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Ki-67 labeling index is a predictive marker for a pathological complete response to neoadjuvant chemotherapy in breast cancer

A meta-analysis

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Abstract

Background: A pathological complete response (pCR) after neoadjuvant chemotherapy (NCT) is a strong indicator of the benefit of therapy and presents an early surrogate for a favorable long-term outcome. It remains unclear whether Ki-67, a marker for tumor proliferation, can function as a predictor of the response to NCT in breast cancer. The objective of this meta-analysis was to compare the pCR rate and clinical outcomes in breast cancer patients with different Ki-67 labeling indexes (Ki-67 LI) who received NCT.

Methods: Clinical studies were retrieved from the electronic databases of PubMed, Embase, Clinical Trials, Wanfang, and the Chinese National Knowledge Infrastructure, from their inception to July 31, 2017. Meta-analysis was performed on pool eligible studies to determine whether Ki-67 LI was associated with the pCR rate and clinical outcomes of breast cancer patients who were treated with NCT. Pooled analyses were performed using fixed effects models. Two reviewers screened all titles and abstracts and independently assessed all articles.

Results: A total of 36 studies involving 6793 patients were included in the meta-analysis. Pooled analysis results revealed that patients with high Ki-67 LI exhibited significantly higher pCR rates (odds ratio [OR] = 3.94, 95% confidence interval [CI]: 3.33–4.67, $P < .001$) but poorer relapse-free survival (OR = 1.99, 95% CI: 1.39–2.85, $P < .001$) than those with low Ki-67 LI, but there was no significant difference in objective tumor response rate.

Conclusion: The meta-analysis reported here demonstrates that pretherapeutic Ki-67 LI is associated with pCR in breast cancer patients undergoing NCT. More phase III randomized clinical trials will be required to confirm our findings.

Abbreviations: HER2 = human epidermal growth factor receptor 2, HR = hormone receptor, Ki-67 LI = Ki-67 labeling index, NCT = neoadjuvant chemotherapy, NR = not reported, OR = odds ratio, OS = overall survival, pCR = pathological complete response, RFS = relapse-free survival, TNBC = triple-negative breast cancer.

Keywords: breast cancer, Ki-67, meta-analysis, neoadjuvant chemotherapy, pathological complete response

1. Introduction

The most common cancer in women in 2016 was breast cancer, which is expected in the near future to account for approximately 29% of all newly diagnosed cancers in females.[1] Neoadjuvant chemotherapy (NCT) has been established as a standard treatment for patients with not only locally advanced breast cancer but also operable breast cancer. The objectives of NCT for operable breast cancers are to downstage tumors, making inoperable tumors operable, to render tumors amenable to breast conserving surgery, and to improve the survival time.[2,3] Biomarkers have been used in the past to monitor cancer treatment and increasing evidence indicates that tumor biomarker levels can help clinicians to assess the effectiveness of NCT.[4–8]

Ki-67 is a nuclear protein expressed during all phases of the cell cycle, except G0, and its expression has been reported to be correlated with the tumor cell proliferation rate. Many studies have investigated immunohistochemical expression of Ki-67 as a prognostic and predictive marker for breast cancer.[9–11] But previous studies did not report completely consistent results regarding the impact of NCT on the status of tumor biomarkers.[12–17]

One of the main objectives of NCT is to achieve a pathological complete response (pCR) because pCR has been found to be associated with longer disease-free and overall survival rates.[18,19] Several studies have associated high levels of Ki-67 with higher pCR rates.[20,21] However, other studies failed to confirm these findings.[22,23] A recently published meta-analysis involving 44 articles that investigated the relationship between Ki-67 expression levels and the pCR rate indicated that a high Ki-67 level was associated with a high pCR rate (OR = 3.10, 95% CI: 2.52–3.81, $P < .001$).[24] However, many of these articles did not explore the relationship between Ki-67 levels and the clinical response, nor did they discuss the prognostic value of Ki-67 in breast cancer. Therefore, the primary purpose of our study was to evaluate the function of pretherapeutic Ki-67 labeling index (LI)

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Table 1

PubMed search strategies.

#1 "breast cancer" OR "breast carcinoma" OR "breast neoplasm" OR "breast tumor" OR "mammary cancer"
#2 "neoadjuvant chemotherapy" OR "preoperative chemotherapy"
#3 "Ki-67"
#4 #1 AND #2 AND #3

as a predictive marker for pCR to NCT using meta-analytical methodology. We also investigated the predictive value of Ki-67 for the clinical response and the prognostic value of Ki-67 in breast cancer patients receiving NCT.

2. Materials and methods

2.1. Literature search strategy

To identify studies involving the association between Ki-67 expression and the pCR in breast cancer, a literature search was conducted among 3 English databases (PubMed, Embase, and Clinical Trials), and 2 Chinese databases (Wanfang and Chinese National Knowledge Infrastructure databases) from their inception to July 31, 2017. We checked these electronic databases using the search terms "Ki-67" and "breast cancer" and "NCT". Additionally, we performed a computerized search of abstracts presented at the Annual Meetings of the American Society of Clinical Oncology (ASCO). Finally, we screened the references in all relevant articles to identify additional articles that were not retrieved during the initial literature search. The search strategy used for PubMed is shown in Table 1.

2.2. Selection criteria

Our meta-analysis included all studies meeting the following criteria: patients were pathologically diagnosed with breast cancer; all patients received NCT; results were stratified according to the level of pretherapeutic Ki-67 expression; pCR was the end point in trials and could be calculated directly; the results were part of an original analysis; papers were published in Chinese or English. We only selected the articles published in peer-reviewed journals and excluded reviews, letters, and meeting abstracts. Patients who received preoperative chemotherapy concomitant endocrine therapy or local treatment were excluded.

2.3. Data extraction

Information from each study was abstracted independently by 2 investigators using a standardized data extraction form, predesigned on the basis of the Cochrane Consumers and Communication Review Group data extraction template. Any disagreement over extracted data was resolved through discussion until the 2 investigators reached a consensus opinion. The following information was recorded for each publication: first author's name, publication year, study type, country of origin, the cut-off value of Ki-67 LI, numbers of patients in study sample, clinical stage, NCT regimens and cycles, molecular subtypes, numbers of patients with "high" Ki-67 LI, and numbers of patients with "low" Ki-67 LI. When key pieces of information were not present in articles, the corresponding author was contacted. In the event that we still could not obtain the whole dataset, the missing information was classified as "not reported". The primary endpoint was the pCR rate of NCT. pCR was defined as complete disappearance of invasive carcinoma in both breast and axillary lymph nodes. Residual ductal carcinoma in situ was included in the pCR category. The objective tumor response was assessed according to modified Response Evaluation Criteria in Solid Tumors.[57] In other words, "complete response" or "partial response" was classified as "response", while "stable" or "progressive disease" as "nonresponse". Relapse-free survival (RFS) was defined as the elapsed time between the date of first diagnosis and the date of the first relapse. Overall survival (OS) was calculated from the date of diagnosis to the date of death or the last follow-up.

2.4. Quality assessment

The initial relevance evaluation was implemented by 2 reviewers through independently screening of titles and abstracts. If either reviewer considered any titles or abstracts met the eligibility criteria, the full text was obtained. The quality and bias risk of the selected papers were critically appraised separately by 2 reviewers. Quality assessment was conducted for each of the eligible studies by using the validated Newcastle–Ottawa Quality Assessment Scale (NOS).[58] This scale is composed of 8 items that assess patient selection, study comparability, and outcome with scores ranging from 0 to 9. In our meta-analysis, studies with a score no <6 were graded as high quality.[59] Eventual consensus governance resolved disagreements.

2.5. Statistical methods

Dichotomous results were summarized as pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) around the point estimates. OR was abstracted or calculated to quantitatively evaluate the association between pretherapeutic Ki-67 LI and the response rate. The overall pooled effect was assessed using the z-statistic with a P-value $\leq .05$ representing statistical significance.

Heterogeneity between the studies was assessed by χ^2 statistics and expressed as an "I^2" value. When $I^2 \geq 50\%$ or the P-value for the I^2 statistic was $<.05$, which indicated significant heterogeneity across the studies, the pooled estimate was calculated using a random effects model and if the data were contrary, a fixed effect model was adopted. In subgroup analysis on the basis of patients' populations, studies were divided into an "Asian population" and a "European population". In the subgroup analysis by cut-off values of Ki-67, studies were classified according to the levels of "$\leq$ 14%," "15% to 29%," and "$\geq$ 30%". And in the subgroup analysis by molecular subtypes, studies were divided into "TNBC," "HER2+," "HR+," "HR–," and "unclassified" (contains all molecular subtypes). All statistical analyses were carried out using RevMan V.5.3 software.

All analyses were based on previous published studies, thus no ethical approval or patient consent was required.

3. Results

3.1. Search results

The search strategy yielded 849 potentially relevant references in the electronic databases. We initially excluded 321 duplicated publications. Upon review of the remaining abstracts, we further removed 433 more articles for reasons of ineligibility. According to the inclusion criteria established for the present study, an additional 59 articles were excluded. We thus finally selected 36 studies,[20–23,25–56] which consisted of a cohort of 6793 patients with breast cancer (shown in the flow diagram).

Table 2

Summary of studies included in the meta-analysis.

Author (year)	Population	Study type	Cut-off value	NCT regimens	Cycles	Molecular subtypes	Ki-67 high level		Ki-67 low level	
							No. of patients	No. of pCR	No. of patients	No. of pCR
Teresa et al, 2017[25]	European	Retrospective	50%	A/T-based	NR	TNBC	107	44	92	16
Wang et al, 2016[26]	Asian	Retrospective	40%	TA	2–6	All	42	14	198	16
Alba et al, 2016[27]	European	Prospective	50%	TA	NR	All	91	36	171	33
Yukie et al, 2016[28]	Asian	Prospective	20%	TA-based	NR	All	78	27	28	4
Gamal et al, 2016[29]	Asian	Retrospective	14%	NR	6–8	All	76	21	25	4
Sasagu et al, 2015[30]	Asian	Prospective	30%	T, FEC	4+4	HER2+	93	67	36	17
Yuan et al, 2015[31]	Asian	Retrospective	13.5%	TAC	6	All	231	34	84	4
Kim et al, 2015[34]	Asian	Retrospective	10%	TA, AC-T	3–6	TNBC	159	31	34	2
Tan et al, 2014[33]	Asian	Retrospective	30%	FEC, AC-T, TEC	2–6	HR–	78	22	105	13
Ingolf et al, 2014[34]	European	Retrospective	15%	NR	NR	All	55	16	22	4
Huang et al, 2013[35]	Asian	Retrospective	14%	FEC, NE	NR	HER2+	70	12	43	2
Ohno et al, 2013[36]	Asian	Prospective	10%	FEC, T-based	4+4	All	299	95	119	11
Cheng et al, 2013[37]	Asian	Prospective	14%	TA, TC	4–6	All	138	42	21	2
Yao et al, 2013[38]	Asian	Retrospective	50%	TA	4	TNBC	25	16	27	2
Ye et al, 2013[39]	Asian	Retrospective	30%	TEC, FEC	2–4	TNBC	45	15	29	4
Jin et al, 2013[40]	Asian	Retrospective	20%	A/T-based	NR	All	197	20	54	4
Saracchini et al, 2013[41]	European	Prospective	20%	AC-T	4+4	HER2+	30	18	8	1
Esserman et al, 2012[42]	European	Prospective	25%	A-based	4	All	61	21	105	12
Zhang et al, 2012[43]	Asian	Retrospective	40%	T-based	2–6	HER2+	49	28	53	17
Grim et al, 2012[44]	European	Prospective	20%	TAC	6	All	39	13	22	1
Peter et al, 2011[45]	European	Retrospective	14%	A/T-based	NR	All	390	113	162	7
Keam et al, 2011[20]	Asian	Prospective	10%	TA	NR	TNBC	77	14	28	0
Li et al, 2011a[21]	Asian	Prospective	50%	TA	4–6	TNBC	27	14	14	2
Li et al, 2011b[46]	Asian	Retrospective	20%	TA	2–6	All	134	15	86	5
Petit et al, 2010[47]	European	Retrospective	20%	FEC	6	HR+	97	22	80	1
Sánchez et al, 2010[48]	European	Prospective	20%	EC/T-based	3–6	All	33	22	36	2
Colleoni et al, 2010[49]	European	Retrospective	20%	NR	NR	All	649	94	134	5
Masuda et al, 2010[50]	Asian	Prospective	50%	A/T-based	4	TNBC	20	10	13	2
Darb et al, 2009[51]	European	Prospective	20%	A/T-based	4	All	21	7	85	5
Guarneri et al, 2009[52]	European	Prospective	15%	TA-based, FEC	1–8	All	155	14	40	1
Zhou et al, 2008[22]	Asian	Retrospective	20%	TA	4	All	56	10	48	7
Wei et al, 2007[23]	Asian	Prospective	25%	FEC	2–8	All	49	10	94	16
Colleoni et al, 2007[53]	European	Prospective	20%	A/T/V-based	6	All	326	36	142	2
Vincent et al, 2004[54]	European	Retrospective	42%	FEC	4	All	27	11	28	4
Mathieua et al, 2004[55]	European	Retrospective	20%	TA-based, FEC	3–4	All	71	9	50	0
Colleoni et al, 2004[56]	European	Prospective	25%	A/T/V-based	3–6	All	210	47	172	14

A = anthracycline, C = cyclophosphamide, E = epirubicin, F = 5-fluorouracil, HER-2 = human epidermal growth factor receptor 2, HR = hormone receptor, NR = not reported, T = taxane, TNBC = triple-negative breast cancer, V = vinorelbine.

All of the 36 selected studies assessed the association analysis between pretherapeutic Ki-67 LI and pCR, 4 of them contained the association analysis between Ki-67 LI and clinical response,[28,31,33,35] 7 of them reported the relationships between pretherapeutic Ki-67 LI and RFS,[20,33,35,43,45,50,51] and 3 of them explored the relationships between Ki-67 LI and OS.[20,35,45] Based on the type of study, there were 17 prospective observational studies, and the 19 remaining studies were retrospective. A summary of the available information included in the present meta-analysis is provided in Table 2. Quality assessment with the NOS, shown in Table 3, demonstrated that the combined scores of selection, comparability, and outcome aspects was >6 in each of the selected studies.

3.2. Clinical and methodological heterogeneity

The included studies utilized either retrospective or prospective observational designs. In addition, they also varied in ways that could affect pCR, including the populations of the study samples, NCT strategies and cycles, proportions of patients with different molecular subtypes, and cut-off values of Ki-67. Therefore, there was considerable clinical and methodological heterogeneity among the included studies.

3.3. Statistical pooling

3.3.1. The pCR rate of patients with high Ki-67 LI was significantly higher than that of patients with low Ki-67 LI. The pooled results from the analysis of the association between pretherapeutic Ki-67 LI and pCR are shown in Figure 1. Since there was low heterogeneity between studies ($\chi^2 = 48.34$, $P = .07$, $I^2 = 28\%$), the fixed effects model was applied to perform the meta-analysis. As shown in Figure 1, the pCR rate of patients with high Ki-67 LI (n = 4305) was significantly higher than that of patients with low Ki-67 LI (n = 2488) (OR: 3.94, 95% CI: 3.33–4.67, $P < .001$), and the OR values of prospective and retrospective studies were 4.02 (95% CI: 3.16–5.12, $P < .001$) and 3.88 (95% CI: 3.06–4.91, $P < .001$) respectively. These results indicated that the pretherapeutic Ki-67 level is indeed a determinant of the pCR rate to NCT in breast cancer.

Table 3

Quality of literature included in the meta-analysis.

Author	Year	Selection (4 points)	Comparability (2 points)	Outcome (3 points)	Total (9 points)
Teresa et al	2017	3/4	2/2	2/3	7/9
Wang et al	2016	4/4	2/2	3/3	9/9
Alba et al	2016	4/4	2/2	2/3	8/9
Yukie et al	2016	4/4	2/2	2/3	8/9
Gamal et al	2016	4/4	2/2	2/3	8/9
Sasagu et al	2015	3/4	2/2	3/3	8/9
Yuan et al	2015	4/4	2/2	2/3	8/9
Kim et al	2015	4/4	2/2	3/3	9/9
Tan et al	2014	4/4	2/2	2/3	8/9
Ingolf et al	2014	4/4	2/2	2/3	8/9
Huang et al	2013	4/4	2/2	3/3	9/9
Ohno et al	2013	4/4	2/2	3/3	9/9
Cheng et al	2013	4/4	2/2	2/3	8/9
Yao et al	2013	3/4	2/2	3/3	8/9
Ye et al	2013	3/4	2/2	2/3	7/9
Jin et al	2013	4/4	2/2	3/3	9/9
Saracchini et al	2013	3/4	2/2	2/3	7/9
Esserman et al	2012	4/4	2/2	3/3	9/9
Zhang et al	2012	3/4	2/2	2/3	7/9
Grim et al	2012	4/4	2/2	2/3	8/9
Peter et al	2011	4/4	2/2	3/3	9/9
Keam et al	2011	4/4	2/2	3/3	9/9
Li et al	2011a	4/4	2/2	2/3	8/9
Li et al	2011b	4/4	2/2	2/3	8/9
Petit et al	2010	4/4	2/2	2/3	8/9
Sánchez-Muñoz et al	2010	4/4	2/2	2/3	8/9
Colleoni et al	2010	4/4	2/2	3/3	9/9
Masuda et al	2010	3/4	2/2	2/3	7/9
Darb-Esfahani et al	2009	4/4	2/2	3/3	9/9
Guarneri et al	2009	4/4	2/2	3/3	9/9
Zhou et al	2008	4/4	2/2	3/3	9/9
Wei et al	2007	4/4	2/2	2/3	8/9
Colleoni et al	2007	3/4	2/2	3/3	8/9
Vincent-Salomon et al	2004	4/4	2/2	3/3	9/9
Mathieua et al	2004	4/4	2/2	3/3	9/9
Colleoni et al	2004	3/4	2/2	3/3	8/9

Taking into account the heterogeneity between studies, we conducted a sensitivity analysis. The pooled results did not differ substantially between the fixed and random effects models. By recalculating ORs with 1 study removed and all others included from the pooled estimate, we assessed the influence of each study on the overall estimate. Influence analysis showed no substantial difference in pooled ORs when any single study was excluded, which indicated that the conclusion was robust.

Then we utilized the fixed effects model to calculate results in a sub-group analysis on the basis of patients' population type and found that the pCR rate was significantly higher in patients with high Ki-67 LI than those with low Ki-67 LI, in both European (22.1% vs 8.0%, OR = 4.90, 95% CI: 3.83–6.28, $P < .001$) and Asian (26.6% vs 11.7%, OR = 3.18, 95% CI: 2.52–4.02, $P < .001$) subgroups (Fig. 2).

Taking into account the effects of different cut-off values of Ki-67 LI on the results, we performed a subgroup analysis based on specified cut-off values. The results showed that patients with high Ki-67 LI were more likely to achieve pCR no matter what the cut-off value; Ki-67 LI was ≤14% (25.1% vs 6.2%, OR = 5.03, 95% CI: 3.45–7.34, $P < .001$), 15% to 29% (17.7% vs 7.0%, OR = 3.76, 95% CI: 2.88–4.91, $P < .001$), or ≥30% (45.9% vs 16.4%, OR = 3.51, 95% CI: 2.69–4.57, $P < .001$) (Fig. 3).

Considering the influence of the molecular subtypes, a subgroup analysis was performed. We found that the pCR rate of patients with high Ki-67 LI was significantly higher than those with low Ki-67 LI even when the included patients were triple-negative breast cancer (31.3% vs 11.8%, OR = 4.65, 95% CI: 2.93–7.38, $P < .001$), HER+ (51.7% vs 26.4%, OR = 3.32, 95% CI: 1.99–5.54, $P < .001$), or unclassified (21.2% vs 8.5%, OR = 3.85, 95% CI: 3.15–4.72, $P < .001$) (Fig. 4).

3.3.2. Patients with Ki-67 LI tended to have a better objective tumor response. We next assessed objective tumor response in 4 studies, which included 717 patients. We performed meta-analysis using the random effects model because of the heterogeneity among studies ($\chi^2 = 8.75$, $P = .03$, $I^2 = 66\%$). We found that patients with a Ki-67 LI tended to have a better objective tumor response (83.8% vs 75.8%, OR = 1.57, 95% CI: 0.72–3.42, $P = .26$; Fig. 5). However, the result did not reach statistical significance.

Because of the significant heterogeneity, we performed a sensitivity analysis and found a substantial difference in pooled OR when the study of Yukie et al[28] was excluded. The adjusted results showed that patients with a high Ki-67 LI had a better objective tumor response than those with a low Ki-67 LI (84.0%

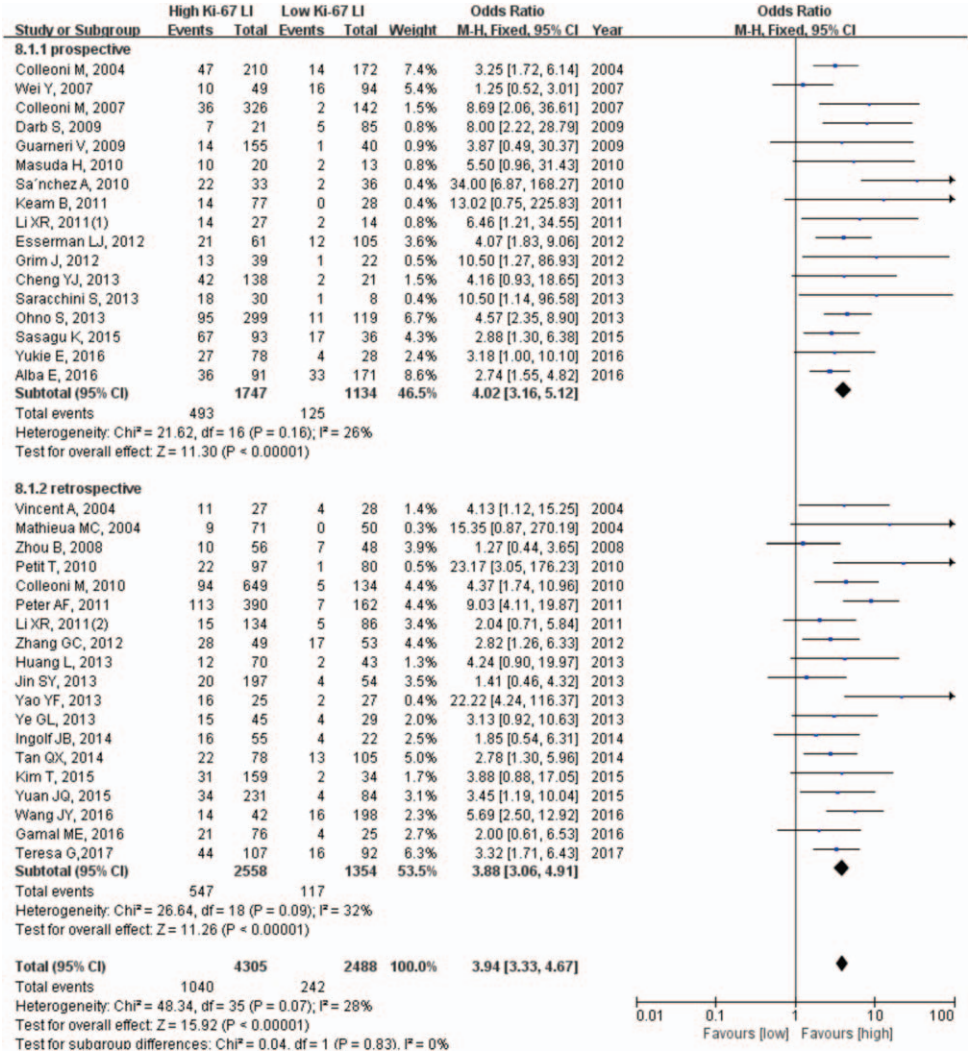

Figure 1. Pooled analysis of Ki-67 LI and pCR. Ki-67 LI = Ki-67 labeling index, pCR = pathological complete response.

vs 73.3%, OR = 2.19, 95% CI: 1.45–3.33, $P < .001$; supplemental Fig. 2, http://links.lww.com/MD/C36).

3.3.3. Patients with a high Ki-67 LI have a poorer RFS. The results of the pooled analysis of the association between pretherapeutic Ki-67 LI and RFS are shown in Figure 6. Patients with a high Ki-67 LI have a poorer RFS than those with a low Ki-67 LI (OR = 1.99, 95% CI: 1.39–2.85, $P < .001$).

3.3.4. Publication bias. In the meta-analysis, funnel plots were generally symmetrical (Fig. 7). These results indicated that publication bias was insignificant across the included studies.

4. Discussion

A recently published meta-analysis reported that a high Ki-67 level was associated with a high pCR rate.[24] Although the selection criteria and pooling methods were not exactly the same, our study came to a similar conclusion. However, in addition we not only explored the predictive value of Ki-67 for NCT in breast cancer, but also investigated its prognostic value. Our results demonstrate that patients with a Ki-67 LI are more sensitive to NCT, have higher pCR rates, and benefit more from NCT compared to those with a low Ki-67 LI ($P < .001$). Conversely, patients with a high Ki-67 LI have a worse RFS.

In a subgroup analysis of patients' population, we found that the pCR rate of patients with a high Ki-67 LI was significantly higher than in patients with a low Ki-67 LI in both European and Asian subgroups. However, it remains unclear whether other factors such as therapy regimens and cycles of NCT, the clinical stage, and tumor location have an impact on Ki-67-based health outcomes. Our study's design did not allow for the evaluation of these relationships, so further research will need to be carried out.

Numerous studies have shown a positive correlation between the expression of Ki-67 and the response to chemotherapy.[60–62] However, threshold values for dividing high and low Ki-67 LI are not clearly defined and vary between laboratories, ranging from 10% to 50%. The St Gallen Consensus Meeting declared that Ki-67 LI is chiefly important for distinguishing between luminal A and luminal B subtypes of breast cancer with a cut-off value of 14%.[63] In a previous study, researchers found that the expression of Ki-67 was the only independent predictor of pCR and also discovered that a Ki-67 value >25% was a significant predictive factor for pCR.[60] The latter results were supported by another study in which a cut-off value of Ki-67 of

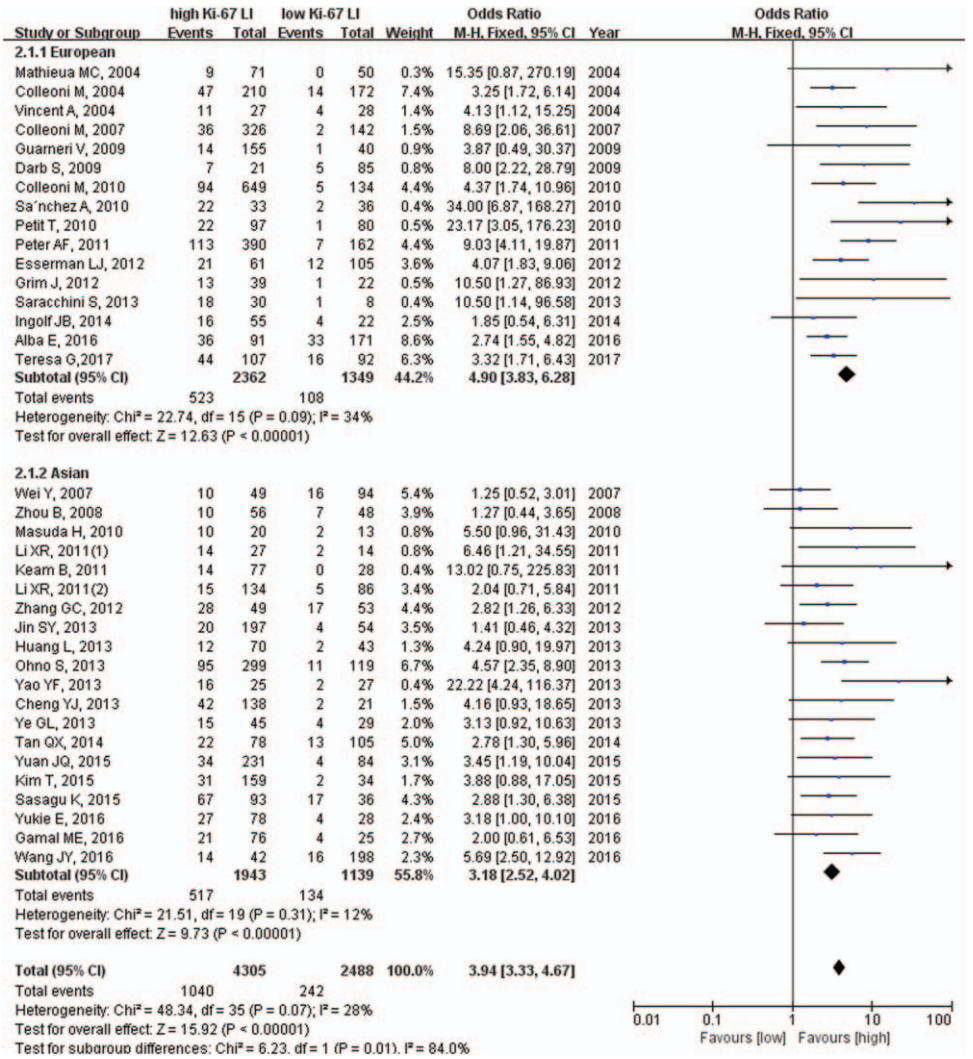

Figure 2. Subgroup analysis of patient population type.

circa 30% was suitable for predicting pCR.[33] Therefore, we performed a subgroup analysis based on this factor with 14% and 30% as the cut-off points and found that the pCR rate of patients with a high Ki-67 LI was significantly higher than in patients with a low Ki-67 LI regardless of whether the cut-off value was ≤14%, 15% to 29%, or ≤ 30%. Interestingly, when we performed a subgroup analysis according to a cut-off value of Ki-67, the heterogeneity among subgroups varied greatly, the I^2 values being 0%, 50%, and 0%, respectively, indicating that the cut-off value of Ki-67 may be one of the sources of heterogeneity.

Patients with different types of breast cancer have different responses to NCT regimens. Previous studies have shown that patients with hormone receptor-positive breast cancer, which were categorized into luminal subtypes, are less likely to achieve pCR.[64,65] In a retrospective study, 240 patients with breast cancer received 4 to 6 weeks of NCT before surgery and it was found that patients with luminal A (1.6%) and luminal B (13.4%) cancer types had the lowest pCR rates followed by the human epidermal growth factor receptor 2 (HER2) overexpression (22.6%) and triple negative (23.8%) forms.[58] This result is consistent with that from another study in which the authors found that the odds of achieving pCR in HER2+ cancers were 3.6 times higher than that in luminal cancers.[66] All of these findings suggest that patients with luminal type tumors gained less benefit from NCT. We next performed a subgroup analysis based on molecular types, and found that the pCR rate of patients with a high Ki-67 LI was significantly higher than those with a Ki-67 LI regardless of the molecular type of cancer. Unfortunately, the vast majority of selected articles (23/36) were not classified into molecular subtypes, so the results do not fully reflect the real clinical situation.

In exploring the relationship between Ki-67 LI and objective remission rates, we found that the Yukie et al's study had a significant impact on outcomes.[28] The study included 183 patients, 120 of whom came from Hyogo College of Medicine, and the others from Yao Municipal Hospital. However, for some reason, further analyses were performed only for patients treated at the Hyogo College of Medicine, which can lead to significant experimental errors. When we excluded this study from the pooled analysis, the results showed that patients with a high Ki-67 LI had a better objective tumor response ($P < .001$). More studies will be needed to confirm this finding.

Several studies have demonstrated that patients who achieve pCR to NCT tend to have improved RFS and OS compared with

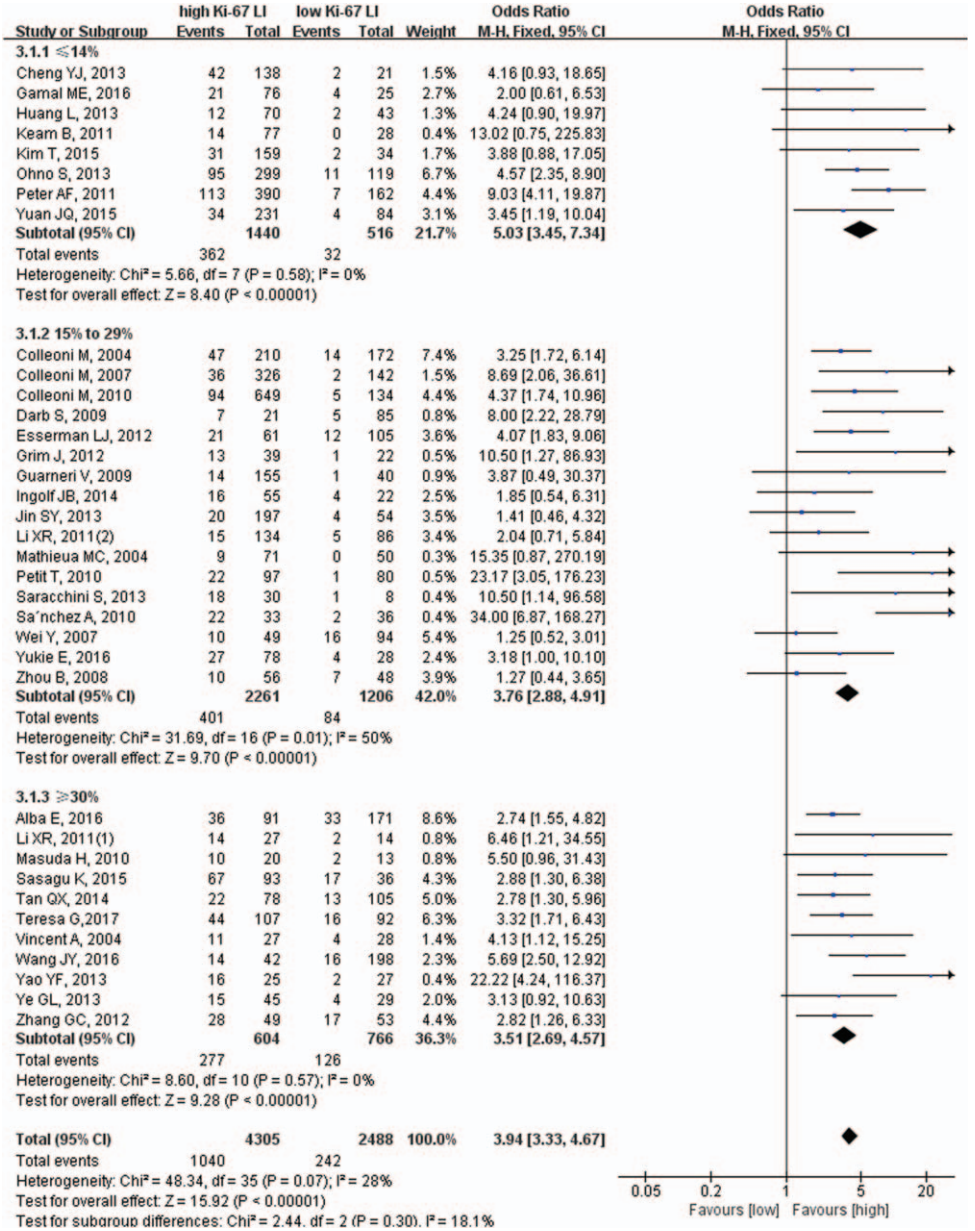

Figure 3. Subgroup analysis of the cut-off value of Ki-67.

those with residual invasive disease.[67,63] However, few studies have explored the relationship between Ki-67 LI and RFS or OS. Our study suggested that high Ki-67 LI was significantly associated with poor RFS ($P<.001$). We explored the relationship between Ki-67 LI and OS using the random effects model and found that patients with a high Ki-67 LI had a worse OS than patients with a low Ki-67 LI (OR = 3.44, 95% CI: 0.57–15.8, $P=.11$, data shown in supplemental fig. 1, http://links.lww.com/MD/C36). But these results may not be reliable due to the small number of included studies (3/36). High Ki-67 LI was significantly associated with a high pCR rate but poor RFS. In other words, patients who did not achieve a pCR to NCT maintained a good prognosis even in the presence of residual disease. The good outcome of these patients was largely dependent on the efficacy of surgery and postoperative therapy. In other words, whether the patients achieved pCR or not, all of them underwent surgery and adjuvant therapy, thus weakening the impact of pCR on survival.

There are several limitations to the present meta-analysis. First, our analysis was based mainly on findings from observational studies, which might contain a higher number of confounding factors than randomized controlled clinical trials. Second, our analysis only contained published studies. Since reports with positive results are more likely to be published than those with negative observations, potential publication bias represents a concern. Furthermore, among the selected studies, the patients' populations and treatment measures differed widely, and the cut-off values for Ki-67 to designate high and low levels varied widely, which may influence the pooled analysis. Therefore, more detailed data such as NCT regimens and cycles are needed for future analyses.

In conclusion, our findings support the hypothesis that Ki-67 LI is associated with the pCR of patients with breast cancer. Ki-67

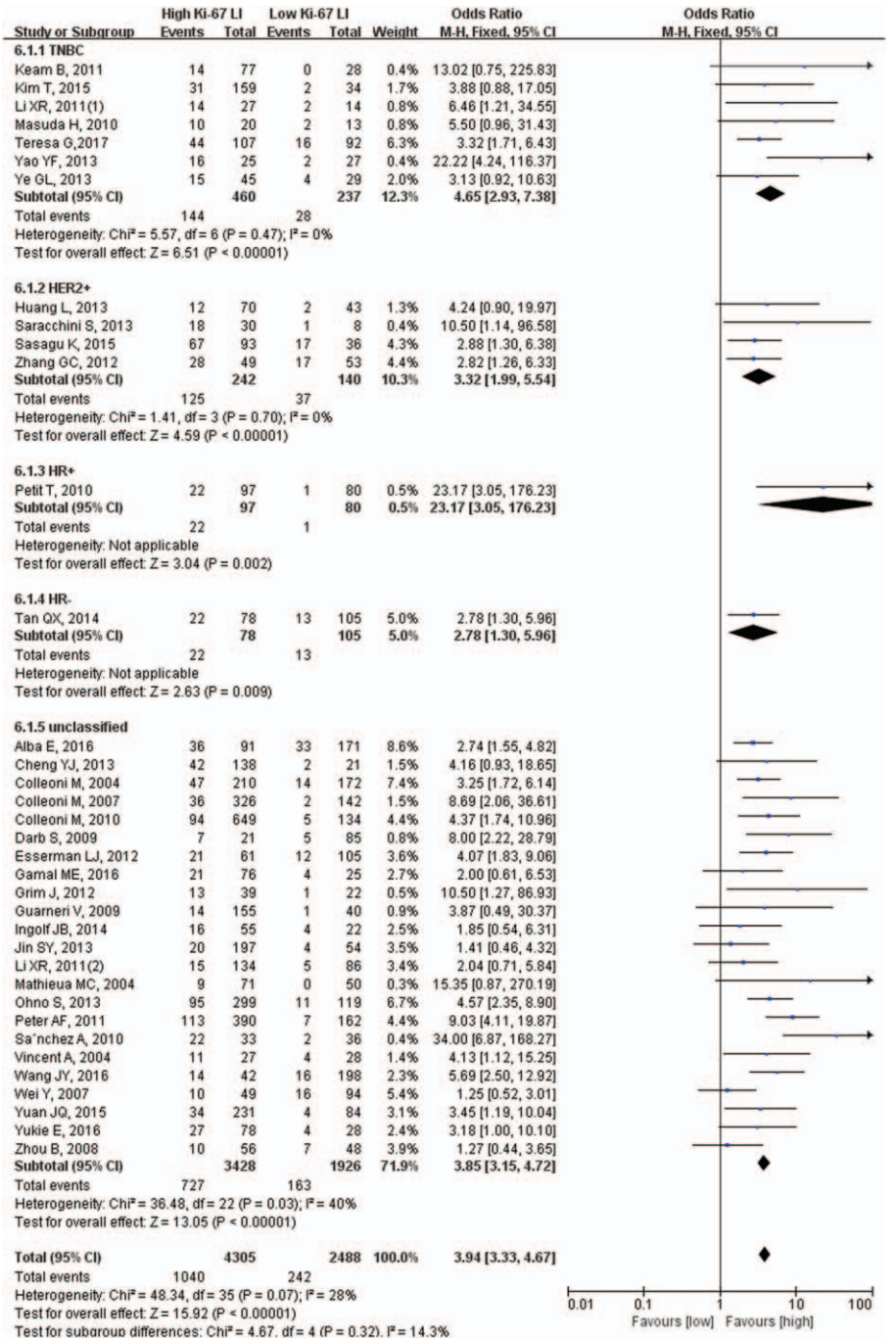

Figure 4. Subgroup analysis of molecular subtypes.

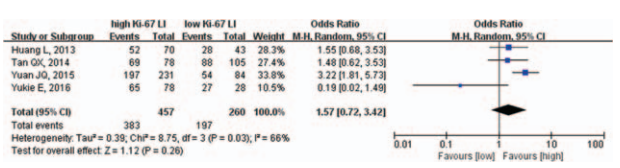

Figure 5. Pooled analysis of Ki-67 LI and objective tumor response. Ki-67 LI = Ki-67 labeling index.

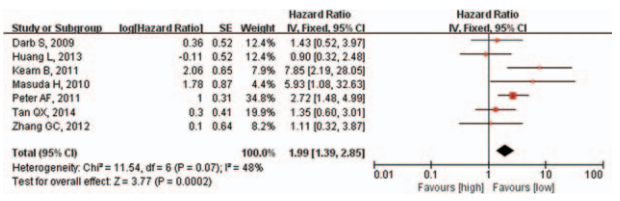

Figure 6. Pooled analysis of Ki-67 LI and RFS. Ki-67 LI = Ki-67 labeling index, RFS = relapse-free survival.

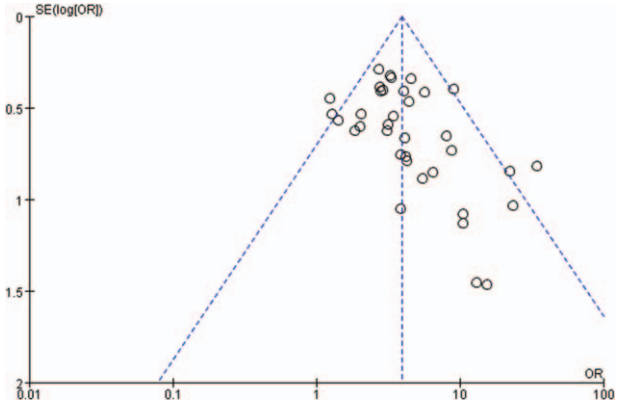

Figure 7. Funnel plot for detection of publication bias.

LI is a crucial predictive biomarker for pCR in patients with breast cancer who received NCT, indicating that this marker could help select patients who will benefit from NCT. However, it is more difficult to translate pathological response results into a clinical benefit. Large-scale prospective and randomized trials will be required before Ki-67 testing can be widely used as a prognostic tool in the clinic.

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