**Original Article**

**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, 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–12 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).

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. 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, nine in Europe, and four in Asia. Two studies included objective response rate, 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.
| Author year       | Country | Study name | Population | Number of PIK3CA mutated patients | Endpoints | Sample type | NAT regime | Sequenced PIK3CA |
|------------------|---------|------------|------------|----------------------------------|-----------|-------------|------------|-----------------|
| Barbareschi et al. 2012 | Italy | N/A | HER2+ | 4 (15.4%) | pCR | FFPE | AH → TH → CMFH | Exon 9/20 |
| Bianchini et al. 2017 | Italy | NeoSphere | HER2+ | 81 (19.4%) | pCR | FFPE | (i) TH; (ii) TPH; (iii) PH; (iv) TP | Exon 7/9/20 |
| Dave et al. 2011  | USA | HER2+ | 80 | 15 (18.8%) | pCR | FFPE | (i) H; (ii) L | NR |
| Ellis et al. 2010 | USA | P024, RAD 2222, ROL | HR+ | 235 | 76 (32.3%) | OR | FFPE | Tamoxifen + Letrozole | Exon 7/9/20 |
| Guaneri et al. 2014 | Italy | CONSORT | HR+/HER2- | 92 | 34 (37.0%) | OR | FFPE | (i) Letrozole; (ii) Letrozole + L | Exon 9/20 |
| Hanusch et al. 2015 | Germany | GBG-70 | HER2+ | 61 | 13 (21.3%) | pCR | NR | Afatinib → TH Afatinib → ACH | Exon 9/20 |
| Harbeck et al. 2016 | Germany | WSG-ADAPT | HR+/HER2+ | 114 | 18 (15.8%) | pCR | NR | (i) T-DM1; (ii) T-DM1 + Tamoxifen or Al; (iii) H + Tamoxifen or Al | |
| Haas et al. 2017 | USA | KRISTINE | HER2+ | 425 | 114 (26.8%) | pCR | NR | (i) T-DM1 + P; (ii) TChbPH | NR |
| Huang 2015 | China | N/A | HER2+ | 77 | 30 (39.0%) | pCR | FFPE | (i) TCH; (ii) TAH | Exon 4/9/20 |
| Hoadley et al. 2016 | USA | CALGB 40601 | HER2+ | 181 | 14 (7.7%) | pCR | NR | (i) TL; (ii) TH; (iii) THL | Exon 9/20 |
| Loibl et al. 2016 | USA | GeparSepto | HER2+ | 291 | 62 (21.6%) | pCR | FFPE | THP | Exon 9/20 |
| Loibl et al. 2016 | USA | GeparTrio | HER2+ | 82 | 31 (37.8%) | pCR | FFPE | (i) TAC; (ii) TAC → NX | NR |
| Loibl et al. 2016 | Germany | GeparQuattro GeparQuinto GeparSixto NeoALTO CHERLOB | HER2+ | 967 | 210 (21.7%) | pCR | FFPE | (i) ACH → TH; (ii) ACh → TL; (iii) THL; (iv) TH; (v) TL; (vi) THB; (vii) TCbHB; (viii) TH → CAH; (ix) TL → CAFL; (x) THL → CAFL | Exon 9/20 |
| Liedtke et al. 2008 | USA | N/A | ALL | 140 | 23 (16.4%) | pCR | NR | (i) FAC; (ii) T → FAC | Exon 1/9/20 |
| Lips et al. 2015 | Netherlands | N/A | TNBC | 140 | 23 (16.4%) | pCR | FTS | (i) AC; (ii) AC → TX; (iii) AC → Xcm + Thiotepa | Exon 9/20 |
| Toomey et al. 2017 | 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 | Germany | TRYPHAENA | HER2+ | 126 | 39 (31.0%) | pCR | NR | (i) FCHP → THP; (ii) FEC → THP; (iii) TCbHP | Exon 7/9/20 |
| Suetal. 2014 | Japan | N/A | HER2+ | 42 | 7 (16.7%) | pCR | FFPE | (i) FAC → T; (ii) TC | Exon 9/20 |
| Yuan et al. 2015 | China | N/A | ALL | 729 | 142 (19.5%) | pCR | FTS | (i) CAH; (ii) AC; (iii) A → T; (iv) A → TC; (v) A → Tc | Exon 9/20 |
| Zhang et al. 2014 | 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 to 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; Ch, 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.
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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). 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). 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).

PIK3CA mutations and response in HR+/HER2- patients

Two studies investigated pCR in HR-/HER2- patients to PIK3CA mutation status (Table 3). 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 2 A summary of pCR incidence among different subgroups

| Categories by PIK3CA and NAT | No. of studies | PIK3CA MT | 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          |
|                             |                | WT        | 76  | 320     | 19          |

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

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

| Categories by PIK3CA and NAT | No. of studies | PIK3CA MT | pCR | Non-pCR | Pooled RR | P | Heterogeneity (I2) (%) |
|------------------------------|----------------|-----------|-----|---------|-----------|---|------------------------|
| Unselected breast cancer     | 3              | 35        | 223 |          | 138       | 0.70 (0.49–0.98) | 0.036 | 0.0                    |
| Unselected HER2+             | 13             | 286       | 636 |          | 1068      | 0.73 (0.66–0.81) | 0.00  | 0.9                    |
| HER2+/HR+                   | 3              | 30        | 147 |          | 237       | 0.50 (0.27–0.93) | 0.028 | 64.4                   |
| HER2+/HR-                   | 2              | 38        | 78  |          | 172       | 0.72 (0.55–0.95) | 0.02  | 0.0                    |
| HER2+ with single trastuzumab | 7             | 46        | 117 |          | 164       | 0.71 (0.54–0.94) | 0.016 | 0.0                    |
| HER2+ with single lapatinib  | 2              | 10        | 57  |          | 47        | 0.76 (0.42–1.37) | 0.363 | 0.0                    |
| HER2+ with dual-targeting treatment | 7             | 196       | 370 |          | 740       | 0.71 (0.62–0.80) | 0.00  | 42.1                   |
| Unselected HR+              | 2              | 9         | 121 |          | 39        | 0.74 (0.22–2.44) | 0.615 | 52.1                   |
| Unselected HR-              | 1              | 2         | 5   |          | 15        | 1.01 (0.29–3.51) | 0.99  | NA                     |
| HR-/HER2-                   | 2              | 10        | 24  |          | 60        | 0.77 (0.44–1.34) | 0.353 | 0.0                    |
| HR+ with neoadjuvant endocrine therapy | 2         | 69        | 40  |          | 138       | 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.
by Response Evaluation Criteria in Solid Tumors) as their outcome (Table 1).\textsuperscript{18,19} PIK3CA status was not related to objective response (RR = 1.03; 95% CI 0.63–1.70), with significant heterogeneity (I\(^2\) = 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.\textsuperscript{16,27,28,31,33,34} There was no heterogeneity among studies. Both PIK3CA exon 9 and

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

| Categories by mutation region | No. of studies | PI3KCA MT (exon 9 or 20) | PI3KCA WT (exon 9 or 20) | Pooled RR | P | Heterogeneity (I²) (%) |
|------------------------------|----------------|--------------------------|--------------------------|-----------|---|----------------------|
| 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. PI3KCA 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. 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 PI3KCA 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 PI3KCA mutation might result in abnormal PI3K pathway activation, which leads to resistance to trastuzumab. Our analysis confirms these results. In all HER2+ patients, PI3KCA 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, and one received no funding. Improved access to unpublished data is needed to overcome the problem of potential bias in results.

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. Activation of the PI3K pathway might lead to resistance to chemotherapy. We found no difference between PIK3CA mutation status and neoadjuvant endocrine therapy response. Heterogeneity was found between two studies. Results of a study by Guarneri et al. indicated that PIK3CA MT might lead to a favorable objective response to endocrine therapy, while Ellis et al. reached a different conclusion. 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. PIK3CA mutations are associated with favorable outcomes in HR+ patients after endocrine therapy. In HER2+ patients, some studies have reported that PIK3CA mutations are not related to prognosis; however, others suggest that PIK3CA mutations are associated with poorer outcomes.

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

| Categories by mutation region | No. of studies | Exon 9 | Exon 20 | Pooled RR | P | Heterogeneity (I²) (%) |
|------------------------------|----------------|--------|--------|-----------|---|----------------------|
| 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, 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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