The PIK3CA Mutation in Advanced Breast Cancer Chemoresistance and Recurrence

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Abstract

Background: The molecular mechanisms facilitating the resurgence of critical breast tumor cells and their potential to engender malignant lesions remain elusive. Several carcinogenic pathways contribute to the progression, chemoresistance, and recurrence of primary breast cancer (BC), among which is the PIK3CA mutation within the PI3K-AKT-mTOR pathway. Drug resistance poses a significant challenge to effective therapy for advanced breast cancer (ABC). This study aimed to assess the PIK3CA mutation as a diagnostic marker for disease prognosis and the prediction of treatment response. Methods: Ninety patients with advanced resistant BC were stratified into two groups: 1) 45 patients with PIK3CA mutation; 2) 45 patients with 'wild-type' PIK3CA. Patient data were collected regarding overall survival and relapse-free status. All patients received comprehensive treatment according to generally accepted standards for their respective subtypes. Results: Seventy percent of lesions associated with chemoresistance and recurrence exhibited high levels of the PIK3CA mutation. Among PIK3CA mutation lesions, 40% were HER2-positive. Analysis of immunohistochemistry data from 30 patients and matched pairings revealed that 18 patients with HER2-positive/PIK3CA mutation exhibited high expression of acquired hormone status, with 35% of patients presenting with ER-negative disease. Conclusions: Women with HER2-positive/PIK3CA mutation lesions demonstrated a threefold higher frequency of subsequent hormone status compared to those with HER2-negative/PIK3CA wild-type lesions. HER2-positive/PIK3CA mutation was associated with ER-negative disease, chemoresistance, and recurrence. These findings underscore the importance of evaluating the protein expression of HER2 and PIK3CA mutations in ABC patients post-diagnosis and intensifying anti-relapse therapy.

Keywords: PIK3CA Mutation, Advanced breast cancer, Chemoresistance, Disease recurrence.

Significance | This study demonstrated PIK3CA mutations' role in chemoresistance and recurrence informs targeted therapies, potentially improving outcomes for advanced breast cancer patients.

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Introduction

The molecular mechanisms underlying the recovery and malignant transformation of crucial breast tumor cells remain elusive. Advanced breast cancer (ABC) research has made significant strides, particularly in efforts to increase the disease-free survival rate of patients (Testa et al., 2020). Among the various genetic alterations implicated in breast cancer, mutations in the phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit alpha (PIK3CA) are notable for their role in chemoresistance and relapse (Hu et al., 2021). PIK3CA mutations can reactivate the development program of dormant tumor cells, thereby increasing recurrence. These mutations arise in response to environmental and chemotherapeutic stressors, leading to transcriptional changes that enhance tumor cell survival and diminish the activation of key

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signaling and cell transit pathways (Alqahtani et al., 2019).

One promising therapeutic approach involves the use of alpelisib in combination with fulvestrant for the treatment of postmenopausal women with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced, or metastatic breast cancer, as detected by an FDA-approved test, following disease progression or after endocrine therapy (Narayan et al., 2021). The molecular mechanisms driving ABC development, chemoresistance, and recurrence are still not fully understood, yet the PIK3CA mutation plays a crucial role in the PI3K-AKT-mTOR pathway (Ferrari et al., 2022).

Somatic mutations in the PIK3CA gene have been ed to various malignancies, including breast cancer, influencing cell proliferation, epithelial-mesenchymal transition (EMT), and stem cell properties (Peng et al., 2022; Dudas et al., 2020; Lytle et al., 2018). These mutations serve as useful biomarkers for selecting quasi-personalized therapies, thereby improving prognosis (Gupta et al., 2019). Drug resistance poses a significant challenge in the effective treatment of ABC. Over the past decade, substantial efforts have been made to investigate the mechanisms of drug resistance in human cancers and to develop strategies to sensitize cancer cells to therapies (Mattioli et al., 2023).

PIK3CA mutations have been shown to function as either tumor suppressor genes or oncogenes, modulating resistance to various treatments such as hormone therapy, anthracyclines, and taxanes (Rasti et al., 2022). Although there is a strong potential for developing PIK3CA mutation-based therapies for clinical use, approximately 70% of patients lack targeted treatment options. Research on PIK3CA mutations and chemotherapeutic resistance is still in its early stages (Rascio et al., 2021). Akt, downstream of PI3K, regulates cell proliferation, metabolism, differentiation, apoptosis, and cancer (Elshazly & Gewirtz, 2022). In patients with overexpressed HER2, PIK3CA mutations have been associated with increased activation of the PI3K/Akt pathway, contributing to treatment resistance (Kaboli et al., 2021).

It is crucial to explore how PIK3CA mutations, as critical genetic alterations in drug resistance mechanisms, impact the response to preoperative breast cancer treatment (Study Assessing the Efficacy and Safety of Alpelisib Plus Fulvestrant, 2023). This review details the functional pathways associated with PIK3CA mutations in ABC and their therapeutic potential for recurrent disease. The objective is to evaluate PIK3CA mutations as diagnostic markers for disease prognosis and predictors of treatment efficacy.

2. Material and Methods

2.1 Study design:

A total of 90 advanced resistant breast cancer patients were included in the study. Patients were divided into two groups: Group 1: 45 patients with PIK3CA mutation and Group 2: 45 patients with 'wild-type' PIK3CA. The overall survival (OS) and relapse-free survival (RFS) of these patients were compared. All patients received comprehensive treatment according to generally accepted standards based on their breast cancer subtype. The Institutional Bioethics Committee of the SNE "National Cancer Institute" of Ukraine approved all patient protocols (Minutes No. 163, June 23, 2020). All patients involved in the study provided informed consent, and identifying information was redacted to maintain confidentiality.

2.2 DNA Isolation and Sequencing:

DNA was isolated from frozen core biopsies using the AllPrep DNA/RNA Mini kit (Qiagen, Hilden, Germany). DNA concentration was measured using the Qubit 2.0 Fluorometer (Quant-iTTM dsDNA BR Assay Kit; Thermo Fisher Scientific, Les Ulis, France).

2.3 Next-Generation Sequencing (NGS):

Next-generation sequencing (NGS) was utilized to detect hotspot mutations in exons 2, 5, 10, 14, and 21 of the PIK3CA gene. The sequencing procedure comprised several stages: first, a 17-cycle PCR amplification using 10 ng of DNA; second, digestion of amplicons using the FuPA enzyme to remove primer sequence extremities; and finally, analysis of sequencing depth (>100 reads), with a requirement of 6% coverage for known single nucleotide variants/mutations and 11% coverage for known insertions or deletions (indels).

2.4 Survival Analysis:

The Kaplan-Meier method was used to evaluate the statistical significance of differences between groups. The log-rank test assessed the risk associations of various clinical, histologic, and pathologic characteristics with hormone status after primary chemotherapy. Hazard ratios (HR) with 95% confidence intervals were calculated. For variables with more than two categories, a P-value of 0.05 was considered statistically significant.

2.5 Data Analysis:

Variants were identified using ANNOVAR and the datasets COSMIC68, dbSNP137, 1000 genomes, ESP6500, and RefGene. Somatic mutations were defined as non-synonymous changes not observed in more than 0.1% of the population (1000 genomes and ESP6500). All somatic mutations were discovered, classified, and evaluated by an expert molecular scientist using available datasets (COSMIC, The Cancer Genome Atlas).

2.6 Prognosis Accuracy:

The prognosis accuracy of the PIK3CA mutation-based classifier was assessed using time-dependent receiver operating characteristic (ROC) analysis with the 'survivalROC' R package.

2.7 Cumulative Incidence and OR Estimation:

The cumulative incidence of relevant hormone status by HER2 and PIK3CA mutation status was estimated using the hormone status

Table 1. Patients distribution based on their immunehistochemistry status in both groups.(p<0,05).

Subtypes	1st group, PIK3CA mutation		2nd group, PIK3CA "wild"	
	n	%	n	%
Lum A	3	6.67±1.18	4	8.89±1.56
Lum B	8	17.78±3.92	17	37.57±5.65
Triple negative	18	40.00±6.93	10	22.44±5.08
Her2/positive	12	26.66±5.32	12	26.66±5.32
Her2/low	4	8.89±1.56	2	4.44±0.84
All	45	100.00	45	100.00

Figure 1. Dynamics of the final proportion of viable tumor tissue (FPVTT)

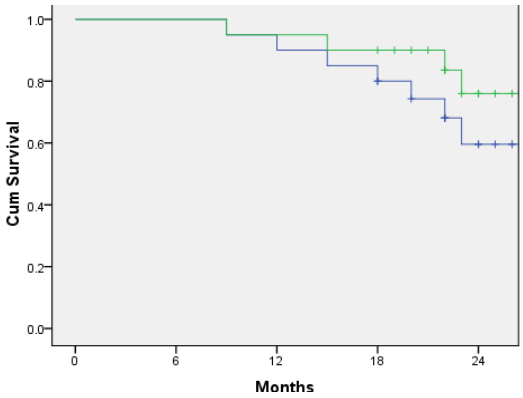

Figure 2. Cumulative survival, 3 years:

1) patients with HER2 negative/PIK3CA "wild" compared to
2) patients HER2 positive/PIK3CA mutation

odds ratios (ORs) for HER2 and PIK3CA mutation status. The relationship between therapeutic effect and biological features was evaluated, defining pathological therapeutic effect grades 2 or 3 as sensitive and grades 0 or 1 as resistant.

Evaluation of Therapeutic Effect:

PIK3CA mutation and its potentially related markers of immunohistochemical (IHC) subtype in breast cancers were assessed to determine their relevance to clinical outcomes after neoadjuvant chemotherapy (NC).

***2.8 Statistical Analysis*:**

All statistical tests were performed using the R program (Version 3.5.0). Categorical variables were determined using the chi-squared ($\chi 2$) test or two-sided Fisher's exact test. The expected cumulative incidence of breast cancer for the study population was calculated using the Hakulinen method from age-specific breast cancer incidence and all-cause mortality rates in the Ukrainian female population.

3. Results

The immunohistochemical findings of biopsies indicated that patients with Lum B1 subtype showed mean percentages of 17.78±3.92% and 37.57±5.65% in the first and second groups, respectively. Among triple negative patients, the first group exhibited 40.00±6.93%, while Her2-positive patients showed similar percentages in both groups (26.66±5.32%). For Her2-low patients, the percentages were 8.89±1.56% and 4.44±0.84% in the first and second groups, respectively (Table 1).

The final proportion of viable tumor tissue (FPVTT), a key indicator of morphological response to therapy, was comparable between the two groups following neoadjuvant chemotherapy. The first group had an FPVTT of 22.9 ± 4.2%, whereas the second group measured 24.8 ± 5.1% ($p>0.05$) (Figure 1).

Analysis revealed that PIK3CA mutation was strongly associated with negative hormone status (OR = 2.53; 95% CI, 1.18-4.16), while HER2 overexpression was ed with positive hormone status (OR = 1.54; 95% CI, 1.19-2.08). Patients with both HER2-positive and PIK3CA mutation had a twofold higher likelihood of negative hormone status compared to those with HER2-negative and PIK3CA wild-type status, with an estimated 15.9% cumulative risk of recurrence at 3 years (Figure 2). Thus, HER2 overexpression and PIK3CA mutation were identified as positive indicators associated with recurrence.

Disease association analysis revealed that combined HER2 overexpression and increased PIK3CA mutation were significantly related to disease progression (LR $\chi 2= 6.12$; OR = 3.98). Conversely, the combination of HER2 overexpression with PIK3CA wild-type status also showed a significant association with relevant hormone status, albeit with a lower odds ratio (LR $\chi 2 = 5.22$; OR = 3.16).

Seventy percent of lesions associated with chemoresistance and recurrence (42 patients) exhibited high levels of PIK3CA mutation. Among these PIK3CA mutations, 40% were HER2-positive and 35% were not.

Furthermore, an analysis of immunohistochemical data from 24 patients showed that 12 patients with HER2-positive/PIK3CA mutation exhibited high expression and acquired hormone resistance, with 26.67% of these patients demonstrating ER-negative status, indicating development of chemoresistance and subsequent recurrence.

4. Discussion

The investigation into the effectiveness of alpelisib in the SOLAR-1 study underscores its role in managing HR-positive, HER2-negative advanced or metastatic breast cancer refractory to aromatase inhibitors. This pivotal trial demonstrated promising outcomes for patients with PIK3CA mutations, leading to FDA priority review status and the clearance of diagnostic tools like the therascreen PIK3CA RGQ PCR Kit by Qiagen Manchester, Ltd. This study aligns with our findings that PIK3CA mutations are pivotal in breast cancer pathogenesis, contributing to disease progression, chemoresistance, and recurrence.

Our results highlight the association between PIK3CA mutations and the transformation of breast cancer cells, promoting aggressive features such as increased microenvironmental stiffness. This correlation supports previous research indicating that PIK3CA mutations portend a poorer prognosis in breast cancer, particularly in triple-negative subtypes. Our observations are consistent with studies by Cho et al., Nedeljković et al., and Fultang et al., which underscore PIK3CA's role in fostering chemoresistance and the development of aggressive phenotypes under environmental stressors typical of triple-negative breast cancer.

Furthermore, recent studies by Kim et al. have implicated PIK3CA mutations in reduced pathologic complete response rates and resistance to neoadjuvant anti-HER2 therapies in HR-positive/HER2-positive breast cancer. These findings raise critical considerations regarding optimal treatment strategies for HER2+/ER+ cancers harboring PIK3CA mutations, given the pathway's involvement in hormonal dependency escape mechanisms.

The prevalence of PIK3CA mutations is notably higher in aggressive primary tumor subtypes like Basal and Luminal B compared to indolent Luminal A subtypes. In patients with advanced breast cancer, these mutations contribute significantly to treatment resistance and disease recurrence, activating latent cancer cells to recurrent competence. Studies, such as those by Ben Rekaya et al., have suggested that PIK3CA mutations may serve as diagnostic indicators to distinguish between aggressive and non-

aggressive breast cancer subtypes, offering potential insights into disease progression and treatment response.

5. Conclusion

Women harboring HER2-positive/PIK3CA mutation lesions exhibited a threefold higher incidence of subsequent hormone status compared to those with HER2-negative/PIK3CA wild-type lesions. This association underscores the propensity of HER2-positive/PIK3CA mutation status to correlate with ER-negative disease, fostering chemoresistance development and subsequent recurrence.

Our study emphasizes the critical need for assessing HER2 protein expression and PIK3CA mutation status in patients with advanced breast cancer (ABC) at the time of diagnosis. Such evaluations are crucial for tailoring anti-relapse therapies effectively. Conversely, patients with PIK3CA wild-type lesions showed chemoresistance and recurrence risks similar to those observed in the general population. These insights highlight the potential clinical utility of molecular profiling in guiding personalized treatment strategies for ABC patients.

Author contributions

M.O.V. conceptualized the study, administered the project, and drafted the original manuscript. M.O.V. and L.A.O. conducted the investigation and contributed to writing and editing the manuscript. S.I.I. and L.A.O. curated the data. D.I.V., L.A.D., and I.O.M. validated the findings. M.O.V., L.A.O., and L.A.D. performed formal analysis. M.O.V. and L.A.D. were responsible for software development. All authors reviewed and edited the manuscript and approved the final version for publication.

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Competing financial interests

The authors have no conflict of interest.

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