded articles about Cyclin E without definite indication of Cyclin E1 (or *CCNE1*) to avoid the possible confounding affect caused by Cyclin E2.

In this meta-analysis, a total of 20 studies were included and 5 survival assessment parameters (OS, PFS, RFS, CSS and DMFS) were measured. The results of meta-analysis indicated that *CCNE1* amplification predicted worse OS rather than PFS and RFS in cancer patients. However, it was noteworthy that the number of the included studies about *CCNE1* amplification for PFS or RFS was relatively small. And in studies adopting multivariate analysis, *CCNE1* amplification carriers showed significantly worse PFS. *CCNE1* overexpression was associated with worse OS, PFS and DMFS rather than RFS and CSS. Nevertheless, of 5 included studies for the relationship between *CCNE1* overexpression and RFS, there were 4 studies with HR and 95% CI reported directly from literatures, and *CCNE1* overexpression predicted poorer RFS in these 4 studies. Therefore, generally, *CCNE1* amplification and overexpression indicated worse prognosis of patients with cancer. Heterogeneity was found to be significant for each analysis group in this meta-analysis, except for the group researching on the correlation of *CCNE1* overexpression with PFS ( $I^2=41%$ ,  $p=0.148$ ). So we carried out detailed subgroup analysis to examine the potential sources of heterogeneity under a broader range of study level circumstances, which showed that for different analysis groups, the causes of heterogeneity were diversified. Contrary to the pooled result of all the involving cancers in each analysis group, in ovarian cancer patients, *CCNE1*

amplification had no prognosis value on OS, and *CCNE1* overexpression also didn't predict worse OS or PFS. It was quite unexpected, as researches about ovarian cancer accounted for the largest share in the included studies of these analysis groups. However, some studies had reported that *CCNE1* amplification or overexpression could select ovarian cancer patients more likely to benefit from CDK2 targeted therapies [69, 70]. And then, because the prognostic value of *CCNE1* was reported to be affected by germline or somatic mutations of *BRCA1/2* in ovarian cancer [48], so we suspected that the mutation rate of *BRCA1/2* in different studies affected the assessment of the prognostic value of *CCNE1* in ovarian cancer. In the future, we believed that the research for the role of *CCNE1* in the prognosis of ovarian cancer needed to be carried adjusting for *BRCA1/2*. The sensitivity analysis showed that no individual study could significantly influence the conclusion in each analysis group of our research, indicating the credibility and stability of our meta-analysis results.

There were several limitations of this meta-analysis, which should be addressed. First, there were a variety of survival assessment parameters, while only 20 articles were included, therefore studies specific to some analysis groups were relatively insufficient, limited data availability for these groups reduced the credibility of our results. Second, we excluded a number of articles for Cyclin E without indication of Cyclin E1 (or *CCNE1*). Although this strategy avoided the possible confounding affect from Cyclin E2, it may inevitably delete some researches exactly about *CCNE1*. Third, despite no significant publication bias was observed in studies researching on the association between *CCNE1* overexpression and OS, publication bias may still exist. Because we did not perform publication bias analysis for the other analysis groups as the quantity of included studies in them was too little; most articles reported positive results, whereas those with negative findings generally were less likely to be published; despite we tried to collect all relevant researches, but inevitably, some studies may still be missing. Fourth, the cut-off definition for *CCNE1* overexpression was not unified among the included studies. Finally, HR and 95% CI in some studies were calculated based on the data extracted from survival curve, which may be less reliable than that reported directly in articles.

Despite the limitations described above, to our knowledge, this was the first meta-analysis about the predictive roles of *CCNE1* amplification and overexpression in the prognosis of various cancers. Our systematic review and meta-analysis suggested that, on the whole, *CCNE1* amplification and overexpression were associated with poor prognosis

in cancer patients, which meant that *CCNE1* could be hopefully applied to clinical work as a prognostic signature for cancers. Additionally, *CCNE1* overexpression showed a more important role in prognosis than *CCNE1* amplification in cancer patients. Unexpectedly, both amplification and overexpression of *CCNE1* had no prognostic prediction ability in ovarian cancer patients. At last, the total number of our included studies was relatively small, more clinical investigations with larger sample size, multicenter, higher quality and prospective design were strongly needed to further validate the prognostic role of *CCNE1* in cancer patients.

## Abbreviations

NR: not reported; EC: endometrial carcinomas; EAC: esophageal adenocarcinoma; UTUC: upper tract urothelial carcinoma; OS: overall survival; PFS: progression free survival, RFS: recurrence free survival, CSS: cancer specific survival; DMFS: distant metastasis free survival; CDK2: cyclin dependent kinase 2; MeSH: Medical Subject Headings; HR: Hazard ratio; 95% CI: 95% confidence interval; K-M curve: Kaplan-Meier curve; NOS: Newcastle-Ottawa Scale; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; FISH: fluorescence in situ hybridization; PCR: polymerase chain reaction; CISH: chromogenic in situ hybridization; IHC: immunohistochemistry; RT-qPCR: real-time quantitative polymerase chain reaction.

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## Supplementary Material

Supplementary tables.

<http://www.jcancer.org/v09p2397s1.pdf>

## Competing Interests

The authors have declared that no competing interest exists.

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