Original Article

PIK3CA mutations and PTEN loss: Effects on neoadjuvant trastuzumab in Vietnamese breast cancer patients

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ABSTRACT

The objective of this study was to ascertain the prevalence of *PIK3CA* mutations and PTEN loss in HER2-positive stage II–III Vietnamese breast cancer patients and evaluate their impact on the disease. This case-control study, conducted from January 2019 to June 2022, included 92 patients initially diagnosed with stage II or III breast cancer, confirmed HER2-positive, and treated with neoadjuvant trastuzumab. Post-mastectomy, patients were grouped into pathological complete response (pCR) and pathological partial response (pPR). Each patient was tested for *PIK3CA* mutations and PTEN loss to examine their correlation with neoadjuvant chemotherapy response. Ninety-two patients completed neoadjuvant chemotherapy and surgery. Among them, 44 (47.8%) achieved complete tumor response, 9 (9.8%) had ductal carcinoma in situ, and 39 (42.4%) had invasive breast cancer. The prevalence of *PIK3CA* mutations was 16.3%, primarily c.3140A>G (p.H1047R) at exon 20. The rate of PTEN loss was 26.1%. There was no observed correlation between *PIK3CA* mutation or PTEN loss and neoadjuvant chemotherapy response, with odd ratios of 1.22 (95% CI: 0.93–1.61) and 0.80 (95% CI: 0.63–1.01), respectively. The study revealed no evident correlation between *PIK3CA* mutations or PTEN loss and the pathological complete response to neoadjuvant chemotherapy. As a result, our findings recommend prudence in the consideration of *PIK3CA* mutations and PTEN loss as prognostic markers for neoadjuvant chemotherapy response in patients with HER2-positive stage II–III breast cancer.

Keywords: *PIK3CA*, PTEN, HER2, Breast cancer, Neoadjuvant chemotherapy

Introduction

Neoadjuvant chemotherapy with the trastuzumab regimen represents a significant advancement in the treatment of breast cancer [1]. Nevertheless, the financial burden associated with this therapeutic approach may impose insurmountable challenges on patients [2]. Consequently, the ability to predict the response to neoadjuvant chemotherapy becomes crucial.

PIK3CA mutations and PTEN loss have been proposed as prognostic indicators for trastuzumab treatment [3]. A study in 2012 on HER2-positive patients revealed a correlation between *PIK3CA* mutations or PTEN loss and a poor response to trastuzumab treatment [4]. However, subsequent studies have not yet demonstrated the benefits of these mutations [5].

At present, the European Society for Medical Oncology (ESMO) consensus does not recommend the utilization of *PIK3CA* mutations or PTEN loss as indicators for trastuzumab neoadjuvant chemotherapy [6]. Additionally, there is a lack of data regarding the prevalence of *PIK3CA* mutations and PTEN loss, as well as their roles in trastuzumab treatment at Ho Chi Minh City Oncology Hospital, despite its significant caseload of breast cancer patients. This study aimed to ascertain the

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Kim LB, Vu HA, Anh LT, Ha HTN, Dieu AN, Diem TDK, et al. *PIK3CA* mutations and PTEN loss: Effects on neoadjuvant trastuzumab in Vietnamese breast cancer patients. J Adv Pharm Educ Res. 2024;14(3):84-9. https://doi.org/10.51847/OEX6hMBb0J

prevalence of *PIK3CA* mutations and PTEN loss in a cohort of HER2-positive stage II–III breast cancer patients and to assess how these genetic alterations are linked to the progression of the illness.

Materials and Methods

This research was conducted retrospectively and used a case-control design. The research obtained assessment and endorsement from the Institutional Review Board at Ho Chi Minh City Oncology Hospital. Before data collection, informed consent was obtained from all participating patients. Patients diagnosed with stage II–III HER2-positive breast cancer were enrolled at Ho Chi Minh City Oncology Hospital between January 2019 and June 2022. The inclusion criteria for this study were as follows: neoadjuvant chemotherapy with trastuzumab, being female, being at least 18 years old, and having a Karnofsky Performance Scale score of 70 or above. The exclusion criteria included cases of bilateral breast cancer and recurrent breast cancer.

The sample size was calculated based on the primary outcome of pCR following neoadjuvant chemotherapy with the trastuzumab regimen. In a study by Quiyun Shi *et al*. it was shown that the rate of pathological complete response (pCR) in instances of breast cancer linked with *PIK3CA* mutation following neoadjuvant chemotherapy was 26.3% [7]. The odds ratio (OR) was calculated to be 0.09, with a 95% confidence interval (CI) ranging from 0.02 to 0.42. Given a statistical power (β) of 0.8 and a significance level (α) of 0.05, the investigation necessitated a minimum sample size of 34 patients.

A total of ninety-two patients were enrolled in this study. The formalin-fixed paraffin-embedded (FFPE) breast biopsy specimens, collected before neoadjuvant chemotherapy, were utilized for immunohistochemical and genetic analyses. Monoclonal antibodies were employed in the Ventana Benchmark XT/Ultra system for the detection of ER [CONFIRM anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Primary Antibody], PR [CONFIRM anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Primary Antibody], HER-2 [VENTANA anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody], Ki67 [CONFIRM anti-Ki-67 (30-9) Rabbit Monoclonal Primary Antibody], and PTEN [VENTANA PTEN (SP218) Rabbit Monoclonal Primary Antibody]. PTEN loss was defined as less than 10% of PTEN expression in tumor cells after immunohistochemistry [8]. *PIK3CA* mutations were investigated in exon 9 and exon 20 using the Sanger sequencing method and the ABI 3500 Genetic Analyzer system (Applied Biosystem). To prevent amplification of the pseudogene region in exon 9, we used the primer pairs PIK-VU10F (5'-GAAAATGTATTTGCTTTGTC'3) and PIK3CA-E10R (5'-AAAGAAACAGAGAATCTCCA-3'). The primer pairs for amplifying exon 20 were PIK3CA-E21F (5'-GTTTCAGGAGATGTGTTACA-3') and PIK3CA-E21R (5'-AGTTCAATGCATGCTGTTTA-3'). Clinical features, including tumor size, T stage, and N stage, as well as pathological features such as histopathology, grading, hormone status, and Ki67 expression, were described. pCR was confirmed when no carcinoma cells were found in any lymph node specimens (ypN0) and no remnant carcinoma (ypT0) or remnant carcinoma in situ (ypTis) were found in all tumor specimens [9].

Statistical analysis

The statistical analyses were performed using R software. The normality of each variable was assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as the mean and standard deviation, while ordinal data are reported as numbers and percentages. The correlation between *PIK3CA* or PTEN and pCR was analyzed using the Student's t-test and OR. A p-value of less than 0.05 was considered significant.

Results and Discussion

From January 1, 2019, to June 30, 2022, 92 patients were recruited for this study. The characteristics of these patients are summarised in **Table 1**. Approximately 79.3% of patients were younger than 60 years old. Overwhelmingly, the tumors examined in this research exceeded a size of 20 mm, with a prevalence of 97.8%. The patients were predominantly classified according to the TNM staging system as stage IIIc (38%).

Table 1. Demographic and clinical characteristics of patients before neoadjuvant chemotherapy

Characteristic	Result (N=92)
Age (year)	53 (43-58.3)
Tumor size (mm)	50 (40-60)
T stage	
T0/Tx	1 (1.1)
T1c	1 (1.1)
T2	26 (28.3)
T3	13 (14.1)
T4b	47 (51.1)
T4c	4 (4.3)
N stage	
N0	21 (22.8)
N1	17 (18.5)
N2a	17 (18.5)
N3a	20 (21.7)
N3c	17 (18.5)
TNM stage	
IIA	9 (9.8)
IIB	12 (13)
IIIA	10 (10.9)
IIIB	26 (28.3)
IIIC	35 (38)

Before neoadjuvant chemotherapy and mastectomy, most tumors were categorized as grade 2, with Ki67 levels of 20% or higher [10]. Over 50% of tumors showed positivity for hormone receptors, mainly estrogen. HER2 expression was mostly scored

as 3+ on immunohistochemistry (97.8%), with only two cases showing HER2 positivity defined by fluorescent in situ hybridization.

Following neoadjuvant chemotherapy and mastectomy, there was a significant reduction in tumor size of approximately 44.03 mm, which differed significantly from the measurements before chemotherapy ($p < 0.001$). The majority of patients achieved no malignant cells within the tumor (ypT0) (47.8%). Among those with remaining carcinoma, invasive grade 2 constituted 62.5%.

Prevalence of PIK3CA mutation and PTEN loss

There were 15 cases of *PIK3CA* mutation, accounting for 16.3%. The most frequently observed *PIK3CA* mutation was c.3140A>G (p.H1047R) in exon 20. Other mutations included c.1633G>A (p.E545K) and c.1624G>A (p.E542K) in exon 9, as well as c.3140A>T (p.H1047L) in exon 20 **(Figure 1)**.

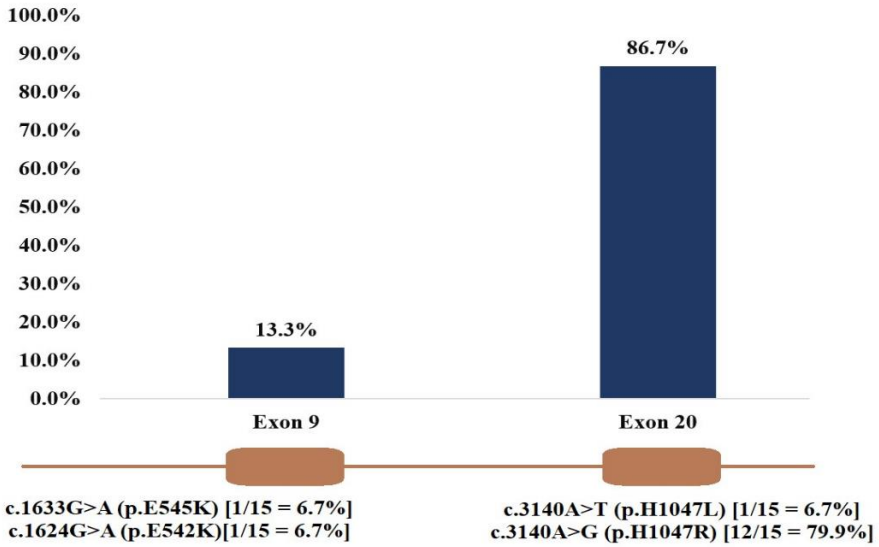
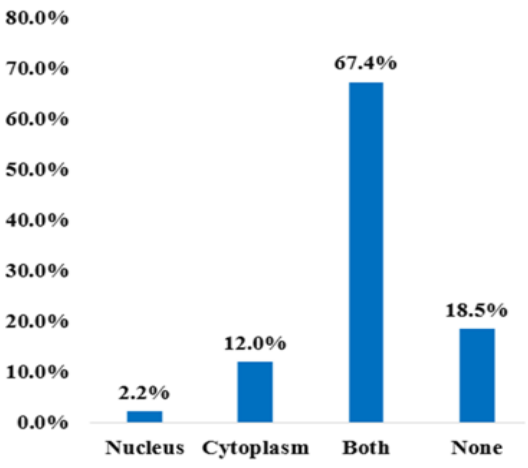
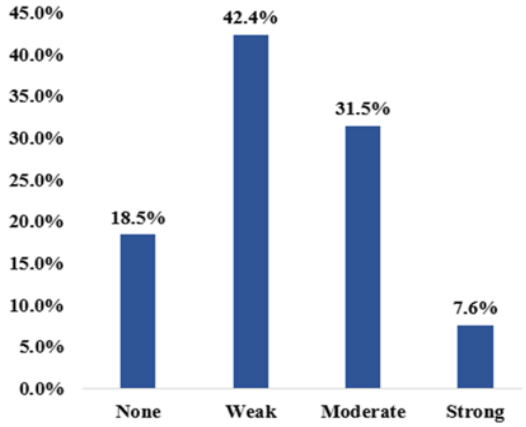


Figure 1. Mutations of the *PIK3CA* gene after sequencing

This research identified 24 cases of PTEN loss. PTEN expression was found in both the cytoplasm and nucleus of 62 patients. Out of the total patients, two patients had PTEN expression exclusively in the nucleus, while 11 other patients showed expression only in the cytoplasm. The intensity of PTEN expression was graded as strong, moderate, and weak in 7, 29, and 39 patients, respectively. In other words, approximately one-fourth of the patients in this study did not exhibit PTEN expression. For those with PTEN expression, the intensity was mostly weak, and the expression occurred both in the nucleus and cytoplasm **(Figure 2)**.



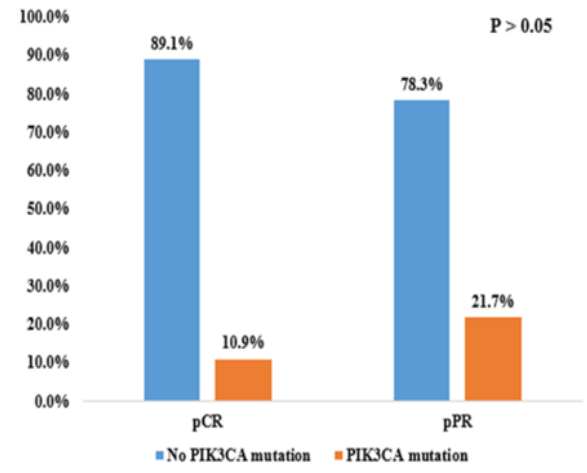
a)



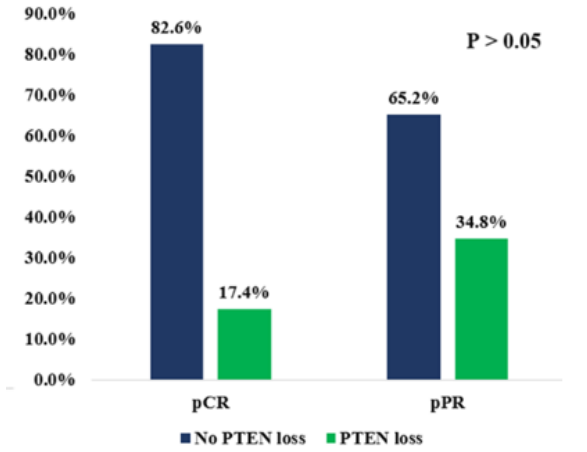
b)

Figure 2. Phenotype (a) and intensity (b) of PTEN expression

Among patients who achieved pPR, the prevalence of *PIK3CA* mutation and PTEN loss was 21.7% and 34.8%, respectively. No significant differences were observed between patients with pCR and pPR. The odd ratios for *PIK3CA* mutation and PTEN loss were 1.22 (95% CI: 0.93–1.61) and 0.80 (95% CI: 0.63–1.01), respectively. No correlations were found between *PIK3CA* mutation or PTEN loss and pCR after neoadjuvant chemotherapy **(Figure 3)**. Consequently, these factors should not be considered prognostic indicators for the response to neoadjuvant chemotherapy, including trastuzumab, in stage II–III HER2-positive breast cancer [11].



a)



b)

Figure 3. Correlation between pCR after neoadjuvant chemotherapy and *PIK3CA* mutation (a) or PTEN loss (b)

In this study, a total of 92 patients with stage II–III HER2-positive breast cancer were included. All of them underwent neoadjuvant chemotherapy, which included trastuzumab. The prevalence of *PIK3CA* mutations was 16.3%, with the majority of mutations located at exon 20 [c.3140A>G (p.H1047R)]. PTEN loss was observed in 26.1% of cases. PTEN expression was predominantly detected in both the nucleus and cytoplasm (62 cases, 67.4%), and the expression was mostly characterized as weak, with 39 cases (42.4%). However, correlation analysis revealed no significant correlations between *PIK3CA* mutation or PTEN loss and pCR after neoadjuvant chemotherapy. The OR was 1.22 (95% CI: 0.93–1.61) for *PIK3CA* mutation and 0.80 (95% CI: 0.63–1.01) for PTEN loss, indicating that these factors do not exhibit strong correlations with pCR in the context of this study.

Most of the tumors in this study had sizes exceeding 20 mm. These tumors were predominantly categorized as T4b, and the clinical stages were predominantly classified as stage III. Other studies have reported breast cancer cases at earlier stages (I or II) [4, 7, 12]. However, the tumor sizes were similar to those in our study. These differences might be attributed to factors such as lymph node metastasis and the lack of regular screening among our patient population [13]. Furthermore, the mean age in our study was under 60 years, which is consistent with findings from other studies [4, 7, 12]. It may suggest that breast cancer at a younger age is not only happening in Vietnamese patients. Most tumors were classified as grade 2, and Ki67 levels were 20% or higher. More than 50% of tumors exhibited positivity for hormone receptors, and almost all showed a HER2 positive score of 3+ on immunohistochemistry. Several studies have described the same immunohistochemistry characteristics as our study in breast cancer patients who received neoadjuvant chemotherapy [12, 14-16].

After neoadjuvant chemotherapy and mastectomy, the prevalence of an absence of residual malignant cells within the tumor was 47.8%. Among those with remaining carcinoma, the majority were classified as ypTMN stage IIA. The histological grades were similar before surgery, with the most being at grade 2. These results were in accordance with trastuzumab treatment before surgery. Trastuzumab has been demonstrated to improve the prevalence of pCR in cases of invasive breast cancer [17].

The prevalence of *PIK3CA* mutations in our study was 16.3%, lower than that reported in other studies. Shi *et al*. reported a prevalence of 46% [7]. Meanwhile, *PIK3CA* mutation rates of 24.2% and 26.7% were reported by Nishimura *et al*. and Reinhardt *et al*., respectively [4, 12]. We hypothesize that these discrepancies might be due to variations in sample sizes and demographic characteristics. Nevertheless, the most frequent type of *PIK3CA* mutation in our study was not different from previous ones [4, 7].

In terms of PTEN loss prevalence, this study recorded a rate of 26.1%, which was lower than some of the previous studies [18, 19]. We suggest possible reasons, including differences in the definition of PTEN loss, tumor size, lymph node metastasis, and the advanced TMN stage of breast carcinoma [20].

Regarding the correlation between *PIK3CA* mutations or PTEN loss and pCR following neoadjuvant chemotherapy in stage II-III HER2-positive breast cancer, our study did not find any significant correlation. These results align with the current medical literature [21]. To the best of our knowledge, considerable uncertainty surrounds the role of *PIK3CA* mutations and PTEN loss as predictors of response to neoadjuvant chemotherapy [20, 22]. This may be due to inconsistencies and inaccuracies in assessment methods across studies, including differences in testing techniques (immunohistochemistry, sequencing), choices of antibodies for immunohistochemistry, scoring systems, cut-off points, and the origin of tumor specimens (primary or metastatic tumors). Therefore, *PIK3CA* mutations or PTEN loss need to be studied in further, detailed research before they can be confidently established as prognostic factors for neoadjuvant chemotherapy outcomes.

Conclusion

PIK3CA mutations and PTEN loss demonstrated no significant correlation with pCR after neoadjuvant chemotherapy. These factors do not appear to play a role in predicting failure to respond to neoadjuvant chemotherapy and should not be utilized

for assessing treatment outcomes in stage II–III HER2-positive breast cancer.

Acknowledgments: We thank the staff of the Center for Molecular Biomedicine at the University of Medicine and Pharmacy in Ho Chi Minh City and the staff of the Pathology Department at Ho Chi Minh City Oncology Hospital for their helpful advice and support.

Conflict of interest: None

Financial support: This study was funded by the University of Medicine and Pharmacy at Ho Chi Minh City under Decision No. 1346/QD-DHYD.

Ethics statement: The research received authorization from the ethics committee at Ho Chi Minh City Oncology Hospital (496/HĐĐĐ-BVUB).

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