World Journal of Surgical Oncology

RESEARCH

Open Access

A study on the impact of neoadjuvant therapy on molecular subtype conversion in breast cancer

Run-Ze Zhang[1†], Dong Liu[1†], Yuan Ke[1†], Wen-Qi Cai[1], Lin-Hui Zheng[1], Chao-Yan Wu[2*] and Hai-Jun Yu[1*]

Abstract

Purpose The aim of this study was to examine molecular subtype conversions in patients who received neoadjuvant therapy.

Methods and materials A retrospective analysis was performed on 316 patients who underwent neoadjuvant therapy at Zhongnan Hospital of Wuhan University between March 2017 and October 2024. The study included data from patients with confirmed pathological residual disease at the primary site post-surgery, alongside complete receptor status and detailed information on the neoadjuvant treatment regimen administered before and after therapy. Univariate and multivariate logistic regression analyses were employed to identify factors influencing molecular subtype heterogeneity before and after neoadjuvant therapy.

Results Of the 316 patients who received neoadjuvant therapy and underwent repeated pathological biopsies, 84 (26.6%) achieved a pathological complete response (pCR). Among the remaining 232 patients with confirmed pathological residual disease after surgery, 85 (36.6%) exhibited conversion of molecular subtypes, with 45 cases (19.3%) leading to alterations in the treatment plan. In breast cancer patients undergoing neoadjuvant chemotherapy (NAC), particularly those with HR-positive tumors prior to NAC, those demonstrating favorable treatment responses on imaging, and those undergoing breast-conserving surgery, molecular subtype heterogeneity before and after NAC was more commonly observed.

Conclusion Neoadjuvant therapy can induce molecular subtype heterogeneity in patients with invasive breast cancer. The identification of factors contributing to this heterogeneity may be associated with variations in biological markers of residual disease post-NAC, sampling discrepancies between core needle biopsy (CNB) and surgical specimens, or the selective mutagenic pressure exerted by chemotherapeutic agents.

Keywords Breast cancer, Molecular subtypes, Neoadjuvant therapy

†Run-Ze Zhang, Dong Liu and Yuan Ke contributed equally to this work.

*Correspondence:
Chao-Yan Wu
grace98765@163.com

Hai-Jun Yu
haijunyu@whu.edu.cn
[1]Department of Radiation and Medical Oncology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China
[2]Department of Integrated Traditional Chinese Medicine and Western Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China

Introduction

Breast cancer is the most frequently diagnosed cancer among women globally, comprising approximately one-quarter of all new cancer cases annually. The incidence of breast cancer varies significantly across different countries. In 2020, China reported the highest number of breast cancer cases, representing 18.4% of the global total [1]; McPherson, Steel, & Dixon [2]. In early-stage breast cancer, the disease is confined to the breast or, in the case of lymph node-positive patients, to the breast and adjacent lymph nodes, all of which can be surgically resected. Stage I and II breast cancers are typically managed with breast-conserving surgery followed by radiation therapy. In contrast, Stage III breast cancer, often requires neoadjuvant chemotherapy (NAC) to reduce tumor size and facilitate breast-conserving surgery [3]; Maughan, Lutterbie, & Ham [4]. Currently, NAC is a cornerstone of breast cancer treatment, with its application expanding, particularly in downstaging primary breast tumors and metastatic axillary lymph nodes. NAC has proven invaluable in locally advanced and inoperable breast cancer, often transforming previously unresectable tumors into resectable ones [5, 6]; Mauri, Pavlidis, & Ioannidis [7],; Wang & Mao [8]. Furthermore, in patients with operable disease, NAC has been shown to modestly increase the rate of breast-conserving surgery, with the proportion rising from 7 to 12%. Consequently, NAC has emerged as a pivotal treatment approach for locally advanced breast cancer, providing opportunities for tumor downstaging, facilitating breast-conserving surgery, and enabling more personalized treatment strategies that ultimately enhance patient prognosis and quality of life [9, 10, 11, 12, 13].

Previous studies have demonstrated that patients achieving a pathological complete response (pCR) following neoadjuvant treatment exhibit significantly improved overall survival (OS) and disease-free survival (DFS), particularly among those with triple-negative and HER2-positive breast cancer [14, 15, 16]. The utilization of immunohistochemical (IHC) biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), is essential for breast cancer subtyping. This information guides treatment decisions, predicts therapeutic outcomes, and aids in assessing patient prognosis [17, 18].

Recent studies have suggested that neoadjuvant therapy may induce changes in hormone receptor status during treatment, a phenomenon known as receptor conversion. These molecular alterations will complicate the therapeutic approach, potentially leading to inappropriate endocrine or HER2-targeted therapy for patients with recurrent or metastatic breast cancer (Sahin, Ayasun, Rizzo, & Guven [19],; van de Ven, Smit, Dekker, Nortier, & Kroep [20]. Molecular subtype conversion is a frequent occurrence during the progression of breast cancer and can be observed across various metastatic sites. These subtype alterations possess predictive value, indicating the need for clinicians to adjust adjuvant treatment plans accordingly. However, the frequency of molecular subtype changes following NAC remains unclear, and no consensus has been reached on this issue.

In this study, we aimed to investigate the changes and frequency in IHC-based molecular subtype in patients who underwent NAC at our institution. We analyzed factors associated with these transitions and identified patient characteristics that increase the probability of molecular subtype changes during NAC. These findings highlight the significance of monitoring subtype conversions to prevent inappropriate treatment. Furthermore, exploring the underlying mechanisms of these transitions will drive the development of precision medicine.

Methods and materials

Patient selection

Upon obtaining approval from the Ethics Committee of Zhongnan Hospital, Wuhan University, we conducted a retrospective review of all female breast cancer patients who underwent neoadjuvant chemotherapy followed by definitive surgery at the Oncology Center of Zhongnan Hospital between March 2017 and October 2024. A total of 233 patients who did not achieve a pathological complete response (pCR) after neoadjuvant chemotherapy were included in the present analysis. Inclusion standard: (a) The patient is a female, over 18 years of age. (b) Underwent core needle biopsy and intraoperative specimen resection with pathological examination at Zhongnan Hospital of Wuhan University. (c) Pathological findings confirmed the diagnosis of invasive breast cancer. (d) Following the NCCN guidelines, she received neoadjuvant therapy according to molecular subtypes. (e) After neoadjuvant chemotherapy (NAC), she underwent breast resection at Zhong nan Hospital of Wuhan University. (f) Both the biopsy and post-surgical specimens were subjected to immunohistochemical analysis. (g) Breast ultrasound was performed prior to both the biopsy and the surgical resection, allowing for accurate measurement of the tumor size before NAC. This study was conducted in accordance with the Declaration of Helsinki and was formally approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (2024318 K). The requirement for written informed consent was waived for this study. All methods were performed in accordance with the applicable guidelines and regulations.

Clinical and demographic information

Clinical data were collected through a review of electronic health records, including patient age at diagnosis, WHO tumor grading prior to neoadjuvant therapy, surgical approach, molecular subtyping before and after

neoadjuvant therapy, and family history of breast cancer. Clinical staging of the tumor, along with the status of breast cancer biomarkers (ER, PR, HER2, Ki67) before and after neoadjuvant chemotherapy, was also reviewed. Estrogen receptor (ER) and progesterone receptor (PR) statuses were assessed using immunohistochemistry (IHC), with a positivity threshold for both ER and PR defined as ≥ 1%. Hormone receptor positivity was classified as ER and/or PR positivity. In accordance with prior literature and clinical practice [21], ER expression was categorized into five groups: negative (ER 0%), low expression (ER 1–10%), moderate expression (ER 10-50%), high expression (ER 50-75%), and very high expression (ER > 75%). PR expression was classified into three categories: negative (PR 0%), low expression (PR 1–10%), and high expression (PR > 10%). Further, details regarding each patient's neoadjuvant treatment regimen, treatment duration, and therapeutic efficacy were collected. Treatment efficacy was evaluated according to the RECIST 1.1 criteria for solid tumors.

Outcomes

The primary outcomes were the proportion of patients exhibiting molecular subtype heterogeneity before and after neoadjuvant chemotherapy (NAC), as well as the proportion of cases in which adjuvant therapy was modified, defined by changes in molecular subtype between the initial pathological biopsy and the post-NAC specimen. Changes in adjuvant therapy were categorized as follows: patients with hormone receptor-positive on the initial biopsy who became HR- after NAC, thereby omitting adjuvant endocrine therapy; patients with hormone receptor-negative on the initial biopsy who became HR + after NAC, thus initiating adjuvant endocrine therapy; patients with HER2 + status on the initial biopsy who became HER2- after NAC, thereby discontinuing adjuvant monoclonal antibody treatment; and patients with HER2- status on the initial biopsy who became HER2 + after NAC, leading to the initiation of adjuvant monoclonal antibody therapy.

The secondary outcome was to analyze and validate the factors associated with molecular subtype heterogeneity before and after NAC. Breast cancer molecular subtypes were classified according to the 2013 St. Gallen consensus (Ignatiadis, Buyse, & Sotiriou [22].

Biomarker testing

Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) analyses were conducted by the Pathology Department of Zhongnan Hospital, Wuhan University. All specimens were fixed in formalin, dehydrated, and embedded in paraffin. After embedding, tissue sections underwent antigen retrieval via heating. Following blocking, primary antibodies were incubated for 1 h, secondary antibodies for 10 min, and DAB substrate was applied for detection, followed by counterstaining and differentiation. The slides were then examined and recorded under a light microscope.

For FISH, paraffin-embedded tissue sections were dewaxed in xylene and rehydrated through a series of ethanol solutions (70%, 85%, and 100%). The tissues were immersed in 30% sulfite for 30 min, followed by three rinses in SSC buffer. Proteinase K digestion was performed for 5 min, after which tissues were rinsed in SSC buffer. The specimens were then treated with 0.1 mol/L HCl for 5 min, dehydrated in ethanol, and incubated in acetone for 3 min. After baking, 10 μL of denatured DNA probe was added for overnight hybridization at 42 °C, followed by a 5-minute rinse in 50% formamide. After additional SSC rinsing and drying, 15 μL of DAPI was applied for 10 min for counterstaining. The tissues were subsequently observed under a fluorescence microscope.

In IHC, positive staining was indicated by dark brown membranous staining. Negative (0) was defined as less than 10% of cells showing no staining. Weak positivity (+) was defined as approximately 10% of cells displaying partial membranous staining. Moderate positivity (++) was characterized by around 10% of cells showing mild to moderate membranous staining. Strong positivity (+++) was defined as more than 10% of cells with intense membranous staining. In FISH, green signals in the nucleus identified the diploid state of chromosome 17, while red signals indicated the presence of the HER2 gene. A red-to-green signal ratio greater than 2, or more than four red HER2 fluorescent signals, indicated positive HER2 gene amplification. We have precisely defined the HER2 positivity threshold by evaluating HER2 protein expression in tumor tissues. IHC scores are categorized into four tiers: 0, 1+, 2+, and 3+. A score of 3 + signifies strong HER2 positivity, directly indicating HER2 - positivity. Scores of 0 and 1 + mean HER2 - negativity. For a score of 2+, we perform ISH testing on the area with the highest HER2 expression detected by IHC. We observe FISH signals with a 100× objective or DSISH signals with a 40× or 60× objective, selecting the region with the highest amplification. We count and calculate the ratio of dual - color signals in at least 20 consecutive tumor cell nuclei. If the ratio of HER2 signals to CEP17 signals is ≥ 2.2, amplification is deemed present (ISH - positive). If the ratio is between 1.8 and 2.2, we recount in 20 more cells or have another pathologist count. A ratio ≥ 2.0 indicates amplification, while < 2.0 means no amplification. This rigorous process ensures accurate HER2 status determination, underpinning the reliability of our subsequent research.

Statistical analysis

Statistical analysis was performed using R version 4.3.2. Descriptive statistics were employed to report the

frequency of molecular subtype changes and the subsequent alterations in treatment regimens. The influence of demographic, disease-related, and treatment-related factors on molecular subtype transitions was assessed using the chi-square test, with results summarized in baseline tables. Variables were screened by excluding those that were either significantly unrelated or those, such as postoperative pathology, that directly reflected molecular subtype. The remaining variables were dichotomized and subjected to both univariate and multivariate logistic regression analyses to evaluate the association between each independent variable and molecular subtype changes. All variables were included in the multivariate logistic regression model to account for all potential influencing factors, ensuring a more precise and comprehensive assessment. Statistical analysis was conducted using R version 4.0.3, with a significance threshold set at $p < 0.05$.

Results

Molecular subtype conversion

During the study period, we identified 316 patients who underwent definitive surgery for breast cancer following neoadjuvant chemotherapy (NAC) and collected both clinical and pathological data. The data filtering process is depicted in Fig. 1. Of these, 86 patients (27.2%) achieved a pathological complete response (pCR). For the remaining 232 patients with residual disease, biomarker testing was repeated on the surgical specimens. A comparison of biomarkers between pre-neoadjuvant and post-surgical specimens revealed that 85 patients (36.6%) experienced a molecular subtype change (Fig. 2).

Among the 232 patients who did not achieve a pathological complete response (pCR) after NAC, the consistency and heterogeneity of molecular subtypes before and after treatment are summarized in Fig. 3. Among the 85 cases with molecular subtype heterogeneity after NAC, 3 out of 11 Luminal A-type cases (27%) transitioned to Luminal B HER2- type. Of the 98 Luminal B HER2- type cases, 32 (33%) transitioned to Luminal A type, and 9 (9%) to Triple-negative type. Among the 46 Luminal B HER + type cases, 1 (2%) transitioned to Luminal A-type, 10 (22%) to Luminal B HER2- type, 7 (15%) to HER2 + type, and 3 (7%) to Triple-negative type. Among the 23 HER2 + type cases, 3 (13%) transitioned to Luminal B HER2- type, 4 (17%) to Luminal B HER2 + type, and 4 (17%) to Triple-negative type. Among the 39 Triple-negative type cases, 2 (5%) transitioned to Luminal B HER2- type and 2 (5%) to HER2 + type. The distribution of cases exhibiting molecular subtype heterogeneity is shown in Fig. 4.

Changes in adjuvant treatment plans

Among the 85 patients with molecular subtype heterogeneity before and after neoadjuvant chemotherapy (NAC), 45 patients (19.4%) experienced a change in their postoperative treatment regimen as a result of molecular subtype alterations (Fig. 5). The proportions of patients whose adjuvant treatment plans were modified based on molecular subtype changes, along with the specific changes in treatment regimens, are detailed in Supplementary Table 1. Of these, 16 patients omitted endocrine therapy, 15 discontinued targeted therapy, 6 initiated endocrine therapy, and 2 added targeted therapy. Additionally, 6 patients received adjuvant regimens that were entirely contrary to their neoadjuvant treatment plans, owing to changes in molecular subtype as indicated by pathology.

Clinical and demographic factors

Among the 316 patients who underwent breast cancer surgery following neoadjuvant chemotherapy (NAC) during the study period, 84 patients (26.6%) achieved a pathological complete response (pCR). For the remaining 232 patients with residual disease, biomarker testing was repeated on the surgical specimens. Baseline demographic, disease-related, and treatment-related factors for the molecular subtype non-transition group and the molecular subtype transition group are summarized in Supplementary Table 2. Statistically significant differences were observed between the two groups in terms of surgical approach, pre-neoadjuvant Ki-67 score, pre-neoadjuvant hormone receptor status, ER status, HER2 status, neoadjuvant treatment efficacy assessment, post-neoadjuvant Ki-67 score, post-neoadjuvant HER2 grading, and post-neoadjuvant ER status. These factors were found to significantly influence molecular subtype changes before and after neoadjuvant therapy.

Factors associated with molecular subtype conversions

In both univariate and multivariate regression analyses (Supplementary Table 3), we examined the relationship between various variables and molecular subtype transitions. Our analysis revealed that, compared to patients undergoing non-breast-conserving surgery, those who underwent breast-conserving surgery after neoadjuvant therapy had a significantly higher likelihood of experiencing molecular subtype changes (adjusted OR = 1.87, 95% CI = 1.01 to 3.46, $p = 0.047$). HR + tumors prior to neoadjuvant therapy were notably more likely to undergo receptor conversion than HR- tumors (adjusted OR = 15.92, 95% CI = 1.17 to 217.43, $p = 0.038$). For patients with HER2 overexpression before neoadjuvant therapy, although the likelihood of molecular subtype transition was increased (adjusted OR = 3.05, 95% CI = 0.89 to 10.50), it did not reach statistical significance.

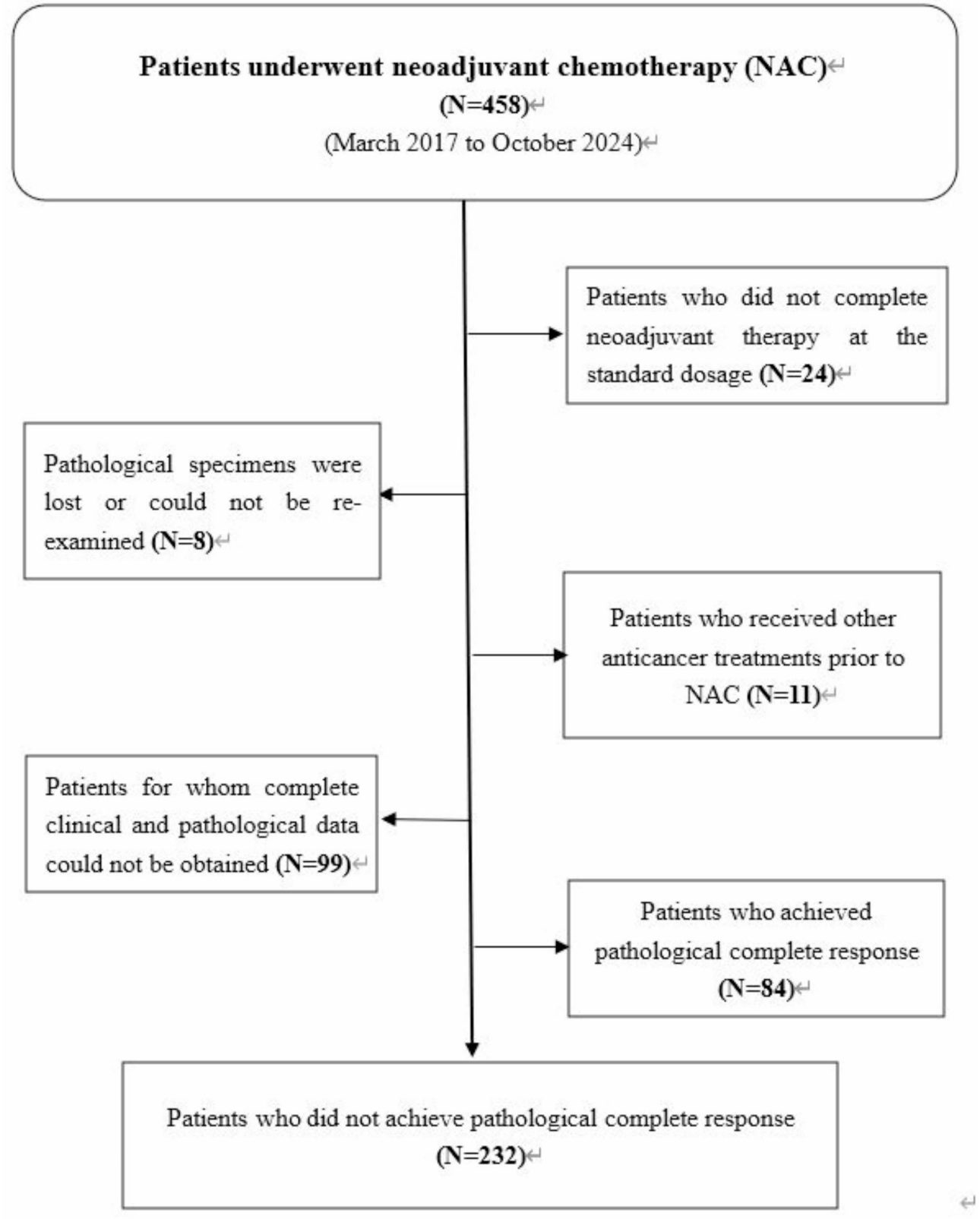

Fig. 1 Screening process of 232 cases of invasive breast cancer who did not achieve pathological complete response after neoadjuvant chemotherapy between 2017 and 2024

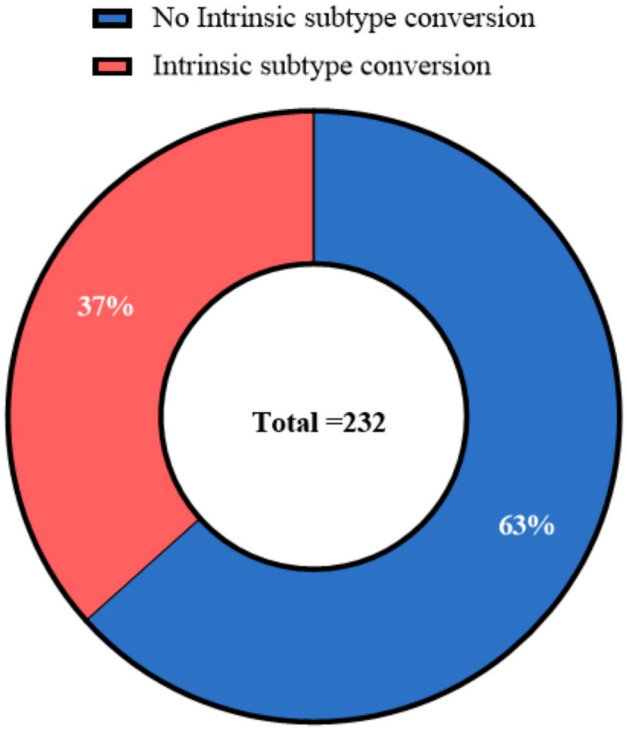

Fig. 2 Proportion of molecular subtype changes in 233 cases of invasive breast cancer that did not achieve pathological complete response after neoadjuvant chemotherapy

Patients who achieved a pathological partial response (PR) after neoadjuvant therapy were significantly more likely to experience molecular subtype changes compared to those with no significant response or tumor progression (adjusted OR = 3.47, 95% CI = 1.49 to 8.06, $p = 0.004$). These findings are visually represented in a forest plot (Fig. 6). In contrast, factors such as age, tumor stage before NAC, WHO grading, pre-treatment Ki-67 score, ER status, PR status, and HER2 grading prior to NAC were not found to be associated with molecular subtype transitions.

Discussion

In our retrospective study, we found that a significant proportion of patients experienced molecular subtype changes following neoadjuvant chemotherapy (NAC), with 36.6% of patients undergoing such transitions. Among patients with altered molecular subtypes,19.4% received a different adjuvant treatment regimen compared to the initial regimen based on pre-NAC biomarker results. We summarized the distribution and frequency of molecular subtype changes after NAC across various pre-treatment molecular subtypes, aiming to guide clinicians in prioritizing repeat biomarker testing for patients with specific subtypes who are more likely to undergo such changes.

Previous studies have primarily focused on the conversion rates of individual receptors [12, 23, 24, 25]; Zhang, Moran, Huo, Haffty, & Yang [26], whereas our study adopts a broader perspective by investigating molecular subtype transitions, which may offer a more intuitive approach for clinicians. Focusing on molecular subtypes rather than receptors enables physicians to more clearly identify, based on clinical breast cancer subtypes, which patients are more likely to experience molecular subtype shifts following neoadjuvant therapy. In our cohort of 232 breast cancer patients who did not achieve a pathological complete response (pCR) after NAC more than one-third (36.6%) exhibited molecular subtype heterogeneity, with varying proportions of subtype changes. Notably, one-third of patients with the Luminal B HER2- subtype transitioned to Luminal A. Furthermore, we confirmed that pre-treatment Ki-67 levels differed between patients with and without molecular subtype changes, consistent with findings by Reiki Nishimura (Nishimura, Osako, Okumura, Hayashi, & Arima [27], and Sasagu Kurozumi [28], who reported a decrease in Ki-67 post-NAC in certain patients. This reduction in Ki-67 indirectly contributed to molecular subtype changes, as multiple studies have demonstrated that treatment can lower Ki-67 expression [27, 28, 29], reflecting a decrease in tumor proliferative activity.

In the Luminal B HER2 + group, nearly one-fifth of patients transitioned to Luminal B HER2-, and approximately 20% of HER2 + patients developed triple–negative breast cancer (TNBC), likely due to a reduction in HER2 expression following NAC. Several studies [30, 31, 32] have documented changes in HER2 expression after NAC. For example, in a multicenter study by Julia Tchou [31], 11 of 207 patients with HER2-negative disease before NAC transitioned to TNBC post-treatment, while 14 out of 52 patients with HER2-positive disease converted to HER2-negative status after NAC. Our findings are consistent with these results, as approximately one-quarter of HER2 + patients converted to HER2- after NAC. This subgroup demonstrated significantly shorter progression-free survival and higher recurrence risks [33], which has important clinical implications for subsequent treatment strategies. However, in our multivariate analysis, there was no statistically significant difference in the HER2 overexpression group before NAC treatment, suggesting that a larger sample size is needed to validate these findings.

We also observed that hormone receptor-positive molecular subtypes were more likely to undergo molecular subtype transitions, a finding confirmed by both univariate and multivariate logistic regression analyses. This aligns with the results of S. van de Ven [20], who reported that changes in hormone receptor expression are associated with NAC. In our study, approximately one-fifth of

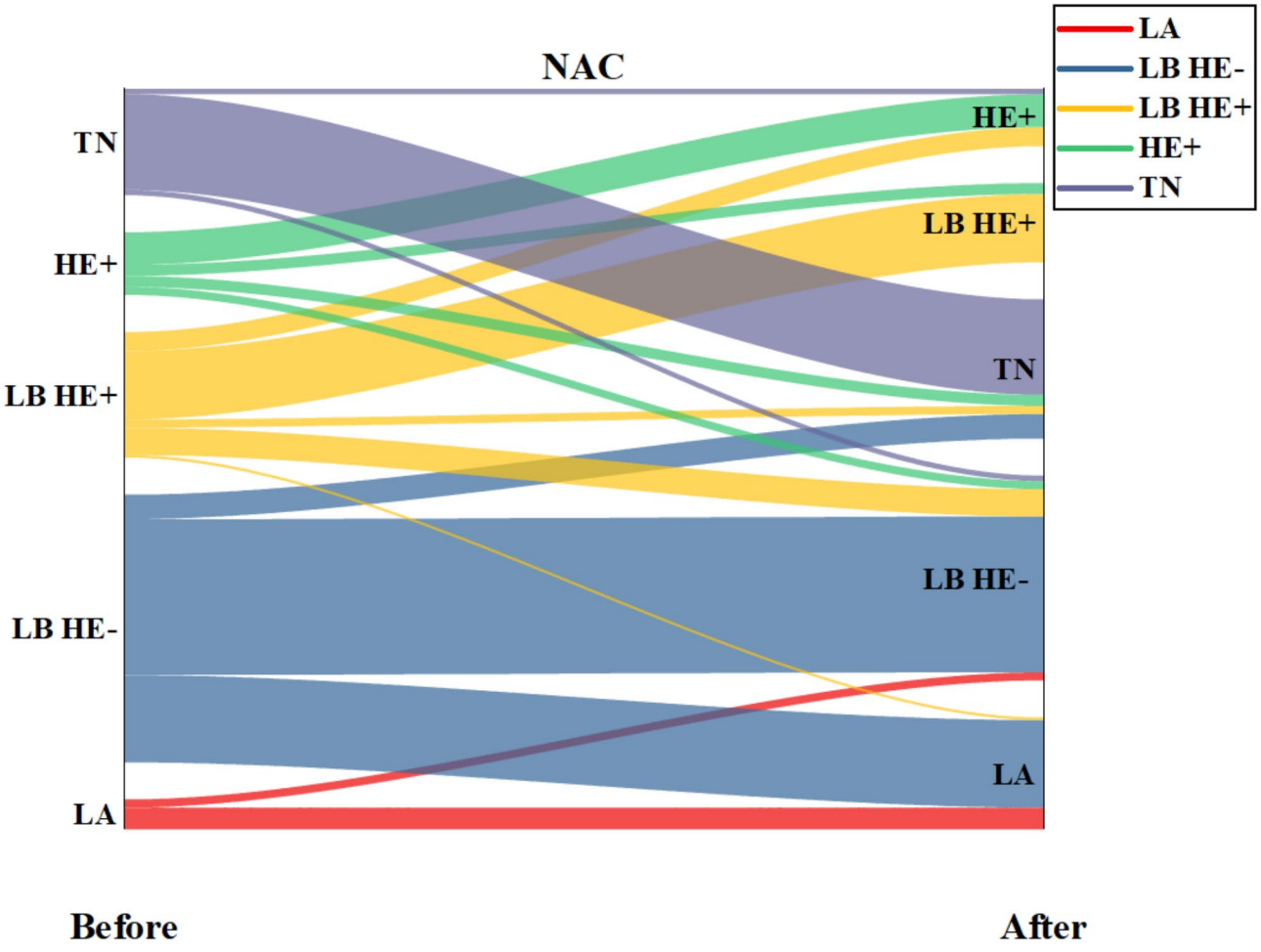

Fig. 3 Sankey diagram of molecular subtype changes in 232 cases of invasive breast cancer that did not achieve pathological complete response after neoadjuvant chemotherapy. NAC: Neoadjuvant chemotherapy; LA: Luminal A-type; LB HE-: Luminal B HER2-; LB HE + Luminal B HER2+; HE+: HER2+; TN: Triple-negative

Luminal B HER2 + patients transitioned to HER2-negative status after NAC, further supporting the connection between hormone receptor expression and NAC. Although we also examined the expression levels of ER and PR, no definitive conclusions could be drawn from the statistical analysis.

In our study, some patients exhibited a shift in molecular subtypes from triple-negative breast cancer (TNBC) to other subtypes post - neoadjuvant therapy. For these patients, treatment adjustments involve combining immune checkpoint inhibitors with chemotherapy, a regimen that significantly boosts TNBC patients' overall survival (OS) (Sharma et al., [34]. For breast cancer patients with altered HER2 expression, treatment modifications include continuing dual - target therapy or intensifying chemotherapy. The latest WSG-TP II trial results show that effective HER2 - targeted therapy can downscale chemotherapy for HR-positive/HER2-positive early - stage breast cancer patients. Patients achieving pCR after neoadjuvant standard endocrine therapy plus dual - target therapy have a 100% five - year overall survival rate [35]. Luminal A - type patients have better clinical outcomes and pathological response rates, while Luminal B - type patients have relatively lower efficacy. Therefore, for Luminal B - type patients, extended endocrine therapy duration or the addition of novel agents like CDK4/6 inhibitors may be considered to further improve survival rates.

Previous studies have demonstrated that tumor heterogeneity, including biopsy selection bias, may contribute to the molecular subtype heterogeneity observed before and after NAC [36, 37]; Robertson, Rönnlund, de Boniface, & Hartman [38]. For instance, in a study by Stephanie Robertson involving 526 patients who underwent direct surgery between 2016 and 2017, the consistency of HER2 immunohistochemistry (IHC) was only 75.4%, and the consistency for Ki-67, using a 20% cutoff, was just 78.8% [38]. The agreement between repeated pre- and post-treatment biopsies for Ki-67 and HER2-IHC was limited, suggesting that variations in biopsy sites within

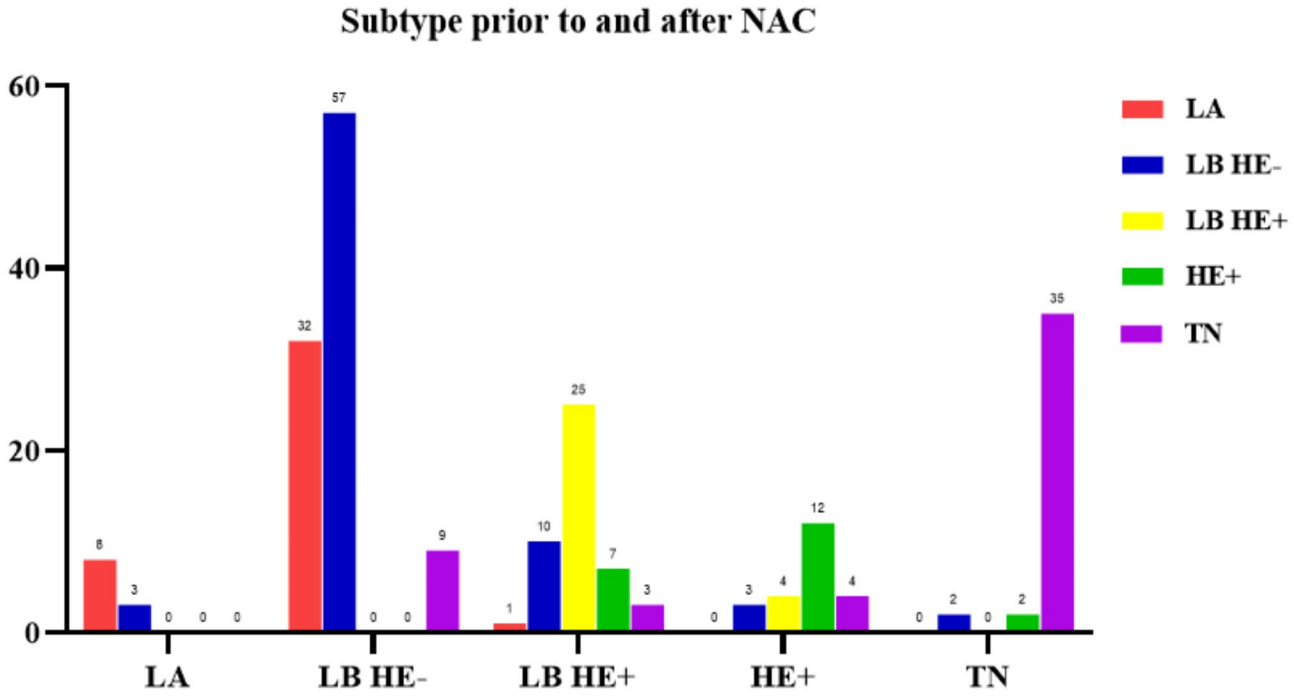

Fig. 4 The specific number of molecular subtype changes in 232 cases of invasive breast cancer that did not achieve pathological complete response after neoadjuvant chemotherapy. NAC: Neoadjuvant chemotherapy; LA: Luminal A-type; LB HE-: Luminal B HER2-; LB HE + Luminal B HER2+; HE+: HER2+; TN: Triple-negative

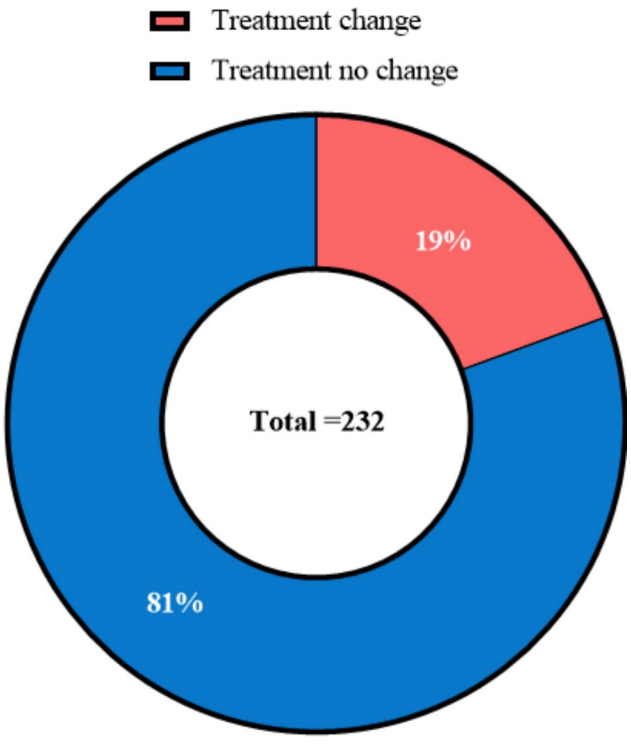

Fig. 5 The proportion of molecular subtype changes after neoadjuvant chemotherapy that led to changes in subsequent treatment

the same tumor may contribute to discrepancies in biomarker reports, thereby leading to molecular subtype heterogeneity. However, in our study, we observed significant differences in the likelihood of molecular subtype heterogeneity after NAC among patients with different initial molecular subtypes. This leads us to conclude that while biopsy selection bias may play a role, the observed changes in molecular subtypes are more closely associated with the specific NAC regimen administered, rather than being solely attributable to biopsy-related factors.

In this study, both the efficacy of NAC and the surgical approach were found to have statistically significant associations with molecular subtype heterogeneity in multivariate logistic regression analysis. Specifically, among patients who did not achieve pathological complete response (pCR), those with partial response (PR) and those undergoing breast-conserving surgery for resection of residual disease were significantly more likely to experience molecular subtype changes after NAC. We hypothesize that this phenomenon may be attributed to the selective pressure exerted by chemotherapy, as the biomarker profiles of tumor cells that survive neoadjuvant treatment may differ from those of the original pre-treatment tumor cells (Venkatesan, Swanton, Taylor, & Costello [39]. This observation aligns with findings from Sudheer Vemuru's retrospective single-center study [32], which reported that receptor conversion was more frequently observed in patients with clinical stage

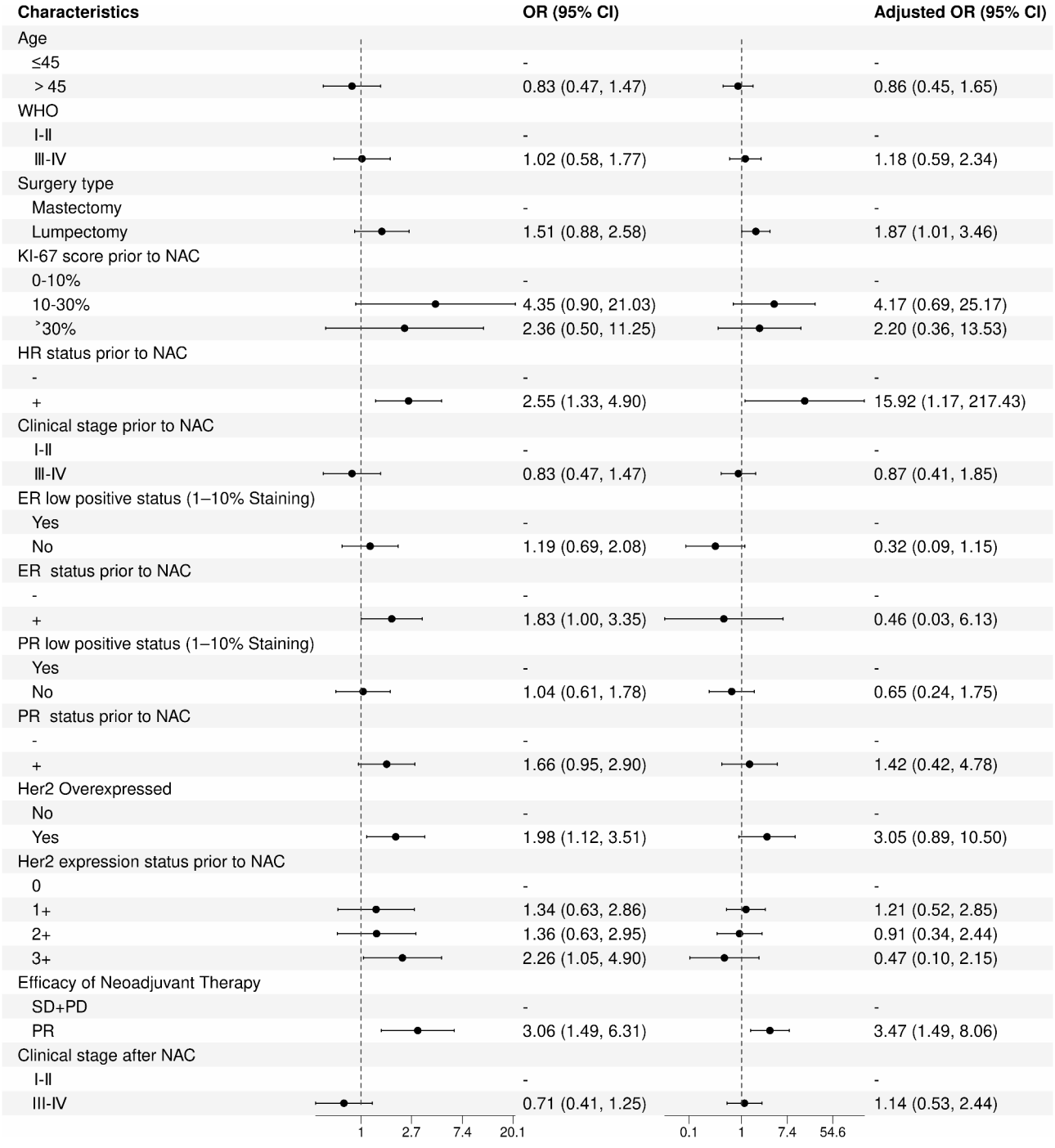

Fig. 6 Univariate and multivariate analysis of influencing factors (Forest plot)

I-II tumors after NAC. This further supports the notion that tumors exhibiting a favorable response to systemic therapy may be more susceptible to molecular subtype heterogeneity.

Although repeated biomarker testing may incur additional costs and potentially delay the initiation of subsequent adjuvant therapy, our findings underscore the clinical significance of this approach. In our study, molecular subtype changes led to alterations in the adjuvant treatment plan for approximately one in five patients, suggesting that postoperative biomarker testing is critical for ensuring appropriate treatment decisions. Given the substantial likelihood of molecular subtype shifts, particularly in hormone receptor-positive and HER2-overexpressing subtypes before NAC, clinicians should be vigilant in conducting timely postoperative

biomarker assessments. This practice is essential for optimizing treatment strategies, as the molecular subtype alterations can lead to significant adjustments in adjuvant therapies, potentially improving patient outcomes and minimizing unnecessary treatments.

We acknowledge that this study had some limitations. First, it was conducted only at a single institution in central China, which may limit the generalizability of the study findings to other regions, populations, or medical settings. Although Zhongnan Hospital is a leading academic medical center with a large number of breast cancer cases, the study sample may not fully reflected the wide diversity of demographic and clinical characteristics among breast cancer patients. Second, as a retrospective study, it was not possible to exclude selection bias. To control for confounding factors, we included all relevant variables (regardless of their significance in univariate analysis) in a multivariable logistic regression model, thereby minimizing the influence of selection bias on the study results.

We hope that this study will stimulate further research and discussion on molecular subtype heterogeneity of breast cancer patients before and after NAC. Specifically, future basic research could explore whether specific NAC regimens can induce consistent changes in tumor cell biomarkers, potentially leading to more personalized therapeutic strategies. Understanding the mechanisms driving molecular subtype transitions during treatment can enhance our knowledge of breast cancer biology and improve clinical decision-making, ultimately optimizing patient outcomes. In the next five years' research on molecular subtype shifts, more studies will increasingly concentrate on different survival outcomes following diverse molecular subtype changes and make significant progress in treatment personalization. As molecular subtype assessment becomes more refined, extending beyond traditional immunohistochemistry and FISH methods, advancements in genomic analysis and liquid biopsy technologies will more precisely characterize residual disease post - neoadjuvant therapy, identifying patients with an initial response but still at high risk of recurrence.

Conclusion

Molecular subtypes of breast cancers changed frequently after NAC and these changes could affect subsequent adjuvant treatment options. Thus, molecular subtypes should be reassessed after NAC using surgical specimens. Understanding the pattern of change in each molecular subtype can provide reference for the treatment and enable individualized management of breast cancer patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-025-03801-6.

Supplementary Material 1

Author contributions

Run-Ze Zhang's contribution to Data curation, Formal analysis, Validation, Writing-original draft. Dong Liu and Yuan Ke's contribution to Methodology. Lin-Hui Zheng and Wen-Qi Cai's contribution to data analysis and Software. Chao-Yan Wu's contribution to Supervision and project administration. Hai-Jun Yu's contribution to Funding acquisition, Investigation, Visualization, Writing-review & editing. All authors reviewed the manuscript.

Funding

This study was partially supported by grants from National Natural Science Foundation of China (82073349), Hubei Provincial Natural Science Foundation of China (ZYYCC2023005), Technology Transfer Fund Project of Zhongnan Hospital of Wuhan University (2022CGZH-IIT003), Science and Technology Innovation Cultivation Fund of Zhongnan Hospital of Wuhan University (CXP12023049), Hubei Provincial Natural Science Foundation of China General Program (2024AFB799), Hubei Provincial Administration of Traditional Chinese Medicine Major Project (ZY2025Z017).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was formally approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (2024318 K). The requirement for written informed consent was waived for this study. All methods were performed in accordance with the applicable guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 February 2025 / Accepted: 8 April 2025
Published online: 22 April 2025

References

1. Luo J, Craver A, Moore K, Stepniak L, King J, Herbert J, Aschebrook-Kilfoy B. Etiology of breast cancer: A perspective from epidemiologic studies. J Natl Cancer Cent. 2022;2(4):195–7. https://doi.org/10.1016/j.jncc.2022.08.004.
2. McPherson K, Steel CM, Dixon JM. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. BMJ. 2000;321(7261):624–8. https://doi.org/10.1136/bmj.321.7261.624.
3. Kerr AJ, Dodwell D, McGale P, Holt F, Duane F, Mannu G, Taylor CW. Adjuvant and neoadjuvant breast cancer treatments: A systematic review of their effects on mortality. Cancer Treat Rev. 2022;105:102375. https://doi.org/10.1016/j.ctrv.2022.102375.
4. Maughan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. Am Fam Physician. 2010;81(11):1339–46.
5. Caudle AS, Yang WT, Krishnamurthy S, Mittendorf EA, Black DM, Gilcrease MZ, Kuerer HM. Improved axillary evaluation following neoadjuvant therapy for patients with Node-Positive breast cancer using selective evaluation of clipped nodes: implementation of targeted axillary dissection. J Clin Oncol. 2016;34(10):1072–8. https://doi.org/10.1200/jco.2015.64.0094.
6. Costa SD, Loibl S, Kaufmann M, Zahm DM, Hilfrich J, Huober J, von Minckwitz G. Neoadjuvant chemotherapy shows similar response in patients with inflammatory or locally advanced breast cancer when compared with

operable breast cancer: a secondary analysis of the GeparTrio trial data. J Clin Oncol. 2010;28(1):83–91. https://doi.org/10.1200/jco.2009.23.5101.
7. Mauri D, Pavlidis N, Ioannidis JP. Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis. J Natl Cancer Inst. 2005;97(3):188–94. https://doi.org/10.1093/jnci/dji021.
8. Wang H, Mao X. Evaluation of the efficacy of neoadjuvant chemotherapy for breast cancer. Drug Des Devel Ther. 2020;14:2423–33. https://doi.org/10.2147/dddt.S253961.
9. Cain H, Macpherson IR, Beresford M, Pinder SE, Pong J, Dixon JM. Neoadjuvant therapy in early breast cancer: treatment considerations and common debates in practice. Clin Oncol (R Coll Radiol). 2017;29(10):642–52. https://doi.org/10.1016/j.clon.2017.06.003.
10. Caputo R, Buono G, Piezzo M, Martinelli C, Cianniello D, Rizzo A, De Laurentiis M. Sacituzumab Govitecan for the treatment of advanced triple negative breast cancer patients: a multi-center real-world analysis. Front Oncol. 2024;14:1362641. https://doi.org/10.3389/fonc.2024.1362641.
11. Chen AM, Meric-Bernstam F, Hunt KK, Thames HD, Oswald MJ, Outlaw ED, Buchholz TA. Breast conservation after neoadjuvant chemotherapy: the MD Anderson cancer center experience. J Clin Oncol. 2004;22(12):2303–12. https://doi.org/10.1200/jco.2004.09.062.
12. Sacchini V, Norton L. Escalating de-escalation in breast cancer treatment. Breast Cancer Res Treat. 2022;195(2):85–90. https://doi.org/10.1007/s10549-022-06685-2.
13. Yau C, Osdoit M, van der Noordaa M, Shad S, Wei J, de Croze D, Symmans WF. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. Lancet Oncol. 2022;23(1):149–60. https://doi.org/10.1016/s1470-2045(21)00589-1.
14. Harbeck N. Neoadjuvant and adjuvant treatment of patients with HER2-positive early breast cancer. Breast 62 Suppl. 2022;1(Suppl 1):S12–6. https://doi.org/10.1016/j.breast.2022.01.006.
15. Rizzo A, Cusmai A, Acquafredda S, Giovannelli F, Rinaldi L, Misino A, Palmiotti G. KEYNOTE-522, IMpassion031 and GeparNUEVO: changing the paradigm of neoadjuvant immune checkpoint inhibitors in early triple-negative breast cancer. Future Oncol. 2022;18(18):2301–9. https://doi.org/10.2217/fon-2021-1647.
16. Rizzo A, Schipilliti FM, Di Costanzo F, Acquafredda S, Arpino G, Puglisi F, Giuliano M. Discontinuation rate and serious adverse events of chemoimmunotherapy as neoadjuvant treatment for triple-negative breast cancer: a systematic review and meta-analysis. ESMO Open. 2023;8(6):102198. https://doi.org/10.1016/j.esmoop.2023.102198.
17. Burstein HJ, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, Gnant M. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen international consensus guidelines for treatment of early breast cancer 2021. Ann Oncol. 2021;32(10):1216–35. https://doi.org/10.1016/j.annonc.2021.06.023.
18. Schipilliti FM, Drittone D, Mazzuca F, La Forgia D, Guven DC, Rizzo A. Datopotamab Deruxtecan: A novel antibody drug conjugate for triple-negative breast cancer. Heliyon. 2024;10(7):e28385. https://doi.org/10.1016/j.heliyon.2024.e28385.
19. Sahin TK, Ayasun R, Rizzo A, Guven DC. Prognostic value of Neutrophil-to-Eosinophil ratio (NER) in cancer: A systematic review and Meta-Analysis. Cancers (Basel). 2024;16(21). https://doi.org/10.3390/cancers16213689.
20. van de Ven S, Smit VT, Dekker TJ, Nortier JW, Kroep JR. Discordances in ER, PR and HER2 receptors after neoadjuvant chemotherapy in breast cancer. Cancer Treat Rev. 2011;37(6):422–30. https://doi.org/10.1016/j.ctrv.2010.11.006.
21. Tarantino P, Jin Q, Tayob N, Jeselsohn RM, Schnitt SJ, Vincuilla J, Tolaney SM. Prognostic and biologic significance of ERBB2-Low expression in Early-Stage breast cancer. JAMA Oncol. 2022;8(8):1177–83. https://doi.org/10.1001/jamaoncol.2022.2286.
22. Ignatiadis M, Buyse M, Sotiriou C. St Gallen international expert consensus on the primary therapy of early breast cancer: an invaluable tool for physicians and scientists. Ann Oncol. 2015;26(8):1519–20. https://doi.org/10.1093/annonc/mdv259.
23. Kasami M, Uematsu T, Honda M, Yabuzaki T, Sanuki J, Uchida Y, Sugimura H. Comparison of Estrogen receptor, progesterone receptor and Her-2 status in breast cancer pre- and post-neoadjuvant chemotherapy. Breast. 2008;17(5):523–7. https://doi.org/10.1016/j.breast.2008.04.002.
24. Tacca O, Penault-Llorca F, Abrial C, Mouret-Reynier MA, Raoelfils I, Durando X, Chollet P. Changes in and prognostic value of hormone receptor status in a series of operable breast cancer patients treated with neoadjuvant chemotherapy. Oncologist. 2007;12(6):636–43. https://doi.org/10.1634/theoncologist.12-6-636.
25. Uzun M, Atag E, Caliskan Yildirim E, Keser M, Semiz HS, Unal OU. Does immunohistochemical marker conversion affect the prognosis in breast cancer patients receiving neoadjuvant chemotherapy? Sci Rep. 2024;14(1):14651. https://doi.org/10.1038/s41598-024-64492-9.
26. Zhang N, Moran MS, Huo Q, Haffty BG, Yang Q. The hormonal receptor status in breast cancer can be altered by neoadjuvant chemotherapy: a meta-analysis. Cancer Invest. 2011;29(9):594–8. https://doi.org/10.3109/07357907.2011.621913.
27. Nishimura R, Osako T, Okumura Y, Hayashi M, Arima N. Clinical significance of Ki-67 in neoadjuvant chemotherapy for primary breast cancer as a predictor for chemosensitivity and for prognosis. Breast Cancer. 2010;17(4):269–75. https://doi.org/10.1007/s12282-009-0161-5.
28. Kurozumi S, Inoue K, Takei H, Matsumoto H, Kurosumi M, Horiguchi J, Oyama T. ER, PgR, Ki67, p27(Kip1), and histological grade as predictors of pathological complete response in patients with HER2-positive breast cancer receiving neoadjuvant chemotherapy using taxanes followed by fluorouracil, epirubicin, and cyclophosphamide concomitant with trastuzumab. BMC Cancer. 2015;15:622. https://doi.org/10.1186/s12885-015-1641-y.
29. Wu YT, Li X, Lu LJ, Gan L, Dai W, Shi YL, Kong LQ. Effect of neoadjuvant chemotherapy on the expression of hormone receptors and Ki-67 in Chinese breast cancer patients: A retrospective study of 525 patients. J Biomed Res. 2017;32(3):191–7. https://doi.org/10.7555/jbr.32.20170059.
30. Guarneri V, Dieci MV, Barbieri E, Piacentini F, Omarini C, Ficarra G, Conte PF. Loss of HER2 positivity and prognosis after neoadjuvant therapy in HER2-positive breast cancer patients. Ann Oncol. 2013;24(12):2990–4. https://doi.org/10.1093/annonc/mdt364.
31. Tchou J, Gottipati S, Goldbach M, Baxter M, Venters S, Balassanian R, Chen YY. Change in biomarker profile after neoadjuvant chemotherapy is prognostic and common among patients with HER2 + Breast cancer. Ann Surg Oncol. 2024;31(12):8093–101. https://doi.org/10.1245/s10434-024-15889-3.
32. Vemuru S, Huang J, Colborn K, Yoon Y, Huynh V, Leonard L, Tevis S. Clinical implications of receptor conversions in breast cancer patients who have undergone neoadjuvant chemotherapy. Breast Cancer Res Treat. 2023;200(2):247–56. https://doi.org/10.1007/s10549-023-06978-0.
33. Uczkowski D, Sekhri A, Gupta S, Gendler L, Misiukiewicz K, Levin M, Guerin B. Treatment of HER2 + breast cancer: a retrospective of disease prognosis with loss of HER2 amplification on residual disease after neoadjuvant treatment in a community hospital setting. Am J Cancer Res. 2023;13(6):2564–71.
34. Sharma P, Stecklein SR, Yoder R, Staley JM, Schwensen K, O'Dea A, O'Shaughnessy J. Clinical and biomarker findings of neoadjuvant pembrolizumab and carboplatin plus docetaxel in Triple-Negative breast cancer: NeoPACT phase 2 clinical trial. JAMA Oncol. 2024;10(2):227–35. https://doi.org/10.1001/jamaoncol.2023.5033.
35. Gluz O, Nitz UA, Christgen M, Kuemmel S, Holtschmidt J, Schumacher J, Harbeck N. Efficacy of endocrine therapy plus trastuzumab and Pertuzumab vs De-escalated chemotherapy in patients with hormone Receptor-Positive/ERBB2-Positive early breast cancer: the neoadjuvant WSG-TP-II randomized clinical trial. JAMA Oncol. 2023;9(7):946–54. https://doi.org/10.1001/jamaoncol.2023.0646.
36. Lorgis V, Algros MP, Villanueva C, Chaigneau L, Thierry-Vuillemin A, Nguyen T, Pivot X. Discordance in early breast cancer for tumour grade, Estrogen receptor, progesteron receptors and human epidermal receptor-2 status between core needle biopsy and surgical excisional primary tumour. Breast. 2011;20(3):284–7. https://doi.org/10.1016/j.breast.2010.12.007.
37. Meattini I, Bicchierai G, Saieva C, De Benedetto D, Desideri I, Becherini C, Nori J. Impact of molecular subtypes classification concordance between preoperative core needle biopsy and surgical specimen on early breast cancer management: Single-institution experience and review of published literature. Eur J Surg Oncol. 2017;43(4):642–8. https://doi.org/10.1016/j.ejso.2016.10.025.
38. Robertson S, Rönnlund C, de Boniface J, Hartman J. Re-testing of predictive biomarkers on surgical breast cancer specimens is clinically relevant. Breast Cancer Res Treat. 2019;174(3):795–805. https://doi.org/10.1007/s10549-018-05119-2.

39. Venkatesan S, Swanton C, Taylor BS, Costello JF. Treatment-Induced mutagenesis and selective pressures sculpt cancer evolution. Cold Spring Harb Perspect Med. 2017;7(8). https://doi.org/10.1101/cshperspect.a026617.