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PIK3CA mutations and their response to neoadjuvant treatment in early breast cancer: A systematic review and meta-analysis

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Abstract

Background: *PIK3CA* mutations frequently occur in breast cancer patients. This study was conducted to evaluate the relationship between *PIK3CA* mutations and neoadjuvant treatment response and to analyze the clinical implications.

Methods: PubMed, Embase, and the Cochrane database were searched for relevant studies in September 2017. The pooled risk ratio (RR) was estimated using fixed effects or random effects models according to heterogeneity among studies.

Results: This meta-analysis included 20 studies with 4392 patients. The pooled RR showed that *PIK3CA* mutation is correlated to lower pathological complete response (pCR) in unselected HER2+ patients (RR = 0.73; 95% confidence interval [CI] 0.66–0.81), thus the predictive value of *PIK3CA* status may be stronger in HER2+/HR+ patients (RR = 0.50; 95% CI 0.27–0.93) and those administered dual-targeting treatment (RR = 0.55; 95% CI 0.39–0.78). In contrast with wild type, either exon 9 (RR = 0.55; 95% CI 0.39–0.78) or exon 20 (RR = 0.71; 95% CI 0.58–0.89) mutations were significantly associated with lower pCR. The predictive value of exon 9 mutations was not significantly greater than exon 20 mutations (RR = 0.76; 95% CI 0.51–1.13).

Conclusion: In early breast cancer, *PIK3CA* mutations seem to identify HER2+ patients who are less likely to reach pCR. The clinical implications of *PIK3CA* mutations tend to vary between exon 9 and exon 20. This mechanism should be explored in further studies.

Introduction

Neoadjuvant treatment (NAT) is a conventional treatment for locally advanced breast cancer.[1] It has been accepted as an important option for early stage breast cancer patients and achieves similar long-term clinical outcomes as adjuvant treatment.[2] The achievement of pathological complete response (pCR) is a valid predictor of good prognosis, especially for triple negative breast cancer (TNBC) and HER2+ patients.[3,4] Although many studies have explored the predictive biomarkers of NAT response, there is no current method to screen patients that may be sensitive to NAT. Promising biomarkers, such as tumor-infiltrating lymphocytes (TILs), *TP53*, and the germline *BRCA* mutation, are under investigation.[5–7]

Activation of the PI3K pathway is common breast cancer,[8] and results from *PIK3CA* mutation or PTEN loss.[9] It has been reported that *PIK3CA* status impacts solid cancer prognosis.[10,11] More than 90% of *PIK3CA* mutations in breast tumors appear in exons 9 and 20.[12]

A number of studies of *PIK3CA* mutation in HER2+ breast cancer have been reported, but have mainly focused on the prognostic value to advanced stage breast cancer. Recently, a pooled analysis of 967 HER2+ breast cancer patients from five randomized trials was conducted.[13] The authors found a significantly lower pCR rate in *PIK3CA*

mutant (MT) compared to wild-type (WT) tumors after neoadjuvant chemotherapy.

While the pCR rate is significantly lower in HER2+ patients, it remains uncertain in hormone receptor positive (HR+) and HR negative (HR-)/HER2- subtypes. The biological functions of exon 9 and 20 mutations may be different,[14] and whether such discrepancies could affect the response to NAT has not been fully elucidated. We conducted a systematic review and meta-analysis of *PIK3CA* related studies of NAT to clarify the possible association between *PIK3CA* mutation and response to breast cancer NAT. Exon 9 or 20 mutations lead to *PIK3CA* mutation; therefore, we conducted subgroup analyses of relevant studies to determine pCR rates between exon 9 and 20 MT and WT tumors.

Methods

Search strategy

Online databases including PubMed, Embase, and the Cochrane database were searched to identify relevant literature published up to September 2017. The following key word combinations were used: "breast cancer," "neoadjuvant," and "PIK3CA." Published studies were included based on the following criteria: (i) English publications; (ii) studies focusing on early stage breast cancer patients and NAT; and (iii) studies with clinical or pathological response outcomes. Studies were excluded if they were: (i) reviews or mechanism studies; or (ii) duplicate studies.

Data extraction

Two reviewers independently extracted the information from all eligible studies. Pathological or clinical response was the end point of interest. The following information was extracted: first author, region, population, sample size, *PIK3CA* mutation incidence, NAT regime, and *PIK3CA* sequence.

Statistical analysis

Fixed effects (Mantel–Haenszel) or random effects (DerSimonian–Laird) models were used to pool risk ratio and 95% confidence interval (CI), according to heterogeneity. The heterogeneity test was verified using Higgins–I2 statistics. If significant heterogeneity was observed (I2 > 50%), a random effects model was used; otherwise, the fixed effects model was used. Publication bias was estimated using an Egger's test with a funnel plot. All *P* values were calculated using a two-sided test and $P < 0.05$ was considered statistically significant. Statistical analyses in our study were carried out using Stata 12.0 (Stata Corp LP, College Station, TX, USA).

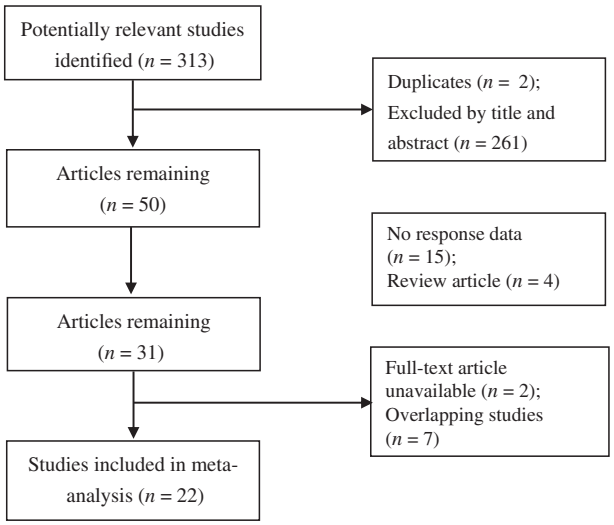

Figure 1 Flow diagram of the systematic search and selection process.

Results

A total of 313 studies were retrieved. After preliminary screening, 263 were excluded by title, abstract, and duplication. Studies with no response data ($n = 15$), overlapping data ($n = 7$), no full text article ($n = 2$), and review articles ($n = 4$) were also excluded. A total of 22 articles referring to 20 studies were included in our meta-analysis (Fig 1).

Study characteristics

As shown in Table 1, 20 studies including 4392 patients were included in our meta-analysis.[13,15–34] Overall, *PIK3CA* mutation incidence in our meta-analysis was 22.4% (range 7.7–39.0%). pCR was 28% for *PIK3CA* MT and 38% for *PIK3CA* WT. Seven studies were conducted in the United States,[17,18,22,24–26,28] nine in Europe,[13,15,16,19–21,29–31] and four in Asia.[23,32–34] Two studies included objective response rate,[18,19] while the others reported pCR as the endpoint in WT versus MT *PIK3CA* tumors. Most of the included studies (12/20) used formalin-fixed paraffin embedded breast samples. Most studies sequenced *PIK3CA* exons 9 and 20, while the remainder also analyzed one or more of exons 1, 4, 7, 9, and 20. Other information, such as NAT regime, first author, study name, and population are illustrated in Table 1. In each subgroup, the pCR rate was higher in *PIK3CA* WT than in MT patients, as illustrated in Table 2.

Table 1 A summary of study characteristics

Author year	Country	Study name	Population	Number of patients	Number of *PIK3CA* mutated patients	Endpoints	Sample type	NAT regime	Sequenced *PIK3CA*
Barbareschi *et al.* 2012[15]	Italy	N/A	HER2+	26	4 (15.4%)	pCR	FFPE	AH → TH → CMFH	Exon 9/20
Bianchini *et al.* 2017[16]	Italy	NeoSphere	HER2+	417	81 (19.4%)	pCR	FFPE	(i) TH; (ii) TPH; (iii) PH; (iv) TP	Exon 7/9/20
Dave *et al.* 2011[17]	USA		HER2+	80	15 (18.8%)	pCR	FFPE	(i) H; (ii) L	NR
Ellis *et al.* 2010[18]	USA	P024, RAD 2222, ROL	HR+	235	76 (32.3%)	OR	FFPE	Tamoxifen + Letrozole	Exon 7/9/20
Guarneri *et al.* 2014[19]	Italy	CONSORT	HR+/HER2-	92	34 (37.0%)	OR	FFPE	(i) Letrozole; (ii) Letrozole + L	Exon 9/20
Hanusch *et al.* 2015[20]	Germany	GBG-70	HER2+	61	13 (21.3%)	pCR	NR	Afatinib → TH Afatinib → ACH	Exon 9/20
Harbeck *et al.* 2016[21]	Germany	WSG-ADAPT	HR+/HER2+	114	18 (15.8%)	pCR	NR	(i) T-DM1; (ii) T-DM1 + Tamoxifen or AI; (iii) H + Tamoxifen or AI	NR
Haas *et al.* 2017[22]	USA	KRISTINE	HER2+	425	114 (26.8%)	pCR	NR	(i) T-DM1 + P; (ii) TCbPH	NR
Huang 2015[23]	China	N/A	HER2+	77	30 (39.0%)	pCR	FFPE	(i) TCH; (ii) TAH	Exon 4/9/20
Hoadley *et al.* 2015[24]	USA	CALGB 40601	HER2+	181	14 (7.7%)	pCR	NR	(i) TL; (ii) TH; (iii) THL	Exon 9/20
Loibl *et al.* 2016[25,26]	USA	GeparSepto	HER2+	291	63 (21.6%)	pCR	FFPE	THP	Exon 9/20
Loibl *et al.* 2016[27]	USA	GeparTrio	HER2+	82	31 (37.8%)	pCR	FFPE	(i) TAC; (ii) TAC → NX	NR
Loibl *et al.* 2016[13,27]	Germany	GeparQuattro GeparQuinto GeparSixto NeoALTTO CHERLOB	HER2+	967	210 (21.7%)	pCR	FFPE	(i) ACH → TH; (ii) ACL → TL; (iii) THL; (iv) TH; (v) TL; (vi) THB; (vii) TCbHB; (viii) TH → CAFH; (ix) TL → CAFL; (x) THL → CAFH	Exon 9/20
Liedtke *et al.* 2008[28]	USA	N/A	ALL	140	23 (16.4%)	pCR	NR	(i) FAC; (ii) T → FAC	Exon 1/9/20
Lips *et al.* 2015[29]	Netherlands	N/A	TNBC	140	23 (16.4%)	pCR	FTS	(i) AC; (ii) AC → TX; (iii) AC → XCb + Thiotepa	Exon 9/20
Toomey *et al.* 2017[30]	Ireland	TCHL (ICORG10–05)	HER2+	74	18 (24.3%)	pCR	FFPE	(i) TCbL; (ii) TCbH; (iii) TCbHL	Exon 1/4/7/9/20
Schneeweiss *et al.* 2014[31]	Germany	TRYPHAENA	HER2+	126	39 (31.0%)	pCR	NR	(i) FECHP → THP; (ii) FEC → THP; (iii) TCbHP	Exon 7/9/20
Sueta *et al.* 2014[32]	Japan	N/A	HER2+	42	7 (16.7%)	pCR	FFPE	(i) FAC → T; (ii) TC	Exon 9/20
Yuan *et al.* 2015[33]	China	N/A	ALL	729	142 (19.5%)	pCR	FTS	(i) CAF; (ii) AC; (iii) A → T; (iv) A → TC; (v) A → TCb	Exon 9/20
Zhang *et al.* 2014[34]	China	N/A	ALL	93	30 (32.3%)	pCR	FFPE	TA	Exon 9/20

Pathological complete response (pCR) was based on Miller and Payne histopathology scoring system. Objective response (OR) was evaluated according Response Evaluation Criteria in Solid Tumors and was defined as complete + partial response. A, anthracycline; AI, aromatase inhibitors; ALL, all subtypes of breast cancer patients; B, bevacizumab; C, cyclophosphamide; Cb, carboplatin; FFPE, formalin-fixed, paraffin-embedded; F, fluorouracil; FTS, frozen tissue sample; G, gemcitabine; H, trastuzumab; HR, hormone receptor; L, lapatinib; M, methotrexate; N, vinorelbine; N/A, not applicable; NR, not reported; P, pertuzumab; T, taxanes; TNBC, triple negative breast cancer; X, capecitabine.

Table 2 A summary of pCR incidence among different subgroups

	PIK3CA status	pCR	Non-pCR	pCR rate (%)
Overall	MT	323	841	28
	WT	1252	2052	38
HR+	MT	39	268	13
	WT	276	731	27
HR-	MT	50	107	32
	WT	247	351	41
HER2+	MT	287	636	31
	WT	1068	1482	42
HER2-	MT	17	132	11
	WT	94	317	23
Exon 9	MT	28	175	14
Exon 20	MT	76	320	19

HR, hormone receptor; MT, mutant; pCR, pathological complete response; WT, wild type.

Meta-analysis

PIK3CA mutations and pathological complete response (pCR) in HER2+ patients

A total of 13 studies of unselected HER2+ patients were used for analysis (Table 3).[13,15,17,20,22–26,30–33] In this study, unselected HER2+ patients are defined as the entire HER2+ population with no restriction to HR status or NAT regime. The fixed effects model was used because of low heterogeneity, except in the HER2+/HR+ subgroup. WT unselected HER2+ patients achieved a higher rate of pCR (RR = 0.73; 95% CI 0.66–0.81) (Fig 2a). There were significant statistical differences in pCR between *PIK3CA* MT and WT after single-targeting trastuzumab treatment (RR = 0.71; 95% CI 0.54–0.94) (Fig 2b), but not after single-targeting lapatinib treatment (RR = 0.76; 95% CI 0.42–1.37). The trend remained significant in the HER2+/HR+ (RR = 0.50; 95% CI 0.27–0.93) and trastuzumab dual-targeting (RR = 0.71; 95% CI 0.62–0.80) subgroups (Fig 2c).

PIK3CA mutations and pCR in unselected hormone receptor positive (HR+) patients

We identified two studies investigating pCR in unselected HR+ patients regarding *PIK3CA* status (Table 3).[28,33] Pooled RR was 0.74 (95% CI 0.22–2.44). The random effects model was used because heterogeneity between the studies (I2 = 52.1%) was found. *PIK3CA* status was not associated with pCR in HR+ patients.

PIK3CA mutations and pCR in unselected HR- patients

Little data of pCR in unselected HR- and PIK3CA mutated patients was available. Liedtke *et al.* reported that *PIK3CA* MT did not influence pCR rate in unselected HR- patients (RR = 1.01; 95% CI 0.29–3.51) (Table 3).[28]

PIK3CA mutations and pCR in HR-/HER2- patients

Two studies investigated pCR in HR-/HER2- patients to *PIK3CA* mutation status (Table 3).[29,33] Pooled RR was 0.77 (95% CI 0.44–1.34). The fixed effects model was used because of low heterogeneity (I2 = 0.0%). *PIK3CA* status was not associated with pCR in HR-/HER2- patients.

PIK3CA mutations and response in HR+ patients with neoadjuvant endocrine therapy

Two studies investigated neoadjuvant endocrine therapy, with objective response rate (partial and complete response

Table 3 A summary of pooled RRs of patients with *PIK3CA* WT and MT

Categories by *PIK3CA* and NAT	No. of studies	*PIK3CA* MT		*PIK3CA* WT		Pooled RR	*P*	Heterogeneity (I2) (%)
		pCR	Non-pCR	pCR	Non-pCR			
Unselected breast cancer	3	35	223	138	563	0.70 (0.49–0.98)	0.036	0.0
Unselected HER2+	13	286	636	1068	1482	0.73 (0.66–0.81)	0.00	0.9
HER2+/HR+	3	30	147	237	447	0.50 (0.27–0.93)	0.028	64.4
HER2+/HR-	2	38	78	172	223	0.72 (0.55–0.95)	0.02	0.0
HER2+ with single trastuzumab	7	46	117	164	280	0.71 (0.54–0.94)	0.016	0.0
HER2+ with single laptinib	2	10	57	47	168	0.76 (0.42–1.37)	0.363	0.0
HER2+ with dual-targeting treatment	7	196	370	740	794	0.71 (0.62–0.80)	0.00	42.1
Unselected HR+	2	9	121	39	284	0.74 (0.22–2.44)	0.615	52.1
Unselected HR-	1	2	5	15	28	1.01 (0.29–3.51)	0.99	NA
HR-/HER2-	2	10	24	60	90	0.77 (0.44–1.34)	0.353	0.0
HR+ with neoadjuvant endocrine therapy	2	69	40	138	67	1.03 (0.63–1.70)	0.901	86.0

P was used to estimate the difference when $P < 0.05$. Unselected were defined irrespective of HER2 status or therapy regime. Pathological complete response (pCR) was based on Miller and Payne histopathology scoring system. Objective response (OR) was evaluated according Response Evaluation Criteria in Solid Tumors and was defined as complete + partial response. HR, hormone receptor; MT, mutant; NA, not applicable; RR, risk ratio; WT, wild type.

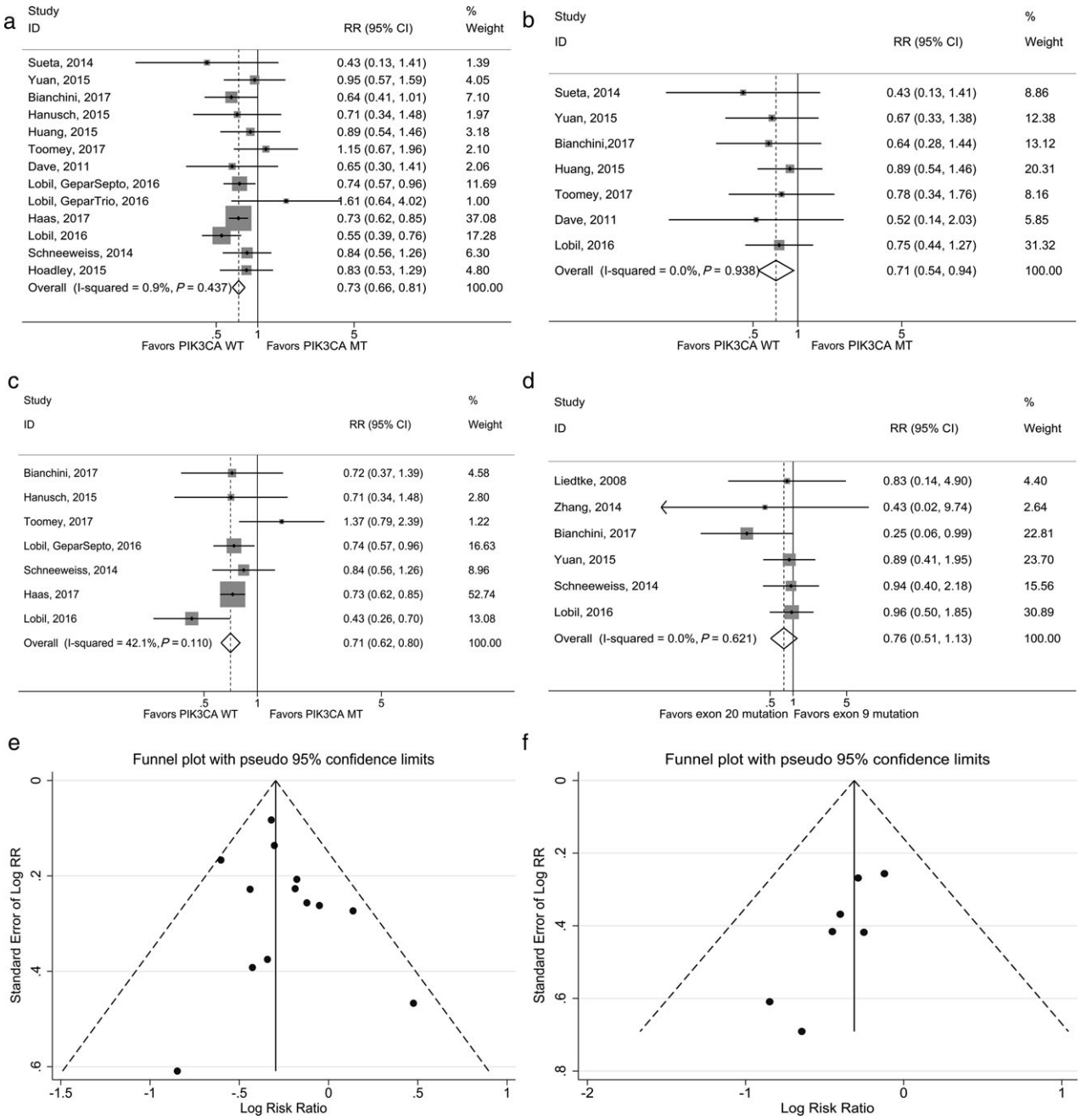

Figure 2 Forest plot of pathological complete response (pCR) of risk ratio (RR) with *PIK3CA* mutation (MT) versus wild type (WT) in (**a**) HER2+ patients, (**b**) in HER2+ patients with restriction to single-targeting trastuzumab treatment, and (**c**) in HER2+ patients with restriction to dual-targeting treatment. (**d**) Forest plot of pCR of RR with exon 9 versus exon 20. Funnel plot for meta-analysis of pCR with *PIK3CA* MT versus WT (**e**) in unselected HER2+ patients (13 studies) and (**f**) in HER2+ patients with restriction to single-targeting trastuzumab treatment (7 studies). *P* value was used to estimate the difference when $P < 0.05$. CI, confidence interval.

by Response Evaluation Criteria in Solid Tumors) as their outcome (Table 1).[18,19] *PIK3CA* status was not related to objective response (RR = 1.03; 95% CI 0.63–1.70), with significant heterogeneity (I2 = 86.0%) (Table 3), thus the random effects model was used (Table 4).

Exon 9 and 20 mutations in *PIK3CA* and pCR

Six studies separately reported pCR between *PIK3CA* exon 9 and *PIK3CA* exon 20 mutations.[16,27,28,31,33,34] There was no heterogeneity among studies. Both *PIK3CA* exon 9 and

Table 4 A summary of pooled RRs of patients with exon 9/20 and WT

Categories by mutation region	No. of studies	*PIK3CA* MT (exon 9 or 20)		*PIK3CA* WT (exon 9 or 20)		Pooled RR	*P*	Heterogeneity (I2) (%)
		pCR	Non-pCR	pCR	Non-pCR			
Exon 9	6	28	175	494	1354	0.55 (0.39–0.78)	0.001	0.0
Exon 20	6	76	320	494	1354	0.71 (0.58–0.89)	0.002	6.4

P was used to estimate the difference when $P < 0.05$. MT, mutant; pCR, pathological complete response; RR, risk ratio; WT, wild type.

20 mutations were significantly associated with lower pCR compared to WT. A comparison between exon 9 and exon 20 mutations was conducted. *PIK3CA* exon 20 mutations may yield a lower pCR (RR = 0.76; 95% CI 0.51–1.13) (Fig 2d, Table 5).

Sensitivity analysis and publication bias

After excluding two studies by Loibl *et al.*, the pooled RR (RR = 0.82; 95% CI 0.63–1.07) was insignificant.[13,27] The other results were significant, suggesting that no single study had any influence on the pooled RR. The funnel plot and Egger's test ($P = 0.014$) showed publication bias in the HER2+ subgroup of single-targeting trastuzumab therapy (Fig 2f), but not in unselected HER2+ patients (Fig 2e).

Discussion

To our knowledge, this is the first systematic review and meta-analysis to determine a relationship between *PIK3CA* mutation and NAT response in early stage breast cancer. Previous preclinical and clinical studies suggest that exon 9 and 20 mutations may differ. However, the predictive value of pCR between exon 9 and 20 mutations is not definitive.

Preclinical studies suggest that *PIK3CA* mutation might result in abnormal PI3K pathway activation, which leads to resistance to trastuzumab.[35] Our analysis confirms these results. In all HER2+ patients, *PIK3CA* MT appears to play a relevant role in defining the likelihood of lower pCR in NAT.

There was obvious publication bias among seven subgroup studies of single-targeting trastuzumab therapy; however, neither heterogeneity nor sensitivity analysis was obvious in this subgroup. Four of the studies were funded by national/academic funding,[17,23,30,33] one was industry-funded,[13] one was funded by both national/academic and industry funding,[16] and one received no funding.[32] Improved access to unpublished data is needed to overcome the problem of potential bias in results.

Hormone receptor and HER2 subtypes represent different diseases that differ in clinical behavior as well as in sensitivity to chemotherapy.[36] The predictive value of *PIK3CA* status in unselected HR+ and HR- patients is unclear. Our pooled analysis of seven studies proved that pCR in the HR+/HER2+ subgroup might be significantly related to *PIK3CA* status. This result indicates a potential interaction between HR and HER2 pathways.

PIK3CA mutations were associated with a lower pCR rate in the HR-/HER2- subgroup, although the difference was insignificant. This might be a result of the relatively small sample size of the HR-/HER2- subgroup, with a relatively low occurrence of *PIK3CA* mutations.[37]

Activation of the PI3K pathway might lead to anti-estrogen resistance.[38] We found no difference between *PIK3CA* mutation status and neoadjuvant endocrine therapy response. Heterogeneity was found between two studies.[18,19] Results of a study by Guarneri *et al.* indicated that *PIK3CA* MT might lead to a favorable objective response to endocrine therapy,[19] while Ellis *et al.* reached a different conclusion.[18] The disparity may result from the different regimes used. In the study by Guarneri *et al.*, HR+/HER2- patients were likely to benefit from additional lapatinib, particularly those with *PIK3CA* mutations; however, neoadjuvant endocrine therapy is still at an early stage.

Prognostic association between *PIK3CA* status and survival among studies remains controversial. Yang *et al.* reported that the prognostic role of *PIK3CA* may differ between various subgroups.[39] *PIK3CA* mutations are associated with favorable outcomes in HR+ patients after endocrine therapy.[40–42] In HER2+ patients, some studies have reported that *PI3KCA* mutations are not related to prognosis;[13,43] however others suggest that *PI3KCA* mutations are associated with poorer outcomes.[44]

Table 5 A summary of pooled RRs of patients between exons 9 and 20

Categories by mutation region	No. of studies	Exon 9		Exon 20		Pooled RR	*P*	Heterogeneity (I2) (%)
		pCR	Non-pCR	pCR	Non-pCR			
Exon 9 and Exon 20	6	28	175	76	320	0.76 (0.51–1.13)	0.169	0.0

P was used to estimate the difference when $P < 0.05$. MT, mutant; pCR, pathological complete response; RR, risk ratio.

In vitro studies found that *PIK3CA* exon 9 and 20 mutations may differ[14], therefore, the clinical implications of exon 9 and 20 mutations on pCR require explanation. pCR was the same between exons 9 and 20 MT. The possible reasons for this result are as follows: (i) exon 9 and 20 mutations were often combined for analysis and some studies did not report the number of *PIK3CA* exon 9 and 20 mutations, which may generate selection bias; (ii) insignificant results between exon 9 and 20 mutations may have resulted from the small sample size of only 203 exon 9 and 396 exon 20 mutations, which is relatively low; and (iii) heterogeneity among patients. The frequency of *PIK3CA* mutation and pCR may vary among different subtypes.

There are some limitations to this analysis. First, because we chose English-based articles we may have overlooked important information published in other languages. Second, clinical heterogeneity may exist among studies, such as age, race, NAT regime, and test method. Different NAT might have a significant impact on pCR, but this could not be concluded as a result of the small study sample. Third, clinical and methodological heterogeneity existed among the studies. Finally, the mutation detection methods were different across the studies, including direct, Sanger, pyrosequencing, and DNA sequencing platforms.

In early stage breast cancer, *PIK3CA* mutations seem to identify HER2+ patients who are likely to achieve a low pCR. The clinical implications of *PIK3CA* mutations might vary between exon 9 and exon 20 mutations after NAT. This mechanism should be explored by further study.

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Disclosure

No authors report any conflict of interest.

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