



Evaluation of Somatic *PIK3CA* Mutations Detected by Next-generation Sequencing in Breast Cancer Cases

Meme Kanserli Olgularda Next-generation Sequencing ile Saptanan Somatik *PIK3CA* Mutasyonlarının Değerlendirilmesi

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Abstract

Objective: There have been new developments in determining the development, treatment, and prognosis of breast cancer. In particular, determining the characteristics of breast cancer at the molecular level has become crucial in the initiation of new therapies. In recent years, the detection of *PIK3CA* mutations in breast cancer, as in many types of cancers, and especially in cases that have become resistant to treatment, is guiding the use of new targeted drugs. Therefore, the aim of this study was to evaluate *PIK3CA* mutations in patients with breast cancer.

Materials and Methods: In this study, *PIK3CA* mutations were detected using next-generation sequencing technology applied to paraffin-fixed, paraffin-embedded samples of primary tumor tissue from 110 breast cancer patients who did not receive neoadjuvant treatment previously. The relationship between *PIK3CA* mutation and tumor molecular subtypes, immunohistochemical estrogen receptor (ER), progesterone receptor (PR), c-erbB2, Ki-67 staining, and human epidermal growth factor 2 (HER2)Neu status detected by fluorescence *in situ* hybridization were investigated.

Results: The *PIK3CA* mutation was found in 21 (19.1%) cases. A significant positive correlation was detected between ER, PR, and luminal A type and *PIK3CA* mutations ($p<0.05$). *PIK3CA* mutation was not observed in any case with triple negative type. No statistically significant correlation was found with other clinicopathological parameters. The most common *PIK3CA* mutation subtypes were H1047R and E542K.

Conclusion: The results of our study showed that *PIK3CA* mutations were observed at significantly higher rates in hormone receptor-positive patients, but *PIK3CA* mutations may be less frequently observed in HER2+ patients.

Keywords: Breast cancer, hormone receptors, HER2Neu, NGS, *PIK3CA* mutations

Öz

Amaç: Meme kanseri gelişimi, tedavisi ve prognozunu belirlemede günümüzde yeni gelişmeler yaşanmaktadır. Özellikle moleküler düzeyde meme kanserinin özelliklerini belirlemek yeni tedavilerin kullanımında çok önemli hale gelmiştir. Son yıllarda *PIK3CA* mutasyonlarının tespiti, birçok kanser tipinde olduğu gibi meme kanserlerinde ve özellikle tedaviye direnç kazanan olgularda hedefe yönelik yeni ilaçların kullanımında yol göstericidir. Bu yüzden bu çalışmada meme kanserli olgularda *PIK3CA* mutasyonları değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada meme kanseri tanısı alan ve neoadjuvan tedavisi bulunmayan 110 olgunun primer tümör dokusuna ait "paraffin-fixed, paraffin-embedded" örneklerinde *PIK3CA* mutasyonları next-generation sequencing ile saptanmıştır. Olgularda *PIK3CA* mutasyonu ile tümör moleküler alt tipleri, immunohistokimyasal östrojen reseptörü (ER), progesteron reseptörü (PR), c-erbB2, Ki-67 boyamaları ile FISH ile saptanan epidermal büyüme faktörü reseptörü 2 (HER2)Neu durumu arasındaki ilişki araştırılmıştır.

Bulgular: Toplam 21 olguda *PIK3CA* mutasyonu (%19,1) bulunmuştur. ER, PR ve Luminal A tip ile *PIK3CA* mutasyonları arasında pozitif anlamlı ilişki saptanmıştır ($p<0,05$). Triple negatif tipte hiçbir olguda *PIK3CA* mutasyonu görülmemiştir. Diğer klinikopatolojik parametreler ile istatistiksel olarak anlamlı ilişki bulunmamıştır. En sık *PIK3CA* mutasyon alt tipleri H1047R, E542K'dir.

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Sonuç: Çalışmamız sonuçları hormon reseptörleri pozitif olgularda anlamlı derecede yüksek *PIK3CA* mutasyonu bulunduğunu ancak HER2+ olgularda da *PIK3CA* mutasyonlarının daha az sıklıkta görülebileceğini göstermiştir.

Anahtar Kelimeler: Meme kanseri, hormon reseptörleri, HER2Neu, NGS, *PIK3CA* mutasyonları

Introduction

Nowadays, classification and management of breast cancer are guided by histopathological grade, stage, metastasis status of the tumor, as well as molecular subtypes that can be determined by overexpression/amplification of immunohistochemical (IHC) histopathological parameters which provide prognostic information and predict response to treatment including hormone receptor profile, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2) and their molecular subtypes which can be determined by Ki-67 Proliferation index (1,2). The evolution of novel escape mechanisms in advanced breast cancer patients and the growing utilization of targeted therapies have altered the course of hormone receptor positive (HR+) breast cancers (3). Although hormone therapy has revolutionized the treatment of breast cancer and outcomes have significantly improved in these patients, optimal management remains a significant challenge (4). A subset of HR+ cases either exhibit resistance to endocrine therapy or develop endocrine-resistant disease (5). In the treatment-resistant setting, it is important to identify molecular somatic alterations. It has become a clinical necessity to identify new predictive biomarkers for current standard therapies and also to find new therapies (4). The present emphasis on molecular pathological testing has become a valuable supplementary prognostic tool, guiding the incorporation of chemotherapy alongside endocrine therapy. The treatment landscape for HR+ breast cancer is experiencing a significant transformation with a variety of targeted individualized therapies, such as phosphoinositide 3-kinase (PI3K) inhibitors. These therapies are now approved for use in combination with endocrine therapies, thanks to the progress made in molecular identification (3). PI3K initiates the activation of AKT and mTOR downstream, creating the PI3K/AKT/mTOR signaling pathway. This pathway plays a crucial role in regulating various cellular activities, often leading to cellular growth, metabolism, proliferation, and survival (6,7). The PI3K/AKT/mTOR pathway is activated in various tumor types, and its therapeutic targeting has garnered significant interest from the research community. The gene encoding the catalytic alpha (α) subunit of PI3K, i.e., *PIK3CA* (OMIM#17184), is prevalently detected activating mutations in breast cancer and *PIK3CA* mutations have been reported in a total of 40% of patients with ER+ breast cancer (8). The prognostic effect of *PIK3CA* mutations may differ between breast cancer subtypes (3). *PIK3CA* has been proposed as a favorable prognostic biomarker and is associated with positive survival outcomes in patients diagnosed with early-stage HR+/HER2- breast cancer (6,9,10). While the frequency of *PIK3CA* mutations may not significantly differ

between early-stage and metastatic HR+/HER2- breast cancer, studies have demonstrated that metastatic breast cancer patients with *PIK3CA* mutations experience worse prognosis and show resistance to chemotherapy (11). In this study, we investigated *PIK3CA* status and its association with some known clinicopathologic features in breast cancer patients using next generation sequencing (NGS) technology.

Materials and Methods

The study included 110 patients who were diagnosed with breast cancer, without a history of neoadjuvant treatment in whom NGS was performed at the Department of Pathology, Aydın Adnan Menderes University Faculty of Medicine, between 2017-2018 were screened. ER, PR, c-erbB2, Ki-67 IHC staining results and also Her2-Neu results determined by fluorescence in situ hybridization (FISH) method, age, TNM stage, Modified Bloom Richardson grade of all cases were recorded.

Histopathologic examination; hematoxylin eosin (HE) stained 4- μ m thick sections prepared from tissues embedded in paraffin blocks after routine tissue follow-up, and fixed in 10% neutral buffered formalin were examined and evaluated under a light microscope (BX51, Olympus, Tokyo, Japan) at x10, x20 and x40 magnifications. From the blocks containing invasive tumoral areas, 4- μ m thick sections were placed on positively charged slides for IHC studies. The study protocol was approved by the Aydın Adnan Menderes University Local Ethics Committee (protocol no: 2023/172, date: 07.09.2023).

IHC Staining and Evaluation

In IHC staining performed using ER, PR, c-erbB-2, Ki-67 stains, DAKO Autostainer Universal Staining System (Autostainer Link 48 DAKO, Glostrup, Denmark) was used. After staining, the sections were observed using a light microscope (Olympus BX51, Tokyo, Japan) at magnifications of 4X, 10X, 20X, and 40X by the same pathologist. The immunostaining scoring was determined based on both the intensity of staining and the percentage of cells that exhibited staining. Staining intensity in the invasive tumoral area was scored ranging from 0 to +3 (no staining: 0, mild: +1, moderate: +2, intense: +3). Percentage of stained cells in the invasive tumor area was scored similarly.

HER2 FISH Application and Evaluation

HER2 amplification was evaluated by FISH method with immunofluorescence microscope using probe sets [*HER2* FISH pharmDx (Dako)].

HER2 FISH was performed on an Olympus BX50 binuclear fluorescence microscope under triple (dapi/red/green) and

dual (red/green) filters and at least 20 tumor cell nuclei were evaluated in each tissue, taking care not to count non-tumor cell nuclei. Of the 20 interphase nuclei analyzed, if the sum of the number of red signals divided by the sum of the number of green signals was less than two, it was considered as “no amplification”, and if it was greater than or equal to two, it was considered as “amplification”.

Next Generation Sequencing

DNA Isolation from Formalin-Fixed Paraffin Embedded (FFPE) Tissues

Tumor areas were marked by the pathologist and 10 µm-thick DNA was isolated using a Qiagen FFPE DNA tissue extraction kit according to the manufacturer’s instructions.

Pre- and Sequencing Stages for NGS

This step was conducted using the MiniSEQ NGS platform (MiniSEQ, MN00676, Illumina, Singapore) using a manufacturer protocol optimized with the QIAseq-targeted Breast Cancer Panel (DHS-005Z, Qiagen, Strasse, Hilden, Germany) (Table 1). The FFPE DNA fragment obtained after isolation, which ranged from 100-150 ng, underwent an end repair process. Target enrichment process and libraries were amplified by polymerase chain reaction (PCR) using the Labcycler from Sensoquest GmbH (Göttinger, Germany). Subsequently, barcoding and library preparation were carried out. The libraries were amplified using PCR on the Labcycler from Sensoquest GmbH and then purified for target enrichment. At the clonal amplification step, the target-enriched library was sequenced on MiniSEQ NGS platforms utilizing a MiniSEQ High Output Reagent Cartridge (Illumina, San Diego, CA, USA).

Data Analysis

The NGS results were subjected to data analysis and quality control using the Qiagen Clinical Insight analysis universal commercial software. After data quality control, variants were imported into the Qiagen Clinical Insight interpretation web interface, which enables data interpretation for predefined variants. The selected variants were analyzed by expert physicians experienced in molecular medicine

to demonstrate, and evaluate the impact of these variants on validation of diagnosis, clinical effects, and treatment protocols using bioinformatics software tools [(CADD(v1.3), Allele Frequency Ensemble, EVS (ESP6500SIV2), RefSeq gene model, JASPAR, Vista Rviewer hg18 or hg19 builds) and clinical trials were analyzed in correlation with the disease phenotype using (Stepford 181112. 001)], PolyPhen-2, 1,000 genome frequency (phase3v5b) softwares. Genomic variations within patients were identified on the Qiagen reporter and QIAGEN Clinical Insights Browser platforms.

Statistical Analysis

Mean and percentage were used for descriptive statistics. Statistical Analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was used for categorical variables and Student’s t-test was used to compare independent data. Results were considered significant at $p < 0.05$.

Results

Our patients with an average age of 46 years (range: 26-78 years) were either over ($n=87$; 79.09%) or under 40 ($n=23$; 20.90%) years old. Clinicopathologic features of the cases are given in Table 2.

ER was positive in 91 (82.7%) and negative in 19 (17.3%) patients. PR was positive in 84 (76.4%) and negative in 26 (23.6%) patients. In the IHC and HER2 FISH study, HER2Neu positivity, and negativity were detected in 33 (30%) and 77 (70%) cases, respectively. Ki-67 proliferation index was above, and below 15% in 56 (50.9%) and 54 (49.1%) patients, respectively. According to these results, respective number of patients were in the Luminal A ($n=63$; 57.3%), Luminal B ($n=28$; 25.5%), triple negative ($n=14$; 12.7%), and Her2Neu positive ($n=5$; 4.5%) groups.

In the PIK3CA mutation analysis evaluated in the NGS study, pathogenic PIK3CA mutation was observed in 21 (19.1%) of 110 cases, while in 89 (80.9%) cases pathogenic mutation was not detected. The most common PIK3CA mutation was detected in Luminal A type cases. The most commonly observed hotspot mutations were p. H1047R (Exon 20) in 12 (57.2%) and p.E542K (Exon 9) in 4 cases (19%). In addition, E726K (Exon 13) mutation was found in 2 (9.5%), p.Q546 (Exon 9) mutation in 2 (9.5%) and p.M1043V (Exon 20) mutation in 1 (4.8%) case.

The distribution of different PIK3CA mutations found in the cases according to molecular subtypes is shown in Table 3.

In our study, a statistically and significantly positive correlation was detected between PIK3CA, ER, PR ($p=0.028$ and $p=0.041$) and Luminal A type ($p=0.039$), in cases where pathogenic PIK3CA mutation was observed, while the correlations between different mutation types in cases with mutations and other molecular types, age, tumor size, grade, stage, metastasis, metastasis site, Ki-67, Her2Neu were not statistically significant ($p > 0.05$).

Table 1. QIAseq-targeted breast cancer panel list

PIK3CA, AR, APC, MLH1, ACVR1B, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRIP1, CASP8, CBF, PALD, CHEK2, HERC1, CDH1, ATR, CCND1, CDK4, CTNNB1, BRCA1, IRAK4, BRCA2, GATA3, MSH2, EGFR, PTEN, EP300, ERBB3, ERCC4, EXOC2, EXT2, FAM175A, ANCC, FBXO32, FGFR1, FGFR2, PBRM1, HOXB13, ESR1, ERBB2, KRAS, MDM2, MED12, MEN1, MRE11A, MUC16, MUTYH, DIRAS3, MYC, NBN, NCOR1, NEK2, NF1, PALB2, PCGF2, RB1, PPM1L, ITCH, RET, SMAD4, RAD51D, RAD50, CSMD1, TP53, PMS2, ATM, SEPT9, MAP2K4, SMAD4, SMARCA4, STK11, SYNE1, TGFB1, TRAF5, ZBED4, VHL, WEE1, PDGFR, EPCAM, MAP3K1, PMS1, XRCC2, XRCC3, CDK6, AKT1, CDKN2A, RAD51C, MSH6, PIK3R1, GEN1, RAD51, TGFB2

Discussion

Great advances have been made in the development, prognosis and treatment of breast cancer in recent years. The molecular classification of breast cancers has brought different treatment options to the agenda, but some cases of these cancers are still treatment-refractory or show disease progression (1). In such cases, the search for new markers that will make hormone therapy and targeted therapies more

predictable continues. Today, with the development and widespread use of NGS technologies, new steps are being taken to predict the response to the treatment outcomes and prognosis for patients with breast cancer (12). Disclosing somatic mutations in cancer cells reveals important results in approaching cases before and after treatment. The use of PI3K inhibitors, which are among targeted therapies, is becoming widespread in breast cancer, the most common cancer observed in women worldwide. PI3K/Akt/mTOR pathway gains importance especially in HR+, HER2-negative metastatic patients refractory to hormone therapy that occurs with various pathogenetic mechanisms. In this case, the use of *PIK3CA* inhibitors such as alpelisib has been approved and the detection of *PIK3CA* mutations in various specimens using various techniques has gained importance (3). There are various publications in the literature on breast cancer and *PIK3CA* mutations (3). These publications focus especially on technical methods and mutation types. There are also studies evaluating *PIK3CA* mutations in breast cancer subtypes. PI3K plays a role in many cellular processes including protein synthesis, cell proliferation and DNA repair (13,14). *PIK3CA*, one of the PI3K enzyme isoforms, has been shown to be associated with cancer development, progression and drug resistance. This relationship has also been shown in breast cancers, especially in HR+/HER2- tumors. Although there are conflicting results in the literature on the use of fresh tumor tissue samples, FFPE tissue samples as well as liquid biopsies in the detection of *PIK3CA* mutation, it is seen that use of all these samples yielded comparably successful rates of mutation detection. There are also comparative studies in the literature on the use of methods such as PCR, Sanger Sequencing and NGS in mutation detection (15). In some studies, mutations were found at slightly higher rates especially when Sanger Sequencing and NGS technologies were used. In our study, FFPE tissue samples were used with NGS method and *PIK3CA* somatic mutation was found in 19.1% of the cases. This rate is similar to the 20-30% *PIK3CA* mutation rates reported in the literature in all breast cancers (16). However, some differences in the methodologies used in the studies cited in the literature, especially those employed in the study of samples after neoadjuvant treatment or in the evaluation of metastatic tissue samples may cause differences in reported *PIK3CA* somatic mutation rates. The results of our study should be considered important in terms of showing

Table 2. Clinopathological characteristics of the cases included in the study

Age (years)	Median (minimum-maximum) 46 (26-78)
Length (cm)	4.5 (1.3-10.5)
Tumor grades (modified Bloom Richardson grading system)	n (%)
Grade 1	2 (2.1%)
Grade 2	60 (63.8%)
Grade 3	32 (34.1%)
Stage (TNM)	
pT1 and pT2	80 (72.7%)
pT3 and pT4	30 (27.3%)
Metastasis	
Yes	44 (40%)
No	66 (60%)
Metastatic region	
Lymph node	35 (79.5%)
Distant organs (bone, lungs, liver etc.)	9 (20.5%)
Molecular subtype	
Luminal A	63 (57.3%)
Luminal B	28 (25.4%)
HER2 positive	5 (4.6%)
Triple negative	14 (12.7%)
TNM: Tumor, node, and metastasis	

Table 3. Distribution of 21 different *PIK3CA* mutations according to molecular subtypes

	p.E542K	p.H1047R	p.Q546	p.E726K	p.M1043V	Total (n=21)
Luminal A (n=63)	2	9	2	2	1	16 (76.2%)
Luminal B (n=28)	1	3				4 (19.1%)
HER2 positive (n=5)	1					1 (4.70%)
Triple negative (n=14)						0
Total	4 (19%)	12 (57.2%)	2 (9.5%)	2 (9.5%)	1 (4.8%)	21

the *PIK3CA* somatic mutation rate on a regional basis in samples retrieved from the patients that did not receive neoadjuvant treatment and studied with NGS technology using primary tumor tissues. According to the molecular classification of breast cancer cases, significant differences emerge between subtypes. The high *PIK3CA* mutation rate in HR+/HER2- tumors, which has been reported in many studies in the literature, was also indicated by the results of our study. According to the results of our study, 76.2% of all cases with *PIK3CA* mutation were in the HR+/HER2- group classified as Luminal A and *PIK3CA* mutation was detected in 25% of Luminal A cases. A significant positive correlation was found between Luminal A type and *PIK3CA* mutation in breast cancer patients. In addition, the results of our study have shown that there is a significant positive correlation between ER and PR positivity in tumor cells and *PIK3CA* mutation. These findings support the relationship between *PIK3CA* mutation and HR+ cases reported in the literature. In the Luminal B group, where *PIK3CA* was detected with the second frequency, the *PIK3CA* mutation rate was 19.1%. Considering that HER2- positive cases were found in this group, apparently *PIK3CA* mutations may be detected at a considerable rate in HR+/HER2+ breast carcinoma cases. Although similar results have been reported in the literature, studies on *PIK3CA* mutation have been mostly focused on HR+/HER2- cases and HR+/HER2+ breast carcinoma cases may be overlooked. It is thought that evaluation of *PIK3CA* mutation should be performed independently of *HER2*, especially in cases refractory to hormone therapy. In our study, although *PIK3CA* mutation was not found in 14 triple-negative cases, mutation was found in 1 out of 5 cases in the HER2+ group, which supports our assessment. In our study, the most frequently observed somatic mutations in *PIK3CA* were p.H1047R and p.E542K. In addition, p.Q546, p.E726K, p.M1043V mutations were also found in *PIK3CA*. These results are similar to the literature. The results of the studies performed hitherto have shown that p.H1047R mutation is the most common mutation, followed by p.E545K mutation (16,8). There are also studies suggesting that these mutation types are differently associated with breast cancer molecular subtypes (3). In our study, although p.H1047R was the most common mutation observed in Luminal A and B subtypes, this relationship was not found to be statistically significant. In recent years, the detection of effective *PIK3CA* mutations has become increasingly important. Therefore, the evaluation of different *PIK3CA* mutations, which can be detected less frequently in a large number of cases may lead to the creation of new and effective treatment options in cases resistant to hormone therapy (4).

Our study shows the importance of *PIK3CA* mutations in hormone receptor-positive cases in breast cancers and draws attention to *PIK3CA* mutations in targeted drug therapies. The limitation of our study is that more patients can be included in our study in order to obtain more meaningful results.

Conclusion

In our study, we have observed that 19.1% of 110 breast cancer patients had *PIK3CA* mutations regardless of subtypes, and 25% of these mutations were in Luminal A type. However, *PIK3CA* mutations were not detected in any patient with triple negative type breast cancer. In addition, considerable rates of *PIK3CA* mutations were detected in HER2+ cases. In our study, similar results with the literature were obtained in the evaluation of *PIK3CA* somatic mutations in FFPE tumor tissue samples using NGS method in a relatively small number of breast cancer patients.

Ethics

Ethics Committee Approval: The study protocol was approved by the Aydın Adnan Menderes University Local Ethics Committee (protocol no: 2023/172, date: 07.09.2023).

Informed Consent: Informed consent is not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.H.E., Concept: İ.H.E., D.G., Design: İ.H.E., D.G., Data Collection or Processing: İ.H.E., Analysis or Interpretation: İ.H.E., Literature Search: İ.H.E., D.G., Writing: İ.H.E.

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References

1. Fusco N, Malapelle U, Fassan M, Marchiò C, Buglioni S, Zupo S, et al. *PIK3CA* Mutations as a Molecular Target for Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer. *Front Oncol* 2021; 11: 644737.
2. Pala EE, Bayol Ü, Cumurcu S, Keskin E. Triple-Negatif/Bazal Benzeri Meme Kanserinin İmmünohistokimyasal Özellikleri [Immunohistochemical characteristics of triple negative/basal-like breast cancer]. *Türk Patoloji Derg* 2012; 28: 238-44.
3. Anderson EJ, Mollon LE, Dean JL, Warholak TL, Aizer A, Platt EA, et al. A Systematic Review of the Prevalence and Diagnostic Workup of *PIK3CA* Mutations in HR+/HER2- Metastatic Breast Cancer. *Int J Breast Cancer* 2020; 2020: 3759179.
4. Mosele F, Stefanovska B, Lusque A, Tran Dien A, Garberis I, Droin N, et al. Outcome and molecular landscape of patients with *PIK3CA*-mutated metastatic breast cancer. *Ann Oncol* 2020; 31: 377-86.
5. Vasan N, Toska E, Scaltriti M. Overview of the relevance of PI3K pathway in HR-positive breast cancer. *Ann Oncol* 2019; 30(Suppl_10): x3-11.
6. Freeland A, Brown LJ, Parker A, Segara D, Portman N, Lau B, et al. Molecular Biomarkers for Contemporary Therapies in Hormone Receptor-Positive Breast Cancer. *Genes (Basel)* 2021; 12: 285.
7. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* 2015; 15: 7-24.

8. Arsenic R, Treue D, Lehmann A, Hummel M, Dietel M, Denkert C, et al. Comparison of targeted next-generation sequencing and Sanger sequencing for the detection of *PIK3CA* mutations in breast cancer. *BMC Clin Pathol* 2015; 15: 20.
9. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, et al. *PIK3CA* mutation associates with improved outcome in breast cancer. *Clin Cancer Res* 2009; 15: 5049-59.
10. Zardavas D, Te Marvelde L, Milne RL, Fumagalli D, Fountzilias G, Kotoula V, et al. Tumor *PIK3CA* Genotype and Prognosis in Early-Stage Breast Cancer: A Pooled Analysis of Individual Patient Data. *J Clin Oncol* 2018; 36: 981-90.
11. Mosele F, Stefanovska B, Lusque A, Tran Dien A, Garberis I, Droin N, et al. Outcome and molecular landscape of patients with *PIK3CA*-mutated metastatic breast cancer. *Ann Oncol* 2020; 31: 377-86.
12. Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, et al. An integrated semiconductor device enabling non-optical genome sequencing. *Nature* 2011; 475: 348-52.
13. Venetis K, Sajjadi E, Haricharan S, Fusco N. Mismatch repair testing in breast cancer: the path to tumor-specific immuno-oncology biomarkers. *Transl Cancer Res* 2020; 9: 4060-4.
14. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* 2015; 15: 7-24.
15. Papaxoinis G, Kotoula V, Alexopoulou Z, Kalogeras KT, Zagouri F, Timotheadou E, et al. Significance of *PIK3CA* Mutations in Patients with Early Breast Cancer Treated with Adjuvant Chemotherapy: A Hellenic Cooperative Oncology Group (HeCOG) Study. *PLoS One* 2015; 10: e0140293.
16. Shimoi T, Hamada A, Yamagishi M, Hirai M, Yoshida M, Nishikawa T, et al. *PIK3CA* mutation profiling in patients with breast cancer, using a highly sensitive detection system. *Cancer Sci* 2018; 109: 2558-66.