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RESULTS OF MULTICENTER TESTING OF PIK3CA SOMATIC MUTATIONS IN HORMONE-RECEPTOR POSITIVE HER2-NEGATIVE ADVANCED BREAST CANCER

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Summary

Aim: Activating somatic mutations in p110α catalytic subunit of PIK3-kinase (PIK3CA) are present in about 40% of breast cancers (BC), and are involved in oncogenesis and cancer growth. PIK3CA inhibitor therapy is approved for patients with advanced HR+/HER2- endocrine-resistant BC with somatic *PIK3CA* mutations. Such targeted therapy improved patient disease-free survival.

Method: Analyses of *PIK3CA* gene mutations were performed in five University Hospital Centers in Croatia. Qualitative detection of *PIK3CA* gene mutations was performed on DNA extracted from 507 breast cancer samples, by the real-time PCR method using Cobas® PIK3CA mutation test.

Results: *PIK3CA* gene mutations were detected in 209 (42.2%) out of 495 successfully analyzed cases. In 12 cases (2.4%), the tumor tissue was not of sufficient quality for analysis. Most mutations were detected in the helical (41.2%) and kinase domains (46.9%) of the PIK3CA protein. The most frequently detected mutations were H1047L/R/Y (46.4%), E545A/D/G/K (26.3%) and E542K (13.9%), followed by N345K (8.1%). *PIK3CA* mutations were detected in 44.8% of primary BC cases and in 39.6% of metastatic lesions (χ2=0.647; P=0.421). The age of the tissue did not significantly affect the percentage of detected mutations (χ^2=0.543; P =0.461). Institutions differed in the number of analyzed cases, but not in the percentage of detected *PIK3CA* mutations (χ2=6.23; df=4; P=0.183) nor in the frequency of the most common mutations (χ2=2.65; df=4; P=0.618).

Conclusion: The frequencies of *PIK3CA* mutations correspond to those reported in the literature. We have shown that the tissue of primary BC as well as metastatic lesions are suitable for analysis, and that the age of the tissue is not a significant obstacle in the analysis.

KEYWORDS: *breast cancer, PIK3CA, mutation*

INTRODUCTION

PI3-kinase (phosphoinositide-3-kinase) is a lipid kinase that activates diverse cellular processes (cell growth, differentiation, proliferation, and survival). The class 1 PI3-kinase family consists of regulatory (p85) and catalytic (p110) subunits,

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each of which has five domains and is encoded by separate genes(1–5). Both subunits function by forming a heterodimer that activates signaling pathways. Mutational studies of p110 subunit alpha (PIK3CA) have identified key mutations in the helical domain and kinase domain of the p110α subunit that results in hyperactivation of the PI3K pathway. Such activating somatic mutations are present in about 40% of breast cancers, and via the AKT/mTOR signaling pathway are involved in oncogenesis and cancer growth(1–5).

The results of the SOLAR-1 phase 3 clinical study showed a significant prolongation of progression-free survival and greater overall response with PIK3CA-inhibitor alpelisib plus fulvestrant therapy compared with placebo-fulvestrant among patients with PIK3CA-mutated, HR+/HER2- advanced endocrine-resistant breast cancer that has relapsed after a period of remission or progression during or after previous endocrine therapy(2). Based on these results, the FDA approved PIK3CA inhibitor therapy in 2019, and the EMA in 2020 for patients with HR-positive/HER2-negative advanced breast cancer who were previously treated with aromatase inhibitors(6). Alpelisib is a non-chemotherapeutic selective inhibitor of α isoform of phosphatidylinositol-3-kinase (PIK3CA) that exhibits antineoplastic activity by blocking Akt-signaling pathway, and, at the same time, induces increased estrogen receptor transcription in breast cancer cells. Among HR+/HER2- advanced breast cancer patients not receiving PIK3CA-targeted therapy, the PIK3CA mutation is a negative prognostic factor(7). Several dozen PI3K inhibitors are currently in clinical trials, and even more prospective trials are currently recruiting patients(8). Also, there are already several methods with different sensitivity and specificity, and companion diagnostic tests that detect different alterations mainly without quantitative information about allele frequency.

The aim of this article was to determine the frequency, nature, and exone distribution of *PIK3CA* gene somatic mutation in the Croatian population of hormone-receptor-positive and HER2-negative advanced breast cancer patients. Given that the therapy with PIK3CA inhibitors had been in clinical use for only a short period of time in Croatia, we will continuously monitor patients in order to obtain more specific clinical data on the effect of the drug.

PATIENTS AND METHODS

Analyses of PIK3CA mutations were performed in the period from July 1, 2020, to December 31, 2021, in five University Hospital Centers (UHC) in the Republic of Croatia: UHC Osijek, UHC Rijeka, UHC Sestre milosrdnice, UHC Split and UHC Zagreb. Qualitative detection of somatic PIK3CA mutations was performed on DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue of postmenopausal patients with advanced hormone-receptor-positive and HER2-negative (HR+/HER2-) breast cancer who met the criteria for treatment with PIK3CA inhibitors. The choice of tissue for analysis depended on its availability from hospital archives and its quality, whether it were primary tumors or their metastases. FFPE tumor tissue was used for analysis in almost all cases except for three cases where circulating cell-free DNA was extracted from blood plasma.

According to the oncologist's assessment, patients who were candidates for therapy with PIK-3CA inhibitors were reffered for testing and signed the informed consent.

DNA extraction procedure

DNA was extracted from FFPE tumor tissue using the cobas® DNA Sample Preparation Kit according to the protocol. FFPET sections containing at least 10% of tumor content are applicable for DNA extraction. When the sample contains less than 10% of the tumor content, it is necessary to make a macrodissection and remove part of the surrounding non-tumor tissue in order to increase the proportion of the tumor in the final section (minimum 30%). Briefly, several 5-10 micron FFPE sections were cut. After deparaffinization with xylene and absolute ethanol, tissue sections were dried and tissue lysis buffer with proteinase K was added and incubated on a dry heat block at 56ºC for at least one hour. After protein digestion, proteinase K is blocked by high temperature (90ºC, 60 minutes). Subsequently, isopropanol is added to the lysis mixture, which is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA binds to the glass fibers while all impurities are removed by centrifugation. The adsorbed nucleic acids were washed two times and then eluted. The amount of genomic DNA was determined spec-

trophotometrically and diluted to a working solution concentration of 2ng/μl.

cfDNA extraction procedure

Cell-free DNA (cfDNA) was extracted from plasma separated by centrifugation of whole blood (EDTA vacutainers), for 3 minutes at 2500 g. The procedure was performed as soon as possible after blood extraction in order to preserve as much cfDNA as possible. For the extraction of nucleic acids we used Roche High Pure PCR Template Preparation Kit. The isolation protocol is very similar to that previously described. cfDNA were lysed using a short incubation with proteinase K (70°C, 10 minutes). The kit contains a special Inhibitor Removal Buffer for heparin-treated materials. Nucleic acids are then selectively bound to glass fibers in filter columns where the bound nucleic acids are purified through a series of quick *washing and centrifugation* steps. A low salt buffer is used to elute nucleic acids from glass fibers. The amount of the DNA was also determined spectrophotometrically and diluted to a working solution concentration of 2ng/μl.

PCR amplification and detection

Amplification and detection of targeted DNA sequences were performed by real-time PCR approach on a Cobas z 480 analyzer (for automated amplification and detection) using the cobas® PIK3CA Mutation Testing kit. The analytical sensitivity (detection rate of selected mutations) for FFPE tissue using this test is 100% (only for exon 4 is 95%), if the DNA sample has at least 5% mutations, with a total input of 50 ng of sample DNA.

The kit contains a pool of primers that define specific sequences from 85 to 155 bp in *PIK3CA* exons 1, 4, 7, 9, and 20, encompassing 17 single amino acid substitutions. An additional primer pair, targeting a conserved 167 bp region in exon 3 of the PIK3CA gene, serves as an internal full process control. Also, the PIK3CA mutant control and the negative control contained in the assay kit were included in each run.

The kit consists of three master mixes (MMX1-2-3) from which the working solution is prepared, calculating that 20 μl of individual MMX and 7 μl of magnesium acetate are needed per well. 25 μl of MMX working solution is transferred to each well in the column of the microwell plate and then 25 μl of sample and positive and negative control are added. Automated detection of mutations is based on technology where a target-specific oligonucleotide probe is labeled with a fluorescent dye and a quenching molecule. After the dye is separated from the quencher by the action of DNA polymerase, the device measures the fluorescence of the characteristic wavelength. At the end of the run, the device automatically reads the result, which is displayed only as the position in the codon, without details of the base substitution that occurred at a specific position in the sequence. Table 1 shows all possible base substitutions in the targeted sequences and their positions in the corresponding codon in the protein (displayed analysis result). Table 1 shows all possible base substitutions in target sequences and the resulting positions of amino acid substitutions in the protein as displayed by the device after analysis (e.g. detected mutations in the sequences 1634 A>C, 1635 G>T, 1634 A>G or 1633 G>A are displayed by the device only as E545X).

Table 1.
List of all single amino acid substitutions that can be detected by real-time PCR using the cobas® PIK3CA mutation test

PIK3CA exon	Nucleic acid sequence	Cosmic ID	PIK3CA mutation
1	263G>A	746	R88Q
4	1035T>A	754	N345K
7	1258T>C	757	C420R
9	1624 G>A 1634 A>C 1635 G>T 1634 A>G 1633 G>A 1636 C>G 1636 C>A 1637 A>T 1637 A>G	760 12458 765 764 763 6147 766 25041 12459	E542K E545X E545A E545D E545G E545K Q546X Q546E Q546K Q546L Q546R
20	3129 G>T 3140 A>T 3140 A>G 3139 C>T 3145 G>C	773 776 775 774 12597	M1043I H1047X H1047L H1047R H1047Y G1049R

Statistical analysis

Data were analyzed by descriptive statistics, and presented as percentages or as median with range values for categorical variables. The $\chi 2$ with

Yates correction or Fisher exact test (when less than five counts were observed) were used to investigate the association between categorical variables. Mann-Whitney test was used to compare the medians of continuous variable (the age) in different groups. Statistical analysis was performed using the SPSS 28.0 (IBM Corp.) statistical software. The two-tailed $P < 0.05$ was considered significant.

RESULTS

Our study included retrospectively collected results of *PIK3CA* somatic mutation analyses from 507 patients with advanced HR+/HER2- endocrine resistant breast cancer. We present the results of a total of 495 successful analyses. In 12 cases (2.4%), DNA extracted from FFPE tissue was not of sufficient quality for analysis and the result was invalid. Five patients were men (1%).

The median age of the patients at the time of testing was 63 years (from 24 to 90 years). *PIK3CA* mutation was detected in three out of five men, who were younger than the women (57 vs. 63 years old). Of the 495 successfully analyzed samples, *PIK3CA* gene mutations were detected in 209 cases (42.2%) (Figure 1a). There was no difference in median age (χ^2=2.23; df=1; P =0.135) or gender (Fisher Exact Test P=0.655) between patients depending on *PIK3CA* mutation status.

Two-thirds of the used FFPE tissue samples came from the last available biopsy were no older than 3 years, and resulted with 37.6% of detected mutations while in archival tissue samples older than 3 years, mutations were detected in 43.2% of cases. Furthermore, in archival FFPE tissue samples older than 10 years, mutations were detected in 51.8% of cases. The oldest material used that was adequate for analysis (with an internal control present) was 19 years old, while the oldest material with a detected mutation was 17 years old. No statistically significant difference was found according to the age of archival FFPE tissue (χ^2=0.543; df=1; P =0.461), even when we took into account only archival tissues samples older than 10 years (χ^2=1.43; df=1; P =0.232).

The number of primary breast cancer and metastatic lesion samples used for DNA extraction was almost identical. *PIK3CA* mutations were detected in 44.8% of primary breast cancer cases, and in 39.6% of metastatic lesions cases. Notably, no statistically significant difference was found according to the type of tissue (primary breast cancer *vs.* metastasis) (χ^2=0.647; df=1; P =0.421).

Most mutations were detected in exons 9 (41.2%), and 20 (46.9%) while less frequent mutations detected in exons 1, 4, and 7 were detected in 11.8% of cases (Figure 1b). Ten patients (4.8%) had double mutations, mainly in exon 9 (six cases), and exon 20 (three cases), associated with mutations in other exons. All reported mutations in less frequent hotspots were single mutations.

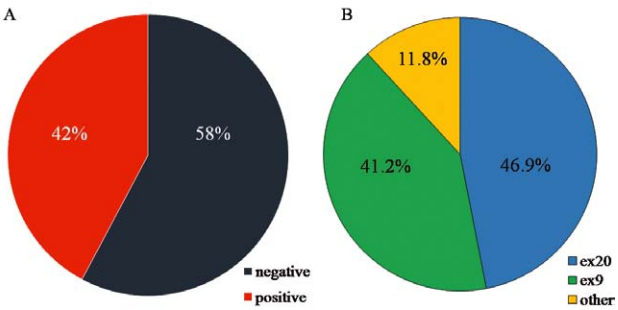

Figure 1. Distribution of detected mutations in p110α catalytic subunit of PIK3-kinase (PIK3CA). A, PIK3CA gene mutation was detected in 209 (42.2%) of 495 analyzed cases of advanced HR+/HER2- endocrine-resistant breast cancer. B, Representation of the most frequently detected mutations in the PIK3CA gene in the studied cohort.

In three cases, due to the lack of a archival FFPE tissue sample, the analysis was performed on DNA extracted from blood plasma, and in two of them a *PIK3CA* mutation was detected.

The distribution of detected variants in the *PIK3CA* gene is shown by a lollipop diagram (Figure 2). The most frequently detected mutation (46.4%) was found in the kinase domain of the p110α subunit, and are responsible for the substitution of the amino acid histidine (H) with leucine (L), arginine (R) or tyrosine (Y) at position 1047 in the protein (H1047L/R/Y). In the helical domain of the p110α subunit the most frequently detected mutations responsible for the substitution of glutamic acid (E) with alanine (A), aspartic acid (D), glycine (G) or lysine (K) at position 545 (E545A/D/G/K; 26.3%) or with lysine at position 542 of the protein (E542K; 13.9%). The detected frequencies of rare mutations are also shown in Figure 2. The most frequently detected mutation was found in exon 4 (c.1035 T>A). This mutation accounts for 8.1 % of all *PIK3CA* mutations and is responsible

for the substitution of asparagine (N) with lysine at position 345 (N345K) in the C2 calcium/lipid-binding domain of the protein and accounts for 8.1 % of all PIK3CA mutations.

Table 2 shows the analysis data of *PIK3CA* mutations detected by individual Institutions involved in the study. Althouhgh the Institutions differ in the number of analyzed cases, but the differences in the percentage of detected *PIK3CA* mutations were not statistically significant (χ2=6.23; df=4; P =0.183). We also did not observe significant differences in the frequencies of the most commonly detected mutations (χ^2=2.65; df=4; P =0.618).

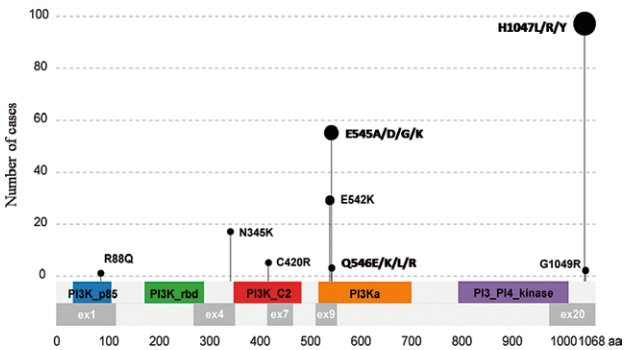

Figure 2. Distribution of detected PIK3CA gene variants. A lollipop diagram shows the detected variants relative to a schematic representation of the gene. Each position with a mutation is marked with a circle, and the length of the line depends on the number of detected cases. The colored boxes represent specific functional domains of the catalytic subunit p110α of PIK3-kinase (PIK3CA): Blue, the p85-subunit binding domain; Green, RAS binding domain (RBD); Red, C2 calcium/lipid-binding domain; Orange, helical domain; Purple, kinase domain. The gray boxes indicate the PIK3CA exons within which the mutations covered by the used commercial kit are located. The numbers on the bottom line represent the amino acid (aa) positions. At the top of the lollipops, detected mutations are labeled as corresponding amino acid substitution.

DISCUSSION

The detection of *PIK3CA* mutations in HR+/HER2- breast cancer samples using the real-time PCR method was carried out in five University Hospital Centers in Croatia. Breast cancer FFPE tissues were mainly used for analysis, and some tissues were older than 10 years, but the time of tissue archiving did not significantly affect the level of detected mutations, which was 42.2%. In a small percentage of samples (2.4%), the extracted DNA was not of good quality, so the analysis showed an invalid result. In order to preserve DNA and obtain a quality sample, appropriate fixation, and processing of the tissue is necessary. Adequate storage of archival material is also important, which is sometimes not entirely possible, especially when oncological material needs to be stored for more than 20 years. The oldest adequately analyzed material was 19 years old. Yi et al.(9) found no significant differences in DNA purity or RNA extracted from FFPE tissue stored for different lengths of time, and more than 99% of samples yielded nucleic acids which could be used to amplify the target gene fragments smaller than 300 bp.

Our results also showed that primary and metastatic lesions are equally suitable for the analysis. Although slightly more mutations were detected in primary lesions, there are no statistically significant differences in the percentage of detected mutations among them (P=0.421). The same results were published by Park et al.(10).

Analysis of *PIK3CA* gene mutations on circulating cell-free DNA (cfDNA) is an option if an FFPE sample is unavailable or inadequate. We analyzed cfDNA from blood plasma in three cases. The mutation was detected in two cases. According to the drug manufacturer's recommenda-

Table 2.

Overview of the most frequent mutations in 209 HR+/HER2- breast cancer cases with PIK3CA mutations detected by the individual Institutions in our cohort

	UHC Osijek (N = 52)	UHC Rijeka N = 48	UHC Split N = 99	UHC Sestre milosrdnice N = 207	UHC Zagreb N = 89
PIK3CA-mutations	23 (42.3%)	21 (43.8%)	50 (50.5%)	75 (35.9%)	40 (44.9%)
Ex 9	9 (39.1%)	7 (33.3%)	20 (40%)	34 (45.4%)	14 (35%)
Ex 20	12 (52.1%)	13 (61.9%)	22 (44%)	31 (41.3%)	21 (52.5%)
Other	2 (3.8%)	1 (4.8%)	8 (12%)	10 (13.3%)	5 (12.5%)

tions, *PIK3CA* mutations are generally stable and thus, both, archival samples and recent or new biopsies can be tested. The analysis can also be performed on ccfDNA, but if no mutation is detected, the analysis should be repeated on the tumor tissue(11). Mosele et al.(4) used the detection of *PIK-3CA* mutation levels in blood plasma after one (66%), two (20%), and three cycles of chemotherapy (14%) to assess patient outcomes. According to the results of Venetis et al.(12), careful selection of samples with adequate DNA quantity and quality allows robust RT-PCR-based testing of the *PIK-3CA* gene in primary tumors as well as matched metastases. In the case of available high-quality material, after optimizing the laboratory workflow, RT-PCR enables a short processing time and cheaper testing.

As we mentioned, *PIK3CA* gene mutations were detected in 42.2% of HR+/HER2- endocrine resistant advanced breast cancers in our cohort. We noticed that the proportions of most common mutations detected by the Institutions are almost equal. However, we did see some differences in the overall percentage of detected *PIK3CA* mutations, but without statistical significance and within the range of detection rates reported in the literature. The difference is most likely due to the smaller number of analyzed samples in certain Institutions. According to published data activating mutations in the *PIK3CA* gene are present in 28-42% of breast cancer cases(2,4,7,11–15). *PIK3CA* mutations were also detected in a significant percentage of HR+/HER2+ breast cancers (22%-31%), as well as in triple-negative breast cancers (16%-18%)(13,15).

Clinical use of the PIK3CA inhibitor, alpelisib, was approved based on the mutations in the *PIK3CA* gene detected in the SOLAR1 study(2). According to the literature, activating mutations in the helical domain (E542K, E545A/D/G/K), and the kinase domain (H1047L/R/Y) were the most frequently detected mutations(13,16). The same mutations have the highest frequency in our cohort . Moreover, most papers show that these mutations in the helical and kinase domains have the strongest potential for malignant transformation(13,16,17) without significant prognostic differences between them(15).

Among the rarer *PIK3CA* mutations in our cohort, the most frequent was N345K in the C2 domain (8.1% of all detected mutations). To our knowledge, this mutation was not covered by the primers used in the SOLAR-1 study. The only mutation detected in that study located within the C2 domain was C420R mutation(17). However, the true therapeutic significance of this mutation is still unclear. The literature reports 2% to 10% of *PIK3CA* missense mutations in the C2 domain, with some deletions that could also be clinically significant(13,16,18). Preclinical results show that N345K mutation could also predict alpelisib benefit, but larger clinical results are awaited(17). The same observation applies to G1049R in the kinase domain(17). All of these rare mutations have shown oncogenic potential in *in vitro* studies (16,17). Regarding the R88Q mutation in the p85 regulatory subunit binding domain (detected in one case), there is currently insufficient clinical data, although it has been assessed as probably damaging by PolyPhen(14,19). Razavi et al.(19) actually identified 12 more previously unknown *PIK3CA* hotspots as significantly mutated in breast cancer, and five of these variants activated the PI3K signaling pathway more potently than the wild-type protein.

In our cohort, 10 patients (4.8%) with double mutations were also recorded. Some papers report about 12% of breast cancer cases with a double *PIK3CA* mutation that showed increased sensitivity to PI3Kα-inhibitors compared to single-mutation cases(20). As in our cohort, most of the observed double mutations occur concurrently with mutations in a helical or kinase domain. Vasan et al.(20) proved that these were true double mutations (clonal) and not the result of tumor cells' polyclonality.

PIK3CA gene mutations are not specific only to breast cancer but have been detected in numerous solid cancers. Moreover, Razavi et al.(19) found 35 hotspot mutations in *PIK3CA* alone affecting 699 tumors, excluding mutations in other subunits. Also, Millis et al.(21) reported a total of 38% *PIK3CA* mutations detected in more than 19,000 patients with various solid tumors such as endometrial cancer (37%), cervical cancer (30%), anal cancer (27%), bladder cancer (22%), colorectal cancer (17%), etc. This certainly opens up the possibility for PIK3CA inhibitors therapy in other neoplasms as well. Several dozen PI3K inhibitors are currently in clinical trials, and even more prospective trials are currently recruiting patients with various tumor types(8).

The PI3K signaling pathway in breast cancer can be activated by modifications (amplification or activating mutations) of molecules participating in the PI3K pathway or by activation of upstream receptor tyrosine kinases(22). More clinical data are needed to define a clear prognostic role of *PIK3CA* mutation in breast cancer. So, Zardavas et al.(15) determined that *PIK3CA* gene mutations predict better survival without invasive disease, but not survival without distant disease or overall survival in early breast cancer. On the other hand, patients with advanced HR+/HER2- breast cancer harboring *PIK3CA* mutation, who are not treated with alpelisib, have resistance to chemotherapy and a poor outcome(4). In contrast, this effect is not as significant in patients with *PIK3CA* mutation in TNBC(4). The reason for this is, of course, the activation of different signaling pathways. However, the addition of α-specific PI3K-inhibitors to standard treatment improved patient outcomes and reduced the estimated risk of disease progression by 35%(2).

Our results, show that in the Croatian population of HR+/HER2- breast cancer patients, there is no difference in the frequency and type of *PIK3CA* gene mutations compared to data published in the literature. There were no statistically significant differences in the percentage of detected *PIK3CA* mutations, nor in the frequency of variants between the institutions. Therefore, our data openedthe possibility of conducting this testing in all molecular laboratories involved in predictive biomarker testing. We have shown that samples of primary cancers as well as metastatic lesions are adequate for analysis. Our results also show that the age of the tissue is not a significant obstacle during the analysis. Of course, newer material is more appropriate, but sometimes disease progression occurs after many years, so there is no new sample. Of course, tumor tissue obtained from a recent biopsy is more suitable, but sometimes disease progression occurs after many years, and biopsying a new tissue sample may not be feasible.

The biggest limitation of our research is the lack of clinical data on the effectiveness of the treatment. Unfortunately, PARP inhibitors were used for too short period of time to obtain relevant clinical effect. Therefore, we will monitor the effect and clinical outcome of PIK3CA-inhibitor treatment to determine whether there are differences in sensitivity to this therapy depending on the mutation.

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Conflict of interest: Authors do not have competing interests to declare.

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Sažetak

REZULTATI MULTICENTRIČNOG TESTIRANJA SOMATSKIH MUTACIJA GENA PIK3CA KOD UZNAPREDOVALOG LUMINALNOG RAKA DOJKE S NEGATIVNIM HER2 RECEPTOROM

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Cilj: Aktivirajuće somatske mutacije u katalitičkoj podjedinici p110α PIK3-kinaze (PIK3CA) prisutne su u oko 40% karcinoma dojke, a uključene su u onkogenezu i rast raka. Terapija inhibitorima PIK3CA odobrena je za bolesnice s HR+/HER2- metastatskim endokrino-rezistentnim rakom dojke sa prisutnim somatskim mutacijama gena PIK3CA. Takva ciljana terapija poboljšala je preživljenje bolesnica bez progresije bolesti.

Metoda: Analize mutacija gena *PIK3CA* provedene su u pet kliničkih bolničkih centara (KBC) u Republici Hrvatskoj. Kvalitativna detekcija mutacija gena *PIK3CA* provedena je na DNA ekstrahiranoj iz 507 uzoraka raka dojke, metodom PCR u stvarnom vremenu koristeći Roche Cobas® PIK3CA mutacijski test.

Rezultati: Od 495 uspješno analiziranih uzoraka mutacije gena *PIK3CA* otkrivene su u 209 bolesnica (42,2%). U dvanaest slučajeva (2,4%) tumorsko tkivo nije bilo zadovoljavajuće kvalitete za analizu. Većina mutacija smještena je u helikalnoj (41,2%) i kinaznoj domeni (46,9%). Najčešće su detektirane mutacije H1047L/R/Y (46,4%), E545A/D/G/K (26,3%), E542K (13,9%) te N345K (8,1%). Mutacije u genu PIK3CA detektirane su u 44,8% slučajeva primarnih karcinoma dojke te u 39.6% metastatskih lezija (χ2=0.647; P=0.421). Starost tkiva nije utjecala na postotak detektiranih mutacija (χ^2=0.543; P =0.461). Broj analiziranih uzoraka se razlikuje među institucijama uključenim u istraživanje, ali to nije značajno utjecalo na postotak detektiranih mutacija (χ2=6.23; df=4; P=0.183) kao niti na učestalost pojedinih mutacija koje su obuhvaćene komercijalnim kitom korištenim (χ2=2.65; df=4; P=0.618).

Zaključak: Učestalosti detektiranih *PIK3CA* mutacija odgovaraju onima navedenima u literaturi. Uočili smo da je tkivo primarnog karcinoma dojke i metastatskih lezija jednakovrijedno za analizu, a starost tkiva ne utječe značajno na rezultat analize.

KLJUČNE RIJEČI: *rak dojke, PIK3CA, mutacija*