

# The role of the phosphatase and tensin homolog status in predicting pathological complete response to neoadjuvant anti-HER2 therapies in HER2-positive primary breast cancer

## A meta-analysis

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### Abstract

**Purpose:** The addition of anti-HER2 therapies to neoadjuvant treatment significantly enhances pathological complete response (PCR) rate in patients with human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Selecting patients unlikely to benefit from neoadjuvant anti-HER2 therapies is increasingly important. In this study, we proposed to assess the role of the phosphatase and tensin homolog (PTEN) as a biomarker in predicting PCR to neoadjuvant anti-HER2 therapies by conducting meta-analysis.

**Methods:** Our team searched Embase, Medline, and the Cochrane Library by the end of September 16, 2018, for trials on patients with HER2-positive breast cancer treated with neoadjuvant anti-HER2 therapies. The associations between PTEN expression and PCR rate were then assessed. Odds ratio (ORs) and hazard ratio (HRs) with 95% confidence intervals (CIs) with 2-sided *P* values were calculated. The Newcastle-Ottawa scale (NOS) was used to estimate the quality of the involved trials.

**Results:** A total of 820 patients from 8 trials were included in this meta-analysis. Overall, the PTEN normal tumors was related to a significant increase in PCR rate (OR 0.55; 95% CI=0.31–0.96; *P*=.04; *I*<sup>2</sup>=54%). In different anti-HER2 agents analysis, the PTEN normal tumors was related to a significant increase in PCR rate in patients treated with trastuzumab alone (OR 0.40; 95% CI=0.24–0.67; *P*=.0005; *I*<sup>2</sup>=15%). Besides, no significant association between PTEN status and PCR rate was detected in patients treated with lapatinib alone (OR 1.90; 95% CI=0.78–4.60; *P*=.16; *I*<sup>2</sup>=0%) or trastuzumab plus lapatinib (OR 1.27; 95% CI=0.27–5.97; *P*=.76; *I*<sup>2</sup>=73%).

**Conclusion:** Based on current evidence, PTEN status could be a suitable biomarker in predicting PCR rate to neoadjuvant anti-HER2 therapies, especially in trastuzumab-treated patients.

**Abbreviations:** CI = confidence interval, DFS = disease-free survival, HER2 = human epidermal growth factor receptor 2, HR = hazard ratio, HR = hormone receptor, Mesh = medical subject heading, NOS = Newcastle-Ottawa scale, OR = odds ratio, OS = overall survival, PCR = pathological complete response, PRISMA = preferred reporting items for systematic reviews and meta-analyses, PTEN = phosphatase and tensin homolog, TDM-1 = ado-trastuzumab emtansine.

**Keywords:** anti-HER2 agents, HER2-positive breast cancer, meta-analysis, neoadjuvant treatment, pathological complete response, PTEN

## 1. Introduction

Neoadjuvant therapies have been demonstrated as an equal effective treatment compared to adjuvant therapy for breast

cancer. Initially, it was deemed that neoadjuvant therapies were only suitable for patients with breast cancer and inflammatory disease or large tumor. Recently, neoadjuvant systemic therapies are also recommended for patients with breast cancer and high-risk operable primary tumor, for example, triple negative, axillary lymph node positive, or HER2 positive. Neoadjuvant therapies enhance the rate of breast conserving surgery, enable observing of treatment reaction, and supply unique opportunity for both pharmaceutical development strategies and individualized therapy.<sup>[1,2]</sup> The benefit of one of the chief neoadjuvant therapies is the probability to evaluate the reaction of tumor to treatment, which could range from no reaction to PCR. In clinical researches, PCR obtained from neoadjuvant therapies is a valuable predictive factor for improved survival.<sup>[3]</sup> Recently, a meta-analysis that comprised breast cancer patients treated with neoadjuvant therapies confirmed that the PCR rate was notably associated with improved long-term survival.<sup>[4]</sup> However, patients who could not achieved PCR from neoadjuvant systemic therapies might face disease progression and need surgery as early as possible. Therefore, select suitable patients who could mostly

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benefit from neoadjuvant systemic therapies and achieve PCR is a problem need to be solved urgently.

HER2 is amplified in approximate 20% to 25% of patients with breast cancer. Tumors of HER2 overexpression have more aggressive characteristic and, if without anti-HER2 agents administration, shorter disease-free survival (DFS) and overall survival (OS) when compared with HER2-normal breast cancers.<sup>[5]</sup> So far, the anti-HER2 agents approved for clinical administration comprised the first generation of trastuzumab and second generation of new HER2/HER3 antibodies such as pertuzumab, small molecule tyrosine kinase inhibitors such as lapatinib, and the new antibody chemotherapy conjugate ado-trastuzumab emtansine (T-DM1).<sup>[6]</sup> Anti-HER2 therapies significantly enhance PCR rate when given in neoadjuvant systemic treatment.<sup>[7–9]</sup> The TECHNO and NOAH were the first studies to prove that patients with HER2-positive breast who achieved PCR from neoadjuvant chemotherapy in combination with trastuzumab had significantly improved DFS and OS compared with those patients who had residual cancer cells after neoadjuvant treatment.<sup>[7–10]</sup> The combination of trastuzumab and lapatinib notably increases PCR rate compared to the anti-HER2 agent used alone. The NSABP B-41 study demonstrated that the combination of chemotherapy with lapatinib and trastuzumab resulted in enhanced PCR rate in both hormone receptor (HR)-positive and -negative patients compared to anti-HER2 agent-treated alone.<sup>[11]</sup> The Neo-ALTT0 study proved that 51.3% of patients treated with lapatinib and trastuzumab in neoadjuvant setting achieved PCR compared to 24.7% with lapatinib plus chemotherapy and 29.5% with trastuzumab plus chemotherapy.<sup>[12]</sup> In the biomarker analysis in CLEOPATRA trial of docetaxel plus trastuzumab plus pertuzumab versus docetaxel plus trastuzumab plus placebo as first-line therapies for patients with HER2-positive metastatic breast cancer, results proved that addition of pertuzumab consistently improved PFS, independent of biomarker expression subgroups.<sup>[13]</sup> Despite this significant benefit, primary anti-HER2 agents resistance is still one of the chief problems.<sup>[14]</sup> If patients could not achieve PCR from neoadjuvant anti-HER2 therapies or even progress for delayed surgery, patients' prognosis may deteriorate. Therefore, selecting patients with HER2-positive breast cancer who would substantially benefit from anti-HER2 agents is essential.

Many potential mechanisms concerning anti-HER2 agents resistance had emerged from preclinical researches. One important mechanism is activated signaling via the PI3K/AKT pathways, which modulate numerous genes that involved in cell survival, differentiation, proliferation, angiogenesis, metastasis, and invasion. Dual phosphatase PTEN mainly dephosphorylates the position D3 of membrane phosphatidylinositol-3,4,5 triphosphate and its loss has been confirmed in 15% to 86% of patients with HER2-positive breast cancer.<sup>[15–19]</sup> Low level or loss of PTEN, which could activate PI3K/AKT signaling pathway, is associated with trastuzumab resistance. Based on preclinical studies, evaluation of PTEN status could be a reliable method in recognizing patients with HER2-positive breast cancer who unlikely to gain benefit from lapatinib- and trastuzumab-based treatment.<sup>[20,21]</sup> However, so far researches have failed to offer conclusive proof on the predictive role of PTEN status in HER2-positive breast cancer in the metastatic, adjuvant, or neoadjuvant settings. A meta-analysis published in 2013 was conducted to assess the association between PTEN loss, PIK3CA mutation, and the effect of trastuzumab-contained therapies in patients with HER2-positive breast cancer and concluded that PTEN status was not related to the response of trastuzumab-

contained neoadjuvant therapies (RR=0.814; 95% CI=0.439–1.074,  $P=.099$ ).<sup>[22]</sup> However, several relative trials published in recent years showed PTEN expression might be correlated with trastuzumab response in neoadjuvant systemic setting. Besides, the role of PTEN in predicting efficacy of other anti-HER2 agents except trastuzumab should also be evaluated. Based on the abovementioned reasons, we took a meta-analysis of published trials that addressed the correlation between PTEN status in pretreatment core biopsies and PCR rate in patients with HER2-positive breast cancer treated with lapatinib, trastuzumab, and pertuzumab alone or in combination in neoadjuvant therapies.

## 2. Methods

### 2.1. Literature search strategy

Systematic review of literatures by the end of September 16, 2018, was conducted to identify studies that discussed the predictive role of PTEN in patients with HER2-positive breast cancer treated with anti-HER2 agents containing lapatinib, trastuzumab, neratinib, T-DM1, and pertuzumab either alone or in combination in neoadjuvant treatment setting. We searched Cochrane, Medline, and Embase database using both Medical Subject Heading (Mesh) terms and free text terms: (“breast neoplasm” OR “breast cancer”) AND (“preoperative therapy” OR “preoperative chemotherapy” OR “neoadjuvant chemotherapy” OR “NAC”) AND (“PTEN” OR “the phosphatase and tensin homolog”) AND (“anti-HER2” OR “HER2-targeted”). The abstracts of identified trials were assessed by 2 reviewers (Chi Zhang and Jiyu Li) independently with a common view for inconsistent studies via discussion. In addition, cross-referencing from pertinent trials on this theme was performed to make sure that we had retrieved all relevant trials. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

### 2.2. Eligibility standard

Qualified trials should meet the requirement of the following standard: (1) study published in language of English; (2) inclusion of patients with HER2-positive breast cancer treated with anti-HER2 agents alone or in combination in neoadjuvant treatment; (3) immunohistochemistry evaluation of PTEN expression in core biopsy before neoadjuvant treatment; and (4) the odds ratio (OR) of PCR in each PTEN expression subgroup (PTEN normal vs PTEN loss) should be informed or can be computed from the data. For each eligible trial, 2 investigators (Chi Zhang and Jiyu Li) gathered the following information independently: study design, year of publication, first author, neoadjuvant chemotherapy strategies, hormone receptor (HR) status, PTEN normal and PCR definitions, and number of PCR in PTEN loss and PTEN normal tumors according to definition in each trial.

### 2.3. Qualitative assessment

We estimate the quality of the involved papers using the Newcastle-Ottawa scale (NOS) ([http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)) for cohort studies. NOS is a validated quality assessment instrument for nonrandomized trials that assesses 3 parameters of study quality (selection, comparability, and outcome), including 8 items (representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at start of study, comparability of cohorts on the basis of the design or analysis, assessment of outcome, was follow-up

long enough for outcomes to occur, and adequacy of follow-up of cohorts). The NOS assigns a maximum score of 4 for selection, 2 for comparability, and 3 for outcome, for a maximum total score of 9.<sup>[2,3]</sup> Two investigators (Chi Zhang and Jiyu Li) conducted the assessment independently. If disagreement happened, the 2 investigators had a discussion until reached consensus. A study with a score  $\geq 6$  was estimated as high-quality study, otherwise were estimated as low quality.<sup>[2,4]</sup> Only studies of high quality will be selected for the final analysis.

#### 2.4. Study aims

The primary aim was to assess the correlation between PTEN status and PCR rate in patients with HER2-positive breast cancer treated with anti-HER2 agents alone or in combination in neoadjuvant therapies. Status of PTEN was deemed as a categorical variable with patients who were separated into PTEN normal versus PTEN loss subgroups according to the boundary defined from each study. The secondary aim was to discuss the correlation between level of PTEN and PCR rate in accordance to different types of anti-HER2 agents therapies. Three analyses according to the different anti-HER2 agents applied were considered: lapatinib alone, trastuzumab alone, and lapatinib plus trastuzumab.

#### 2.5. Statistical analysis

The correlation between the level of PTEN and PCR rate was assessed by analyzing the possibility of PCR between PTEN normal versus PTEN loss tumors treated with anti-HER2 agents either alone or in combination. Patients who obtained PCR in each PTEN status subgroups were calculated and then separated by different anti-HER2 agents. OR and 95% confidence intervals (CI) with 2-sided *P* values were calculated. The OR of PCR was computed as the amount of patients achieving PCR in the PTEN normal tumors divided by the amount of patients achieving PCR in the PTEN loss tumors. OR  $< 1$  illustrated higher PCR rate possibility occurred in the PTEN normal tumors. The inconsistency occurrence among trails was determined via statistics of  $I^2$ , which is applied to determine the percentage of entire variation attributed to heterogeneity among trials.<sup>[2,5]</sup> The analysis of funnel plots was used to determine the potential publication bias.<sup>[2,6]</sup>  $P < .05$  was regarded significant. The statistical analysis, forest plots analysis, and funnel plot analysis were conducted applying RevMan statistical software 5.0.

### 3. Results

#### 3.1. Description and quality assessment of included trials

Based on critical search strategies and inclusion criteria, 8 eligible trials comprising 820 patients with HER2-positive breast cancer who received anti-HER2 agents alone or in combination in the neoadjuvant treatment setting were concluded in this meta-analysis (Fig. 1; Table 1).<sup>[15,27-33]</sup> Three studies were single-center studies,<sup>[28,30,33]</sup> and the other 5 were multicenter studies.<sup>[15,27,29,31,32]</sup>

The quality of the 8 involved studies was estimated by NOS. The scores were all  $\geq 6$  (Table 2). This proved that all this 8 studies were high-quality trials.

#### 3.2. Association between PTEN and PCR rate in the entire study population

For all the 8 eligible studies, PTEN normal tumors was associated with remarkably increased PCR rate (OR 0.55; 95% CI=0.31–

0.96;  $P = .04$ ). Nevertheless, notable heterogeneity has arisen ( $I^2 = 54\%$ ;  $P = .03$ ), approving massive inconsistency across trials (Fig. 2). A low possibility of publication bias is showed by funnel plot analysis (Fig. 3).

#### 3.3. Association between PTEN and PCR rate on the neoadjuvant anti-HER2 therapies

**3.3.1. Trastuzumab.** Trastuzumab was included in the neoadjuvant treatment among all of the 8 studies, and 6 of the 8 studies assessed the correlation between PTEN and PCR rate in patients with HER2-positive breast cancer receiving trastuzumab alone.<sup>[15,28-30,32,33]</sup> The patients in 3 studies received trastuzumab weekly (4 mg/kg of loading dose followed by 2 mg/kg),<sup>[15,30,32]</sup> while in 2 studies trastuzumab was treated every 3 weeks (8 mg/kg loading dose followed by 6 mg/kg).<sup>[28,29]</sup> Besides, 1 study did not clarified the exact usage of trastuzumab.<sup>[33]</sup> OR for PCR in the PTEN normal tumors was 0.40 (95% CI=0.24–0.67;  $P = .0005$ ) with no notable heterogeneity observed ( $I^2 = 15\%$ ;  $P = .32$ ) (Fig. 4). This result indicated that PTEN normal tumors was associated with remarkable increased PCR rate in patients treated with neoadjuvant trastuzumab alone therapies.

Except these 6 studies, concluded patients received trastuzumab alone in the neoadjuvant anti-HER2 treatment, 1 study assessed the correlation between PTEN and PCR rate in patients treated with trastuzumab (8 mg/kg of loading dose followed by 6 mg/kg per 3 weeks for 18 weeks) plus pertuzumab (840 mg of loading dose followed by 420 mg per 3 weeks for 18 weeks).<sup>[31]</sup> In this step, we add this study to the abovementioned 6 studies in which trastuzumab was treated alone in neoadjuvant anti-HER2 therapies. OR for PCR in the PTEN normal tumors was 0.44 (95% CI=0.28–0.69;  $P = .0004$ ) with no notable heterogeneity observed ( $I^2 = 8\%$ ;  $P = .37$ ) (Fig. 5). This result indicated PTEN normal tumors was associated with remarkable increased PCR rate in patients treated with neoadjuvant trastuzumab alone or in combination with pertuzumab therapies.

#### 3.4. Lapatinib plus trastuzumab

Four studies assessed the correlation between PTEN and PCR rate in patients treated with trastuzumab and lapatinib.<sup>[15,27,29,32]</sup> Among the 4 studies, neoadjuvant trastuzumab was given (4 mg/kg of loading dose followed by 2 mg/kg per week) with concomitant lapatinib (1500 mg/d) in 2 studies,<sup>[15,32]</sup> trastuzumab was given (4 mg/kg of loading dose followed by 2 mg/kg per week) with concomitant lapatinib (1000 mg/d) in 1 study,<sup>[27]</sup> and trastuzumab was given (8 mg/kg of loading dose followed by 6 mg/kg per 3 weeks) with concomitant lapatinib (1000 mg every day) in another 1 study.<sup>[29]</sup> OR for PCR rate in the PTEN normal tumors was 1.27 (95% CI=0.27–5.97;  $P = .76$ ). Notable heterogeneity has arisen ( $I^2 = 73\%$ ;  $P = .01$ ) (Fig. 6). This result indicated PTEN status was not associated with PCR rate in patients treated with neoadjuvant lapatinib plus trastuzumab therapies.

#### 3.5. Lapatinib

Two studies assessed the correlation between PTEN and PCR rate in patients treated with neoadjuvant lapatinib as a single anti-HER2 agent plus chemotherapy.<sup>[29,32]</sup> Neoadjuvant lapatinib was administered 1000 mg or 1500 mg every day for 18 weeks, respectively. OR for PCR rate in the PTEN normal tumors was 1.90 (95% CI=0.78–4.60;  $P = .16$ ). No notable heterogeneity

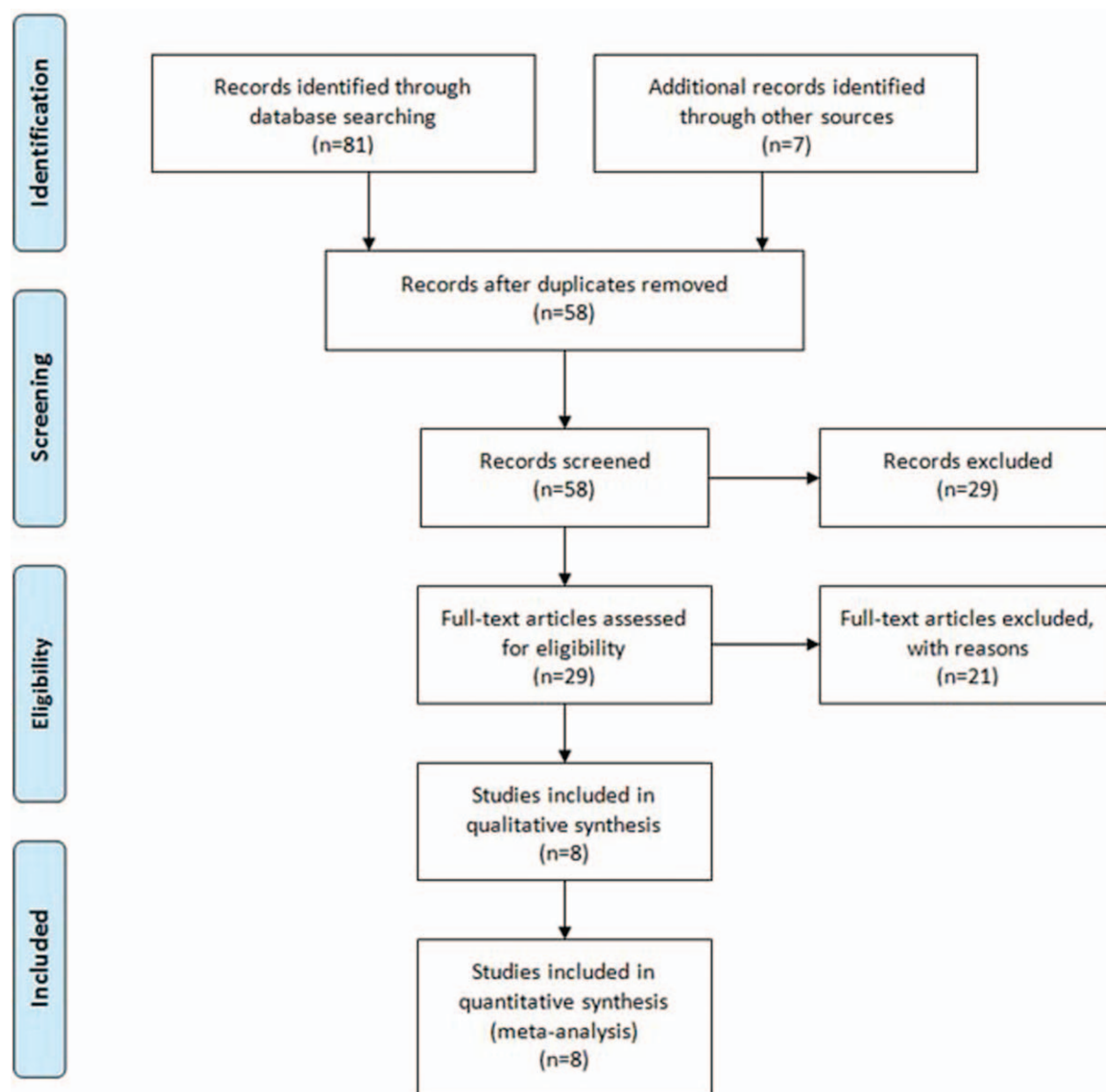


Figure 1. The literature search process.

ity has arisen ( $I^2=0\%$ ;  $P=.42$ ) (Fig. 7). This result indicated PTEN status was not associated with PCR rate in patients treated with neoadjuvant lapatinib alone therapies.

#### 4. Discussion

Individualized therapies play a critical role in tumor treatment. So far, anti-HER2 agents which mainly comprised trastuzumab, T-DM1, lapatinib, neratinib, and pertuzumab are recommended for patients with HER2-positive breast cancer. However, majority of these patients could not benefit from anti-HER2 treatment. This trickle problem urges us to probe in the suitable biomarkers to direct individualized anti-HER2 treatment, differentiate those patients who are likely to gain essential benefit from neoadjuvant treatment and distinguish those who are unlikely to gain response from toxicities. A meta-analysis evaluated the relationship between PIK3CA mutations, an activation factor of PI3K/AKT signaling pathway, with neoadjuvant treatment response. The data demonstrated that PIK3CA mutation is associated with lower PCR rate in HER2-

positive breast cancers (RR=0.73; 95% CI=0.66–0.81), the predictive role of PIK3CA mutation in PCR rate is stronger in HR-positive/HER2-positive patients (RR=0.50; 95% CI=0.27–0.93) and in those treated with dual anti-HER2 therapies (RR=0.55; 95% CI=0.39–0.78), prompting the PIK3CA mutation induced of PI3K/AKT pathway activation might contribute to anti-HER2 therapies resistance.<sup>[34]</sup> Another relative meta-analysis evaluates the relationship between PTEN loss, PIK3CA mutation, and the effect of trastuzumab-contained therapies in patients with HER2-positive breast cancer. The data proved neither loss of PTEN (RR=0.687, 95% CI=0.439–1.074), PIK3CA mutation (RR=1.114, 95% CI=0.453–2.735), nor activation of PI3K/AKT pathway (RR=0.787, 95% CI=0.417–1.484) was correlated with the effect of trastuzumab-contained neoadjuvant therapies. Loss of PTEN was not related to the DFS in trastuzumab-contained adjuvant therapies (RR=1.096, 95% CI=0.706–1.700). In metastatic or recurrent patients, loss of PTEN was notably associated with poorer response to trastuzumab-contained salvage therapies (RR=0.682, 95% CI=0.550–0.846). The author concluded that in patients with



**Table 1**

**Summary of the main features of the included trails.**

Study	No. of patients	initial tumor stage or size	Neoadjuvant chemotherapy and endocrine therapy	Neoadjuvant HER2-targeted agents	High PTEN definition	PTEN position for IHC	PCR definition	HR status (%)	PCR RATE (%)		
									T%	L%	T+L%
Rimawi et al 2017	59	Stage II/III	Without chemotherapy. Endocrine therapy if HR positive.	12 wk of L (1000mg every day) plus T (4 mg/kg loading dose followed by 2 mg/kg per wk)	H-score (0–300; multiplying the percentage and intensity scores) ≥ 100	NR	ypT0is	ER+:52 PR+:37	NA	NA	24
Loibl et al 2016	108	T3–4	4EC-4T* or 4EC-4T*X or 4EC-4T*-4X	24 or 36 wk of T (8 mg/kg loading dose followed by 6 mg/kg per 3 wk)	Aqua data (0–100) ≥ 60	Cytoplasm and/or nuclear	ypT0 ypNO	HR+:54	31	NA	NA
Dave et al 2011	61	T3–4	4T*	15 wk of T (4 mg/kg loading dose followed by 2 mg/kg per wk); 6 wk of L (1500 mg every day) plus 12 wk of T.	>3	Nuclear	ypT0is ypNO	NR	31	NA	63
Toomey et al 2017	45	Stage IC–III	6T*C*	18 wk of T (8 mg/kg loading dose followed by 6 mg/kg per 3 wk); L (1000mg every day) plus T; L.	Immunostaining (0: absent, 1 +: weak, 2+: intermediate, 3+: strong) is 2/3+	Cytoplasm	ypT0is ypNO	ER+:42 PR+:64	57	17	44
Yonemori et al 2009	44	Stage IIA–IIIB	OE-T* or AT*-T* or AC-T*	T (4 mg/kg loading dose followed by 2 mg/kg per wk)	Cytoplasmic staining	Cytoplasm	ypT0is ypNO	ER+:89 PR+:93	14	NA	NA
Schneeweiss et al 2014	113	Stage IIA–IIIC	6T*C* or 3CEF-3T*	18 wk of T (8 mg/kg loading dose followed by 6 mg/kg per 3 wk) plus P (840 mg loading dose followed by 420 mg per 3 wk)	Modified H-score (0–400; multiplying the percentage and intensity scores) ≥ 200	Cytoplasm	ypT0is	NR	62	NA	NA
Nuciforo et al 2015	349	Stage I–III	12T*	18 wk of L (1500 mg every day); T (4 mg/kg loading dose followed by 2 mg/kg per wk); L (1000 mg every day) plus T.	H-score ≥ 50	Cytoplasm	ypT0is ypNO	HR+:50	31	21	46
Sueta et al 2014	41	Stage I–III	6T*C* or 4CEF-4T*	T	H-score ≥ 60	Cytoplasm and/or nuclear	ypT0is ypNO	ER+:46 PR+:24	61	NA	NA

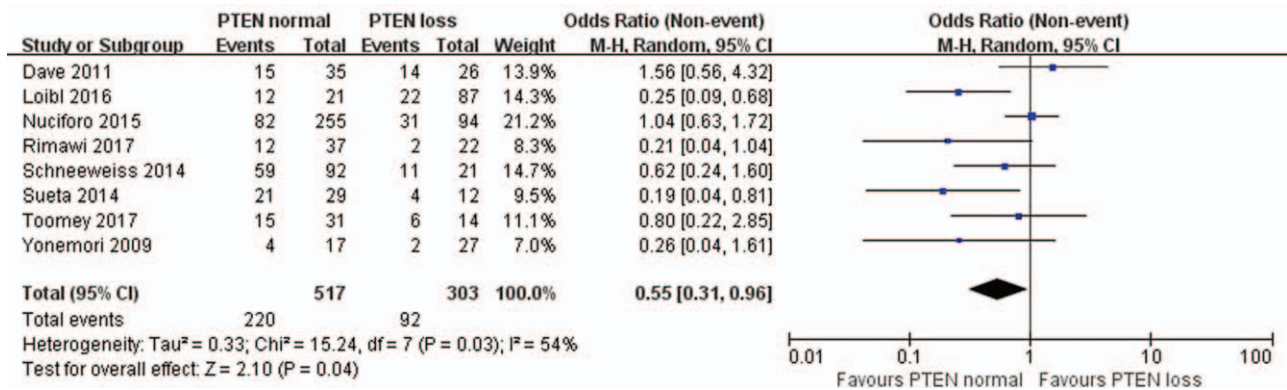
\* = carboplatin, C = cyclophosphamide, E = epirubicin, F = fluorouracil, IHC = immunohistochemistry, L = lapatinib, NA = not applicable, NR = not reported, P = pertuzumab, T\* = docetaxel or paclitaxel, T = trastuzumab, X = capecitabine.

**Table 2**

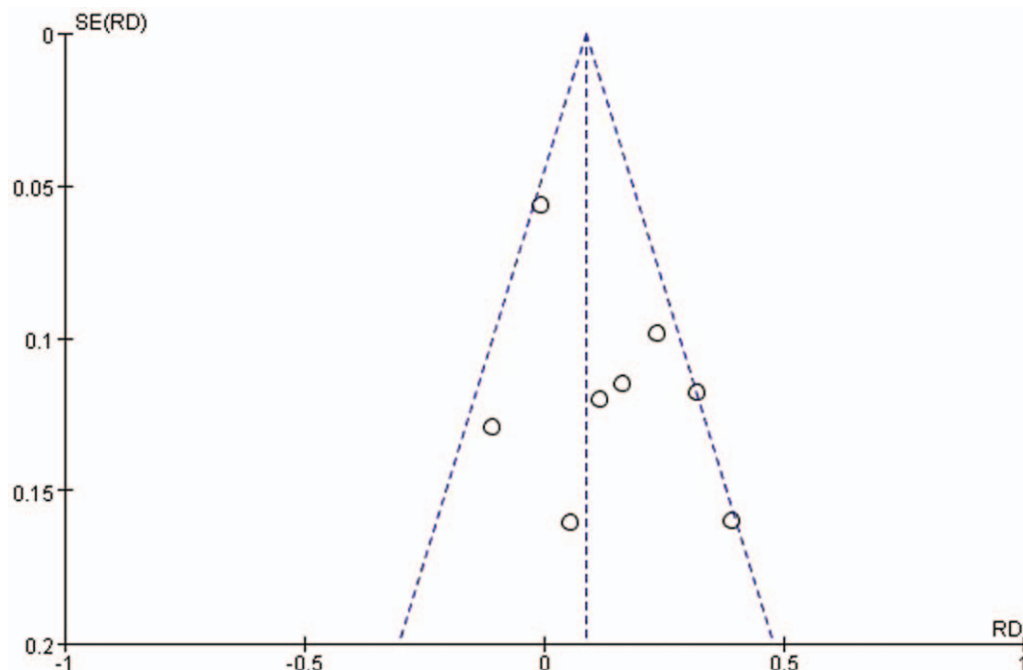
**Newcastle–Ottawa scale for quality assessment.**

Study	Selection			Comparability		Outcome		Total score	
	Exposed cohort	Non-exposed cohort	Ascertainment of exposure	Outcome of interest	control for factor	Assessment of outcome	Follow-up long enough		Adequacy of follow-up
Rimawi et al 2017	*	*	*	*	**	*	*	*	9
Loibl et al 2016	*	*	*	*	**	*	*	*	8
Dave et al 2011	*	*	*	*	*	*	*	*	8
Toomey et al 2017	*	*	*	*	**	*	*	*	7
Yonemori et al 2009	*	*	*	*	*	*	*	*	9
Schneeweiss et al 2014	*	*	*	*	*	*	*	*	8
Nuciforo et al 2015	*	*	*	*	*	*	*	*	7
Sueta et al 2014	*	*	*	*	**	*	*	*	9

\*A study can be awarded a maximum of 1 star for each item within the selection and outcome categories. A maximum of 2 stars can be given for comparability.



**Figure 2.** Forest plot from the random-effect model meta-analysis for relation between phosphatase and tensin homolog status and neoadjuvant anti-HER2 treatment efficacy in with HER2-positive breast cancer. The square box size represents the weight that each trial contributed in this meta-analysis. The total estimate and confidence interval are marked by a diamond. The symbol on the right of the solid line manifest OR>1 and symbol on the left manifest OR<1. HER2=human epidermal growth factor receptor 2, OR=odds ratio.



**Figure 3.** Funnel blot was displayed to observe potential publication bias.

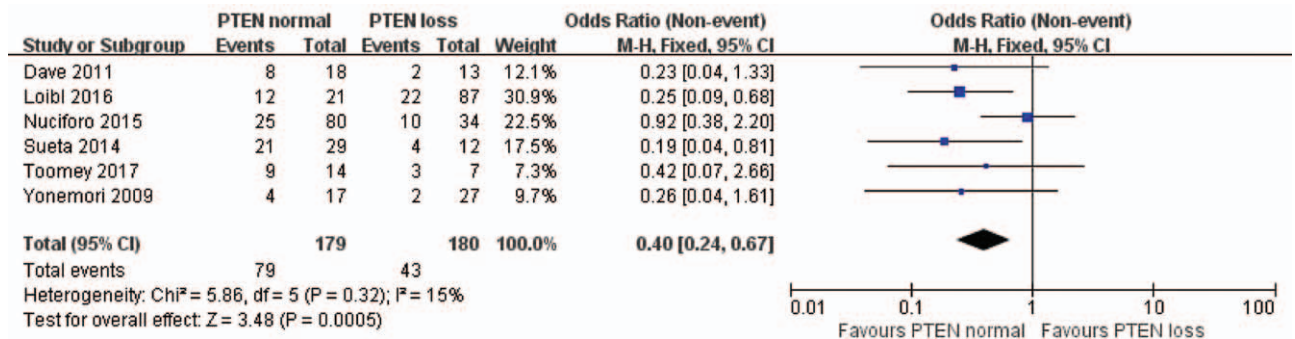


Figure 4. Forest plot from the fixed-effect model meta-analysis for relation between phosphatase and tensin homolog status and neoadjuvant trastuzumab treatment efficacy in patients with human epidermal growth factor receptor 2-positive breast cancer.

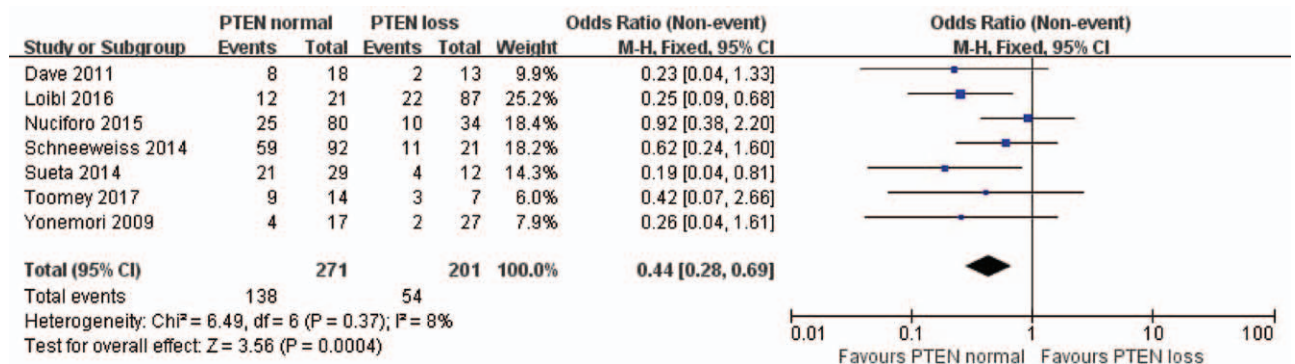


Figure 5. Forest plot from the fixed-effect model meta-analysis for relation between phosphatase and tensin homolog status and neoadjuvant trastuzumab plus pertuzumab treatment efficacy in patients with human epidermal growth factor receptor 2-positive breast cancer.

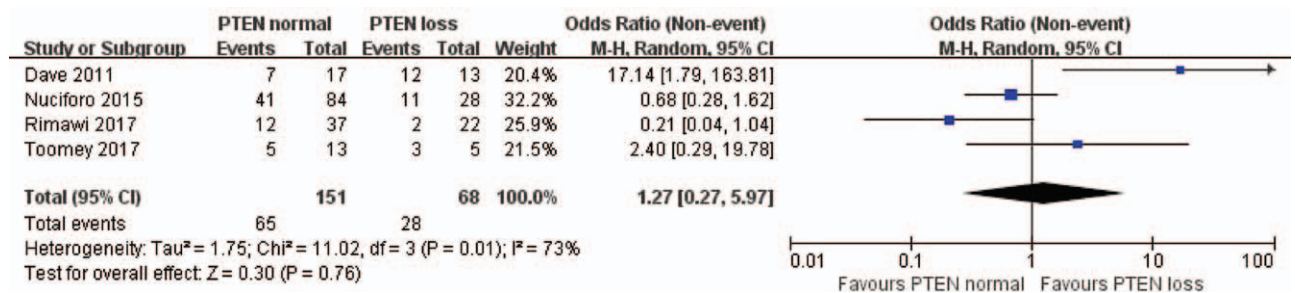


Figure 6. Forest plot from the random-effect model meta-analysis for relation between phosphatase and tensin homolog status and neoadjuvant trastuzumab plus lapatinib treatment efficacy in patients with human epidermal growth factor receptor 2-positive breast cancer.

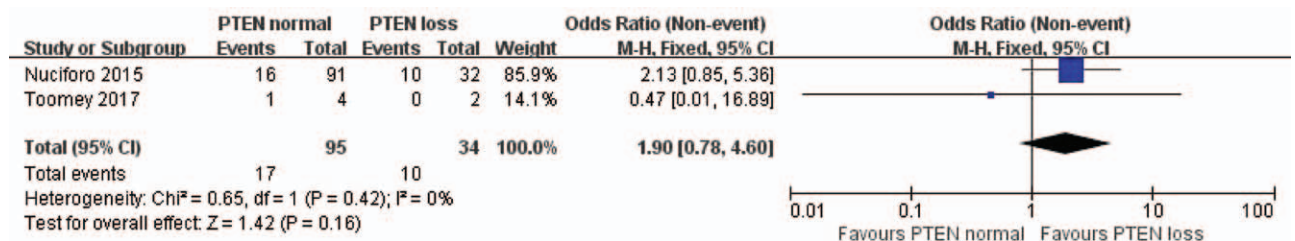


Figure 7. Forest plot from the fixed-effect model meta-analysis for relation between phosphatase and tensin homolog status and neoadjuvant lapatinib treatment efficacy in patients with human epidermal growth factor receptor 2-positive breast cancer.

HER2-positive metastatic or recurrent breast cancer, loss of PTEN could predict resistance to trastuzumab-contained salvage therapies.<sup>[24]</sup> Different from the previous research, our meta-analysis collected more trials published in recent years and is the first time to evaluate the role of PTEN on predicting PCR in patients with HER2-positive breast cancer treated with neoadjuvant anti-HER2 therapies containing trastuzumab, pertuzumab, and lapatinib alone or in combination. The finding showed that the high level of PTEN is significantly correlated to enhanced PCR rate in patients treated with anti-HER2 therapies in the entire study population (OR=0.55; 95% CI=0.31–0.96;  $P=.04$ ), but remarkable heterogeneity arise ( $I^2=54%$ ;  $P=.03$ ). Different anti-HER2 agents analysis revealed in the trastuzumab alone-treated population, PTEN normal tumors is also significantly correlated to increased PCR probability (OR=0.40; 95% CI=0.24–0.67;  $P=.0005$ ) without significant heterogeneity ( $I^2=15%$ ;  $P=.32$ ) and this result is distinct from previous relative meta-analysis. However, when it comes to patients treated with lapatinib alone, PTEN loss tumors is not associated with PCR rate (OR=1.90; 95% CI=0.78–4.60;  $P=.16$ ). And no association between PTEN status and PCR rate was detected in patients treated with trastuzumab plus lapatinib (OR=1.27; 95% CI=0.27–5.97;  $P=.76$ ) with significant heterogeneity ( $I^2=73%$ ;  $P=.01$ ). The analysis between different anti-HER2 agents showed that the significant heterogeneity in the entire study population was chiefly induced by data from trastuzumab plus lapatinib-treated population and lapatinib alone.

Lapatinib is a reversible dual kinase inhibitor against HER2 and EGFR and has activity in patients with HER2-positive breast cancer when given either as first-line treatment or after failure with trastuzumab therapy.<sup>[35]</sup> A meta-analysis which assessed the efficacy of adding lapatinib to trastuzumab plus chemotherapy in neoadjuvant setting on PCR rate showed the PCR rate was 55.7% and 38.36% in the trastuzumab plus lapatinib and the trastuzumab alone arms, respectively (OR=1.94; 95% CI=1.44–2.60).<sup>[36]</sup> As the mechanisms of anti-HER2 agents demand the adjustment of critical signaling pathways that modulate the transforming functions of HER2,<sup>[37,38]</sup> activation of PI3K/AKT pathway, as results of low level or loss of PTEN, is associated with resistance to trastuzumab.<sup>[39,40]</sup> Several researches targeting the PI3K/AKT pathway performed biomarkers analysis and proved that patients with loss or low level of PTEN had a poor response to trastuzumab treatment and might derive enhanced benefit from PI3K/AKT pathway-targeted agents.<sup>[33,41]</sup> But mechanisms of lapatinib are not well established. In one research, the effect of lapatinib was not affected after downregulation of PTEN via RNA interference in HER2-positive breast cancer cell lines, thus demonstrating that these cell lines were still sensitive to lapatinib despite loss of PTEN.<sup>[42]</sup> Conversely, a recent research using genetic approach in breast cancer cell lines demonstrated that PTEN was proved to be a predictive biomarker of lapatinib sensitivity.<sup>[20]</sup> Bhuvanesh<sup>[15]</sup> proved that different from trastuzumab, lapatinib influences cell proliferation via MAPK signaling pathway. The authors also demonstrated that trastuzumab seemed to mainly influence cell survival but had less action on cycle kinetics, nevertheless lapatinib seem to influence cell cycle kinetics through MAPK signaling pathway but had less influence on cell survival. Therefore, trastuzumab and lapatinib have different antitumor mechanisms and therefore may have different response predictive biomarkers. In our meta-analysis, patients with PTEN loss tumors were demonstrated to have poorer response to trastuzumab therapies. But PTEN loss tumors were not associated with PCR rate in lapatinib-contained therapies.

Our study have some limitations. We did not include relative trials in which detailed data could not obtained. Additionally, we might overestimated the relationship between PTEN expression and PCR rate in patients with HER2-positive breast cancer treated with anti-HER2 agents because negative results may not be punished by researches. What is more, we included trials with evaluation of PTEN by immunohistochemistry. To our knowledge, prognostic biomarkers based on immunohistochemistry could supply inconsistent or even contradictory results, because of different processing methods and antibodies, along with different categorization and scoring systems. Besides, we consider that other elements (e.g.: different hormone receptor status and chemotherapy strategies) in these trials have different influence on PCR rate; therefore, these elements should also be taken into account. Unfortunately, we could not extract enough data from these trials for further analysis. Therefore, more meticulous trials should be conducted.

In summary, PTEN normal tumors were associated with high PCR rate in patients with HER2-positive breast cancer treated with anti-HER2 agents in neoadjuvant therapies setting. Undoubtedly, these results should be confirmed by more prospective and randomized clinical trials. However, we provide new insights that support PTEN as a potential predictive biomarker of anti-HER2 therapies in neoadjuvant treatment setting.

### Author contributions

Jiyu Li, Xingsong Tian, and Xuening Duan designed the study. Chi Zhang, Yin Guo, and Jiyu Li collected the data and sample materials. Chi Zhang and Ying Guo assembled the databases and analyzed the data. Chi Zhang, Yin Guo, and Jiyu Li interpreted the data. All authors participated in writing the manuscript. All authors read and approved the final manuscript.

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