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Evaluation of PIK3CA Gene Mutations in Breast Cancer Patients Treated by Trastuzumab

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ABSTRACT

Background: The phosphatidylinositol 3-kinases are known as a family of lipid kinases, playing a role in various cellular processes. A member of this family is PIK3CA which is frequently mutated in breast cancer. In this study, the association between H1047R, E542K and E545K mutations, and therapy resistance was investigated in Iranian breast cancer patients treated by Trastuzumab.

Methods: Resistance and sensitive groups were chosen from Iranian patients treated by Trastuzumab. A PCR-RFLP approach was designed for detecting the H1047R mutation. Mutations in positions of codons E542K and E545K were detected using PCR-based DNA Sanger sequencing assay. The overall survival and disease-free survival were assessed.

Results: A significant relationship was observed between the presence and absence of H1047R mutation and the overall survival (P = 0.025). In addition, there was a significant relationship between the response to Trastuzumab and some clinicopathological features, including the age and the status of ER/PR receptors (P-value<0.05). E542K and E545K mutations were not observed in the patients.

Conclusion: It can be said that probably H1047R mutation is a prognostic marker in the Trastuzumab-based therapy resistant breast cancer. Further studies can be performed to evaluate this mutation before using Trastuzumab to predict the effectiveness of this treatment.

INTRODUCTION

Breast cancer (BC) is the most common cancer and the 2nd cause of cancer death in women. In 2020, BC was the most commonly diagnosed cancer (estimated 2.3 million new cases, representing 11.7% of total cancer cases) in women and the leading cause of cancer death (6.9% of total cancer cases) in 185 countries.[1] About 20-30 percent of patients are HER2 receptor positive. This type of BC develops faster than the other types.[2] HER2 gene amplification is considered an important predictive factor in the response to Trastuzumab targeted therapy. However, studies show that some HER2-positive patients are Trastuzumab-resistant.[3]

The phosphatidylinositol 3-kinases are known as a family of lipid kinases, involved in various cellular processes, including cell proliferation and regulation of cell survival. Potential changes in the PI3K signaling pathway have a role in the formation of different cancers including breast, colorectal, uterine, and glioma.[4,5] Under normal circumstances, PI3K plays a crucial role in translating extracellular growth

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signals into the intracellular actions that facilitate growth by coupling macromolecule biosynthesis with the initiation of cell-cycle progression.[4] The structure of PIK3CA is consisted of two catalytic and regulatory subunits, p110α and p85a, respectively. The catalytic subunit has five domains containing helical, and kinase domains encoded by the PIK3CA gene which is reported to have somatic mutations in a variety of human tumors.[6] PIK3CA gene contains 21 exons, and 80 percent of mutations are located in 3 regions, two mutations in exon nine located at 542 and 545 helixes, and one at codon number 1047 in the kinase region of exon 20.[7] The aberrant PIK3CA signaling pathway has been studied in Trastuzumab-resistant patients and there is evidence that links resistance to mutations of this gene. Therefore, this gene mutation may be a potential prognostic and predictive biomarker for Trastuzumab treatment. Research suggests that 25 percent of HER2-positive patients had PIK3CA mutation. Although frequent studies have confirmed the correlation of PIK3CA gene mutations and drug-resistant, more studies should be carried out on the correlation between this mutation and resistance to Trastuzumab. Since the occurrence of mutation is different due to race and geographical location, more studies in other populations are required to confirm this connection.[8-10]

This case-control study aims to evaluate the mutations of PIK3CA exon 9 and 20 in the tumor tissue of HER2-positive patients receiving Trastuzumab and to determine if these mutations correlate with the survival of the patients.

METHODS

Study Population and Selection Criteria

Seventy-three FFPE samples from patients with invasive ductal carcinoma were investigated, including 38 samples of Trastuzumab-resistant patients and 35 samples of responsive patients. All patients were HER2-positive. The Trastuzumab-resistant group consisted of patients who showed events like local recurrence, metastasis, or death within a year of treatment.[11,12] The control group included Trastuzumab-responsive patients with no signs of local recurrence, metastasis, or death within three years of taking Trastuzumab. In addition, patients diagnosed with metastasis, stage IV, were excluded from the study. Some other clinicopathological features of the patients, including patient age at BC onset, tumor grade, tumor stage, ER and PR expression, the number of invaded lymph nodes, and treatment regimen, were evaluated. Patient's time to event was also recorded. Patient tissues were provided by the BC Research BioBank (BCRC-BB).[13] The Ethical Committee of Motamed Cancer Institute of Iran (IR.ACECR.IBCRC.REC1395.12) approved this study and informed consent was obtained from all of the patients.

DNA Isolation and PCR Amplification of Target Regions

Five sections of two µm of FFPE tumor were applied for DNA extraction. Sections were from >80% tumor area. For samples less than 80% tumor area, FFPE blocks were manually dissected to get the required percentage of the tumor. According to the manufacturer protocol, DNA extraction was performed using FFPE DNA kit black (Analytik Jen, Jena, Germany).

Primers were designed using Gene Runner (V.6.5.52x beta) for H1047R, E542K, and E545K mutations (Table 1).

Table 1. The sequence of primers designed to detect H1047R, E542K, and E545K mutations

	Primers	Sequence	Tm	GC%
PIK3CA Exon 20 (126 bp)	Forward	5′-GGAGTATTTCATGAAACAAATGAATGATGC**G**-3′	61.5	35.5
	Reverse	5′-GAGCTTTCATTTTCTCAGTTATCTT-3′	54.9	32
PIK3CA Exon 9 (107 bp)	Forward	5′-AGGGAAAATGACAAAGAACAGC-3′	63.1	40.9
	Reverse	5′-TTTAGCACTTACCTGTGACTCCA-3′	62.5	43.5

Regarding the exon 20, a mismatched nucleotide (A to G, in the fourth nucleotide position at the 3′ end) was used in the forward primer to provide a restriction endonuclease recognition site for the FspI restriction enzyme. In mutant form, the substitution of CAT to CGT results in the loss of the FspI restriction site. Therefore, cleavage did not occur in the mutated form due to loss of enzyme recognition site, and only the 126-bp fragment was identified, while two fragments of 96 and 30bp were identified in wild type form of exon 20 through the restriction site. A 107bp product was amplified by PCR regarding exon 9.

Amplification of the 126-bp fragment of exon 20 and 107bp fragment of exon 9 was performed using a VeritiPro Thermal Cycler device by the manufacturer (Applied Biosystems) as follows. The initial cycle of

denaturation at 95°C for 2min was followed by 35 cycles of template denaturation at 95°C for 30sec. Primer annealing occurred at 60°C for 30sec, and extension at 72°C for 30sec, and a final extension at 72°C for 5min. Exon 9 containing E542K and E545K mutations PCR was performed as above.

The reaction contained 200ng DNA, 0.2mM dNTPs (A, G, C, and T), 0.5µM forward and reversed primers separately, 1.5mM $MgCl_2$, 1x buffer prepared from 10x, and 1 Unit AccuPOL (Ampliqon, Denmark). The DNA products were validated by agarose gel electrophoresis after optimization.

RFLP

Considering the cut site of FspI (Thermo Fisher Scientific), the wild-type gene results in a 96- and a 30-bp fragment. However, if there is a H1047R mutation, the FspI does not cut the region, and the gene will be an intact 126-bp fragment. All products were investigated by gel electrophoresis.

Sequencing

Sanger sequencing by big dye Terminator Kit (Applied Biosystems, USA) was performed to analyze the validity of enzyme activity on H1047R mutated gene. Furthermore, to evaluate E542K and E545K mutations, sequencing was performed using specific primers.

Statistical Analysis

Kaplan-Meyer method was used to estimate overall survival and disease-free survival. The effect of the presence or absence of mutation was investigated as a prognostic factor. The total survival time was calculated from the beginning of surgery until death or the last follow-up, and the disease-free survival was calculated from the beginning of surgery until relapse or the last follow-up. Clinical data were analyzed and the survival curve was plotted using the Kaplan-Meyer method. Statistical analysis of data and survival graphs were obtained by SPSS 22 software. Probability values<0.05 were considered statistically significant.

Evaluation of the relation between treatment response and patient clinicopathological information was done using Chi-square test. Furthermore, the possibility of a relation between the studied mutations and the response to Trastuzumab treatment was investigated by the Chi-square test in the sensitive and resistant groups.

RESULTS

Clinicopathological Characteristics of BC Patients

The medical records of all patients included in this study were evaluated for clinicopathological characteristics. Seventy-three women (the mean age = 47±20) were recruited in this study. Out of 73 patients included in the study, 38 (52%) were resistant to Trastuzumab and 35 (48%) were sensitive to Trastuzumab. The correlation between Trastuzumab response and clinicopathological features in the patients was determined as showed in Table 2. According to the Chi-square test, there is a significant relationship between the response to Trastuzumab and some clinicopathological characteristics including the age of BC onset and ER and PR receptor status.

Table 2. Comparison of correlations between Trastuzumab response and different variables in the study

		Response to Trastuzumab				Chi-square test P-value [a]
		Sensitive (n=35)		Resistance (n=38)		
		Frequency	Percent	Frequency	Percent	
Age at diagnosis [b]	<40	14	40%	6	18.2%	0.048
	>40	21	60%	27	81.8%	
Grade [b]	Grade I	2	5.7%	5	13.5%	0.367
	Grade II	23	65.7%	19	51.4%	
	Grade III	10	28.6%	13	35.1%	
Stage [b]	Stage I	2	5.7%	0	0%	0.133
	Stage II	11	31.4%	11	40.7%	
	Stage III	22	62.9%	16	59.3%	
Estrogen Receptor [b]	Negative	10	28.6%	25	65.8%	0.001
	Positive	25	71.4%	13	34.2%	
Progesterone Receptor	Negative	14	40%	28	73.7%	0.004
	Positive	21	60%	10	26.3%	
Lymph Node [b]	Negative	7	20%	4	11.8%	0.393
	1-3	11	31.4%	7	20.6%	
	4-9	12	34.3%	14	41.2%	
	>9	5	14.3%	9	26.5%	

[a] at a significance level of 0.05.
[b] Some data is missing for this variable.

PI3KCA Mutation Analysis of Exon 9 and 20

The PCR product and digestion of exon 20 and 9 were validated by 1% agarose gel electrophoresis (Figure 1 and 2). Moreover, the enzyme activity was validated by sequencing in exon 20 (Figure 3).

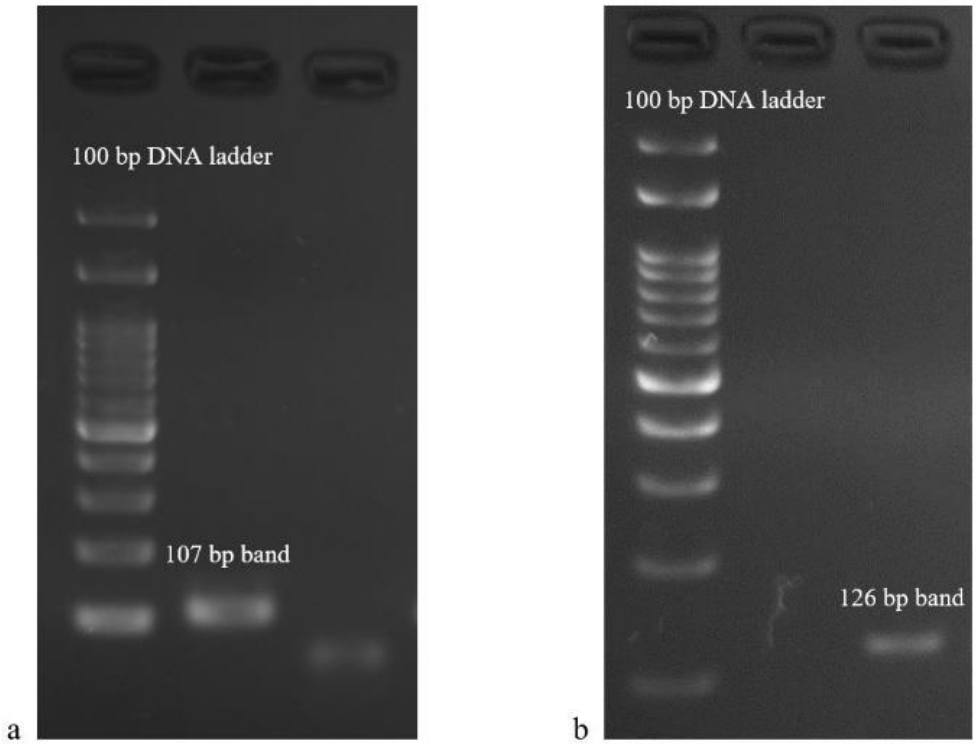

Figure 1. PCR amplification of exon 9 (107 bp) and exon 20 (126 bp) in 1% agarose gel electrophoresis; a) Lane1: a 100 bp to 2,000 bp DNA ladder (Invitrogen, CAT 15628019); Lane2: PCR product of exon 9; Lane3: negative control, distilled water was used instead of template DNA; b) Lane1: a 100 bp to 2,000 bp DNA ladder (Invitrogen, CAT 15628019); Lane2: negative control, distilled water was used instead of template DNA, lane3: PCR product of exon 20.

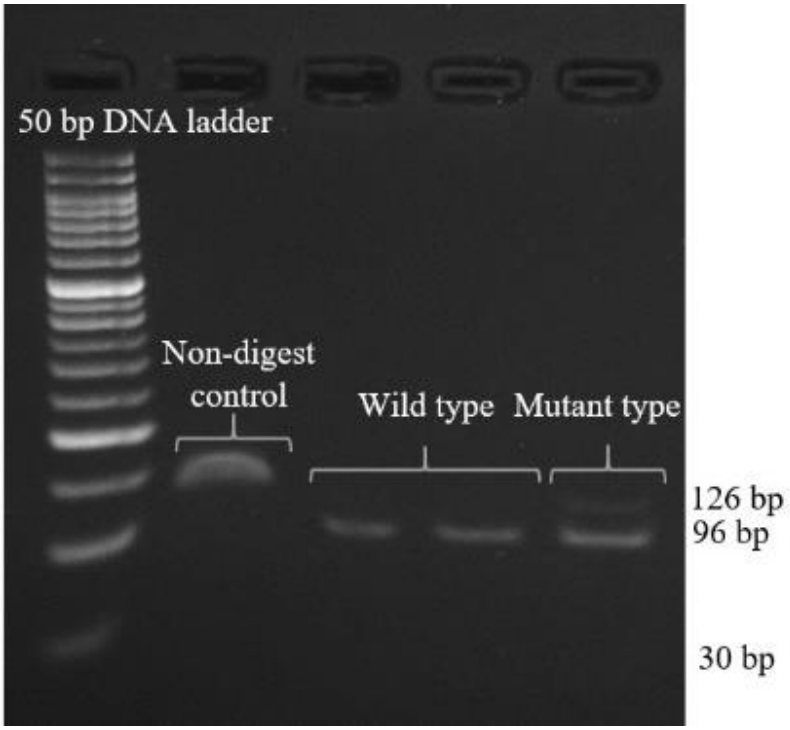

Figure 2. Enzymatic digestion of the exon 20 PCR products in 1% agarose gel electrophoresis; Lane1: a 50 bp to 1,000 bp DNA ladder (Thermo Scientific™, CAT SM0371); Lane2: non-digest control; Lane 3 and 4: a wild type cut by restriction enzyme FspI (Thermo Fisher Scientific) into two fragments of 96 bp and 30 bp; Lane 5: heterozygous PIK3CA H1047R

mutation, the uncut fragment of 126 bp coexisting with two fragments 96 bp and 30 bp, cut by restriction enzyme FspI (Thermo Fisher Scientific).

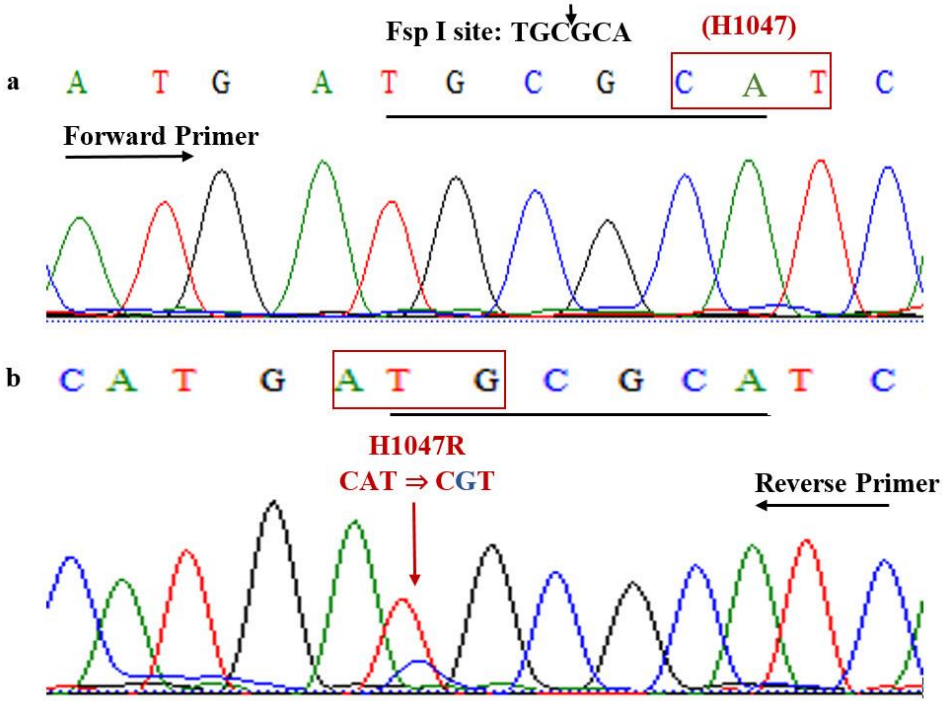

Figure 3. The result of Sanger sequencing, a) exon 20 Wild type and b) exon20 H1047R mutation

Overall Survival and Disease-free Survival in Patients

The presence of H1047R mutation was associated with the overall survival of patients in this study (P=0.025) but no significant relationship was observed in the effect of mutation on disease-free survival of the patients (P=0.118). Figure 4 shows the overall survival curve of the patients by month.

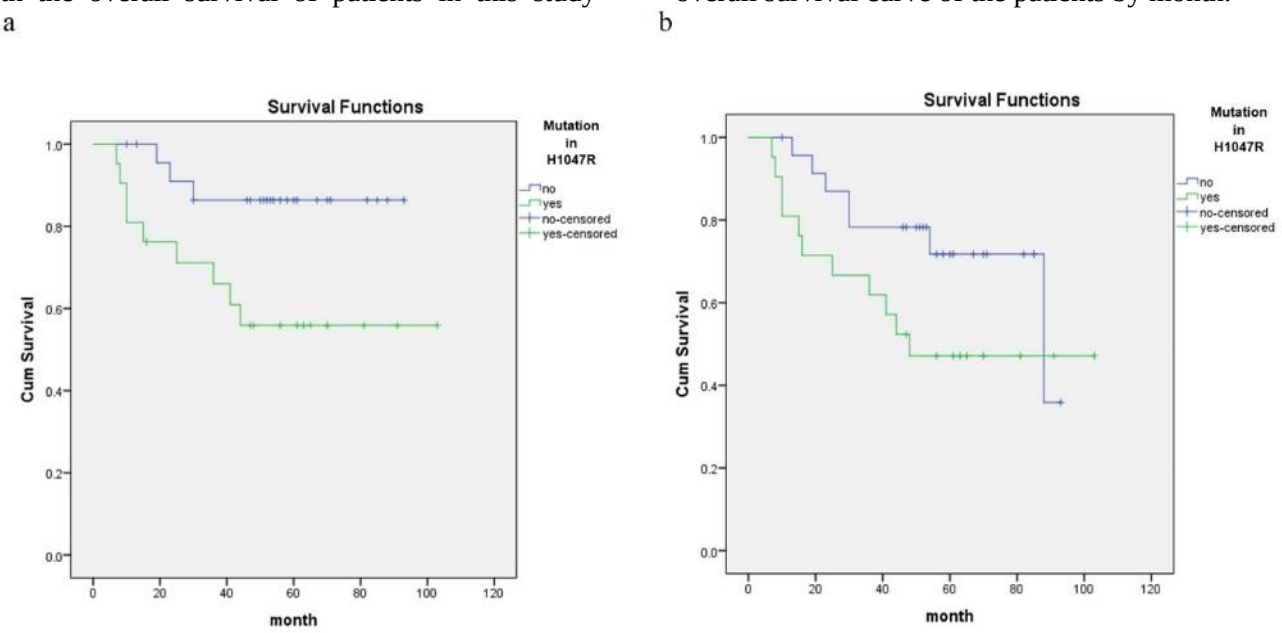

Figure 4. Kaplan-Meyer overall survival and disease- free survival curves according to H1047R mutation. Probability values < 0.05 were considered statistically significant; a) Overall survival probability function curve, b) disease- free survival probability function curve

Relationship Between Mutation and Trastuzumab Response

The possibility of a relationship between mutation in H1047R codon in exon 20 and the response to Trastuzumab in both sensitive and resistant groups was investigated. According to the Chi-square test, no significant relationship was observed between mutations in H1047R codon and the response to

treatment. In relation to exon 9, since no E542K and E545K mutations were observed in any of the patients, the desired correlation could not be examined (Table 3).

Table 3. Comparison of correlation between Trastuzumab response and mutations in H1047R, E542K and E545K codons

		Response to Trastuzumab				Chi-square Test
		Sensitive (n=35)		Resistance (n=38)		
		Frequency	Percent	Frequency	Percent	P-value [a]
H1047R mutation in exon 20	Yes	14	40%	14	36.8%	0.782
	No	21	60%	24	63.2%	
E542K mutation in exon 9	Yes	0	0	0	0	-
	No	35	100%	38	100%	
E545K mutation in exon 9	Yes	0	0	0	0	-
	No	35	100%	38	100%	

[a] at a significance level of 0.05.

DISCUSSION

Assessment of the responses to anti-HER2 therapies has been investigated by different studies applying a variety of techniques in various communities. Although some results indicate a significant correlation between Trastuzumab response and PI3K/AKT mutations, the results of some others reveal no correlation.[11]

Trastuzumab resistance is an important clinical issue in BC. In the current study, we evaluated the association of PIK3CA exon 9 and 20 mutations and Trastuzumab resistance and survival. Seventy-three patients (mean age = 47±20) were included in this study. Out of 73 patients included in the study, 38 (52%) were resistant to Trastuzumab and 35 (48%) were sensitive to Trastuzumab.

Based on the results of the present study, a significant relationship (P=0.025) was observed between H1047R mutation and overall survival which is consistent with previous research.[14] In a study by Francisco *et al.* in 2010, mutations in the PIK3CA gene were associated with shorter survival times (P=0.015) of patients with BC.[14]

Our results showed that there was a significant relationship between the response to Trastuzumab treatment and some clinicopathological features including age of BC onset and ER and PR receptor status (P-value<0.05). Furthermore, the possibility of a relationship between mutation in H1047R codon in exon 20 and the response to Trastuzumab treatment was investigated in two sensitive and resistant groups. No significant association was found between the studied mutation and treatment resistance (P-value>0.05), which is consistent with the findings of previous studies.[15,16] In this regard, Loi S *et al.*, by assessing mutations in PIK3CA gene in 705 HER2-Positive BC samples, reported that mutations in PIK3CA were not statistically significantly associated with Trastuzumab resistance.[16] In a meta-analysis study, Wang *et al.* also concluded that in patients with HER2-positive BC, the mutation in PIK3CA was not associated with the response to Trastuzumab-based therapy.[15] Furthermore, in an Italian study, it was shown that there was no correlation between PIK3CA gene mutations and Trastuzumab resistance.[17] Although the results of these studies show that there is no significant relationship between these mutations and the response to treatment, the results of some other studies confirm this relationship. Cizkova M. *et al.* reported a relatively high prevalence of PIK3CA gene mutations and their prognostic impact on HER2-positive BC patients. Furthermore, they suggested that these mutations result in resistance to Trastuzumab. According to the aforementioned results, personalized therapy is a possible option for BC patients in case of the diagnosis of these mutations.[18] Also, in another study on HER2-positive patients, Cizkova M. *et al.* confirmed that patients with PIK3CA mutations show a lower response to treatment compared to patients with wild-type tumors which suggest the dominant role of PIK3CA mutation in Trastuzumab resistance.[19]

According to the findings of previous studies, the rates of E542K and E545K mutations were lower than those of the H1047R mutation in the studied patients.[20,21] In this study, regarding exon 9, since E542K and E545K mutations were not detected in any of the patients, the desired correlation could not be examined as large sample sizes were not available. Since a small sample size may affect the absence of desired mutations in exon 9, definite conclusions cannot be made.

Although using Trastuzumab for BC treatment in HER2-overexpressing patients has been effective so far, the resistance in others needs more research to find predictive biomarkers for personalized treatment.[22, 23]

CONCLUSION

Recognition of drug-resistant mutations leads to choosing precise treatment strategies and stopping spending time and money on ineffective treatments. This points to the considerable role of personalized medicine. The results showed that H1047R mutation is a possible prognostic factor for resistance to Trastuzumab therapy, according to the significant correlation between H1047R mutation and overall survival of patients.

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CONFLICT OF INTEREST

None.

ETHICAL CONSIDERATIONS

The Ethical Committee approved the study. The ethics code is IR.ACECR.IBCRC.REC1395.12.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-49. doi: 10.3322/caac.21660.
2. Dowsett M, Cooke T, Ellis I, Gullick W, Gusterson B, Mallon E, et al. Assessment of HER2 status in breast cancer: why, when and how? European Journal of Cancer. 2000;36(2):170-6. doi: 10.1016/s0959-8049(99)00264-6.
3. Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. New England Journal of Medicine. 2020;382(7):610-21. doi: 10.1056/NEJMoa1914510.
4. Goncalves MD, Hopkins BD, Cantley LC. Phosphatidylinositol 3-kinase, growth disorders, and cancer. New England Journal of Medicine. 2018;379(21):2052-62. doi: 10.1056/NEJMra1704560.
5. Ikenoue T, Kanai F, Hikiba Y, Obata T, Tanaka Y, Imamura J, et al. Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer research. 2005;65(11):4562-7. doi: 10.1158/0008-5472.CAN-04-4114.
6. Gkeka P, Evangelidis T, Pavlaki M, Lazani V, Christoforidis S, Agianian B, et al. Investigating the structure and dynamics of the PIK3CA wild-type and H1047R oncogenic mutant. PLoS computational biology. 2014;10(10):e1003895. doi: 10.1371/journal.pcbi.1003895.
7. Bellacosa A, Etro D, Neri LM. Mutations of the PIK3CA gene in ovarian and breast cancer. The Women's Oncology Review. 2005;5(4):223-5. doi: 10.3109/14733400500463606.
8. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer biology & therapy. 2004;3(8):772-5. doi: 10.4161/cbt.3.8.994.
9. Pérez-Tenorio G, Alkhori L, Olsson B, Waltersson MA, Nordenskjöld B, Rutqvist LE, et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clinical Cancer Research. 2007;13(12):3577-84. doi: 10.1158/1078-0432.CCR-06-1609.
10. Pogue-Geile KL, Song N, Jeong J-H, Gavin PG, Kim S-R, Blackmon NL, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. Journal of Clinical Oncology. 2015;33(12):1340. doi: 10.1200/JCO.2014.56.2439.
11. Nahta R, Esteva FJ. HER2 therapy: molecular mechanisms of trastuzumab resistance. Breast Cancer Research. 2006;8(6):1-8. doi: 10.1186/bcr1612.
12. Lavaud P, Andre F. Strategies to overcome trastuzumab resistance in HER2-overexpressing breast cancers: focus on new data from clinical trials. BMC medicine. 2014;12(1):1-10. doi: 10.1186/s12916-014-0132-3.
13. Majidzadeh-a K, Kaviani A, Esmaeili R, Farahmand L, Shojamoradi MH, Zare AA, et al. Iranian Breast Cancer Bio-Bank: the activity and challenging issues. Cell and tissue banking. 2013;14(1):11-20. doi: 10.1007/s10561-012-9293-5
14. Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, et al. PTEN, PIK3CA, p-AKT, and p-p70S6K status: association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. The American journal of pathology. 2010;177(4):1647-56. doi: 10.2353/ajpath.2010.090885.
15. Wang Y, Liu Y, Du Y, Yin W, Lu J. The predictive role of phosphatase and tensin homolog (PTEN) loss, phosphoinositol-3 (PI3) kinase (PIK3CA) mutation, and PI3K pathway activation in sensitivity to trastuzumab in HER2-positive breast cancer: a meta-analysis. Current medical research and opinion. 2013;29(6):633-42. doi: 10.1185/03007995.2013.794775.
16. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen P-L, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. Journal of the National Cancer Institute. 2013;105(13):960-7. doi: 10.1093/jnci/djt121.
17. Barbareschi M, Cuorvo LV, Girlando S, Bragantini E, Eccher C, Leonardi E, et al. PI3KCA mutations and/or

PTEN loss in Her2-positive breast carcinomas treated with trastuzumab are not related to resistance to anti-Her2 therapy. Virchows Archiv. 2012;461(2):129-39. doi: 10.1007/s00428-012-1267-2.
18. Cizkova M, Susini A, Vacher S, Cizeron-Clairac G, Andrieu C, Driouch K, et al. PIK3CA mutation impact on survival in breast cancer patients and in ERα, PR and ERBB2-based subgroups. Breast Cancer Research. 2012;14(1):1-9. doi: 10.1186/bcr3113.
19. Cizkova M, Dujaric M, Lehmann-Che J, Scott V, Tembo O, Asselain B, et al. Outcome impact of PIK3CA mutations in HER2-positive breast cancer patients treated with trastuzumab. British journal of cancer. 2013;108(9):1807-9. doi: 10.1038/bjc.2013.164.
20. Martínez-Sáez O, Chic N, Pascual T, Adamo B, Vidal M, González-Farré B, et al. Frequency and spectrum of PIK3CA somatic mutations in breast cancer. Breast Cancer Research. 2020;22:1-9. doi: 10.1186/s13058-020-01284-9.
21. 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. International Journal of Breast Cancer. 2020;2020. doi: 10.1155/2020/3759179.
22. Pohlmann PR, Mayer IA, Mernaugh R. Resistance to trastuzumab in breast cancer. Clinical cancer research. 2009;15(24):7479-91. doi: 10.1158/1078-0432.
23. Mukohara T. PI3K mutations in breast cancer: prognostic and therapeutic implications. Breast Cancer: Targets and Therapy. 2015;7:111. doi: 10.2147/BCTT.S60696.

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