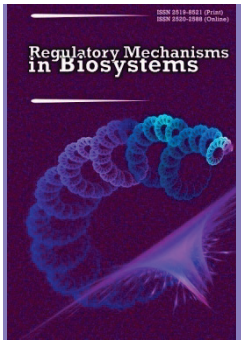

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Biological mechanisms of resistance to trastuzumab and ways to overcome them: Modern problems of clinical oncology

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In 2022, 2.3 million new cases of breast cancer were registered in the world, which accounted for 11.6% of the total number of malignant neoplasms. Depending on the tumor's molecular profile, the prognosis for patients can be different. One of the most aggressive types is HER2-positive breast cancer. Trastuzumab, a recombinant humanized monoclonal antibody against HER2, is used to treat such tumors. Congenital or acquired resistance to trastuzumab is one of the essential problems in clinical oncology. Our study aimed to investigate the resistance mechanisms to trastuzumab and ways to overcome them. This drug influences several directions of oncogenesis at the same time. The fundamental mechanisms of action of trastuzumab are inhibition of HER2 ectodomain shedding, inhibition of angiogenesis, degradation of HER2 protein and its internalization, inhibition of DNA repair, influence on the phosphatidylinositol 3-kinase pathway, cell cycle and antibody-dependent cellular cytotoxicity. The biological mechanisms of resistance to trastuzumab are based on vascular mimicry and hypoxia, the appearance of breast cancer stem cells, activation of alternative signaling pathways, metabolic changes, alternative molecular variants of HER2, changes in the processes of immune regulation, heterogeneity of expression and stability of the HER2 protein. In modern clinical oncology, trastuzumab is used as an original product and as antibody-drug conjugates. Trastuzumab emtansine and trastuzumab deruxtecan are approved for the treatment of patients with HER2-positive breast cancer, including those with low HER2 expression. This literature review identified the biological resistance mechanisms to trastuzumab and ways to overcome them. The implementation of new targeted drugs in combination with trastuzumab is the way to personalized treatment. It can significantly improve the survival of patients with HER2-positive breast cancer.

Keywords: trastuzumab; resistance; immune response; antibody drug conjugates; signaling pathways.

Introduction

According to Globocan, 2.3 million new cases of breast cancer were registered in 2022, which accounted for 11.6% of the total number of malignant neoplasms. In 157 out of 185 countries, breast cancer is the leading cause of cancer among women (Kolomiiets & Moskalenko, 2023; Bray et al., 2024). This disease is quite heterogeneous and has different molecular profiles due to the expression of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) (Roy et al., 2023). The HER2 gene encodes the last receptor type and belongs to the EGFR receptor family. In addition to HER2 receptors, the family includes EGFR/HER1, HER3, and HER4. Their role is to promote signaling through the RAS-MAPK and PI3K-AKT pathways. ErbB receptors in domains I/L1, II/CR1, III/L2, and IV/CR2 are in the extracellular environment. During signal transduction, the cytosolic kinase dimerizes and is autophosphorylated. As a result, the signaling pathway spreads inside the cell (Vahidian et al., 2019).

Commonly, HER2 is expressed in small amounts on the surface of liver cells, ovaries, lungs, and kidneys. However, hyperexpression is observed in the case of breast cancer, which is confirmed by the results of an immunohistochemistry. Metastatic breast cancer with high HER2 expression is associated with poor overall survival and poor prognosis (Wang et al., 2023).

Trastuzumab is a recombinant humanized monoclonal antibody against HER2. This drug is a development of Genentech Inc. (California, USA). Trastuzumab is one of the most effective targeted drugs for the treatment of patients with hyperexpression of HER2. At the same time, it does not affect tumors with an average level of these receptors. In combination with chemotherapy, trastuzumab has been shown to increase progression-free survival (PFS) and overall survival (OS) in people with metastatic breast cancer (Dempsey et al., 2023).

Despite significant progress in the treatment of HER2-positive BC and the effectiveness of trastuzumab, overcoming acquired resistance to monoclonal antibodies is a highly relevant question. The resistance is associated with the progression of the disease and the reduction of OS. Therefore, knowledge of the mechanisms of development of acquired resistance is the key to creating new directions in the therapy of breast cancer and overcoming resistance to trastuzumab.

Our study aimed to investigate the mechanisms of resistance to trastuzumab and ways of overcoming them.

Biological mechanisms of action of trastuzumab

The antitumor effect of trastuzumab is complicated. This drug simultaneously influences several directions of oncogenesis. Its action can be direct or indirect. The direct action is associated with a direct action on receptors, inhibition of the release of the HER2 ectodomain, and disruption of the signal passage inside the cell. Indirect action is manifested in the ability of trastuzumab to activate the immune response through antibody-dependent cellular toxicity (Mandó et al., 2021). This drug has several antitumor effects with a targeted mechanism of action. The main ones will be discussed below.

One of the essential reasons for developing resistance to trastuzumab is the appearance of a truncated type of HER2. This phenomenon is observed due to proteolytic cleavage in the area of the extracellular domain. As a result, interaction with monoclonal antibodies becomes impossible due to the absence of a binding part. The extracellular domain (ECD) can be determined in the blood serum of patients and is an essential predictor of the prognosis of the disease. An increase in the level of this biological marker in women with metastatic breast cancer indicates an unfavorable prognosis. Serum HER2-ECD is considered a promising and convenient method for monitoring the effectiveness of trastuzumab treatment (Ding et al., 2020).

Trastuzumab inhibits the migration of endothelial cells and reduces the density of the vascular wall. This negatively affects angiogenesis as a whole and leads to an increase in the antitumor effect. Yang et al. (2022) investigated the relationship between trastuzumab resistance and angiogenesis. It was established that B-chains of alpha-crystallin are formed in tumor cells and resistant to the influence of monoclonal antibodies. They promote the formation of endothelial cell tubes by activating the pathological mTOR pathway. Rapamycin (mTOR inhibitor) helps to stop the formation of endothelial tubes.

Trastuzumab leads to degradation and internalization of the HER2 protein on the surface of tumor cells with overexpression of these receptors. The basis of this process is the stimulating effect of tyrosine kinase-ubiquitin ligase c-Cbl, which ubiquitates and cleaves HER2 (Klapper et al., 2000). The motility of cancer cells highly expressing HER2 is affected by Endo II (endocytic adapter protein endophilin A2). The level of expression of this protein may be an indicator of efficacy or resistance to trastuzumab therapy. Endo II blockade causes defects in HER2 internalization and reduces the efficacy of monoclonal antibody therapy (Baldassarre et al., 2017).

Many theories attempt to explain the molecular mechanisms of DNA repair inhibition. Baselga et al. (2023) established that the consequence of radiation exposure can be increased effectiveness of trastuzumab. Modern studies support this theory, as trastuzumab is recommended to be used competitively with radiation therapy (Li et al., 2024).

Salvestrini et al. (2023) demonstrated that trastuzumab inhibits EGF-induced lipid heterodimer EGFR-HER2. In turn, this leads to disruption of MβCD and blocking of HER2-mediated activation of AKT. The effect is very similar to the principle of action of trastuzumab. As a result, MβCD and trastuzumab synchronously negatively affect AKT activation. The scientists concluded that the targeted action of monoclonal antibodies consists of inhibition of the EGFR-HER2 heterodimer, inhibition of AKT phosphorylation, and cell division.

Poly (ADP-ribose) polymerase (PARP) blockers, together with AKT serine-threonine kinase inhibitors, enhance the antitumor effect of trastuzumab in patients with HER2 overexpression (Li et al., 2022).

The PI3K signaling pathway is associated with the development of many malignant neoplasms, including breast cancer. Some current studies aim to study the mechanisms of regulation of the PI3K signaling pathway in patients with HER2 overexpression. In particular, Liu et al. (2023) discovered the role of miR-18a-5p receptors on the surface of tumor cells and their role in the progression of breast cancer. It was established that they counteract adhesion, proliferation, migration of tumor cells, and activation of the pathological PI3K/AKT pathway.

HER1, HER2, HER3, and HER4 are involved in HER signaling, to which different ligands can attach. As a result, membrane receptor tyrosine kinases activate the PI3K/AKT pathway. Currently, studies are being conducted in which inhibitors of the pathological PI3K/AKT/mTOR pathway are evaluated to increase trastuzumab therapy's effectiveness (Miricescu et al., 2020).

Trastuzumab inhibits the proliferation of tumor cells by affecting the G1 phase of the cell cycle. This effect is due to the inhibition of the expression of specific proteins and the cleavage of the cyclin-dependent kinase (CDK) blocker p27 (Kip1). Kurozumi et al. (2015) discovered that a low level of p27 (Kip1) is one of the predictors of a complete response to neoadjuvant chemotherapy with trastuzumab in patients with breast cancer. Similar results were obtained during the study of p27 expression in patients with early stages of HER2-positive breast cancer. A low level of p27 expression is associated with a good response to trastuzumab (Filipits et al., 2018). ADCC is one of the essential mechanisms of action of trastuzumab, which can influence the condition of the immune system. For example, natural killer cells (NK cells) that express FcγRIIIA are activated. The relationship between ADCC and these receptors is direct. Fc-receptor-dependent mechanisms contribute to the activation of antitumor immunity and improve the functioning of cytotoxic antibodies. According to its mechanism of action, trastuzumab binds to tumor cells. It is then recognized by the Fc receptors of ADCC effector cells (e.g., macrophages, NK cells, and granulocytes). As a result, the cytotoxic activity of these immune cells and the death of cancer cells are observed (Guti et al., 2023). ADCC activity increases in the blood serum of patients with breast cancer against the background of trastuzumab administration. In addition, the number of NK cells and cytotoxic proteins increases significantly. C leads to modification of antibodies and inhibition of proteins such as histone deacetylase (HDAC) and caspases (CD112R and TIGIT) (Nami et al., 2018; Stenger et al., 2024).

Biological mechanisms of resistance to trastuzumab

Overcoming resistance to trastuzumab is one of the essential tasks in oncology. According to the results of studies by Fogazzi et al. (2022), only a third of women receiving targeted therapy demonstrated a good response. Others have disease progression, suggesting congenital or acquired resistance to trastuzumab. The basis of this phenomenon may be detachment from Cbl, dysregulation of HER2, and change in the endocytosis process. In addition, the expression of TGFα receptors leads to the activation of dissociation mechanisms and makes complete contact of trastuzumab with the tumor cell impossible (Liu et al., 2021). About 70% of women with metastatic HER2-positive breast cancer have experienced disease progression during the first year of treatment (von Arx et al., 2023; Marra et al., 2024). The main mechanisms that cause this situation are discussed below.

An essential mechanism for the development of resistance to trastuzumab is altered angiogenesis. Hori et al. (2019) introduced a particular term to describe this phenomenon – vasculogenic mimicry (VM). According to this theory, tumor cells can transform from an epithelial to a vascular phenotype. These cells are characterized by the presence of periodic acid Schiff (PAS), which is a surface antigen of endothelial cells. However, they do not express classical markers such as CD31 and are incapable of tube formation. This mechanism of angiogenesis is an adaptive process that helps the tumor to survive in conditions of hypoxia.

Morales-Guadarrama et al. (2021) observed the phenomenon of VM in HER2-positive patients. Tumor tissue from patients receiving trastuzumab had a significantly higher channel with VM than samples from the control group. A similar picture was observed when comparing samples before and after neoadjuvant therapy. Scientists explained this phenomenon by activating alternative pathological pathways and oncogenic receptors, such as IGF-1R, FGFR2, EGFR, and VEGF2. Vasculogenic mimicry ensures the survival of tumor cells in conditions of hypoxic stress. This phenomenon allows them to adapt to the surrounding conditions, grow and spread. All of the above factors, in combination with unfavorable clinical and pathological characteristics, lead to the progression of breast cancer (Chavoshi et al., 2022).

Some studies have confirmed that the leading cause of breast cancer is cells that express CD24−/low/CD44+. They are named breast cancer stem cells (BCSCs). Currently, BCSCs are considered to be the driving force behind metastasis and resistance to trastuzumab (Santisteban et al., 2009). These cells are resistant to radiation and cytotoxic agents. They are capable of self-renewal, self-regulation, and re-differentiation, which ensures aggressive tumor growth and disease progression. BCSCs change fundamental processes in such a way as to increase metabolism, develop resistance to autophagy and apoptosis, and induce transcription of stem cells. In addition, their action is aimed at disrupting detoxification mechanisms and activating proteins involved in drug transport. All processes are accompanied by a malfunction of many signaling pathways (Espinosa-Sánchez et al., 2020).

BCSCs may be affected by trastuzumab because they overexpress HER2. An experimental *in vitro* study confirmed that trastuzumab increases the number of BCSCs and promotes the development of resis-

tance. BT474 spheroids and 2D shapes represented cell cultures. The population of spheroid cells expressed HER2 differently. It was observed that the population of cells with a higher expression of HER2 increased intensively after using trastuzumab. The consequence of this phenomenon was resistance to trastuzumab (Rodríguez et al., 2018).

Stem cells are associated with specific signaling pathways and receptors on the surface of tumor cells. For example, reduced receptor tyrosine kinase (EPHA5) expression indicates resistance to trastuzumab and a poor prognosis. This phenomenon is due to the enhancement of BCSC-like properties of tumor cells. At the same time, the number of cells with the CD44+/CD24low phenotype increases. So-called mammospheres with increased expression of CD133+ and NANOG are formed. The expression of E-cadherin decreases. Activation of PTEN/AKT and Notch-1 signaling pathways was observed. During the experiment, Li et al. (2019) confirmed that downregulation of EPHA5 in HER2-positive breast cancer cells initiates resistance to trastuzumab. EPHA5 can be considered a predictor of response to trastuzumab therapy. Using inhibitors of the Notch1 pathway can overcome resistance and enhance the antitumor response. Several scientific groups have supported this approach. Notch signaling has been shown to stimulate the growth of HER2-positive breast cancer cells, promoting the BCSCs phenotype (Maruthachalam et al., 2022; Ray et al., 2024). Osipo et al. (2008) confirmed the effectiveness of the above therapeutic strategy. They used trastuzumab in combination with Notch-1 small interfering RNA (siRNA). This combination of drugs inhibited the growth of trastuzumab-resistant and sensitive cells.

The following molecule with an inextricable connection with stem cells is cyclin-dependent kinase 12 (CDK12). In many cases, simultaneous overexpression of HER2 and CDK12 is observed. This phenomenon correlates with insensitivity to trastuzumab and a poor prognosis. The resistance mechanism is based on activation of the Wnt signaling pathway and self-renewal of BCSCs (Li et al., 2021). Blocking the activity of CDK12 with danaciclib allows us to overcome resistance to trastuzumab (Lu et al., 2024). In metastatic BCSCs, signaling is more active compared to non-stem tumor cells. Geldanamycin, a heat shock protein (HSP-90) inhibitor, increases sensitivity to trastuzumab and influences the Wnt signaling pathway (Willert et al., 2006).

Hyperexpression of HER2 on the surface of breast cancer cells stimulates the secretion of inflammatory cytokines such as interleukin-6 (IL-6). In turn, IL-6 initiates the JAK1-STAT3 signaling pathway. The consequence of this cascade reaction is the activation of STAT3 in HER2-positive breast cancer cells (Hartman et al., 2011; Sakai et al., 2023). A stimulatory effect of HER2 on STAT3 signaling has been demonstrated in several studies. The consequence of this effect is the stimulation of BCSCs and resistance to trastuzumab. For example, Marotta et al. (2011) studied IL-6/JAK2/STAT3 signaling and concluded that this process is particularly active in CD44+CD24− BCSCs. In addition, blocking JAK2 leads to a reduction in the population of BCSCs and restoration of sensitivity to trastuzumab.

Dogan et al. (2018) studied the role of telomerase in stimulating the development of BCSCs. The telomerase matrix antagonist imetelstat was used to restore sensitivity to trastuzumab. The effect of the drug resulted in the impossibility of maintaining the process of mammosphere formation and the reduction of the population of BCSCs (Koziel et al., 2015).

Resistance to trastuzumab can be caused not only by the properties of the tumor but also by the tumor microenvironment. The tumor is surrounded by different types of cells, such as fibroblasts, mesenchymal stem cells, immune and endothelial cells. Their number is directly related to the degree of inflammation. Mesenchymal stem cells induce exosomal AGAP2-AS1, which is overexpressed in patients with trastuzumab resistance. In turn, AGAP2-AS1 increases the production of carnitine palmitoyltransferase 1 and stimulates the formation of a complex with human antigen R, which later increases the expression of genes associated with BSCSs (Han et al., 2021). It is worth noting that the role of stem cells in resistance to trastuzumab was studied not only for breast cancer. In HER2-positive gastric tumors, resistance was due to the expression of the spiral protein GSE1. GSE1 overexpression is associated with rapid metastasis to regional lymph nodes and lower survival. Blocking these receptors allows the restoration of sensitivity to trastuzumab and improves patient outcomes (Wang et al., 2021).

Therefore, the induction of BCSCs is an essential mechanism of resistance to trastuzumab. Chemotherapy and radiation therapy have a limited effect on BCSCs, so the question arises about the development of alternative drugs with targeted action in order to restore sensitivity to trastuzumab (Qiu et al., 2021).

Based on the results of several studies, a connection between resistance to trastuzumab and disturbances in the MAPK and PI3K/Akt/mTOR signaling pathways has been established. Abnormal signaling begins through other HER receptors, including HER1, HER3, and HER4. As a result, the effect of trastuzumab is weakened due to the inability to completely block signals (Sanz-Álvarez et al., 2023).

Cataldo et al. (2023) studied the efficacy of combining trastuzumab with a PI3K inhibitor to overcome resistance. A cell line of PTEN-deficient or PIK3CA-mutant breast cancer cells was used for this. Scientists have found that combining PI3K inhibitors (such as alpelisib) with trastuzumab is an effective strategy to overcome resistance. The following study was conducted in a mouse model and showed satisfactory results. In contrast to chemotherapy, alpelisib has a better safety profile and, in combination with trastuzumab, may prove to be a new therapeutic strategy for the treatment of patients with HER2-positive breast cancer.

DiGiovanna et al. (2005) established a relationship between abnormal EGFR expression and HER2 activation. Hyperexpression of EGFR was found in 35% of HER2-positive breast cancer samples. Another study confirmed that such patients have a poor prognosis (Li et al., 2022).

Pathological MAPK, PTEN, PI3K, Akt, and mTOR signaling pathways can be affected by non-HER receptor tyrosine kinases. For example, overexpression of insulin-like growth factor I (IGF-1R) receptors is observed in 50% of cases of breast cancer. Patients with this variant of tumors have a worse prognosis. Resistance to trastuzumab is caused by direct activation of HER2. IGF-1R combines with HER-2 to form a heterodimer that can lead to HER2 phosphorylation. Blocking IGF-1R receptors disrupts the process of heterodimer formation and restores sensitivity to trastuzumab (Yan et al., 2022).

Luo et al. (2021) found that IGF2/IGF-1R/IRS1 signaling is abnormally activated in patients with Herceptin-resistant BC. This phenomenon is associated with disturbances in the FOXO3a-miRNA negative feedback inhibition process.

The expression of MET receptors and its ligands can contribute to resistance to trastuzumab. This occurs through prolonged activation of AKT and inhibition of p27 induction (Shattuck et al., 2008). Another molecular mechanism of resistance to monoclonal antibodies is the increased expression of EphA2 receptors, which leads to increased signaling by the PI3K/Akt and MAPK pathways. Targeting EphA2 receptors is currently considered a promising way to overcome resistance to trastuzumab (Veiga et al., 2024).

Erythropoietin receptors (EpoR) may also contribute to drug resistance. Martins-Branco et al. (2024) evaluated the impact of erythropoietin-stimulating agents on prognosis in patients with early stages of HER2-positive breast cancer. Scientists have established that the combination of this group of drugs with trastuzumab is safe and does not increase the number of side effects.

Liu et al. (2016) elucidated the molecular mechanisms of the effects of the catecholamine/β_2-adrenergic receptor (β_2-AR) signaling pathway on the efficacy of trastuzumab treatment. β_2-AR expression is negatively correlated with response to monoclonal antibody therapy. Catecholamines counteract the antiproliferative effect of trastuzumab. They upregulate MUC-1 and miR-21 expression by activating STAT3 and Her2. This causes phosphatase deficiency and activation of the Akt and PI3K pathways. In addition, catecholamines are able to inhibit miR-199a/b-3p and activate the mTOR pathway. The level of expression of β_2-AR receptors can be a prognostic marker of the course of breast cancer. In addition, β-blocker propranolol enhances the effectiveness of trastuzumab therapy.

Estrogen receptors play a significant role in developing innate or acquired resistance. Most HER-positive tumors express estrogen and progesterone receptors; they are hormone-positive. There is a cross-talk between the hormonal pathway and HER2 (Tommasi et al., 2024). That is why the decision to combine hormonal therapy and targeted therapy is justified. Clinical studies confirm that such a scheme is equal to the traditional one when a target drug and chemotherapy are prescribed for the first line of

therapy (Liang et al., 2024). Other elements of influence to overcome resistance to trastuzumab may be the use of inhibitors of cyclin-dependent kinase 4/6, Akt, PI3K, programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1) (Pegram et al., 2023).

Metabolic disorders can significantly influence resistance to trastuzumab. HER2 overexpression is associated with increased heat shock factor 1 (HSF1) synthesis. Its trimer HSF-1 leads to the activation of lactate dehydrogenase A (LDH-A) synthesis, which takes part in anaerobic glycolysis. A decrease in the expression of HSF1 and LDH-A manifests the effect of trastuzumab on HER2-positive tumors. However, artificial hyperexpression of these markers leads to the development of resistance to trastuzumab (Gumilar et al., 2023).

The t-Darpp protein is another factor that causes resistance by affecting metabolism. It activates pathological IGF-1R signaling by heterodimerizing with EGFR or HER2 and stimulates glycolysis. In general, the basis of the molecular mechanism of resistance is an increased dependence on oxidative phosphorylation and glycolysis (Denny et al., 2015).

Resistant BC cells demonstrate dependence on ATP synthase function and highly express these genes. Gale et al. (2020) used the ATP synthase inhibitor oligomycin A, which, in an *in vivo* experiment, led to the regression of tumor cells resistant to trastuzumab. That is why targeted drugs that affect ATP synthase and glycolysis may be promising therapeutic directions (Wang et al., 2021; Akter et al., 2024).

An essential factor in the aggressiveness of HER2 is the influence of fatty acids. Fatty acid synthase (FASN) catalyzes the intracellular synthesis of palmitate from malonic-CoA and acetyl-CoA. Blocking FASN upregulates the transcriptional repressor HER2 and leads to a decrease in HER2 surface expression. In turn, HER2 enhances the endogenous formation of fatty acids, stimulating the overexpression of FASN. The targeted drug denifanstat, capable of blocking FASN, is currently undergoing clinical trials (Menendez et al., 2024).

Ferraro et al. (2021) established that fatty acids are involved in the process of metastasis of HER2-positive breast cancer to the brain. This process is mediated by fatty acid-binding protein 7, which promotes lipid accumulation. Trastuzumab can inhibit FASN expression, but due to a metabolic switch from the process of endogenous lipogenesis to the external capture of fatty acids, this process can be compensated. Increased absorption of long-chain fatty acids contributes to primary resistance.

Lipid metabolism and HER2 pathogenesis may be influenced by lipid raft-resident protein (MAL2). The MAL2-mediated molecular mechanism of action leads to increased HER2 signaling and plasma membrane retention of HER2 in breast cancer cells. During the interaction of HER2 and MAL2, a protein complex HER2/Ezrin/NHERF1/PMCA2 is formed, leading to a low intracellular calcium concentration. This process is especially pronounced in cells of HER2-positive breast cancer resistant to trastuzumab. MAL2 is critical for HER2 signaling and membrane stability in tumor cells. Therefore, MAL2 can be considered a biological marker and a potential therapeutic target. Trastuzumab, in combination with the FASN inhibitor cerulenin, induces apoptosis (Jeong et al., 2021).

The HER2 protein can have several molecular variants that impair its ability to bind trastuzumab. Additional anti-HER2 drugs that block these alternative variants are used therapeutically. For example, the carboxy-terminal fragment of HER2 (p95HER2) lacks the trastuzumab-binding extracellular domain. It is formed in alternative translation or initiation of mRNA encoding HER2. Another variant of its origin is the synthesis of the extracellular domain by the ADAM10 metalloprotease. The ability to form homodimers provides p95HER2 with the ability to become resistant to trastuzumab. Patients with p95HER2 overexpression have poor PFS and OS, so this biological marker may be considered a therapeutic target (Sperinde et al., 2018).

In 2–9% of cases of the total HER2, there is a spliced variant – HER2Δ16 with missing exon 16. HER2Δ16 induces cell transformation several times more strongly. Homodimers provide resistance to trastuzumab with strengthened disulfide bonds. In addition, HER2Δ16 can model the tumor microenvironment. Ectonucleotide pyrophosphatase-phosphodiesterase 1 (ENPP1) acts as a regulator of the immune cold microenvironment. Blocking HER2Δ16-derived ENPP1 leads to increased T-cell infiltration and inhibition of tumor growth. HER2Δ16-mediated activation of ENPP1 is associated with aggressive HER2-positive breast cancer. Thus, ENPP1 can be considered one of the targets to overcome resistance to trastuzumab (Attalla et al., 2023). Castagnoli et al. (2019) established that HER2Δ16 dimers activate PI3K/AKT, MAPK, and FAK signaling pathways, ensuring breast cancer cell proliferation and migration. In 89% of patients with at least one metastatic lymph node, an oncogenic variant of HER2 – HER2Δ16 is detected. In an experiment on cancer cell lines, forced overexpression of HER2Δ16 promotes receptor dimerization and resistance to trastuzumab. Vo et al. (2023) found that miRNA expression abrogates resistance to trastuzumab, as it inhibits HER2Δ16-mediated carcinogenesis in breast tissue. Micro-RNA is represented by a sequence of 20–25 nucleotides, which at the post-transcriptional level regulate gene expression.

During treatment with trastuzumab, changes in the processes of immune regulation can occur, which leads to the appearance of resistance. Chaganty et al. (2018) found that trastuzumab upregulated PD-L1 in HER2-overexpressing breast cancer cell culture when co-cultured with human peripheral blood. However, it alone cannot affect the level of PD-L1 expression. IFNγ-neutralizing antibodies can permanently block the PD-L1 activation process. At the same time, PD-L1 can be stimulated, enhancing the release of IFNγ. Essential sources of IFN-γ are T cells and NK cells. Recruitment of immune effector cells and trastuzumab-mediated upregulation of PD-L1 are potential mechanisms for overcoming resistance. Combining trastuzumab with PD-1/PD-L1 inhibitors is a promising therapeutic strategy for the treatment of patients with HER2-positive breast cancer (Zhao et al., 2024).

Another molecular mechanism responsible for developing acquired resistance to trastuzumab is the inhibition of antibody-dependent cellular cytotoxicity of NK cells (ADCC). Darwich et al. (2021) found that the blood serum of trastuzumab-resistant BC patients contains a high concentration of the inflammatory protein chitinase 3-like 1 (CHI3L1). Recombinant CHI3L1 inhibits innate NK cell cytotoxicity and ADCC by preventing proper polarization of the microtubule center. An experiment on mice established that introducing CHI3L1 leads to a decrease in the infiltration of NK and T cells and an increase in the number of macrophages. The combination of these factors has a negative impact on the prognosis of the disease. The use of CHI3L1 inhibitors potentiates the antitumor effect of trastuzumab (Ma et al., 2021).

As a result of prolonged exposure to trastuzumab, the expression of genes responsible for resistance increases. Zazo et al. (2020) studied CCL5, which belongs to the cytokine family. It activates the ERK signaling pathway and is considered a mediator of trastuzumab resistance. The higher the expression of CCL5, the lower the chance of a complete response following neoadjuvant therapy. In addition, impaired regulatory T-cell function may mediate poor response to targeted therapy (De Angelis et al., 2020).

The basis of resistance to trastuzumab may be increased regulation of complement regulatory proteins. Neutralization of the complement regulatory proteins CD55, CD59, and CD46 enhances the complement-mediated activity of pertuzumab and trastuzumab in HER2-positive breast cancer cells. Usually, the human body's cells are insensitive to human complement, but tumors increase their regulation and contribute to the emergence of resistance to trastuzumab. There is an inverse relationship between CD55 and CD59 levels in tumor cells and patient survival. The higher the expression, the worse the prognosis (Mamidi et al., 2013).

Another reason for resistance to trastuzumab is the heterogeneous expression of HER2 itself (Ocaña et al., 2020). Tanei et al. (2024) investigated the effect of heterogeneity on patient survival. Two hundred fifty-one patients with HER2-positive breast cancer took part in the study. They were all divided into two groups. Forty-six people were included in the group with high heterogeneity and 205 - with low heterogeneity. The group of patients with high HER2 heterogeneity had more distant metastases and worse survival. The scientists concluded that high HER2 heterogeneity in patients with HER2-positive breast cancer is associated with a poor prognosis. In most cases, low response to trastuzumab therapy is associated with high HER2 heterogeneity (Hou et al., 2023).

Trastuzumab resistance may be caused by HER2 protein stability, which HSP90 indirectly regulates. The complex formed by the interaction of HSP90 and HER2 leads to its conformation and stabilization. Park et al. (2024) studied the effect of HVH-2930, a novel C-terminal inhibitor of

HSP90, in overcoming resistance to trastuzumab. Inhibition of HSP90 was found to restore sensitivity to targeted therapy with trastuzumab. In their previous study, scientists explained the mechanism of restoration of sensitivity. HSP90 inhibitors promote the degradation of full-length HER2 and truncated p95HER2. In addition, they disrupt the dimerization of receptors of the HER2 family (Park et al., 2020).

Ye et al. (2021) investigated the effectiveness of the combination of lapatinib (an analog of trastuzumab) with the second-generation HSP90 inhibitor ganetespib. As a result of the therapy, early apoptotic cell death processes and cell division stops at the G1 phase were intensified in the breast cancer tissue. In addition, inhibition of the Ras/MEK/ERK and PI3K/Akt pathways was observed. The combination of lapatinib and ganetespib disrupts STAT3 transcription, which restores sensitivity to lapatinib. Currently, HSP90 inhibitors are being produced as nanoparticles (Li et al., 2023).

Bon et al. (2023) found that resistance to trastuzumab can be caused by the formation of complexes between HER2, cyclic AMP-regulated phosphoprotein (DARPP-32), and dopamine. DARPP-32 protein and its shortened variant t-DARPP are expressed on the surface of tumor cells in patients with HER2-positive breast cancer. Their effect consists in long-term activation of the Akt pathway and stimulation of the proliferation of cells resistant to trastuzumab. In addition, DARPP-32 and t-DARPP proteins stimulate the formation of complexes between HER2 and HSP90, which also negatively affects the treatment results with monoclonal antibodies.

MUC4 is a high molecular weight glycosylated protein that plays a role in adhesion and signaling during cell growth and division. This protein is expressed on the apical surfaces of the epithelium of various tissues, including the mammary gland. Dreyer et al. (2021) found that the expression levels of this protein in metastatic lesions are significantly higher compared to the primary tumor, indicating selective overexpression of MUC4 in metastases. A mouse study demonstrated that MUC4 deletion can effectively inhibit the metastasis of HER2-positive breast cancer. The effect of MUC4 is to mask receptors for trastuzumab binding epitopes. In addition, this glycoprotein stimulates PI3K and MAPK signaling through alternative pathways, disrupting the interaction of HER2 and its regulatory binding molecules. Abnormal TNF-α activity is the primary cause of MUC4 overexpression. In addition to MUC4, other glycoproteins, such as hyaluronan and MUC1, can mask the HER2 protein in the tumor microenvironment. A high level of MUC4 expression is thought to be associated with a poor response to targeted therapy and a poor prognosis for patients (Mercogliano et al., 2017).

Resistance to antibody drug conjugates of trastuzumab

In modern clinical oncology, trastuzumab is used as an original product and in antibody drug conjugates. One of the first trastuzumab-based drug antibody conjugates is trastuzumab emtansine (T-DM1), which was approved in 2013 for the treatment of patients with metastatic breast cancer. The phase III KATHERINE study evaluated the efficacy and safety of T-DM1 compared with trastuzumab in patients with HER2-positive breast cancer and residual disease after neoadjuvant chemotherapy and HER2-targeted therapy. Patients receiving T-DM1 showed a better response to treatment compared to the trastuzumab group. However, they had a significantly higher incidence of grade ≥ 3 side effects, particularly thrombocytopenia (Huang et al., 2021).

Hunter et al. (2020) explained the molecular mechanism of the advantages of T-DM1 compared to trastuzumab. Conjugates combine the properties of trastuzumab directly (blocking HER2-mediated signaling and induction of ADCC) and cause cell death through the phenomenon of mitotic catastrophe, which develops due to the inability to form the mitotic spindle and disruption of tubulin depolymerization. However, T-DM1, like trastuzumab, is also characterized by congenital or acquired resistance. The mechanisms of resistance are similar. For example, loss or reduction of surface HER2 expression and masking of the glycoprotein MUC4. In the KRISTINE phase III study, the influence of tumor cell heterogeneity of patients with breast cancer on T-DM1 response was evaluated (Hurvitz et al., 2018). Intratumoral heterogeneity, manifested by varying degrees of HER2 expression, is associated with poor response to therapy with drug conjugates to trastuzumab.

The cause of resistance to T-DM1 may be dysregulation of signaling pathways such as PTEN and PIK3CA. There can be several mechanisms of resistance at once. The development of one or another mechanism depends on the drug administration, the patient's general condition, and the stage of the disease. Impaired transport of the HER2-T-DM1 complex, high rate of HER2-T-DM1 recycling, and defective lysosomal degradation of T-DM1 may also indirectly affect the therapeutic effect of T-DM1 (Monteiro et al., 2024).

Trastuzumab deruxtecan (DS-8201) is another trastuzumab-based antibody-drug conjugate drug. This complex includes antibodies against HER2, a tetrapeptide, and an inhibitor of cytotoxic topoisomerase I. Trastuzumab deruxtecan is approved by the FDA for the treatment of patients with HER2-positive breast cancer, including those with low HER2 expression (Lee et al., 2022; Modi et al., 2022). Mechanisms of resistance to trastuzumab deruxtecan are similar to trastuzumab and trastuzumab emtansine.

Conclusions

Trastuzumab is an essential drug with a targeted effect with multiple mechanisms of action on tumor growth. Congenital or acquired resistance to trastuzumab is one of the main problems in clinical oncology. The investigation of molecular resistance mechanisms and the development of modern drugs capable of influencing them is the way to personalized treatment. Expanding knowledge about trastuzumab and the molecular genetic component of resistance will allow tyrosine kinase inhibitors, immune checkpoint inhibitors, and antibody drug conjugates to be included in routine therapeutic regimens for treatment patients with HER2-positive breast cancer.

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