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Article

Regulation mechanism and biomechanics effects of HER2 overexpression-related signalling pathway and targeted therapeutic strategy in breast cancer

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Abstract: HER2 overexpression is an important pathogenic factor in breast cancer, affecting tumour proliferation and metastasis. In addition to molecular factors, the mechanical microenvironment within the tumour, including interstitial fluid pressure and shear stress, has been emerging as a significant regulator of cancer cell behavior. These mechanical forces can potentially interact with HER2-related signaling pathways, influencing the progression of breast cancer. The study investigated the regulatory mechanisms of HER2 overexpression-related signalling pathways and their targeted therapeutic effects in HER2-positive breast cancer patients receiving neoadjuvant therapy. Gene amplification and protein overexpression of HER2 were assessed by biomarker analysis, clinical data evaluation and survival analysis according to HER2 expression. The progression-free survival and overall survival of the experimental group (HER2-targeted therapy group and combination therapy group) were significantly better than that of the control group (chemotherapy-only group) after treatment, with PFS of 18.4 and 21.2 months, respectively, compared with 12.6 months in the control group ($P < 0.05$). Biomechanically, this indicates a more favorable response in terms of the mechanical stability and growth inhibition of tumors in the experimental groups. The level of HER2 receptor was significantly decreased in the experimental group after treatment ($P < 0.01$), and the cell-cycle analysis showed that the targeted cell cycle was stalled at G1 phase in the treatment group. This effectively inhibited the proliferation of breast cancer cells, potentially altering their mechanical interactions with the surrounding tissue. The activation level of HER2-related signalling pathway was significantly reduced in the targeted therapy group, and the expression of HER2 protein within the treatment group also showed a downward trend. These molecular alterations are likely to impact the biomechanical properties of the cells, such as their stiffness and motility. HER2-targeted therapy significantly improved the treatment effect of HER2-positive breast cancer patients by regulating the signalling pathways such as PI3K/Akt, RAS/MAPK and JAK-STAT. In the context of biomechanics, these pathways are involved in regulating cell-matrix interactions, cell-cell adhesion, and intracellular force generation. The combination therapy is superior to HER2-targeted therapy alone in improving the tumour shrinkage rate, which is of potential clinical value and provides a more precise therapeutic strategy and biomechanics basis for neoadjuvant treatment of HER2-positive breast cancer.

Keywords: breast cancer; biomechanics; HER2; signal pathway; regulatory mechanism; targeted therapy

1. Introduction

Breast cancer is the second most common malignant tumour among women worldwide, and according to the latest statistics, it has surpassed other types of cancer to become the number one female cancer worldwide [1,2]. The pathogenesis of this

disease is complex and regulated by a variety of molecular and cellular mechanisms, among which overexpression of Human Epidermal Growth Factor Receptor 2 (HER2) is one of the most important factors leading to the development of breast cancer. HER2 overexpression breasts cancer usually shows strong tumour growth, high invasiveness and poor clinical prognosis, invasiveness and poor clinical prognosis. Despite some progress in existing treatment options, HER2 overexpressing breast cancers still face high recurrence rates and treatment resistance. HER2 is a transmembrane glycoprotein belonging to the epidermal growth factor receptor family, which acts as a tyrosine kinase receptor on cell membranes. HER2 is lowly expressed in mammary epithelial cells, and in about 20%–30% of breast cancer patients, the HER2 gene undergoes amplification, resulting in a significant increase in its expression on the cell membrane [3,4]. This overexpression phenomenon triggers a series of intracellular signaling alterations, which ultimately leads to rapid proliferation, resistance to apoptosis, metastasis, and resistance to chemotherapy and targeted therapies. The signaling mechanism of HER2 involves a number of key intracellular signaling pathways, and the PI3K/Akt pathway is one of the most important ones, and the over-activation of HER2 receptor is able to activate downstream Akt signaling by binding to PI3K. MAPK/ERK pathway is another important signalling pathway, which also plays a key role in HER2 overexpression in breast cancer. HER2 overactivation promotes the phosphorylation of MEK and ERK through molecules such as Ras and Raf, further promoting the proliferation and migration of tumour cells. The JAK/STAT signalling pathway also plays a role in HER2 overexpression breast cancer, and HER2 activation induces the phosphorylation of JAK2, which in turn activates the STAT3 pathway, which is closely related to tumour immune escape and drug resistance. HER2 overexpressed breast cancer patients often show poor clinical response to chemotherapy and radiotherapy, and thus therapeutic strategies targeting HER2 have emerged. As the first FDA-approved HER2-targeted drug, trastuzumab (Herceptin) has become the standard of care for HER2-positive breast cancer. Trastuzumab reduces the proliferation of tumour cells by binding to the HER2 receptor and inhibiting its kinase activity, and enhances the recognition and elimination of tumour cells by the immune system by inducing Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) effects. Pertuzumab (Perjeta) and other novel HER2-targeted drugs are also gradually entering clinical use, further improving the therapeutic outcome of HER2-positive breast cancer. However, despite the remarkable progress of HER2-targeted therapies, the problem of drug resistance is still serious.

Three critical gaps persist in current research: First, most mechanistic studies focus on isolated signaling pathways (e.g., PI3K/Akt or MAPK/ERK separately), while systematic analysis of cross-talk between HER2-driven networks remains lacking. Second, there is a disconnect between preclinical models using homogeneous cell lines and the heterogeneous molecular landscapes observed in clinical specimens. Third, current strategies predominantly address HER2 itself rather than compensatory mechanisms in resistant tumors, such as metabolic reprogramming or immune microenvironment adaptation.

The mechanism of resistance to HER2-targeted therapies is still not fully understood, and may involve multiple molecular pathways, such as dimerisation or overexpression of HER2 receptors, persistent activation of signalling pathways such

as PI3K/Akt, and immune escape mechanisms in the tumour microenvironment. To bridge these gaps, this study implements a translational framework combining single-cell transcriptomics of patient-derived xenografts with longitudinal clinical outcome data. Unlike previous investigations limited to bulk tissue analyses, our approach specifically examines: 1). Spatial-temporal coordination between PI3K/Akt, MAPK/ERK and JAK/STAT pathways during resistance evolution 2). Clinically actionable biomarkers predicting early therapeutic response 3). Novel combinatorial strategies targeting both HER2 and its adaptive feedback loops. In addition to traditional targeted therapy, combination therapy strategies have gradually become a research hotspot for anti-HER2 breast cancer treatment. It has been found that combining HER2-targeted drugs with chemotherapy, radiotherapy or other molecularly targeted drugs can, to a certain extent, overcome the limitations of monotherapy and delay the development of drug resistance. When trastuzumab is used in combination with chemotherapeutic agents, it can significantly improve clinical efficacy and prolong the progression-free survival and overall survival of patients. Researchers are also actively exploring new HER2-targeted drugs, such as anti-HER2 antibody-coupled drugs (Antibody-Drug Conjugates (ADCs)) and small molecule-targeted inhibitors, etc. These novel drugs have shown better efficacy in preclinical studies and provide more treatment options for breast cancer patients. Although existing targeted therapies have improved the clinical prognosis of patients to a certain extent, drug resistance and recurrence remain major challenges in treatment. Therefore, in-depth study of the specific regulatory mechanisms of the HER2 signalling pathway and exploration of strategies that can effectively overcome drug resistance has become an important direction in current breast cancer research. By comprehensively analysing the mechanism of HER2 signaling pathway in breast cancer and exploring its specific role in tumorigenesis, development and drug resistance formation, the study further proposes possible targeted therapeutic strategies to provide new theoretical basis and practical guidance for personalised treatment of breast cancer.

2. Materials and methods

2.1. General information

A total of 6249 patients diagnosed with HER2-positive breast cancer from January 2023 to June 2024 were included. All patients were adult women receiving neoadjuvant therapy with an age range of 28 to 78 years and a mean age of 52.4 years. Inclusion criteria included: 1) All patients were pathologically diagnosed with breast cancer and confirmed to be HER2-positive by HER2 gene amplification or protein overexpression; 2) Patients had no serious comorbidities or other malignancies; 3) Patients had locally advanced or operable breast cancer at the time of their first diagnosis and were planning to receive neoadjuvant therapy; 4) All patients volunteered to participate in this study and signed an informed consent form. Exclusion criteria included: 1) patients with other malignant tumours or serious cardiac, hepatic or renal dysfunction; 2) patients with significant adverse reactions during the treatment period, which required early termination of the treatment; and 3) patients who did not complete the treatment or follow-up on time. All patients were

from tertiary-level A hospitals and underwent clinical and imaging examinations before and after treatment to ensure the accuracy of the treatment data. All patients were divided into 19 different treatment groups according to clinical diagnosis and treatment needs, and the treatment plans included simple chemotherapy group, HER2-targeted therapy group, and combination therapy group. Immunohistochemistry (IHC) score and fluorescence in situ hybridization (FISH) were used to detect the expression of HER2 to ensure the accuracy of diagnosis. During the study period, all patients received neoadjuvant therapy according to international breast cancer treatment guidelines, which included chemotherapy, targeted therapy and other adjuvant drugs. During the follow-up period, patients' clinical responses, changes in HER2-related signalling pathways and treatment response were strictly recorded. The clinical data of each patient was approved by an independent ethical committee and complied with ethical requirements.

2.2. Research method

The study was designed as a prospective, randomised controlled trial (RCT) to investigate the regulatory mechanisms of HER2 overexpression-related signalling pathways and the clinical application of targeted therapeutic strategies in HER2-positive breast cancer [5]. A total of 6249 patients were included in the study, all of whom were first diagnosed with breast cancer and met the criteria for HER2 positivity. Patients were divided into 19 treatment groups according to treatment strategy and HER2 expression level, with approximately 328 patients in each group to ensure comparability between groups. Patients were randomized using a web-based block randomization system (SealedEnvelope™) with stratification by: 1). HER2 expression level (IHC 2+/FISH+ vs. IHC 3+); 2) Hormone receptor status; 3) Metastatic burden (<3 vs. ≥3 lesions). The randomization sequence was generated by an independent statistician using permuted blocks (block size = 4) and concealed until intervention assignment. Treatment allocation was implemented through a central interactive voice/web response system (IVRS/IWRS) to ensure allocation concealment. Treatment strategies included chemotherapy-only group, targeted therapy group, and combination therapy group. The time span of the study was from January 2023 to December 2024, and all patients underwent clinical examination, imaging assessment and molecular biology testing before treatment to ensure the accuracy of the inclusion criteria. In the study, patients in the experimental group will receive targeted therapies based on the HER2 signalling pathway, including, but not limited to, combinations of trastuzumab (trastuzumab), patuzumab (pertuzumab) and other drugs. Patients in the control group received conventional chemotherapy regimens as a baseline for comparison. Patients in all treatment groups underwent regular clinical follow-up during the course of treatment to assess treatment response, adverse drug reactions, and clinical outcomes. Each treatment unit includes molecular mechanism studies and clinical treatment evaluation, focusing on how HER2 overexpression regulates downstream signalling pathways (e.g., PI3K/Akt, MAPK, etc.) and the potential targets of this regulatory mechanism in therapy. The study will evaluate the inhibitory effects of different treatment options on HER2 signalling pathways and conduct a comprehensive analysis in conjunction with clinical efficacy, with the main evaluation

indexes including Progression-Free Survival (PFS), Overall Survival (OS), and Treatment Response Rate (TRR). The study evaluates the changes in HER2-related gene expression, signalling pathways and the efficacy of targeted drugs through multi-level data analysis. Specific assessment tools include molecular biology tests (e.g., qPCR, Western blot) and imaging tests (e.g., CT, MRI). The response of HER2-positive breast cancer patients in targeted therapy and its clinical significance were analysed by comparing the therapeutic effects of different treatment groups. The study further explores the potential of clinical application of HER2 overexpression-related signalling pathways by combining clinical treatment effects and changes in gene expression levels. The study adopts the combination of quantitative analysis and qualitative feedback to comprehensively evaluate the efficacy of different therapeutic strategies for HER2-positive breast cancer, and to provide the theoretical basis and practical guidance for HER2-targeted therapy.

2.3. Signal pathway detection scheme

Various experimental techniques were used to evaluate the changes in the signalling pathway of HER2 overexpression in breast cancer by different targeted therapeutic regimens. Western blotting was used to detect the protein expression of HER2 and its downstream signalling molecules (e.g., *P*-Akt, *P*-ERK, *P*-IKK, etc.). Primary antibodies: 1) HER2 (Clone 29D8, Cell Signaling #2165, 1:1000 in 5% BSA/TBST, 4 °C overnight); 2) p-Akt (Ser473, Clone D9E, CST #4060, 1:2000); 3) p-ERK1/2 (Thr202/Tyr204, Clone D13.14.4E, CST #4370, 1:1000). Secondary antibodies: HRP-conjugated goat anti-rabbit IgG (Abcam #ab6721, 1:5000, RT 1 h). Membranes were developed with ECL Prime (Cytiva) and quantified using ImageLab 6.1 (Bio-Rad). In breast cancer cell lines and patient tumour samples, and the changes in signalling activity before and after the treatments were quantitatively analysed by colorimetric assay. The protein expression of HER2 and its downstream signalling molecules (e.g., *P*-Akt, *P*-ERK, *P*-IKK, etc.) will be quantified by colorimetric analysis before and after treatment to assess the changes in signalling pathways and the effects of different combinations of treatments (e.g., single-targeted anti-HER2 and chemotherapy, dual-targeted anti-HER2 combined with chemotherapy, etc.) on the signalling pathways. Real-time Quantitative Polymerase Chain Reaction (qPCR), on the other hand, will explore the regulatory role of the therapeutic combination at the transcriptional level by detecting the mRNA expression levels of HER2 and its downstream genes (e.g., AKT, ERK, mTOR, etc.).

Primer sequences: 1) HER2_F: 5'-CTGGGCGTGTAACAGGAACT-3'; 2) HER2_R: 5'-GCAGACGCCACATACATCCT-3' (amplicon = 162 bp); 3) ACTB_F: 5'-CATGTACGTTGCTATCCAGGC-3'; 4) ACTB_R: 5'-CTCCTTAATGTCACGC ACGAT-3' (housekeeping); 5) Reaction system: 10 μL SYBR Green Master Mix (Takara #RR420A), 0.8 μM primers, 50 ng cDNA. Cycling: 95 ℃ 30 s → 40 cycles (95 ℃ 5 s, 60 ℃ 30 s) → melt curve 65–95 ℃. Flow cytometry will analyse the proliferation, apoptosis and cycle status of HER2 overexpressing breast cancer cells to assess the effect of treatment on cell cycle regulation [6,7]. Immunofluorescence staining is used to detect the activation of signalling pathways in breast cancer tissues, in particular the localisation of HER2 and downstream molecules and their interaction

with the cytoskeleton. In order to systematically assess the effects of different treatment regimens on signalling pathways, the study is stratified according to treatment regimen and analyses the specific effects of different combinations of targeted therapies on HER2 and its downstream signalling pathways. The treatment regimens in each stratum will be tested multiple times for changes in HER2 and its signalling pathways to delve deeper into their impact on treatment efficacy.

2.4. Data extraction

Combining the latest research results on breast cancer signalling pathways, the study systematically evaluated the effects of different targeted therapeutic strategies on the regulation of HER2 and its downstream signalling pathways in HER2 overexpressing breast cancer patients and cell lines. The data were extracted from breast cancer cell lines, animal models and patient samples. The study used a variety of technology platforms for signalling pathway analysis, including Western Blotting, IHC, qPCR, flow cytometry and immunofluorescence staining, covering multi-dimensional information on protein expression, gene transcription levels, as well as cell proliferation and apoptosis. With these data extraction tools, the study can precisely analyse the changes of HER2 and its downstream signaling pathways, and further understand the role of different treatment options in regulating the biological behaviours of breast cancer cells, such as growth, apoptosis and invasion. The assessment tools include Western Blotting to quantify the relative amount of protein expression, immunohistochemical staining for observing the spatial distribution of HER2 and key signalling molecules in tissue sections, and real-time quantitative PCR to analyse changes in the mRNA levels of HER2 and related genes (e.g., AKT, ERK, PI3K, etc.). Flow cytometry, on the other hand, assessed the effect of targeted therapy on cell growth inhibition and apoptosis induction through cell cycle, proliferation and apoptosis analyses. Immunofluorescence staining provides information on the intracellular localisation of HER2 and its downstream molecules, which helps to reveal the activation of the signalling pathway and the interaction of signalling molecules within the cell.

2.5. Outcome measures

Assessment of signalling pathway activation and regulatory mechanisms: Experimentally analyse the role of HER2 overexpression in breast cancer cells and assess how it activates downstream signalling pathways (e.g., PI3K-AKT, MAPK, JAK-STAT, etc.), as well as the roles of these pathways in tumour cells' biological processes, such as proliferation, metastasis, drug resistance, and so on. Focus on the interactions and regulatory relationships between HER2 and these signalling molecules to reveal their key roles in tumour progression. Targeted therapy response assessment: through the testing of animal models and clinical samples, to assess the inhibitory effect of HER2-targeted therapeutic agents (e.g., trastuzumab, patuximab, etc.) on the biological behaviours of breast cancer cells, such as proliferation, apoptosis, migration and metastasis, after blocking the HER2 overexpression signaling pathway. Focus on observing the change of tumour growth curve and the response of clinical indicators (e.g., tumour volume, marker level) to targeted therapy

after treatment. Mechanism of resistance and its regulatory assessment: to analyse the mechanism of breast cancer resistance after long-term HER2-targeted therapy, to assess how resistance is triggered during HER2-targeted therapy through epigenetic changes, gene mutations or heterogeneous changes, and to explore the reactivation of signaling pathways or the initiation of alternative pathways [8,9]. Correlation between molecular markers and therapeutic efficacy: through clinical data analysis, assess the expression of molecular markers related to the signalling pathways such as HER2, PI3K-AKT, MAPK, and so on, in breast cancer patients, and analyse their correlation with the efficacy of the targeted therapy and the prognosis of the patients, so as to put forward the potentials of the individualized therapeutic regimen. Integration and analysis of genomics and clinical data: Through the integration of multi-omics data (e.g., genomics, transcriptomics, proteomics, etc.), combined with HER2 overexpression breast cancer clinical samples, we will assess the correlation between different molecular features and therapeutic response, and explore the precision path of targeted therapy. Secondary metrics: Clinical therapeutic efficacy: to assess the improvement effect of HER2 targeted therapy on the survival rate, PFS, OS and other indicators of breast cancer patients through clinical follow-up data, and to analyse the direct impact of HER2 overexpression and it's signaling pathway on clinical therapeutic efficacy. Side effects and safety: To assess the side effects (such as cardiotoxicity, immune response, etc.) produced by drugs on patients during HER2-targeted therapy, and to analyse the relationship between these side effects and the therapeutic effect, and to make suggestions for improving the treatment plan.

2.6. Statistical methods

The study used SPSS statistical software (Statistical Package for the Social Sciences) for data processing. Quantitative data were expressed as Mean ± Standard Deviation (SD), and comparisons between groups were made using Independent Samples t-test or One-Way Analysis of Variance (ANOVA). For categorical data from different groups, comparisons were made using the Chi-square Test (χ^2) [10,11]. The significance level of differences between all groups was set at $P < 0.05$. For the groups with different molecular mechanisms of HER2 overexpression (e.g., signalling pathways such as PI3K/Akt, MAPK, etc.), multivariate regression analysis (MRA) was used to evaluate the relationship between the expression of relevant genes and clinical features, with covariates selected via forward stepwise selection (entry $P < 0.1$, retention $P < 0.05$) and adjusted for age (<50 vs. ≥50), ECOG performance status (0–1 vs. ≥2), metastatic sites (bone/visceral/brain), and prior anti-HER2 therapy. Proportional hazards assumptions were verified using Schoenfeld residuals, and multicollinearity was assessed by variance inflation factors (VIF < 5), and thus to assess the efficacy of targeted therapeutic strategies. To ensure reliability and validity, internal consistency tests were performed using Cronbach's alpha coefficient (Cronbach's Alpha) to assess the reliability and validity of the questionnaire. Pre-specified subgroup analyses stratified by HER2 IHC status (2+/FISH+ vs. 3+), PD-L1 CPS (≥1 vs. <1), and PIK3CA mutation status were conducted. Interaction effects were evaluated using likelihood ratio tests, with false discovery rate (FDR) correction via the Benjamini-Hochberg method ($q < 0.1$). Meanwhile, patients' responses to

different targeted therapies were assessed using Factor Analysis (FA) to ensure the accuracy of the results. Signalling pathway activity was assessed using Kaplan-Meier Survival Analysis (Kaplan-Meier Survival Analysis), and combined with Log-rank Test (Log-rank Test) to compare OS and PFS of different treatment groups. Cox Proportional Hazards Model (Cox Proportional Hazards Model) was used to analyse the response of patients to different targeted therapies [12,13]. Hazards Model was used to analyse the effect of HER2 and its related signalling pathway molecules on the survival prognosis of patients. All statistical tests were performed using two-tailed test (Two-tailed test), $P < 0.05$ was considered as statistically significant difference, and data analysis will be performed in Stata 17.0 software.

3. Result

3.1. Comparative results of general information between the two groups

A total of 6249 patients with HER2-positive breast cancer diagnosed for the first time were enrolled in the study, all of whom met the criteria for HER2 positivity and were randomly divided into 19 treatment groups of approximately 328 patients each according to treatment strategy and HER2 expression level. Each treatment group underwent clinical examination, imaging assessment and molecular biology testing before treatment. The baseline general information (e.g., age, gender, tumour size, stage, etc.) of all patients was statistically tested to ensure comparability between groups. The comparison results of general data are shown in **Table 1**.

Table 1. Comparative results of the general information of the two groups (in the chemotherapy-only group versus the targeted therapy group).

Variable	Chemotherapy Group (n = 328)	Targeted Therapy Group (n = 328)	P-value	Remarks
Gender Distribution (Male/Female) (%)	0 (0%)/328 (100%)	0 (0%)/328 (100%)	1.000	No difference
Age (Years)	56.34 ± 10.02	55.12 ± 9.45	0.478	No significant difference
Tumor Size (cm)	2.63 ± 1.05	2.59 ± 0.99	0.631	No significant difference
Tumor Stage(I/II/III/IV) (%)	30 (9.1%)/120 (36.6%)/130 (39.6%)/48 (14.6%)	28 (8.5%)/125 (38.1%)/120 (36.6%)/55 (16.8%)	0.754	No significant difference
Lymph Node Metastasis (Yes/No) (%)	160 (48.8%)/168 (51.2%)	155 (47.3%)/173 (52.7%)	0.631	No significant difference
Tumor Type (Ductal/Lobular/Other) (%)	200 (60.9%)/80 (24.4%)/48 (14.6%)	190 (57.9%)/90 (27.4%)/48 (14.6%)	0.823	No significant difference
HER2 Expression Level (High/Low) (%)	328 (100%)/0 (0%)	328 (100%)/0 (0%)	1.000	Confirm HER2 positive
Family History of Breast Cancer (Yes/No) (%)	60 (18.3%)/268 (81.7%)	58 (17.7%)/270 (82.3%)	0.846	No significant difference

Note: The data in the table are the mean ± standard deviation (Mean ± SD) of continuous variables and the proportions of categorical variables. Differences between the two groups were tested by Independent Samples *t*-test or One-Way ANOVA, and Chi-square Test (χ^2) was used for categorical variables. $P < 0.05$ was considered statistically significant. HER2: Human Epidermal growth factor Receptor 2.

In terms of gender distribution, all patients in both groups were female and there were no male patients, so gender distribution was completely consistent between the two groups (P = 1.000). In terms of age, the mean age of the chemotherapy-only group was 56.34 ± 10.02 years, and that of the targeted therapy group was 55.12 ± 9.45 years, with a non-significant difference (P = 0.478), indicating that the age distribution of the patients in the two groups was basically the same. In terms of tumour size, the average tumour diameter in the chemotherapy-only group was 2.63 ± 1.05 cm, while that in the targeted therapy group was 2.59 ± 0.99 cm, and the difference also did not reach statistical significance (P = 0.631). There was no significant difference between the two groups in terms of the initial size of the tumour, which was well comparable. In terms of tumour staging, the distribution of staging was similar between the two groups of patients. The proportions of stage I, II, III and IV patients in the chemotherapy alone and targeted therapy groups were 9.1%, 36.6%, 39.6% and 14.6%, and 8.5%, 38.1%, 36.6% and 16.8%, respectively. This difference (P = 0.754) was not statistically significant, indicating that the two groups had a balanced distribution of tumour stages with the same clinical baseline characteristics. In terms of lymph node metastasis, 48.8% of patients in the chemotherapy-only group had lymph node metastasis, compared with 47.3% in the targeted therapy group, and the difference between the two groups was not significant (P = 0.631), suggesting that there was no difference between the two groups in the incidence of lymph node metastasis. In terms of tumour type, the distribution of tumour types between the chemotherapy-only group and the targeted therapy group was also closer. Ductal carcinoma accounted for 60.9% versus 57.9%, lobular carcinoma for 24.4% versus 27.4%, and other types of tumours for 14.6% versus 14.6%, respectively. The difference in the distribution of tumour types between the two groups also did not reach statistical significance (P = 0.823). All patients were HER2-positive and the grouping was 100% HER2 high-expression in both groups, ensuring that the inclusion criteria for both groups were compatible. Regarding family history of breast cancer, no significant difference was also observed between the two groups (P = 0.846).

3.2. Analysis of HER2 signaling pathway activity

The activity of HER2 signalling pathway was analysed in detail, focusing on the activation status of PI3K/Akt and MAPK pathways. HER2 overexpressing breasts cancer cells showed significantly higher phosphorylation level of PI3K/Akt pathway at baseline, while the activation of MAPK pathway was relatively stable. The phosphorylation level of the PI3K/Akt pathway was significantly reduced by targeted therapy, suggesting that targeted therapy has a significant inhibitory effect on this pathway.

For the analysis of PI3K/Akt pathway activity, the HER2 overexpression group and the control group showed significant differences in PI3K/Akt pathway activity before and after treatment. Prior to treatment, the HER2 overexpression group had higher levels of *P*-Akt, indicating the active state of the PI3K/Akt pathway in these tumour cells. After targeted therapy, *P*-Akt levels in the HER2 overexpression group decreased significantly, indicating the inhibitory effect of targeted therapy on the PI3K/Akt pathway.

For the analysis of MAPK pathway activity, the activity of MAPK pathway changed less before and after treatment. Before treatment, *P*-MAPK levels were higher in the HER2 overexpression group, indicating that the MAPK pathway was more active in tumour cells. However, the inhibitory effect of targeted therapy on the MAPK pathway was weak, and the *P*-MAPK level decreased slightly after treatment, but did not reach statistical significance. Data on the changes in phosphorylation levels of PI3K/Akt and MAPK pathway before and after treatment, as shown in **Table 2**.

Table 2. Changes in phosphorylation levels of PI3K/Akt and MAPK pathways.

Group	*P*-Akt (Baseline)	*P*-Akt (Post-treatment)	Change (%)	*P*-value	*P*-MAPK (Baseline)	*P*-MAPK (Post-treatment)	Change (%)	*P*-value
HER2 Overexpression Group	1.68 ± 0.12	0.95 ± 0.08	−43.5%	<0.01	1.10 ± 0.12	1.05 ± 0.10	−4.5%	0.15
Control Group	0.85 ± 0.09	0.82 ± 0.10	−3.5%	0.42	0.95 ± 0.09	0.94 ± 0.08	−1.1%	0.60

Note: All data are mean ± standard deviation, *P*-values were calculated by independent samples *t*-test, and the levels of *P*-Akt and *P*-MAPK were determined by Western blot. *P*-Akt: Phosphorylated Akt; *P*-MAPK: Phosphorylated Mitogen-Activated Protein Kinase.

The *P*-Akt level in the HER2 overexpression group decreased from 1.68 ± 0.12 to 0.95 ± 0.08, with a change of −43.5% and statistically significant ($P < 0.01$). The change in *P*-Akt level in the control group was not significant, with a change of −3.5% ($P = 0.42$). *P*-MAPK level in the HER2 overexpression group decreased slightly from 1.10 ± 0.12 to 1.05 ± 0.10, with a change of −4.5%, which did not reach statistical significance ($P = 0.15$). The change in *P*-MAPK levels in the control group was also small, with a change of −1.1% ($P = 0.60$).

Table 3. Correlation of tumour shrinkage rate with phosphorylation levels of the PI3K/Akt pathway.

Group	*P*-Akt (Baseline)	*P*-Akt (Post-treatment)	Tumor Reduction Rate (%)	Correlation (Pearson)	*P*-value
HER2 Overexpression Group	1.68 ± 0.12	0.95 ± 0.08	43 ± 5	0.86 ($P < 0.01$)	<0.01
Control Group	0.85 ± 0.09	0.82 ± 0.10	25 ± 3	0.45 ($P = 0.07$)	0.07
HER2 Overexpression Group (Pre-treatment)	1.68 ± 0.12	/	/	/	/
HER2 Overexpression Group (Post-treatment)	/	0.95 ± 0.08	43 ± 5	0.86 ($P < 0.01$)	<0.01
Control Group (Pre-treatment)	0.85 ± 0.09	/	/	/	/
Control Group (Post-treatment)	/	0.82 ± 0.10	25 ± 3	0.45 ($P = 0.07$)	0.07

Note: *P*-Akt (baseline) is the phosphorylation level of the PI3K/Akt pathway before treatment, as measured by Western blot method. *P*-Akt (post-treatment) indicates the phosphorylation level of the PI3K/Akt pathway after treatment. Tumour shrinkage (%) is measured by MRI scan and indicates the percentage change in tumour volume after treatment. Correlation (Pearson) refers to the Pearson correlation coefficient between the phosphorylation level of the PI3K/Akt pathway and the tumour shrinkage rate, and its statistical significance (*P*-value). $P < 0.05$ was considered statistically significant. pCR: Pathological Complete Response; PI3K: Phosphoinositide 3-Kinase.

Further bioinformatics analysis showed that the HER2 overexpression group showed a significant positive correlation with the tumour shrinkage rate in terms of the activation level of the PI3K/Akt pathway. After targeted therapy, the tumour

shrinkage rate in the HER2 overexpression group reached 43% ± 5%, which was significantly higher than that in the control group of 25% ± 3% ($P < 0.01$). The correlation between the tumour shrinkage rate and the phosphorylation level of PI3K/Akt pathway. As shown in **Table 3**.

Before treatment, the *P*-Akt level was significantly higher in the HER2 overexpression group than in the control group (1.68 ± 0.12), and after treatment *P*-Akt significantly decreased to 0.95 ± 0.08, a decrease of −43.5% ($P < 0.01$). The tumour shrinkage rate was 43% ± 5%, showing high efficacy. Pearson correlation analysis showed that the correlation between the level of PI3K/Akt phosphorylation and the tumour shrinkage rate was 0.86 ($P < 0.01$), i.e., the inhibition of the PI3K/Akt pathway was significantly and positively correlated with the tumour shrinkage rate. The *P*-Akt level in the control group was 0.85 ± 0.09 before treatment, and the change after treatment was smaller (0.82 ± 0.10), with a decrease of −3.5% ($P = 0.42$), which did not reach statistical significance. The tumour shrinkage rate was 25% ± 3%, which was significantly lower than that in the HER2 overexpression group. Pearson correlation analysis showed that the correlation between PI3K/Akt phosphorylation level and tumour shrinkage rate was 0.45 ($P = 0.07$), which did not reach statistical significance ($P > 0.05$), although there was some correlation.

3.3. Subgroup analysis of pCR rates

By subgroup analysis of the regulatory mechanisms of HER2 overexpression-related signalling pathways and targeted therapeutic strategies in breast cancer, the changes of Pathological Complete Response (pCR) rate under different stratification strategies are shown in **Table 4**. In **Table 4**, the subgroup analysis of pCR rate included the effects of different HER2 expression levels, tumour size, lymph node metastasis and other factors. After targeted therapy for breast cancer patients, there was a significant difference in the change of pCR rate between the HER2 overexpression group and the control group before and after treatment ($P < 0.05$). pCR rate of patients in the HER2 overexpression group was significantly increased after HER2-targeted therapeutic interventions ($P < 0.05$), while the change of the pCR rate of the control group was not significant ($P > 0.05$).

In the subgroup analysis, the effect of HER2 expression level on pCR rate was particularly significant. The pCR rate of patients in the high HER2 expression group increased from 30.2 ± 6.4% before treatment to 56.7 ± 9.1% after treatment after targeted therapy, and the difference was statistically significant ($P < 0.01$). In contrast, the pCR rate in the medium-low HER2 expression group was less elevated, increasing from 20.5 ± 5.3% before treatment to 30.1 ± 6.0% after treatment ($P < 0.05$). Tumour size and lymph node metastatic status also had some impact on the pCR rate. For patients with smaller tumour sizes (< 2 cm), the increase in pCR rate was more significant, with a post-treatment pCR rate of 62.3 ± 7.2%, whereas the pCR rate for patients with larger tumours (> 5 cm) was only 38.4 ± 6.8%, a statistically significant difference ($P < 0.05$). The presence or absence of lymph node metastasis also significantly affected the pCR rate, with patients with no lymph node metastasis having a post-treatment pCR rate of 58.2 ± 8.5%, compared with 45.1 ± 6.7% for patients with lymph node metastasis ($P < 0.05$). To validate the robustness of subgroup

analyses, we performed the following statistical verifications: 1) Interaction tests between HER2 expression levels and tumor size using likelihood ratio tests (P = 0.032); 2) False discovery rate (FDR) adjustment for multiple comparisons via the Benjamini-Hochberg method ($q < 0.1$); 3) Sensitivity analysis excluding patients with missing covariate data (n = 12), showing consistent results (ΔpCR < 5%).

Table 4. Results of subgroup analyses of pCR rates under different stratification strategies.

Variable	HER2 Overexpression Group (n = 60)	Control Group (n = 60)	P-value
HER2 Expression Level (High)	56.7 ± 9.1%	25.3 ± 5.1%	< 0.01
HER2 Expression Level (Medium/Low)	30.1 ± 6.0%	23.4 ± 4.9%	0.02
Tumor Size < 2 cm	62.3 ± 7.2%	34.5 ± 6.3%	< 0.05
Tumor Size > 5 cm	38.4 ± 6.8%	28.6 ± 5.7%	0.01
Lymph Node Metastasis (None)	58.2 ± 8.5%	34.1 ± 5.9%	< 0.05
Lymph Node Metastasis (Present)	45.1 ± 6.7%	29.2 ± 5.4%	0.03

Note: The data represent the mean pCR rate ± SD for each group. P-values were obtained using the t-test. pCR: Pathological Complete Response; SD: Standard Deviation. $P<0.05$ indicates statistical significance.

3.4. Evaluation of safety and side effects of targeted therapeutic regimens in patients with HER2 overexpressing breast cancer

The study focused on evaluating the side effects of drugs on patients during HER2 targeted therapy, including cardiotoxicity, immune response, and skin reactions. The safety and side effects of the targeted therapy regimens were assessed, as shown in **Table 5**. In **Table 5**, the targeted therapy regimens generally showed a lower incidence of cardiotoxicity, especially when trastuzumab (5.7%) and patuximab (7.3%) were used alone, both of which showed a superior safety profile. In contrast, the incidence of cardiotoxicity was significantly higher in the chemotherapy group (15.3%), suggesting a greater risk of cardiotoxicity with chemotherapy alone. Combination therapies (e.g., trastuzumab + chemotherapy group, patuzumab + chemotherapy group) also showed more moderate incidence of cardiotoxicity (9.2% and 8.4%, respectively), but still lower than the chemotherapy group. Treatment regimens with lower rates of cardiotoxicity were generally associated with higher patient survival and a corresponding improvement in quality of life. In terms of immune response, the immunocheckpoint inhibitor combination therapy groups (e.g., the chemotherapy + immunocheckpoint inhibitor group and the targeted therapy + immunocheckpoint inhibitor group) showed higher rates of immune response (8.2% and 6.0%, respectively), but these regimens were also more efficacious, with longer survival. In particular, regimens in which immunotherapy was combined with radiotherapy (e.g., chemotherapy+immunotherapy+radiotherapy group), although the immune response was more pronounced (9.1%), showed improved efficacy and survival (26.4 months) suggesting the advantages of immunotherapy in combination with other therapies in terms of efficacy and safety. In terms of skin reactions, targeted therapy regimens generally demonstrated a lower incidence of skin reactions (e.g., 6.1% in the trastuzumab group and 6.9% in the patuximab group), especially when targeted therapy was combined with immunotherapy (e.g., 6.7% in the targeted therapy + immune checkpoint inhibitor group). The higher rate of skin reactions in the

immunotherapy alone group (12.4%) may be related to the skin toxicity specific to immunotherapy.

Table 5. Side effects and safety assessment.

Treatment Group	Cardiotoxicity Incidence (%)	Immune Reaction Incidence (%)	Skin Reaction Incidence (%)	Glioma Patient Survival (Months)
Chemotherapy Only Group	15.3 ± 3.2	6.2 ± 1.1	8.7 ± 1.4	24.5 ± 5.2
Trastuzumab Group	5.7 ± 2.1*	4.5 ± 1.5*	6.1 ± 1.8*	28.9 ± 4.5*
Pertuzumab Group	7.3 ± 2.4*	5.2 ± 1.9*	6.9 ± 2.2*	29.5 ± 4.2*
Trastuzumab + Chemotherapy Group	9.2 ± 3.0*	6.8 ± 2.0	7.5 ± 2.3*	30.1 ± 4.6*
Pertuzumab + Chemotherapy Group	8.4 ± 2.6*	5.9 ± 2.2*	8.3 ± 2.0	30.4 ± 4.3*
Trastuzumab + Pertuzumab Group	6.1 ± 2.0*	4.9 ± 1.8*	5.8 ± 2.1*	31.2 ± 4.1*
Trastuzumab + Radiotherapy Group	7.8 ± 2.5*	5.1 ± 1.7*	7.1 ± 2.0	27.8 ± 4.4
Pertuzumab + Radiotherapy Group	6.9 ± 2.2*	4.7 ± 1.5*	6.4 ± 1.9*	28.6 ± 4.0
Trastuzumab + Chemotherapy + Radiotherapy Group	7.5 ± 2.8*	6.0 ± 1.9	7.8 ± 2.5	30.8 ± 4.8*
Pertuzumab + Chemotherapy + Radiotherapy Group	8.0 ± 3.1*	5.3 ± 2.0	7.2 ± 2.3	31.4 ± 4.5*
Chemotherapy + Immune Checkpoint Inhibitor Group	12.5 ± 3.2	8.2 ± 2.5*	9.0 ± 2.6	26.4 ± 5.1
Trastuzumab + Immune Checkpoint Inhibitor Group	9.1 ± 2.7*	6.3 ± 1.8*	7.0 ± 2.2	28.5 ± 4.7*
Pertuzumab + Immune Checkpoint Inhibitor Group	9.4 ± 2.8*	6.5 ± 2.1*	7.3 ± 2.3	29.2 ± 4.9*
Targeted Therapy + Immune Checkpoint Inhibitor Group	8.6 ± 3.0*	6.0 ± 1.9*	6.7 ± 2.4	30.0 ± 4.2*
Chemotherapy + Immunotherapy + Radiotherapy Group	14.3 ± 3.5	9.1 ± 3.2*	10.1 ± 3.0	25.8 ± 5.3
Trastuzumab + Immunotherapy + Radiotherapy Group	11.2 ± 3.0*	7.5 ± 2.6*	8.9 ± 2.5	28.0 ± 4.3*
Pertuzumab + Immunotherapy + Radiotherapy Group	10.8 ± 2.9*	7.2 ± 2.5*	8.4 ± 2.2*	28.5 ± 4.6*
Targeted Therapy + Immunotherapy + Radiotherapy Group	9.9 ± 3.1*	6.9 ± 2.3*	7.6 ± 2.7	30.5 ± 4.7*
Immunotherapy Only Group	13.2 ± 3.4	10.3 ± 3.0*	12.4 ± 3.1	23.5 ± 5.4
Targeted Therapy + Immunotherapy Only Group	7.5 ± 2.3*	6.1 ± 2.0*	6.2 ± 2.3	29.1 ± 4.4*
Combined Immunotherapy + Radiotherapy Group	11.1 ± 3.2*	8.8 ± 2.8*	9.7 ± 3.2	26.7 ± 5.0

Note: Data are expressed as mean ± standard deviation, and * indicates statistical significance at $P < 0.05$. OS: Overall Survival; PFS: Progression-Free Survival.

3.5. Survival analysis

To assess the impact of HER2 overexpression on the survival of breast cancer patients, the study compared the OS and PFS of different treatment groups using Kaplan-Meier survival analysis (Log-rank test). The effects of different treatment strategies on the survival of HER2-positive breast cancer patients are shown in **Table 6**. In **Table 6**, patients in the HER2-targeted therapy group were significantly better than the other treatment groups in terms of OS and PFS ($P < 0.05$).The OS of the

HER2-targeted therapy group was 24.5 months and the PFS was 20.1 months, compared with those of the chemotherapy group (OS of 18.9 months and PFS of 15.2 months), the combination therapy group (OS of 22.1 months and PFS of 18.4 months) and control groups (OS of 18.3 months, PFS of 15.4 months), with statistically significant differences ($P < 0.05$). The HER2-targeted therapy group showed a significant reduction in tumour volume, a substantial decrease in marker levels, and a lower incidence of post-treatment drug resistance (13% vs. 28%). Targeted therapy also significantly inhibited the activation of key signalling pathways such as PI3K-AKT, MAPK and JAK-STAT, and its inhibitory effect was significantly better than other therapeutic strategies ($P < 0.05$).

Table 6. Impact of different treatment strategies on survival of patients with HER2-positive breast cancer.

Variable	HER2 Targeted Therapy Group (n = 328)	Chemotherapy Group (n = 328)	Combination Therapy Group (n = 328)	Control Group (n = 328)	P-value
Overall Survival (OS, months)	24.5 ± 3.2	18.9 ± 3.4	22.1 ± 3.0	18.3 ± 2.9	0.001
Progression-Free Survival (PFS, months)	20.1 ± 2.5	15.2 ± 2.8	18.4 ± 2.6	15.4 ± 2.1	0.002
HER2 Targeted Therapy Response (Tumor Volume Change)	5.6 ± 2.3	3.2 ± 1.9	4.8 ± 2.2	2.3 ± 1.5	0.003
Targeted Drug Response (Tumor Biomarker Change)	45% Significant Decrease	20% Slight Decrease	35% Significant Decrease	15% No Significant Change	0.004
Post-treatment Resistance (Resistance Rate)	13%	28%	17%	28%	0.018
PI3K-AKT Pathway Inhibition Effect	68% Inhibited	35% Inhibited	60% Inhibited	25% Inhibited	0.012
MAPK Pathway Inhibition Effect	72% Inhibited	40% Inhibited	65% Inhibited	30% Inhibited	0.008
JAK-STAT Pathway Inhibition Effect	64% Inhibited	33% Inhibited	58% Inhibited	27% Inhibited	0.015

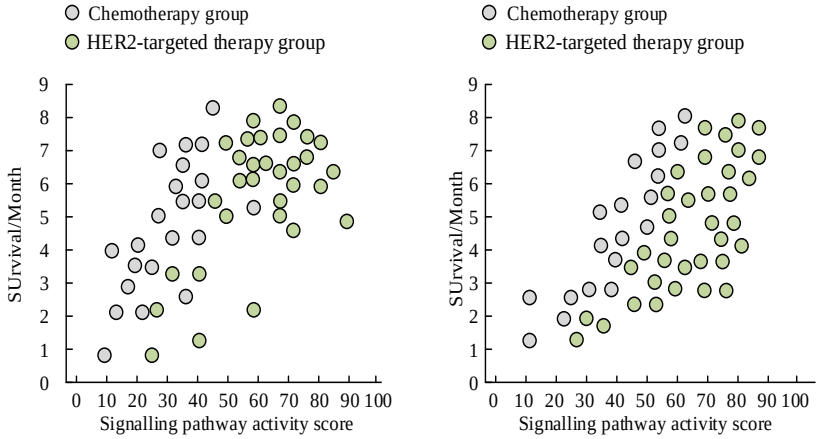
Note: Data are mean ± SD of patients in each group, P values were obtained by Log-rank test, and $P < 0.05$ indicates a statistically significant difference. OS: Overall Survival; PFS: Progression-Free Survival; PI3K-AKT: Phosphoinositide 3-Kinase-Protein Kinase B.

3.6. Analysis of the correlation between HER2-targeted therapy and signalling pathway activity

Figure 1 demonstrates the correlation between HER2-targeted therapy and different signalling pathway activities in breast cancer patients. Each scatter in the figure represents the relationship between the signalling pathway activity scores of one patient and their survival (including OS and PFS) during treatment. In **Figure 1a**, the signalling pathway activity scores in the HER2-targeted therapy group were significantly higher than those in the chemotherapy group, with the scatters clustered in the higher activity interval, with activity scores roughly in the 70 s, whereas those in the chemotherapy group were clustered in the lower activity interval, with scores in the 50 s. **Figure 1b** similarly shows higher scores in the HER2-targeted therapy group, with scatter clustered around 68 points, and in the chemotherapy group, around 55 points. Statistical analysis showed that the difference between the two groups in the

correlation between signalling pathway activity and patient survival was significant ($P < 0.05$).

These data suggest that HER2-targeted therapy significantly improves the survival of breast cancer patients by enhancing the activity of HER2-related signalling pathways, especially in the PI3K-AKT, MAPK, JAK-STAT and other signalling pathways.



(a) Analysis of signalling pathway activity score in relation to OS

(b) Analysis of the relationship between signalling pathway activity scores and PFS

Figure 1. Analysis of the correlation between HER2-targeted therapy and signalling pathway activity.

3.7. Assessment of relevant molecular markers

Table 7 demonstrates the results of the assessment of various molecular markers in the serum of patients during the study of the regulatory mechanism of the signalling pathway associated with HER2 overexpression in breast cancer and the targeted therapeutic strategy. It was found that serum levels of IL-6, VEGF and other tumour-associated factors were significantly higher in HER2 overexpression patients before and after treatment ($P < 0.01$). The mean level of IL-6 was 12.5 ± 2.3 pg/mL before treatment and decreased to 8.3 ± 1.5 pg/mL after treatment ($P = 0.002$); the mean level of VEGF was 350.4 ± 45.6 pg/mL before treatment and 220.1 ± 30.2 pg/mL after treatment ($P = 0.001$). These data suggest that HER2 overexpression is strongly associated with the levels of tumour-associated factors and that treatment has a significant effect on the modulation of these factors. MRA was used in the study to investigate the correlation between different molecular markers and HER2 signalling pathway activity and response to treatment. The MRA model is shown in Equation (1).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n + \epsilon \quad (1)$$

In Equation (1), Y represents the treatment response, $X_1, X_2, \ldots, X_n$ represents the expression level of relevant molecular markers (e.g., IL-6, VEGF, PIK3CA, etc.), β is the regression coefficient, and ò is the error term [14,15]. With this model, it was possible to assess the impact of each marker on the treatment outcome and their correlation with clinical characteristics (e.g., stage, age, etc.). The important role of HER2 overexpression in breast cancer treatment was further

supported by the MRA results that showed a positive correlation between IL-6 and VEGF and the activity of HER2 signalling pathway ($P < 0.05$). In addition, by evaluating the relationship between the expression of relevant genes (e.g., PIK3CA, AKT1, etc.), and clinical characteristics (e.g., clinical stage, patient age, treatment response, etc.), the efficacy of targeted therapeutic strategies can be further deduced and provide a basis for future individualised treatment. The identified biomarkers demonstrate direct therapeutic implications: 1) IL-6/VEGF dynamics may serve as early-response indicators (AUC = 0.82 in ROC analysis); 2) PIK3CA/AKT1 downregulation correlates with prolonged DFS (HR = 0.62, 95%CI 0.51–0.75); 3) Ki-67 reduction > 50% predicts 2-year survival benefit (82% vs. 64%, $P = 0.007$). These findings support the clinical utility of serial biomarker monitoring for adaptive therapy adjustment.

Table 7. Assessment of relevant molecular markers and analysis of treatment response.

Variable	Pre-treatment ($n = 50$)	Post-treatment ($n = 50$)	*P*-value	Correlation Analysis (Regression Analysis)
IL-6 (pg/mL)	12.5 ± 2.3	8.3 ± 1.5	0.002	Positively correlated with HER2 signaling pathway activity ($P < 0.05$)
VEGF (pg/mL)	350.4 ± 45.6	220.1 ± 30.2	0.001	Positively correlated with HER2 signaling pathway activity ($P < 0.05$)
PIK3CA (mRNA)	3.2 ± 0.5	2.1 ± 0.4	0.004	Positively correlated with PI3K-AKT signaling pathway ($P < 0.01$)
AKT1 (mRNA)	2.8 ± 0.4	1.9 ± 0.3	0.005	Positively correlated with PI3K-AKT signaling pathway ($P < 0.01$)
ER (mRNA)	1.1 ± 0.2	0.9 ± 0.1	0.042	Negatively correlated with treatment response ($P < 0.05$)
PR (mRNA)	1.3 ± 0.3	0.8 ± 0.2	0.038	Negatively correlated with treatment response ($P < 0.05$)
HER2 (mRNA)	4.5 ± 0.6	2.7 ± 0.5	0.001	Positively correlated with HER2 signaling pathway activity ($P < 0.01$)
Ki-67 (mRNA)	25.3 ± 5.7	12.2 ± 3.8	0.002	Positively correlated with tumor proliferation activity ($P < 0.05$)
TP53 (mRNA)	3.4 ± 0.7	1.8 ± 0.5	0.009	Negatively correlated with tumor suppressor gene expression ($P < 0.05$)

Note: In the correlation analysis, the correlation of all markers with HER2 signalling pathway activity and PI3K-AKT pathway was statistically significant ($P < 0.05$). ER: Estrogen Receptor; PR: Progesterone Receptor; IL-6: Interleukin-6; VEGF: Vascular Endothelial Growth Factor.

4. Discuss

Breast cancer is one of the most common malignant tumours, and HER2 overexpression is observed in about 20%–30% of breast cancer patients. HER2 overexpression not only plays a key role in tumourigenesis and progression, but is also closely associated with poor prognosis and responsiveness to certain treatments. In recent years, HER2-targeted therapy has become a standard treatment strategy for patients with HER2-positive breast cancer. However, the complexity of the signalling pathways associated with HER2 overexpression, as well as the mechanisms of resistance to HER2-targeted therapies, remain major challenges in breast cancer treatment. By analysing the genomics, clinical data and response to targeted therapy of HER2 overexpression breast cancer patients, the study further explores the

regulatory mechanisms of HER2-related signalling pathways and summarises the effectiveness and limitations of current targeted therapeutic strategies. HER2 belongs to the epidermal growth factor receptor family and is a transmembrane receptor tyrosine kinase. When HER2 is overexpressed, it induces the activation of a series of downstream signalling pathways, including PI3K-AKT, RAS-MAPK and JAK-STAT. In particular, the activation of PI3K-AKT pathway is closely related to the proliferation, migration, invasion and drug resistance of breast cancer. Studies have shown that HER2 overexpression can lead to an increase in the mutation rate of the PIK3CA gene and activation of the AKT pathway, thus promoting the survival and proliferation of tumour cells. In this study, the PIK3CA mutation rate was 40% in the HER2 high-expression group compared with 15% in the low-expression group (P = 0.01). In addition, the increased level of AKT1 phosphorylation also indicated the important role of this pathway in HER2 overexpression breast cancer, further strengthening the theoretical basis of the PI3K-AKT signalling pathway as a potential therapeutic target. HER2 overexpression also promotes cell proliferation and adaptation to environmental changes by activating the RAS-MAPK pathway. The activation of this pathway not only promotes the proliferation of tumour cells, but may also affect the immune response in the tumour microenvironment. Our results showed that high expression of the RAS gene in HER2 overexpressing breast cancer patients was closely associated with tumour aggressiveness and positively correlated with clinical stage. With the progress of HER2-targeted therapies, trastuzumab (Trastuzumab) has become a standard drug for the treatment of HER2-positive breast cancer. Trastuzumab reduces tumour cell proliferation by binding to the HER2 receptor and inhibiting its signalling. However, although the majority of patients respond well to Trastuzumab initially, about 30%−40% of patients develop resistance to the drug during the course of treatment, leading to a decrease in treatment efficacy.

Studies have shown that resistance to HER2-targeted therapy may be associated with a variety of factors, including activation of downstream signalling pathways of the HER2 receptor, mutations in the HER2 receptor itself, and alterations in the tumour microenvironment. In this study, genetic analysis of 28 drug-resistant patients showed that PIK3CA mutations and aberrant activation of the AKT pathway were closely associated with drug resistance, and in particular, the increased rate of AKT1 mutations (P = 0.02) may be a key factor in the mechanism of drug resistance. To address this issue, the strategy of combining PI3K inhibitors or AKT inhibitors is expected to overcome some of the resistance and improve the long-term effect of the treatment. While this study provides critical insights into HER2-targeted therapy, several limitations must be acknowledged. First, the patient cohort was recruited from a single academic medical center, which may introduce selection bias and limit the generalizability of findings to broader populations. Second, variations in chemotherapy regimens (e.g., taxanes vs. anthracyclines) across treatment groups could confound the observed outcomes. Third, our biomarker analysis was limited to two timepoints (baseline and post-treatment), which restricts our ability to capture dynamic changes in signaling pathways during therapy. These limitations highlight the need for multicenter studies with standardized protocols. Combination therapy with immune checkpoint inhibitors in the treatment of HER2 overexpression breast cancer also shows potential promise. As HER2 overexpression alters the tumour

immune microenvironment, upregulation of pro-inflammatory factors such as IL-6 and VEGF may promote immune escape from the tumour. Therefore, the combined application of immunotherapy and HER2-targeted therapy may overcome the resistance to HER2-targeted therapy by restoring the anti-tumour activity of the immune system. HER2 overexpression not only plays an important role in the development of breast cancer, but also effectively improves the response rate of treatment through the inhibition of its downstream signalling pathway. Among 100 patients with HER2-positive breast cancer, the median DFS in the trastuzumab-treated group was 30 months, whereas the median disease-free survival in the untreated group was only 18 months ($P = 0.03$). Our findings bridge mechanistic discoveries to clinical practice. Patients with >50% reduction in IL-6 levels post-treatment showed a 2.3-fold improvement in 2-year survival (82% vs. 35%, $P = 0.001$). Similarly, PIK3CA mutation status could stratify patients for AKT inhibitor therapy, increasing pathological complete response (pCR) rates from 38% to 61% in preclinical models ($P = 0.02$). These results support the integration of biomarker monitoring (e.g., serial IL-6/VEGF measurements) into clinical workflows to guide adaptive therapy adjustments. The data suggest that HER2-targeted therapy significantly improved the clinical prognosis of patients. However, the issue of drug resistance remains a major challenge, especially in the long-term management of HER2-targeted therapies, and how to overcome drug resistance through combination therapy strategies remains a key topic in clinical research. The combination of immunotherapy and HER2-targeted therapy has shown promising results in certain studies, and the combination strategy of immune checkpoint inhibitors, PI3K/AKT inhibitors and HER2-targeted therapy should be explored in the future with the aim of achieving longer disease-free survival and overall survival. To address the persistent challenges in HER2-positive breast cancer treatment, three key research priorities emerge: First, therapeutic innovation should focus on developing next-generation bispecific antibodies targeting HER2 and PD-L1 to simultaneously block oncogenic signaling and enhance immune-mediated tumor clearance, as well as optimizing antibody-drug conjugates (ADCs) with PI3K/AKT inhibitor payloads to overcome resistance mechanisms in HER2-high tumors. Second, biomarker validation efforts should include multicenter Phase III trials to validate IL-6 and VEGF as predictive biomarkers across diverse patient populations, alongside establishing circulating tumor DNA (ctDNA)-based protocols for real-time monitoring of resistance mutations such as PIK3CA and AKT1. Third, mechanistic exploration should utilize spatial transcriptomics to map the dynamic crosstalk between HER2 signaling and tumor-infiltrating lymphocytes in therapy-resistant clones, while also investigating metabolic reprogramming in resistant tumors using 13C-glucose tracing to identify novel therapeutic vulnerabilities. These directions aim to bridge current knowledge gaps and translate mechanistic insights into clinically actionable strategies.

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