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ORIGINAL

Trastuzumab Response in Iraqi Women with Her2 Positive Breast Cancer: The Role of PIK3CA Oncogene Mutations at E542k and E545k

Respuesta al trastuzumab en mujeres iraquíes con cáncer de mama Her2 positivo: El papel de las mutaciones del oncogén PIK3CA en E542k y E545k

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Abstract

Introduction: Breast cancer (BC) frequently carries PIK3CA mutations. These frequently include the helical domains and exon 10 of the protein kinase, which can activate it. In addition to being expensive, trastuzumab, a human epidermal growth factor receptor 2 antagonist, is ineffective in 20% to 25% of patients with related Her2-positive BC. The response to treatment is significantly influenced by genetic differences. The study's goal was to determine whether the presence of PIK3CA polymorphism at sites on E542k and E545k would affect Iraqi patients with Her2-positive breast cancer's propensity to respond to trastuzumab (TRS) or not. in addition to cardiac toxicity.

Method: The Department of Oncology/Diwaniya University Hospital recruited 60 Her2-positive Iraqi BC women who had been receiving TRS for at least 1 year. Patients were divided into non-responders and responders using the Response Evaluation Criteria in Solid Tumors (RECIST). After DNA amplification by polymerase chain reaction, the DNA was sequenced using the Sanger method to detect polymorphisms at positions E542k and E545k.

Result: Data analysis revealed that there was no statistically significant association between Her2 promoter region E542k and E545k polymorphism and the propensity to respond positively to TRS. Reactive and unresponsive females had a very significant difference in the frequency of the combination of mutations.

Key words: Breast cancer, Her2 positive, Trastuzumab, Genetic polymorphism.

Resumen

Introducción: El cáncer de mama (CM) es frecuentemente portador de mutaciones en PIK3CA. Éstas suelen incluir los dominios helicoidales y el exón 10 de la proteína cinasa, que pueden activarla. Además de ser caro, el trastuzumab, un antagonista del receptor 2 del factor de crecimiento epidérmico humano, es ineficaz en el 20% al 25% de las pacientes con CB Her2-positivo relacionado. Las diferencias genéticas influyen significativamente en la respuesta al tratamiento. El objetivo del estudio era determinar si la presencia del polimorfismo PIK3CA en los sitios E542k y E545k afectaría a la propensión de las pacientes iraquíes con cáncer de mama Her2-positivo a responder o no al trastuzumab (TRS), además de a la toxicidad cardiaca.

Metodología: El Departamento de Oncología del Hospital Universitario de Diwaniya reclutó a 60 mujeres iraquíes con cáncer de mama Her2 positivo que habían estado recibiendo TRS durante al menos 1 año. Las pacientes se dividieron en no respondedoras y respondedoras utilizando los Criterios de Evaluación de la Respuesta en Tumores Sólidos (RECIST). Tras la amplificación del ADN mediante la reacción en cadena de la polimerasa, se secuenció el ADN con el método Sanger para detectar polimorfismos en las posiciones E542k y E545k.

Resultados: El análisis de los datos reveló que no existía una asociación estadísticamente significativa entre el polimorfismo E542k y E545k de la región promotora de Her2 y la propensión a responder positivamente al TRS. Las hembras reactivas y no reactivas presentaron una diferencia muy significativa en la frecuencia de la combinación de mutaciones.

Palabras clave: Cáncer de mama, Her2 positivo, Trastuzumab, Polimorfismo genético.

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Introduction

Women in Iraq are most commonly diagnosed with breast cancer (BC)[1]. Breast cancer is believed to be the leading cause of cancer death in women[2-3] and is responsible for approximately 25% of all new cancer cases in women worldwide[1-2]. Breast cancer, which can affect one or both breasts, is known as breast cancer. Fat, ducts and glands make up the breast. Breastfeeding gives birth to babies and produces milk to breastfeed them. Both secondary (metastatic) or primary BC are possible[4]. The major health problem, cancer, tends to develop drug resistance, increasing efforts to develop new protocols for cancer treatment[5]. The HER2 ECD transmembrane protein is targeted by humanized antibodies known as TRS. This was initially approved for the treatment of HER2-positive breast cancer. The extracellular domain of the HER2 receptor can be attacked to produce ADCC or to antagonize HER2 receptors, which have an anti-tumor effect. The method of anti-HER2 therapy has been shown to involve blocking upstream signaling pathways and vasculature, activating cell cycle arrest or death, and impairing genome repair[6].

TRS is used independently or in conjunction with other treatments. The HER2 protein, present on the surface of certain cancer cells, is a target of this drug[7]. This could improve the immune system's ability to fight cancer cells. TRS is a monoclonal substance and a potent HER2 inhibitor, also known as Herzuma[8-9]. Researchers are currently evaluating available PI3K antagonists; investigate their potential relevance in HER2+ breast cancer; and in this article provide an additional comprehensive article on current studies of PI3K inhibitors in HER2+ disease. Researchers are also evaluating the status of screening for somatic PIK3CA mutations and looking for information gaps that could prevent the successful use of PI3K drugs in chemotherapy for HER2+ prostate cancer. The PI3K signaling pathway is necessary for tumor development, replication, or survival[10]. PI3K is used to transmit signals from oncogenesis receptor tyrosine kinases (RTKs), which include insulin-like growth receptor 1, platelet-derived neurotrophic receptor, and female epidermal proliferation regulatory receptors 2 (HER2). Its serine-threonine kinase AKT is attracted to membranes through this phosphorylation of (PIP2) (PIP3), which is achieved by activation of 3-kinase. AKT and mTOR (mammalian target of rapamycin) complexes are stimulated by RTK-based signaling. AKT inhibits apoptosis while promoting growth, epithelial-mesenchymal transformation, infiltration, metastasis, or vasculature. The absence of heterozygosity, inhibitory mutations and epigenetic inactivation is often thought to enhance the effect of PI3K stimulation[11]. The catalytic subunit (PI3K) is encoded by the PIK3CA oncogene. The PI3K/AKT/mTOR signaling pathway is more frequently activated in cancers with PIK3CA gene mutations, including breast cancer[12].

Among the mutations found in the PIK3CA gene are the E542k and E545k mutations. At positions 545 and 542 of the protein sequence it causes lysine (k) to replace glutamic acid (E). In breast cancer, this mutation causes an increase in PI3K activity, which in turn triggers the activation of downstream signaling pathways[13].

HER2-positive breast cancer is often treated with targeted therapy, such as TRS. Although it has demonstrated great effectiveness, cardiac side effects may occur. Decreased left ventricular ejection fraction (LVEF) is the main symptom of cardiac toxicity associated with TrRS. During treatment, cardiac biomarkers such as B-type natriuretic peptide (BNP) and troponin I can be used to monitor cardiac function[14].

Since it takes 12 months for a patient's response to TRS treatment to become apparent, this is a long period of time to determine whether or not the patient is responding. During this period, in addition to the expensive but clinically ineffective treatment, the patient may also be exposed to risky side effects. Therefore, to avoid exposing patients to the toxicity of this drug without any clinical benefit, we should study this allele as a determinant marker of response in the future for those receiving TRS treatment. The aim of the present study was to determine whether the presence of single nucleotide polymorphisms in the pik3ca gene at positions E542k and E545k can influence the propensity of Iraqi patients to become non-responders or responders. Previous studies have examined the influence of single nucleotide polymorphisms in PIK3CA on the propensity to become positive in TRS non-responders.

Patients and methods

This article was part of a research project that ran from December 10, 2021 to August 8, 2022. Sixty patients with BC who tested positive for Her2 were included in the study and classified according to the revised version 2010 (ACO)/ (RECIST). Standard BC modifications[15]. The patients were from the Iraqi Oncology Department of Diwaniya Teaching Hospital. This department provides services to a wide variety of Iraqi communities, including urban, rural and inner-city areas of various provinces. The ethical clearance number (RECAUBCP7102021A) was agreed on July 10, 2021 by the Scientific and Ethics Committee of the Baghdad P-University School of Pharmacy and the Medical Department of Rheumatology of Baghdad University Hospital. Additionally, each participant provided written informed consent.

Patients' selection

Sixty-nine patients with Her2-positive breast cancer who met the following inclusion criteria received trastuzumab alone as sole therapy during the study. However, only sixty-three patients agreed to participate in the study, and only sixty of them met all the requirements.

The inclusion criteria:

- Women under 18 years of age with a history of HER2-positive breast cancer determined by biopsy.
- After the full twelve-month period, patients received TRS.
- Before starting treatment with TRS, normal cardiac, renal and hepatic function should be monitored in all patients[16].
- Disease evaluation: clinical, radiological or endoscopic using radiographs, plain films, computed tomography (CT), ultrasound (US) and magnetic resonance imaging (MRI). Patients were also asked to complete the TRS. the first dose was administered as an intravenous infusion of 8 mg/kg over 90 minutes. After a continuous 12-month period of intravenous infusion of 6 mg/kg over 30-90 minutes every three weeks with no history of missed doses.

The exclusion criteria:

- People with breast cancer and other concomitant neoplastic disease.
- Less than a year has passed since treatment with TRS.
- Has already undergone radiotherapy.
- Infections such as those caused by bacteria, HIV and tuberculosis.
- History of heart disease.

Patients' classification:

After 12 continuous months of TRS treatment, patients were divided into non-responders and responders according to (RECIST) criteria[14], as shown in **figure 1**. Stable disease is one in which no new lesions appear. appears. No lesion changes in size by more than 20% and no new lesions appear. All visible or radiographic tumors must disappear completely to be considered a complete response. If the maximum radius of metastatic lesions decreases by 30% or less without the appearance of new lesions, this is considered a partial response. Response was defined as stable disease, complete response, or partial response within 12 months (clinical benefit = stable disease + complete response + partial response). People who met the criteria for disease progression, defined as the appearance of new lesions or an increase in the size of existing lesions at a rate of at least 20%, were classified as "non-responders", while those who met the criteria the criteria for responding were classified as "responders". Patients were divided into two groups based on their responses. Thirty BC patients who did not respond to TRS formed the first group (group A). 30 BC patients who responded well to TRS formed the second group (group B).

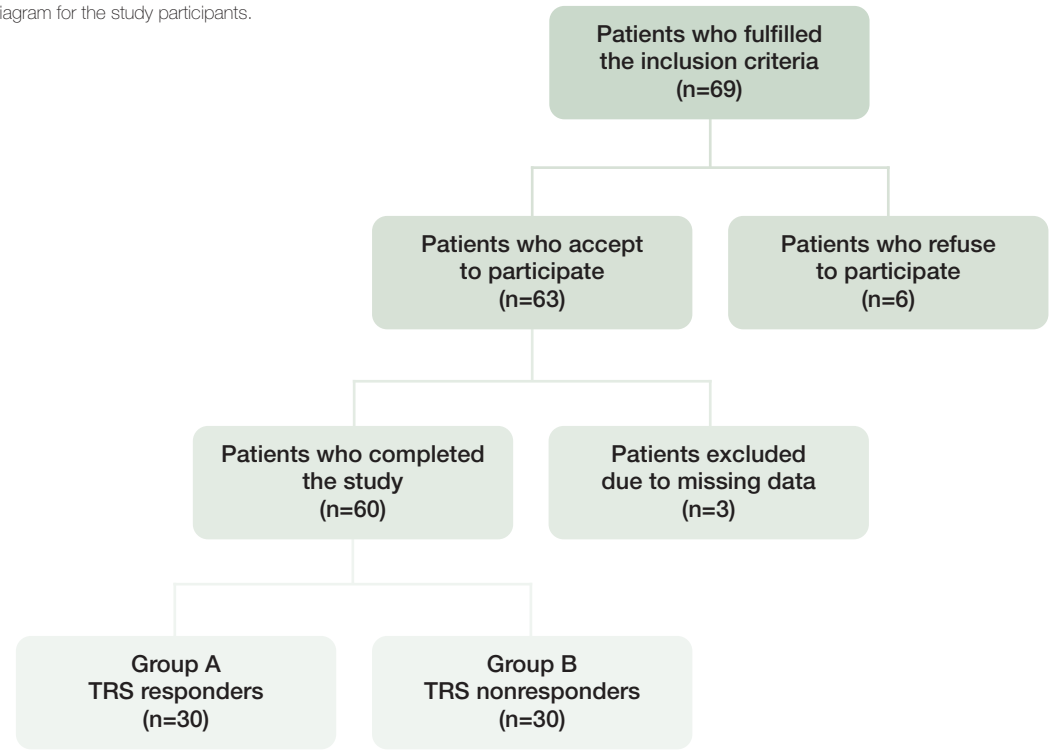
Data collection

Simple patient interviews using an information sheet specially designed for this study allowed information on disease duration, age and weight to be collected. By dividing the weight in kilograms by the square of the height in meters, the body mass index (BMI) can be calculated[17].

Sample collection and preparation

Collect a venous blood sample (5 ml) from the patient's forearm. Then, deoxyribonucleic acid was extracted from blood (2 ml) using an ethylenediaminetetraacetic acid tube. After centrifugation for 10 min, the remaining blood (3 ml) was poured into a gel tube. The remaining serum was collected and stored in an Eppendorf tube at -20°C until all samples were obtained. Creatine kinase-MB, cardiac

Figure 1: Flow diagram for the study participants.



troponin I, and cardiac troponin T were measured using an enzyme immunoassay. One China; Cat. The device was used to measure and identify cardiac troponin I, troponin T, and creatine kinase-MB levels. NO. (fitted ELIZA kit) CSB-SL0536hu, CSB-SI1747hu and CSB-SI1746hu. ELIZA[18] is used in this test. Direct DNA purification is offered by the \"Promega ReliaPrepTM Blood gDNA Miniprep System\" for DNA extraction. A hybrid thermal cycler and polymerase chain reaction were used for enlargement[19].

Statistical analysis

The data was verified using the SPSS program for Windows 26.0. Constant fluctuations are called the standard error of the mean or mean value. Alleles and genotypes are described using frequencies and percentages[18]. There is a possibility that more than 0.05 is considered statistically significant. The Shapiro-Wilk test was used to verify the normality of the results[19]. The t test is used for distributed data to determine when there is a significant difference in parameters and statistical characteristics between the responding group and the non-responding group. To examine the change in the mean between more than two groups, the analysis of variance test is used. And if an important difference between the three sample means or a significant difference between the three-sample means was detected through the analysis of variance, a post hoc analysis was used. To determine the extent to which the percentages of the test groups varied, Fisher or chi-square tests were used. If any of the expected values in a 2 x 2 comparison are greater than 5, the Fisher test is used. Phi correlation is used to quantify the probability of non-response and the relationship between genotypes.

Results

Demographics and clinical characteristics of the study groups.

Table I summarizes the statistical characteristics of the study groups. Patients in the present study consented. Additionally, there were differences in smoking levels between non-respondents and respondents (P). After twelve months of Herceptin administration, non-responders and responders showed differences in creatine kinase MB, TN I and T (value 0.04).

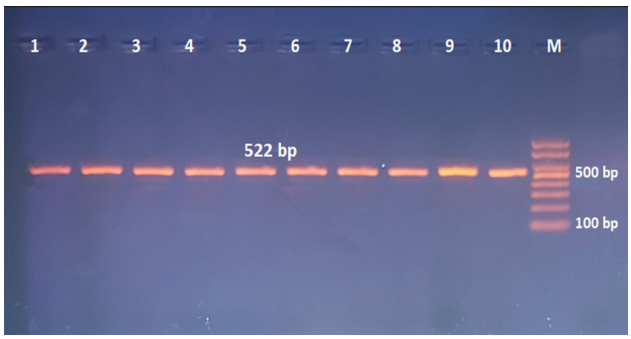
DNA concentration

The extracted DNA concentration ranged from 20 to 30 µg/ml across all samples.

PCR amplification Result

Figure 2 shows the PIK3CA magnification of the samples. PIK3CA is a magnification of samples divided by electrophoresis on 1% agarose gel and stained with ethidium bromide.

Figure 2: Enlargement of the human PIK3CA gene was obtained by 1% agarose gel electrophoresis and ethidium bromide staining. Conductive marker M:100bp. the 522 bp PCR products in lanes 39 to 57.



Analysis of Sanger sequence data

A study of the single nucleotide polymorphism of PIK3CA. The PCR products were directly sequenced. There were homozygous wild-type and heterozygous mutant genotypes of the E542K mutation.

Figure 3: Sequence analysis of the leading strand of the E542K mutation. The lower sequence represents the heterozygous mutant genotype, while the upper sequence represents the homozygous wild type.

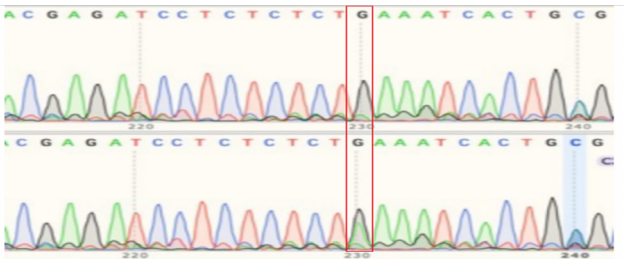
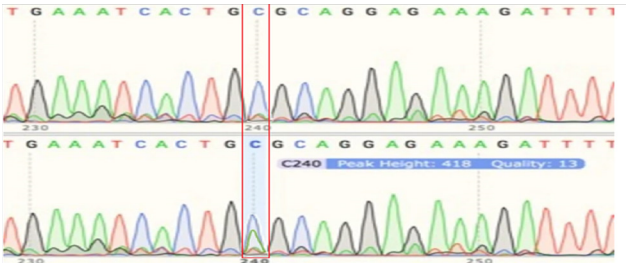


Table I: Demographics and clinical characteristics of the study groups.

Variables		Responder group (N=30)	Non-Responder group (N=30)	p-value
Age (yrs.)		51.86 ±8.148	48.76 ±8.601	0.1[a]
BMI (Kg/m²)		63.37 ±8.277	63.20 ±9.297	0.1[a]
Smoking status (N%)	Non smoker Smoker	5(16.7%) 25(83.3%)	12(40.0%) 18(60.0%)	0.04*[c]
CK_-MB ng/ml	Baseline After 12 months	10.912 ±2.404 12.012 ±2.419	10.989 ±2.370 11.458 ±2.075	0.9 0.3
Tn T Pg/ml	Baseline After 12 months	158.696 ±185.82 130.55 ±32.381	134.652 ±26.429 149.590 ±38.88	0.4 0.04*
Tn I Pg/ml	Baseline After 12 months	626.66 ±169.029 671.177 ±151.33	732.489 ±195.09 1008.93 ±150.66	0.183 0.0001*

Results are reported as means ±SD or frequency (percentage).CK_MB Creatine kinase MB. Tn-troponin a: 2-sample independent t-test. b: chi-square test. C: Fisher's exact test.

Figure 4: Sequence analysis of the leading strand of the E545K mutation. The lower sequence represents the heterozygous mutant genotype, while the upper sequence represents the homozygous wild type.



According to mutation analysis, 20% of unresponsive women had the E542K mutation, compared to only 33% of responders, with no apparent difference. Non-responding women (23 points, 33 percent) and responsive women (33 points, 33 percent) had almost identical distributions of the E545K mutation, but there was a significant difference (OR = 8 points 83, CI to 95 percent = 1 point 0-76). points). 96, p= 0 point 049). **Table II** shows that the combination of mutations was significantly more common in unresponsive women (40%) than in responsive women (6.67%) (OR = 9.33, 95% CI = 1.87 to 46, 68, p = 0.007).

Discussion

Breast cancer, currently the most common form of cancer worldwide, continues to increase in incidence every year. 2.3 million new cases are expected in 2020. Human epidermal growth factor receptor 2 (HER-2) plays an important role in breast cancer and serves as a diagnostic marker and target for genetic engineering[20-21].

The demographic information and clinical characteristics of the study group are shown in **table I**. That is, a non-responder group (N=30) and a responder group (N=30). The table contains several variables with corresponding p-values, which indicate the statistical significance of the differences observed between the two groups. The first two variables, age and BMI, showed no statistically significant differences between the non-responder and responder groups, as indicated by the p-value of 0.1. In terms of clinical characteristics, baseline values of creatine kinase-MB (CK-MB), troponin T (TnT) and Tn I showed no significant differences between the two groups, as demonstrated by the p-value of 0.9, 0, 4 and 0.183. Everything is fine. However, troponin I (TnI) levels at 12 months showed a significant difference between groups with a p-value of 0.0001. Due to higher doses of anthracyclines (mainly in combination with trastuzumab), non-responders at 12 months had higher mean baseline TnI values (1,008.93 pg/ml) than responders (671.177 pg/ml) in previous study.: Elevated cTn levels have also been shown to be a predictor of cardiac toxicity in breast cancer patients[22]. T and I cTn isotypes are very important for the assessment and diagnosis of ACS risk as well as other causes such as chemotherapy. It is a sensitive and specific indicator of myocardial damage[23]. Cardiac biomarkers such as NT-pro BNP, CK-MB or myoglobin have no proven value in predicting the course of cardiovascular events. The challenge is to effectively treat JK while minimizing toxicity[24]. In the present study, the presence of E542K and E545K mutations was significantly associated with trastuzumab resistance (OR = 9.33, 95% CI = 1.87 to 46.68, p = 0.007). This means that carriers of this mutation have a 9.33 times greater risk of not responding to trastuzumab compared to wild-type carriers. These findings are consistent with many previous studies conducted around the world.

Cizkova and colleagues[25] reported that the prevalence of PIK3CA gene mutations in patients with HER2-positive breast cancer is relatively high and has prognostic implications, and that these mutations lead to resistance to trastuzumab. Vasan *et al*.[26] showed that the presence of a double PIK3CA mutation in the same allele led to increased PI3K activity and enhanced downstream signaling, cell proliferation and tumor growth. Huang *et al*.[27] showed that most human gain-of-function mutations of p110α and p85 occur at the interface between them or at residues located between the p110α kinase domain and other domains of the catalytic subunit. These mutations increase Her2 activity and contribute to resistance to trastuzumab. Additionally, Burns et al. [28] showed a significantly worse response to trastuzumab in a cohort of 55 breast cancer patients with low PTEN levels and the presence of oncogenic PIK3CA mutations. In contrast, many other studies have reported no association. In this context, Loi et al. [29], evaluating PIK3CA gene mutations in 705 HER2-positive breast cancer samples, reported that PIK3CA gene mutations

Table II: Mutational profile of E542K and E545K in responsive and non-responsive women with her-2 new breast cancer for trastuzumab.

Mutation	Non-responsive (n=30)	Responsive (n=30)	p-value	OR (95%CI)
E542K				
Wild type	24 (80%)	29 (96.67%)	0.076	1.0
Mutant	6 (20%)	1 (3.33%)		7.25 (0.81-64.46)
E545K				
Wild type	23 (76.67%)	29 (96.67%)	0.049	1.0
Mutant	7(23.33%)	1 (3.33%)		8.83 (1.0-76.96)
E542K/E545K				
Wild type	18 (60%)	28 (93.33%)	0.007	1.0
Mutant	12 (40%)	2 (6.67%)		9.33 (1.87-46.68)

were not significantly associated with trastuzumab resistance. In a meta-analysis study, Wang et al. [30] also concluded that in patients with HER2-positive BC, PIK3CA mutation was not associated with response to trastuzumab-based therapy. Finally, an Italian study showed that there was no association between mutations in the PIK3CA gene and resistance to trastuzumab[31].

Interestingly, there might even be a link between PIK3CA mutation and response to trastuzumab. A systematic review of breast cancer clinical trials including 2,587 breast cancer cases from 12 independent studies found that patients with tumors harboring a PIK3CA mutation have better clinical outcomes than those with a wild-type PIK3CA gene[32].

These conflicting results strongly suggest that trastuzumab resistance is multifactorial and that the impact of PIK3CA mutations on the clinical course of breast cancer appears to vary depending on the background of other genomic alterations such as HER2 status. hormonal status and other genetic and epigenetic influences.

The possible fundamental component of increased resistance to trastuzumab in women with E542K and E545K alterations is suggested by the work of the PIK3 protein. It has been well established that the p110α catalytic subunit of PI3K is encoded by the quality PIK3CA, which phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) at the third position of the inositol ring, producing PIP3, after inscription into the cellular film by part of the receptor -mediated activation. In this way, Akt is phosphorylated, stimulating a signaling cascade involving the mammalian target of rapamycin (mTOR) and downstream effectors[33]. Hotspot transformations within helical or kinase spaces hyperactivate the PI3K/Akt pathway downstream of the ErbB family[34]. among which H1047R, E545K and E542K are the most common transformation sites, accounting for approximately 63% of all PIK3CA modifications[35]. In vitro information informed that the unusual execution of the PI3K/Akt pathway through the effect of PIK3CA changes was involved in resistance to anti-HER2 targeted trastuzumab[36].

Conclusion

After 12 months of treatment with trastuzumab, both troponin I and T increased significantly in non-responders compared to responders, whereas CK-MD had no such association with response to trastuzumab. Troponins I and T are cardiotoxic markers, but also have excellent predictive value for trastuzumab resistance in Her-2-positive breast cancer. Resistance to trastuzumab is significantly related to the E545K mutation, either present alone or in combination with the E542K mutation.

Competing interests

Authors have declared that they have no competing interests.

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