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Prognostic significance of PIK3CA immunoexpression and mutation status in triple-negative breast cancer

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MAIN POINTS

- PIK3CA immunoexpression was predominantly low in TNBC, with only 20% of cases showing moderate–high expression, indicating that overexpression is limited to a distinct biological subset.
- Moderate–high PIK3CA expression was significantly associated with improved overall survival, while all deaths occurred in the negative-expression group (p=0.043).
- No meaningful correlation was found between PIK3CA expression and conventional clinicopathological parameters.
- PIK3CA mutations were detected in 33.3% of high-expression cases, indicating only a partial alignment between protein expression and mutation status.
- The observed association between PIK3CA expression and overall survival should be interpreted cautiously and requires validation in larger, adequately powered cohorts.

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ABSTRACT

Aim: Triple-negative breast cancer (TNBC) is an aggressive subtype with limited targeted therapies. This study investigated PIK3CA protein expression in TNBC and, exploratorily, assessed PIK3CA mutations in a subset of tumors with high expression.

Materials and Methods: Sixty-five TNBC cases were retrospectively analyzed. PIK3CA expression was assessed by H-score and grouped for survival analysis as negative versus moderate–high. Six high-expression cases underwent mutation analysis. Associations with clinicopathological parameters, overall and disease-free survival were evaluated using Kaplan–Meier and log-rank tests.

Results: PIK3CA expression was negative in 80.0% (52/65) of cases, while moderate–high expression was observed in 20.0% (13/65). No significant associations were observed between PIK3CA immunohistochemical expression and clinicopathological features (p>0.05). PCR-based mutation analysis was performed on the moderate–high expression subgroup. 33.3% (2/6) of the tested high-expression cases harbored PIK3CA mutations. All deaths occurred in the negative-expression group; overall survival was significantly better in the moderate–high expression group (p = 0.043). However, no death events were observed in the moderate–high subgroup (n = 13), and this finding should be interpreted cautiously because of the low number of events. No significant difference in disease-free survival was found between groups (p = 0.588).

Conclusion: PIK3CA protein expression was not associated with conventional clinicopathological prognostic parameters in TNBC. Nevertheless, moderate–high PIK3CA expression was associated with better overall survival, indicating a hypothesis-generating rather than an independent prognostic effect. Moreover, despite the absence of significant differences at the immunohistochemical level, PIK3CA mutations were detected by PCR in a limited subset of high-expression cases, highlighting a potential discordance between protein expression and molecular alterations. These findings warrant validation in larger, multicenter studies.

Keywords: TNBC, PIK3CA, Immunohistochemistry, Prognosis, Survival

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INTRODUCTION

Breast cancer is one of the most common malignancies among women worldwide. According to the 2022 GLOBOCAN data, it accounts for 23.8% of all cancer types in women, with an annual incidence of 46.8 per 100,000 women and a mortality rate of 12.7 per 100,000 women. Breast cancer remains a major public health concern globally, affecting women in both developed and developing countries [1].

Triple-negative breast cancer (TNBC), an aggressive subtype of breast carcinoma, accounts for approximately 15–25% of all breast cancer cases [2]. Compared with other molecular subtypes, TNBC is characterized by a more aggressive clinical course, with a higher risk of distant metastasis and poorer prognosis [2]. Under current treatment approaches, the me-

dian overall survival is approximately 10.2 months; the five-year survival rate is reported as 65% for patients with regional disease, dropping to 11% in cases with distant metastatic spread [3,4].

Despite advances in diagnostic techniques and the development of targeted therapeutic options, the biologically heterogeneous nature of the disease continues to necessitate a personalized treatment approach. In this context, the identification of novel molecular biomarkers that may enhance our understanding of tumor biology is of great importance for improving clinical management.

Activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway plays a pivotal role in regulating key cellular processes, including cell growth, proliferation, survival, and metabolism. The PIK3CA gene encodes the p110α catalytic subunit of class IA PI3Ks and frequently harbors oncogenic mutations. PIK3CA mutations are commonly detected in breast cancer, with higher prevalence in estrogen receptor-positive (ER+) tumors, HER2-negative tumors, and tumors with low proliferation. However, the relationship between PIK3CA gene mutations and protein-level expression remains insufficiently clarified, and current evidence in the literature shows inconsistent findings [5–7]. Importantly, mutation status does not necessarily translate into protein abundance detectable by immunohistochemistry, as PIK3CA expression may be influenced by transcriptional and post-transcriptional regulation, protein stability, and intratumoral heterogeneity. This discordance may be particularly relevant in TNBC, where available data are more limited and heterogeneous. Therefore, assessing PIK3CA at the protein level by immunohistochemistry may provide complementary information beyond mutation status, including the extent and spatial distribution of expression within the tumor.

Evidence in the literature suggests that PIK3CA mutations may be associated with a more favorable prognosis in certain breast cancer subgroups; however, this effect appears to vary depending on tumor subtype, treatment modality, and additional molecular parameters [8]. Moreover, a substantial proportion of studies reporting favorable prognostic associations are derived from hormone receptor–positive cohorts, which may limit the direct applicability of these findings to TNBC. In this context, evaluating PIK3CA not only at the genetic level but also in terms of its immunohistochemical expression profile may provide valuable prognostic and predictive insights.

The present study aimed to evaluate PIK3CA protein expression in TNBC using immunohistochemistry and to examine its associations with clinicopathological characteristics and survival outcomes, including overall and disease-free survival. PIK3CA mutation analysis was also performed in a subset of tumors exhibiting high protein expression to preliminarily assess the relationship between protein expression and mutation status. These findings may generate hypotheses regarding the prognostic significance of PIK3CA in TNBC.

MATERIALS AND METHODS

Patient selection

This study included 65 patients who were histopathologically diagnosed with triple-negative breast cancer (TNBC) (ER−, PR−, HER2−) at our center between 2015 and 2024 and who had available formalin-fixed, paraffin-embedded (FFPE) tissue samples suitable for immunohistochemical evaluation. Eligible cases were required to have sufficient clinicopathological data and follow-up information in the hospital records and to have undergone surgical and/or systemic treatment at our institution.

Patients diagnosed with breast cancer of molecular subtypes other than TNBC (i.e., Luminal A, Luminal B, or HER2-positive) [9], those lacking adequate tissue for immunohistochemical or molecular analysis, cases with incomplete clinical data or follow-up information, patients diagnosed solely with in situ carcinoma, and those presenting with metastatic disease at initial admission were excluded from the study. Patients with a history of any other malignancy prior to diagnosis—except for non-melanoma skin cancer—were not included in the study.

Ethical approval for this study was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (Date: 09.05.2024; Approval No: E-60116787-020-523657).

Immunohistochemical and molecular analysis

PIK3CA expression was evaluated immunohistochemically and scored using the H-score method, with staining intensity graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The H-score is obtained by multiplying the percentage of tumor cells showing each staining intensity by the corresponding intensity value. The total score ranges from 0 to 300. For categorical interpretation, scores were classified as follows: negative (0–30), moderate (31–100), and high (101–300) according to previously published cut-off values [10] to ensure methodological consistency and comparability with the existing literature. To increase statistical power, scores were additionally regrouped into two categories for analysis: negative (0–30) and moderate–high (31–300) by combining the moderate and high groups to avoid sparse subgroup sizes and improve the robustness of statistical analyses [10].

Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissues using the cobas® DNA Sample Preparation Kit in accordance with the manufacturer's instructions. PCR-based mutation analysis was performed to detect hotspot mutations in the PIK3CA gene. Specifically, mutations in exon 9 (E542K, E545K) and exon 20 (H1047R) were assessed.

The PCR reaction was prepared in a total volume of 25 µL and consisted of DNA template, primers, dNTP mix, $MgCl_2$, Tris-HCl buffer, and Taq DNA polymerase. Thermal cycling conditions included an initial denaturation step, followed by

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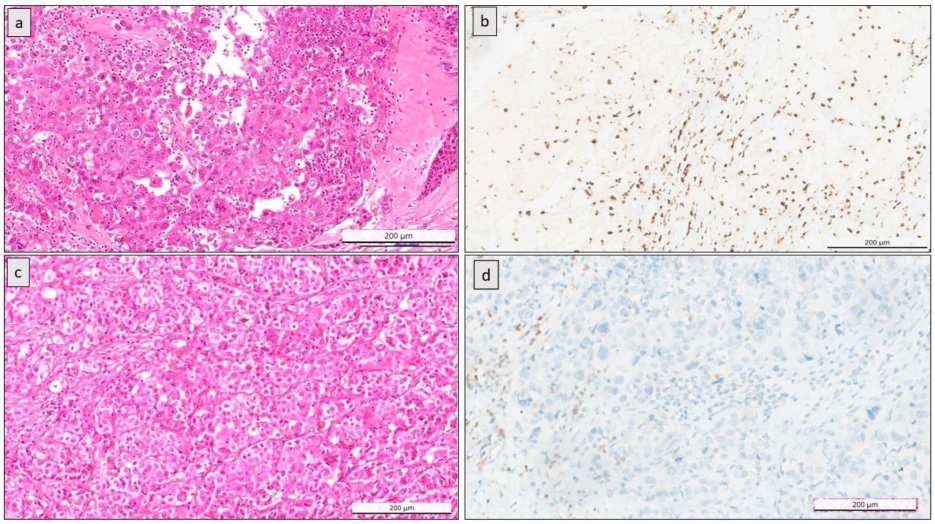

Figure 1. Representative histopathological and immunohistochemical features of triple-negative breast carcinoma. (a) High-grade invasive carcinoma (H&E, ×100). (b) PIK3CA-positive nuclear immunohistochemical staining (×100). (c) High-grade invasive carcinoma (H&E, ×100). (d) PIK3CA-negative immunohistochemical staining (×100).

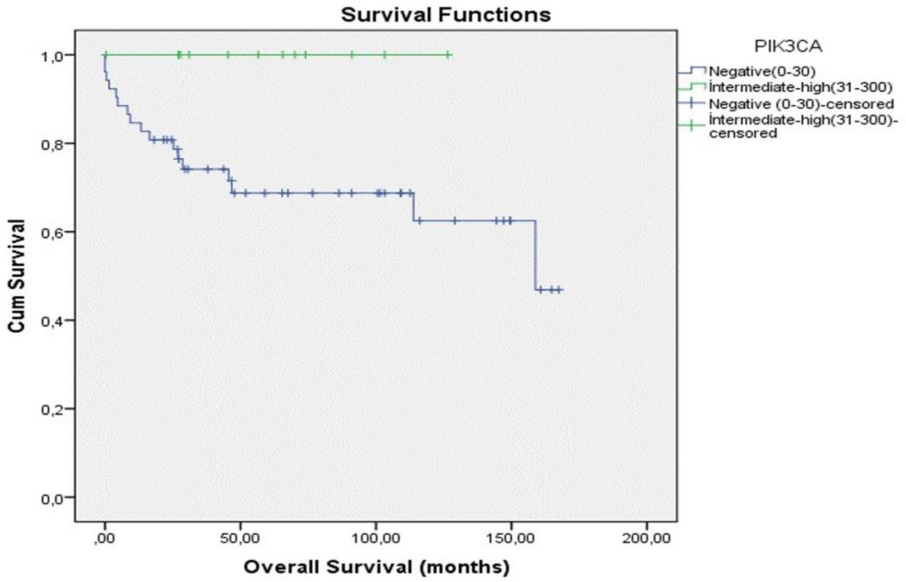

Figure 2. Overall survival curves according to PIK3CA expression levels. Patients with moderate–high expression (31–300) showed better survival than those with negative/low expression (0–30) (Log-rank, χ^2=4.106; df=1; p=0.043).

35 cycles of denaturation, annealing, and extension, and a final extension step optimized for target amplification.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). The Chi-square test or Fisher's exact test was used to compare categorical variables, as appropriate. Survival analyses were conducted using Kaplan–Meier survival curves, and differences between curves were assessed using the log-rank test. A p-value of <0.05 was considered statistically significant. Multivariable Cox regression analysis was considered but not performed due to the limited number of events and complete separation.

RESULTS

Clinicopathological characteristics

The mean age was 52.4 ± 13.3 years (range: 28–87 years), and 67.7% were postmenopausal.

Based on age distribution, 60% of the cases were aged ≥55 years and 40% were <55 years. The mean overall survival time was 63.3 ± 49.8 months, and the median overall survival was 46.8 months.

Histopathologically, 90.8% (n = 59) of cases were diagnosed as invasive carcinoma of no special type (NST), 3.0% (n=2) as invasive lobular carcinoma, and 6.2% (n=4) as metaplastic carcinoma.

Tumors were located in the right breast in 46.9% of cases and

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Table 1. Clinicopathological characteristics of the study cohort.

Parameter		n (%)
Age groups	<55 years	26 (40.0)
	≥55 years	39 (60.0)
Histologic type	Invasive carcinoma of no special type (NST)	59 (90.8)
	Invasive lobular carcinoma	2 (3.1)
	Metaplastic carcinoma	4 (6.2)
Type of surgery	Mastectomy	31 (47.7)
	Excision	31 (47.7)
	Tru-cut biopsy only	3 (4.6)
Tumor size	1--20 mm	19 (29.2)
	21--50 mm	37 (56.9)
	≥50 mm	9 (13.8)
Histologic grade	Grade 2	23 (36.5)
	Grade 3	40 (63.5)
Tumor localization	Right breast	30 (46.9)
	Left breast	34 (53.1)
Lymphovascular invasion	Absent	31 (49.2)
	Present	14 (22.2)
	Unknown	18 (28.6)
Extranodal extension	Absent	48 (73.8)
	Present	7 (10.8)
	Unknown	10 (15.4)
Perineural invasion	Absent	53 (81.5)
	Present	3 (4.6)
	Unknown	9 (13.8)
Ductal carcinoma in situ (DCIS)	Absent	34 (52.3)
	Present	23 (35.4)
	Unknown	8 (12.3)
Neoadjuvant therapy	No	28 (43.1)
	Yes	14 (21.5)
	Unknown	23 (35.4)
Multicentricity	Absent	34 (52.3)
	Present	10 (15.4)
	Unknown	21 (32.3)
Tumor focus	Unifocal	43 (66.2)
	Bifocal	15 (23.1)
	Multifocal	7 (10.7)
Sentinel lymph node metastasis	Absent	31 (49.2)
	Present	14 (22.2)
	Unknown	18 (28.6)
Survival status	Alive	48 (73.8)
	Deceased	17 (26.2)

in the left breast in 53.1% of cases. The mean tumor size was 1.78 ± 0.88 cm. 66.2% (n=43) of patients had unifocal tumors, 23.1% (n=15) had bifocal tumors, and 10.7% (n=7) had multifocal tumors.

Histological grade was assessable in 63 cases: 63.5% were grade 3 and 36.5% were grade 2; no grade 1 tumors were identified. A ductal carcinoma in situ (DCIS) component was present in 35.4% of cases. Lymphovascular invasion (LVI) and perineural invasion (PNI) were observed in 26.2% and 4.6% of cases, respectively.

Axillary lymph node status was available for 45 patients. Of these, 68.9% (n=31) had no lymph node metastasis, and 31.1% (n=14) had lymph node metastasis. Extranodal extension was detected in 10.8% of cases.

Based on immunohistochemical evaluation, PIK3CA expression was negative (0–30) in 80% of cases, moderate (31–100) in 6.2% of cases, and high (101–300) in 13.8% of cases (Figure 1).

Regarding surgical characteristics, mastectomy was performed in 47.7% of patients, excisional biopsy in 47.7%, and core needle biopsy alone in 4.6%. Both core needle and surgical excision specimens were available for evaluation in 63.1% of patients. Sentinel lymph node biopsy was performed in 30.8% of cases, with an average of two nodes removed. Neoad-

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Table 2. Comparison of clinicopathological parameters according to PIK3CA expression level in breast carcinoma.

Parameter	PIK3CA Negative (0--30) n (%)	PIK3CA Moderate--high (31--300) n (%)	p
Histologic Type†			
Invasive carcinoma of no special type (NST)	48 (73.8)	11 (16.9)	
Invasive lobular carcinoma	2 (3.1)	0 (0)	
Metaplastic carcinoma	2 (3.1)	2 (3.1)	
Age (years)			0.167
≥55	29 (44.6)	10 (15.4)	
<55	23 (35.4)	3 (4.6)	
Tumor Size (mm)			0.385
1--20	13 (20.0)	6 (9.2)	
21--49	32 (49.2)	5 (7.7)	
≥50	7 (10.8)	2 (3.1)	
Lymph Node Metastasis			0.846
Absent	25 (38.5)	6 (9.2)	
Present	11 (16.9)	3 (4.6)	
Unknown	15 (23.1)	3 (4.6)	
Stage			0.190
Early stage	29 (44.6)	9 (13.8)	
Advanced stage	6 (9.2)	0 (0)	
Menopausal Status			0.412
Premenopausal	16 (24.6)	5 (7.7)	
Postmenopausal	36 (55.4)	8 (12.3)	
Extranodal Extension			0.868
Absent	38 (58.5)	10 (15.4)	
Present	6 (9.2)	1 (1.5)	
Unknown	8 (12.3)	2 (3.1)	
DCIS Component			0.427
Absent	25 (38.5)	9 (13.8)	
Present	21 (32.3)	2 (3.1)	
Unknown	6 (9.2)	2 (3.1)	
Local Recurrence			0.632
Absent	47 (72.3)	11 (16.9)	
Present	2 (3.1)	1 (1.5)	
Unknown	3 (4.6)	1 (1.5)	
PNI			0.727
Absent	43 (66.2)	10 (15.4)	
Present	2 (3.1)	1 (1.5)	
Unknown	7 (10.8)	2 (3.1)	
Multicentricity			0.837
Absent	27 (41.5)	7 (10.8)	
Present	9 (13.8)	1 (1.5)	
Unknown	16 (24.6)	5 (7.7)	
Neoadjuvant Therapy			0.296
No	24 (36.9)	4 (6.2)	
Yes	11 (16.9)	3 (4.6)	
Unknown	17 (26.2)	6 (9.2)	

† p value not calculated due to uneven distribution of histologic subtypes.

juvant therapy was administered to 21.5% of patients (Table 1).

Among the 44 patients with available staging data, 86.4% had early-stage disease (Stage I–II) and 13.6% had advanced-stage disease (Stage III–IV). Local recurrence was observed in 4.6% (n=3) of patients. Among the 48 patients with available postoperative metastasis data, 25% developed distant metastasis. Metastatic sites were the liver (72.7%; n=8), the brain (9.1; n=1), the colon (9.1; n=1), and the vertebrae (9.1; n=1).

Association between PIK3CA expression and clinicopathological variables

No statistically significant association was found between PIK3CA immunostain expression levels and various clinicopathological parameters, including age group, tumor laterality, histological grade, lymph node metastasis, tumor size, menopausal status, type of surgery, sentinel lymph node

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Table 3. Basic clinicopathological features and PIK3CA mutation status of the breast carcinoma cases.

Case	Age (years)	Tumor size (cm)	Sentinel LN metastasis	Local recurrence	PIK3CA mutation status
1	53	5.0	No	No	Positive (H1047R)
2	50	1.6	No	No	Negative
3	38	5.7	No	No	Positive (E545K)
4	40	2.5	Yes	No	Negative
5	50	1.2	No	No	Negative
6	57	1.5	No	Unknown	Negative

Notes: - Sentinel LN: Sentinel lymph node; - "Unknown" indicates unavailable data.

biopsy status, neoadjuvant therapy, multicentricity, number of tumor foci, tumor lymphovascular invasion (LVI), perineural invasion (PNI), extranodal extension, presence of ductal carcinoma in situ (DCIS), and local recurrence (all $p>0.05$) (Table 2).

Molecular findings

PIK3CA mutation analysis was performed by PCR on six cases exhibiting high PIK3CA expression levels (101–300). Mutations were detected in 33.3% (n=2) of these cases, and 66.7% (n=4) were mutation-negative. Statistical comparison across expression categories could not be performed because mutation testing was limited to the high-expression subgroup. Clinicopathological features of these cases are summarized in Table 3.

Survival analysis

The impact of PIK3CA expression levels on survival outcomes was assessed using the Kaplan–Meier method, and differences between groups were analyzed using the log-rank test. For analytical robustness, PIK3CA expression scores were dichotomized into two groups: negative (0–30) and moderate–high (31–300). In the negative-expression group, 32.7% of patients were deceased, whereas 0% (n = 0/13) in the moderate–high-expression group were deceased. Analysis of overall survival stratified by PIK3CA expression level (0–30 vs. 31–300) revealed a statistically significant difference between the groups (Log-Rank test: $\chi^2 = 4.106$, df = 1, $p = 0.043$). Patients with moderate–high PIK3CA expression demonstrated better overall survival compared with those with negative expression (Figure 2). However, this finding should be interpreted with caution, given the small sample size of the moderate–high expression subgroup and the absence of death events in this group, which may limit the robustness of the survival comparison and increase the likelihood of an unstable estimate. Accordingly, multivariable Cox proportional hazards regression, adjusting for established prognostic factors, was considered but could not be performed reliably because of the limited number of events and complete separation (i.e., zero death events in the moderate–high expression group). Therefore, these results should be regarded as hypothesis-generating and warrant validation in larger, adequately powered cohorts.

With respect to disease-free survival (DFS), three recurrences were recorded in total: two occurred in the negative-expression group and one in the moderate-expression group. No recurrences were observed among patients with high expression levels. The DFS analysis based on PIK3CA expression categories (0–30 vs. 31–300) did not show a statistically significant difference between the groups (Log-Rank [Mantel–Cox] test: $\chi^2 = 0.293$, df = 1, $p = 0.588$). The mean DFS time was 159.8 ± 5.3 months (CI: 149.4–170.3) for the PIK3CA-negative group (0–30), and 116.8 ± 9.3 months (CI: 98.5–135.1) for the moderate–high expression group (31–300). Median DFS could not be estimated due to censoring of long-term survivors. No statistically significant association was observed between PIK3CA expression level and disease-free survival.

DISCUSSION

Chemotherapeutic agents that induce DNA damage, which are frequently used in the treatment of TNBC, have been shown to stimulate AKT phosphorylation (Protein Kinase B, PKB) through DNA damage–responsive protein kinases. In this context, several studies have demonstrated that the pro-apoptotic effects of DNA-damaging drugs are synergistically enhanced when combined with PI3K inhibitors [11–13]. These findings suggest that mutations within the PI3K pathway may shed light on the molecular basis of chemoresistance in TNBC, particularly with respect to resistance mechanisms that evolve in response to conventional chemotherapy.

Our cohort had a predominantly postmenopausal, older-age profile. While prior studies (mostly not specific to TNBC) have reported higher PIK3CA mutation rates in older and postmenopausal women, the extent to which this observation applies to TNBC remains less well established [14,15].

In the present study, the mean age of the 65 patients included was 52.4 ± 13.3 years, with approximately two-thirds (67.7%) being postmenopausal. Moreover, 60% of the patients were aged 55 years or older. This demographic profile aligns with previous reports indicating that PIK3CA mutations occur more frequently in older and postmenopausal women. Previous studies in breast cancer (not specifically TNBC) have reported higher PIK3CA mutation rates in older and postmenopausal women [14,15].

In TNBC, the reported frequency of PIK3CA mutations

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generally ranges between 5% and 16% [5], although rates exceeding 20% have been described in enriched, single-center cohorts—such as the 23.7% mutation rate reported in a PLOS ONE cohort [13]. For broader, non-selected TNBC populations, the most reliable clinically accepted prevalence remains within the 8–16% range [5].

Literature indicates that activating mutations in the PIK3CA gene are observed in approximately 18–40% of breast tumors, predominantly clustered within exon 9 and exon 20 hotspot regions [14,16]. Although these prevalence estimates are largely derived from mixed breast cancer cohorts rather than TNBC-specific series, they provide a general framework for the distribution of PIK3CA hotspot mutations. In the present study, PIK3CA mutations in exon 9 were detected in two mutation-positive cases. These alterations are known to activate the PI3K/AKT signaling pathway, thereby promoting tumor development [16,17]. Findings related to endocrine resistance (e.g., associations with anti-estrogen therapy resistance in Luminal B disease) are not directly applicable to TNBC, given the lack of hormone receptor expression; therefore, these reports are mentioned here primarily to highlight subtype-specific clinical consequences of PI3K pathway activation [15]. Similarly, evidence from HER2-positive breast cancer regarding improved efficacy with combined PI3K inhibition and HER2-targeted therapy reflects a non-TNBC treatment context [18]. In TNBC, where standard management relies heavily on chemotherapy (and, in selected settings, immunotherapy), PI3K pathway alterations may instead be most relevant to chemotherapy response and resistance mechanisms, warranting TNBC-focused validation.

In TNBC, emerging evidence suggests that chemoresistance may be linked to PI3K/AKT pathway activation, and synergistic effects have been reported when PI3K inhibitors are combined with chemotherapy [19,20]. Nevertheless, the precise prognostic significance of PIK3CA mutations and the PI3K/AKT pathway across different molecular subtypes remains unclear [21]. Overall, the PI3K/AKT pathway is recognized as both a prognostic and predictive biomarker and is increasingly gaining prominence in the landscape of targeted therapeutic strategies [22].

In our study, the mean overall survival was 63.3 months and the median survival was 46.8 months, broadly consistent with previously published estimates. For contextual reference, the SOLAR-1 trial reported overall survival of 53–64 months in patients with PIK3CA-mutant, hormone receptor–positive/HER2-negative metastatic breast cancer [23]. Given the HR-positive population and endocrine-therapy backbone of SOLAR-1, these outcomes should be interpreted as background rather than a direct comparator to a TNBC cohort. Histologically, most cases (90.8%) were invasive ductal carcinoma (IDC), consistent with the distribution reported in the literature [24]. The relatively low frequency of lobular carcinoma (3%) and the higher proportion of metaplastic carcinoma (6.2%) may be attributable to the TNBC-focused cohort in this study. The notably elevated rate of metaplastic carcinoma likely reflects its well-established association with triple-negative phenotype and inherently aggressive biological behavior [2].

When tumor characteristics were examined, the rates of multifocality and bilaterality were 13.8% and 15.4%, respectively. In the literature, multifocality has been reported in the range of 10–15%, while bilaterality has been reported between 2–11% [22]. The high proportion of grade 3 tumors (63.5%) in our cohort further supports the inherently aggressive biology of TNBC. The lymph node metastasis rate of 22.2% was at the lower end of the range reported in previous studies [25], whereas the extranodal extension rate of 10.8% was clinically remarkable. Notably, 86.4% of patients were diagnosed at an early stage (Stage I–II), which is higher than the 70–80% reported in the literature [3], and may reflect improved access to healthcare services and increased awareness. The local recurrence rate was 4.6%, which was lower than previously reported rates, whereas distant metastasis developed in 25% of patients. This finding underscores the high metastatic potential of TNBC, even in cases diagnosed at an early stage, likely due to the biologically aggressive nature of certain subtypes [26].

Based on immunohistochemical evaluation, PIK3CA expression was negative (H-score 0–30), moderate (H-score 31–100), and high (H-score 101–300) in 80%, 6.2%, and 13.8% of TNBC cases, respectively. This distribution indicates that PIK3CA overexpression is restricted to a relatively small subset of TNBC rather than representing a universal molecular feature of this subtype. Previous studies have predominantly linked PIK3CA overexpression to biologically aggressive breast cancer phenotypes, particularly basal-like, HER2-positive, and triple-negative non-basal tumors [10]. PIK3CA protein expression does not necessarily correlate with PIK3CA gene mutation status, which may be explained by post-transcriptional and post-translational regulatory mechanisms influencing protein expression and stability [13]. Interestingly, our findings demonstrated a more favorable overall survival among patients with moderate-to-high PIK3CA expression, contrasting with studies reporting associations with adverse outcomes [24]. This suggests that PIK3CA expression may identify a biologically distinct, potentially more indolent TNBC subset with prognostic relevance, in line with recent evidence demonstrating survival heterogeneity across TNBC subgroups [19]. Collectively, these data highlight that the prognostic significance of PIK3CA may vary across breast cancer subtypes and underscore its potential utility as a stratification biomarker in TNBC. Validation in larger, multi-center cohorts is warranted.

This study investigated the impact of PIK3CA protein expression levels on tumor biology and the disease course of breast cancer. Our findings demonstrated that PIK3CA expression was low in most cases, with only 13 of 65 patients (20%) show-

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ing moderate or high expression. Notably, no mortality or recurrence was observed among patients with high PIK3CA expression; however, given the small subgroup size and the low number of events, this observation should be interpreted cautiously and considered hypothesis-generating rather than evidence of a definitive favorable prognostic effect. The statistically significant difference in mortality rates between the negative and moderate–high expression groups further reinforces this observation.

In our study, PIK3CA expression did not show a significant association with conventional clinicopathological variables, including age, tumor size, stage, histological grade, menopausal status, lymph node involvement, surgical approach, or recurrence. This finding suggests that PIK3CA expression may provide additional prognostic information beyond traditional clinicopathological parameters and could reflect aspects of the tumor's molecular behavior; however, its independent prognostic value could not be established in the absence of multivariable analyses.

Although the detection of PIK3CA mutations in a subset of TNBC cases with high immunohistochemical expression supports a potential molecular association, the limited sample size precludes definitive conclusions; therefore, these should be interpreted as hypothesis-generating. Previous studies have demonstrated that PIK3CA protein expression does not consistently correlate with underlying gene mutations and may arise through mutation-independent mechanisms [10,19,20]. PCR-based mutation analysis was performed on six cases exhibiting high PIK3CA expression to preliminarily investigate the association between protein expression and mutation status. Due to technical constraints at the time of analysis, PIK3CA mutation testing could only be performed in these six cases. This was not a pre-specified selection strategy. Therefore, this mutation analysis should be considered exploratory and interpreted cautiously given the limited sample size and restricted generalizability.

In the present study, PIK3CA mutations were identified in only two cases exhibiting high protein expression, suggesting that elevated PIK3CA expression in TNBC is not uniformly driven by genetic alterations. Alternative regulatory mechanisms, including post-translational modifications, altered protein stability, and activation of the PI3K/AKT pathway independent of direct genetic mutations, have been reported in breast cancer [11,21]. Therefore, the clinical relevance of PIK3CA in TNBC should be evaluated through an integrated assessment of protein expression and molecular alterations rather than mutational status alone [19,20].

Limitations

This study has several limitations. First, the relatively small sample size and uneven distribution of cases across PIK3CA expression subgroups reduced statistical power. In particular, the moderate–high expression subgroup was small, and the absence of deaths in this group may have led to an unstable survival estimate; therefore, the observed overall survival difference should be interpreted with caution. Survival analyses were limited to univariate Kaplan–Meier estimates, as multivariable Cox proportional hazards regression adjusting for established prognostic factors could not be reliably performed due to the limited number of events and complete separation (i.e., zero events in one group). Second, its single-center, retrospective design limits the generalizability of the findings and may introduce selection bias.

Due to the retrospective nature of the study, the possibility of missing clinical or pathological data cannot be excluded. Although PIK3CA mutation analysis was performed, it was limited to cases with high expression levels. Importantly, mutation testing could be performed in only six patients due to limited technical resources at the time of analysis. Mutation testing was restricted to a small subset of high-expression cases as an exploratory, feasibility-driven assessment. Therefore, the lack of mutation testing in low-expression cases limits comparisons across expression subgroups and may introduce selection bias in mutation–expression analyses. Furthermore, the potential discordance between PIK3CA protein expression and gene mutation status adds complexity to the interpretation. Finally, some comparisons in the Discussion necessarily drew on evidence from non-TNBC breast cancer cohorts, which may limit the direct applicability of those contextual comparisons to TNBC. The inclusion of less common histological TNBC subtypes, such as lobular and metaplastic carcinomas, may have introduced biological heterogeneity. Although their inclusion reflects real-world clinical practice and does not materially alter the main findings, future studies focusing exclusively on invasive carcinoma of no special type (NST) that include larger, histologically homogeneous cohorts are warranted to validate and refine these observations. Therefore, the findings should be considered preliminary and hypothesis-generating, requiring validation in larger, multicenter prospective studies with comprehensive molecular profiling and adequately powered multivariable survival modeling [10,20].

Taken together, our findings underscore the potential utility of PIK3CA expression as a biomarker for more refined risk stratification in TNBC and further support the incorporation of molecular markers into routine pathological evaluation to facilitate more precise and personalized therapeutic decision-making. However, its independent prognostic value and clinical utility require confirmation in larger, well-characterized cohorts.

CONCLUSION

In conclusion, alterations in PIK3CA and the PI3K/AKT pathway play a key role in breast cancer. In this study, PIK3CA expression was predominantly low in TNBC cases, with high expression observed only in a small subset of patients. Notably, no mortality was detected among patients with moderate-to-high expression, suggesting a potential as-

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sociation with improved survival.

The absence of correlation between PIK3CA expression and conventional clinicopathological parameters indicates that this biomarker may be linked to more complex molecular mechanisms. Furthermore, the mismatch between protein expression and mutation status highlights that gene analysis alone may not be sufficient.

These findings support the potential prognostic value of PIK3CA expression in TNBC; however, larger multicenter studies are needed to validate these results.

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