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Combinational Treatment of Doxorubicin With Neoadjuvant Docetaxel for Different Subtypes of Patients With Breast Cancer

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Abstract

Aim: The aim of this study is to characterize the effect of chemotherapy drug doxorubicin with neoadjuvant drug docetaxel for different molecular subtypes. **Methods:** A total of 83 patients with late-stage breast cancer were chosen to undergo treatment and compared to these patients to the combinational treatment to identify the molecular characteristics that can predict the responses. **Results:** Total response rate is 81.9% (68/83 patients). Among them, 7 patients show pathological complete response of 8.4%, 12 patients show clinical complete response of 14.5%, 49 patients show partial response of 59%, and 15 patients show stable disease of 18.1%. The comparison among different subtypes of breast cancer, including luminal A, luminal B, basal-like, and ERBB2^{+} subtypes, did not show statistical significant differences to the treatment of combinational treatment for the complete response rate, including pathological complete response and clinical complete response. Comparing with luminal A and luminal B subtypes, the ERBB2^{+} and basal-like subtypes have better complete response and response rate rates. The disease-free survival rate and overall survival rate at 29 months after treatment did not show statistical significant differences among different subtypes of patients with breast cancer. **Conclusion:** The molecular subtypes of breast cancer can predict responses to the combinational treatment of doxorubicin with docetaxel, and ERBB2^{+} and basal-like subtypes have better response rate and complete response rate. There is correlation of estrogen receptor and KI-67 level changes with response rate as well, where KI-67 high patients are more sensitive to the treatment.

Keywords

breast cancer, cavity radiosurgery, radiosurgery, 3d conformal radiotherapy, stereotactic body radiation therapy

Abbreviations

cCR, clinical complete response; CTX, cyclophosphamide; DFS, disease free survival; ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor type 2; IHC, immunohistochemistry staining; NAC, N-acetylcysteine; OS, overall survival; PD, progressive disease; pCR, pathological complete response; PR, partial response; RR, response rate; SD, stable disease; TNBC, triple-negative breast cancer.

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Introduction

For breast cancer study, it is well established that estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor type 2 (HER2), and KI-67 levels are critical markers for the guidance of chemotherapy and targeted therapy strategies, and their levels correlate with the response rate (RR) to the treatment.[1-4] However, it is not clear that during the neoadjuvant therapy for patients with late-stage breast cancer, what molecular markers can be used to predict the responses to the treatment.[5] In order to characterize the correlation between molecular subtypes and the RR to the combinational treatment of doxorubicin with neoadjuvant docetaxel, we performed immunohistochemistry staining (IHC) and fluorescence in situ hybridization (FISH) to determine the expression levels of ER, PR, HER2, and KI-67 among subtypes of patients with breast cancer tumor. Furthermore, we sought to identify the RR to this combinational treatment strategy among different subtypes of patients with breast cancer and clinical pathological correlation with these subtypes of late-stage breast cancer.

Material and Methods

Patients

The present study protocol was approved by the ethics xommittee of Renmin Hospital, Hubei University of Medicine (No. 2011-023). All patients provided written informed consent prior to enrollment in the study. We recruited female patients with breast cancer who undergo surgery operations. The key eligibility criteria were consented patients as diagnosed for the first time for breast cancer who are younger than 70 years old, the diameter of the tumor size equal or larger than 4 cm, and with signed consent for the chemotherapy treatment with neoadjuvant. Those patients with severe heart, liver, or kidney diseases or other malignant tumor were excluded from this study. Patients who did not finish more than 4 cycles of treatment were also excluded from the analysis.

Trial Design

Before the surgery, the patients were treated with combination of 75 mg/m^2 docetaxel, 90 mg/m^2 epirubicin, and 500 mg/m^2 cyclophosphamide (CTX) for 3 weeks as a cycle. Preoperative neoadjuvant chemotherapy regimen (ie, TEC regimen): docetaxel 75 mg/m^2, epirubicin 90 mg/m^2, CTX 500 mg/m^2, 1 cycle every 3 weeks, is given by intravenous injection. After 2 cycles, the clinical efficacy is determined. If the disease progresses, change the chemotherapy regimen or surgery, otherwise continue chemotherapy. Modified radical mastectomy for breast cancer was performed 10 to 14 days after completing 2 to 6 cycles of chemotherapy. Give dexamethasone injection 20 mg intravenously 30 minutes before docetaxel neoadjuvant chemotherapy. Each cycle of chemotherapy was treated with recombinant human granulocyte colony-stimulating factor throughout the course of treatment to prevent and treat leukocyte deficiency. All patients underwent modified radical mastectomy for breast cancer, of which 2 had skin grafts. Those who did not complete the course of chemotherapy before surgery continued chemotherapy after surgery. After chemotherapy, ER-positive patients were given tamoxifen before menopause and letrozole was given after menopause. All HER-2-positive patients were recommended to be treated with trastuzumab, but only 15 patients agreed to the treatment. Trastuzumab was administered at a dose of 8 mg/kg (the first day), followed by an intravenous infusion over 90 minutes, followed by an intravenous infusion at 6 mg/kg once every 3 weeks. The clinical responses were assessed after 2 cycles. If the disease progresses, the patient will be changed to other chemotherapy treatment or undergo surgery; otherwise, they will be treated with the same combinations. After 4 to 6 cycles of treatment, total mastectomy will be performed 10 to 14 days after the end of the treatment.

The biopsy samples were collected via the Mammotome system with ultrasound guidance. Eighty-three patients with advanced breast cancer underwent 2 to 6 cycles of neoadjuvant chemotherapy to evaluate the tumor efficacy and study the relationship between neoadjuvant chemotherapy and efficacy. We studied the relationship between tumor efficacy and the changes in ER, PR, HER-2, and Ki-67 expression status before and after neoadjuvant chemotherapy. The collected sample before and after treatment were examined via IHC for the levels of ER, PR, HER2, and KI-67. If the tumor samples show as HER2^{++}, they will be further examined via FISH. The KI-67 levels were characterized for percentage of positive cells among all the cells in the field, and percentage lower than 14% is determined as low expression levels, while percentage equal to or higher than 14% is determined as high KI-67 expression levels. Eighty-three patients with advanced breast cancer in this group were followed up for 29 months to evaluate disease-free survival (DFS) and overall survival (OS) and to study the relationship between molecular subtypes and curative effects and prognosis.

The evaluation of treatment response is determined according to RECIST 1.1 (2009) as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Within CR category, it is further categorized as pathologic complete response (pCR) and clinical complete response (cCR). The total RR is determined as combination of CR plus PR. Pathologic complete response is defined as no primary tumor cell and no invasive cancer cell at lymph node.

Statistics

SPSS version 16.0 statistical software was used for analysis, and χ^2 test was used for counting data. Counting data were tested using χ^2. Disease-free survival and OS were compared between groups using Kaplan-Meier survival analysis. The difference was statistically significant at $P < .05$.

Results

Patients

From 2011 to 2014, a total of 83 patients were enrolled. Among these patients, the subtypes of breast cancer disease have no

Table 1. Clinical Pathological Characteristics and Subtypes.

	Luminal A	Luminal B	ERBB2$^+$	Basal-like
Age				
<35	2	4	3	5
≥35	9	22	24	14
Menopausal status				
Premenopausal	10	23	23	16
Postmenopausal	1	3	3	3
Involved lymph node				
Positive	7	20	24	14
Negative	4	6	3	5
Pathological effect grade				
1a or 1b	3	5	5	0
2 or 3	8	21	22	19
Metastasis status				
Invasive ductal carcinoma	11	23	24	17
Other	0	3	3	2
Total	11	26	27	19

Table 2. Clinical Responses and Subtypes.

	Luminal A	Luminal B	ERBB2$^+$	Basal-like
Complete response (CR)				
Pathological complete response (pCR)	0	1	3	3
Clinical complete response (cCR)	0	1	6	5
P value (CR) luminal A vs		$P = .488$	$^*P = .029$	$^*P = .013$
P value (CR) luminal B vs			$^*P = .033$	$^*P = .038$
Partial response (PR)	7	16	16	10
P value (RR) luminal A vs			$^*P = .047$	$^*P = .047$
P value (RR) luminal B vs			$^*P = .033$	$^*P = .038$
Stable disease (SD)	4	8	2	1
Progressive disease (PD)	0	0	0	0

statistical relevance to the age, size of the tumor, involved lymph node, menopausal status, pathological grade, or metastasis status ($P > .05$; Table 1). We separated the examined patients into 4 groups based on their molecular signatures: luminal A subtype, ER$^+$, PR$^+$, HER2$^-$, and KI-67 low; luminal B subtype, ER$^+$, PR$^+$, HER2$^-$, and KI-67 high; ERBB2$^+$ or HER2$^+$ subtype, ER$^-$ and PR$^-$; and Basal-like/triple-negative ER$^-$, PR$^-$, HER2$^-$ subtype. Both luminal A and luminal B subtypes are hormone-receptor (ER, PR) positive. And KI-67 is a proliferation marker which correlates with tumor growth rate. Basal-like subtypes are more common in women with BRCA1 mutations who do not respond to hormone-based therapy or targeted therapy against HER2 overexpression. Among these patients, there are 11 (13.2%) patients who are luminal A subtype, 26 (31.3%) patients who are luminal B subtype, 27 (32.5%) patients are who are ERBB2$^+$ subtype, and 19 (22.9%) patients who are basal-like subtype. Luminal A cancers are often low-grade, tend to grow slowly, and they have the best prognosis in the clinic. Therefore, this subtype has the least number of patients undergoing neoadjuvant therapy comparing with the other 3 groups. There are a total of 14 (16.9%) patients who are younger than 35 years old and 69 (83.1%) patients who are 35 years or older, while 72 (86.7%) patients are premenopausal and 10 (12.0%) patients are postmenopausal. When we analyze the severity level of these patients, there are 13 (15.7%) patients who are at stage 1a or 1b grade, while 70 (84.3%) who are at stage 2 or 3. There are a total of 65 (78.3%) patients having involved lymph nodes and 75 (90.4%) patients defined as invasive ductal carcinoma (Table 1).

Clinical Responses to Neoadjuvant Therapy

Among the 83 patients who enrolled in this study, there are 71 patients who has SD finished 4 to 6 cycles of chemotherapy and 12 patients who has SD finished 2 to 3 cycles of the treatment. The total RR is 81.9% (68/83 patients). Among them, the pCR rate is 8.4% (7/83), cCR rate is 14.5% (12/83), partial RR is 59% (49/83), and SD rate is 18.1% (15/83; Table 2). There is no PD (Table 2). Comparing different subtypes of breast cancer, including luminal A, luminal B, ERBB2$^+$, and basal-like subtypes, there is no statistically significant difference of either pCR or cCR between these groups after neoadjuvant therapy. Subtypes luminal A comparing with luminal B and subtypes ERBB2$^+$ comparing with basal-like have no statistical differences for CR ($\chi^2 = 1.000$, $P = .488$, and $\chi^2 = .757$, $P = .382$, respectively). Luminal A and luminal B subtypes have statistical differences with ERBB2$^+$ and basal-like subtypes for CR rates ($\chi^2 = 0.38$, $P = .029$; $\chi^2 = 0.14$, $P = .013$; $\chi^2 = 0.039$, $P = .023$ and $\chi^2 = 0.010$, $P = .008$, respectively). In addition, there is statistical difference of both luminal A and luminal B subtypes comparing with either ERBB2$^+$ or basal-like subtypes for the overall RR ($\chi^2 = 0.047$, $P = .047$ and $\chi^2 = 0.047$, $P = .047$; $\chi^2 = 0.039$, $P = .033$ and $\chi^2 = 0.058$, $P = .038$, respectively). There is no statistical difference in RR between luminal A and luminal B subtypes (Table 2).

To further delineate the relationship between hormone receptor levels and KI-67 levels with clinical responses, we analyzed these marker changes before and after the combinational treatment of chemotherapy drugs with neoadjuvant docetaxel. There are 5 patients who change from ER negative to positive, while 9 patients change from ER positive to negative, while there are 2 patients who change from PR negative to positive and 5 patients change from PR positive to negative, no patient changes from HER2 negative to positive, while 3 patients change from HER2 positive to negative, and 8 patients change from KI-67 negative to positive and 21 change from KI-67 positive to negative (Table 3). Among them, only KI-67

Table 3. Hormone Receptor Status, KI-67 Levels, and Subtypes.

	Before chemotherapy	After chemotherapy
Estrogen receptor (ER)		
Negative	40	44
Positive	36	32
Progesterone receptor (PR)		
Negative	45	31
Positive	48	28
ERBB2		
Negative	49	27
Positive	52	24
KI-67		
Negative	25	51
Positive	38	38

Table 4. Clinical Responses and ER, KI-67 Status Changes.

	CR	PR	SD
Estrogen receptor (ER)			
Negative to positive	0	2	3
Positive to negative	1	8	0
KI-67			
Negative to positive	0	5	3
Positive to negative	9	10	1

Abbreviations: CR, complete response; PR, progesterone receptor; SD, stable disease

Table 5. Clinical Responses and KI-67 Status Before Treatment.

	pCR	cCR	PR	SD
KI-67				
Negative	1	2	16	10
Positive	6	10	33	5

Abbreviations: cCR, clinical complete response; pCR, pathologic complete response; PR, progesterone receptor; SD, stable disease.

levels showed statistical significant difference to the treatment of neoadjuvant therapy. This is due to the fact that both doxorubicin and docetaxel target proliferating cells including cancer cells through different mechanisms. Doxorubicin intercalates DNA and inhibits topoisomerase II during DNA replication, and docetaxel binds and stabilizes microtubules that prevent mitotic cell division. Furthermore, both ER and KI-67 level changes are correlated with RR ($\chi^2 = 7.031$, $P = .030$ and $\chi^2 = 4.581$, $P = .031$, respectively; Table 4), but not PR or HER2 level change (data not shown). When analyze the levels of KI-67 before the treatment, there is statistical significant difference between CR (pCR + cCR) and PR + SD groups ($\chi^2 = 3.975$, $P = .046$; Table 5). This indicates KI-67 levels are correlated with better outcome of the patient to the neoadjuvant treatment, due to the fact the KI-67 high cells have higher proliferation rate.

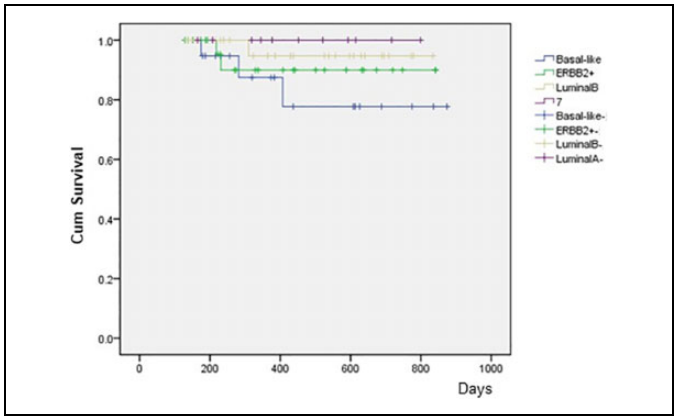

Figure 1. Overall survival (OS) and subtypes.

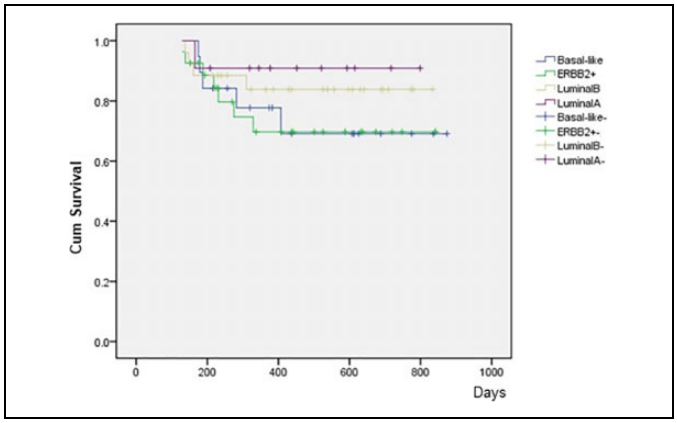

Figure 2. Disease-free survival (DFS) and subtypes.

Disease-Free Survival and OS

A total of 29 months were followed up after the patients undergo neoadjuvant therapy, and there is no statistical significant difference in survival rate among different subtypes of breast cancer of luminal A, luminal B, ERBB2$^+$, HER2$^+$, and basal-like subtypes, including both OS ($\chi^2 = 3.390$, $P = .335$) and DFS ($\chi^2 = 2.183$, $P = .535$; Figures 1 and 2). There is no statistical difference among different subcategory for the OS ($\chi^2 = 2.801$, $P = .423$; Figure 3), while there is statistical significant difference for DFS ($\chi^2 = 8.588$, $P = .035$), and cCR is superior to PR ($\chi^2 = 4.064$, $P = .044$; Figure 4).

Discussion

As an important method for the treatment of late-stage breast cancer, neoadjuvant therapy provides patients the opportunity to undergo treatment and for operation, with increased OS and DFS for some of the patients with pCR or cCR.[6,7] However, some of the patients showed SD to the neoadjuvant chemotherapy treatment, or even PD. In this case, other treatment strategies instead of neoadjuvant chemotherapy might be better choice for these patients. Therefore, the improvement in clinical responses to the neoadjuvant chemotherapy, especially the

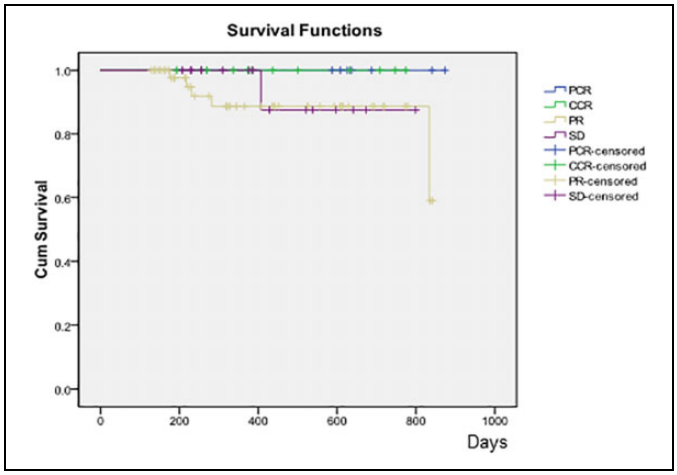

Figure 3. Overall survival (OS) of subcategory.

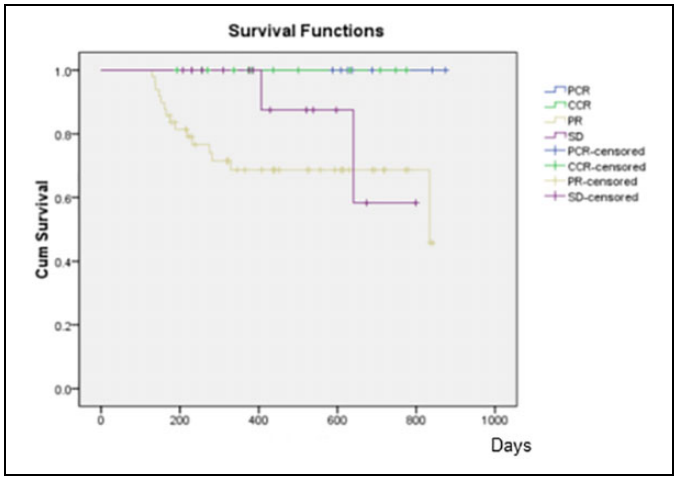

Figure 4. Disease-free survival (DFS) of subcategory.

identification of prediction markers that are critical for the RR to the treatment, is critical to guide the treatment of patients with late-stage breast cancer.

Currently, there are 4 different subtypes of breast cancers according to the expression levels of ER, PR, ERBB2, and KI-67, including luminal A, luminal B, ERBB2$^+$ and basal-like subtypes. It is recommended that different patients with breast cancer should be treated differently according to these subtypes.[8] While it is well established for the molecular signature and subtypes for breast cancer, new molecular portraits and types are emerging for both primary and metastatic breast cancer.[4,9]

It is reported previously that the basal-like subtype of breast cancer is correlated with the age of the patient, pathological grade, and menopausal status, while other groups showed the opposite result.[2,10] There are several different studies that focus on the ER, PR, HER2, and KI-67 level changes after the neoadjuvant chemotherapy treatment, while the conclusion remains elusive.[11,12] For example, it is reported previously that the levels of ER/PR and Ki-67 are significantly changed after chemotherapy, and the level changes correlate with RR to neoadjuvant chemotherapy. Berry *et al* found that in 6644 patients with breast cancer with lymph node metastasis, there was a significant difference between ER-positive and ER-negative patients after adjuvant chemotherapy, and it was confirmed that ER-negative patients had better prognosis than ER-positive patients after chemotherapy.[13] While other studies with similar design did not show significant changes in ER/PR level after neoadjuvant chemotherapy, Lee *et al*[14] pointed out that there was no correlation between the change of ER and PR molecular marker expression level before and after neoadjuvant chemotherapy and whether they experienced chemotherapy. Piper *et al*[15] found that there was no significant change in the expression of ER and PR in patients with breast cancer before and after neoadjuvant chemotherapy. The experiment of Adams *et al*[16] and Arens *et al*[17] also supports this result. Whether N-acetylcysteine (NAC) affects the expression of HER-2 has not been put forward by the authoritative and generally accepted theory, and there are different opinions in academic circles. In a study of 118 patients with breast cancer by Burcombe *et al*, 7.6% of the patients changed HER-2 expression after NAC, of which 4.2% changed from HER-2 positive to negative, while 3.4% changed from HER-2 negative to positive.[18] Shet *et al* reported that the expression of HER-2 in patients with breast cancer before and after NAC did not change significantly.[19] Similarly, our study didn't show statistical differences in clinical pathological features among different subtypes of breast cancer. Furthermore, we didn't observe differences in the neoadjuvant chemotherapy treatment for the complete RR, including either pCR or cCR, when comparing luminal A with luminal B subtypes, or comparing ERBB2$^+$ with basal-like subtype. None of the subtypes showed PD after the treatment. Comparing with luminal A and luminal B subtypes, the ERBB2$^+$ and basal-like subtypes showed better CR and RR rates.

It has been shown previously that KI-67 can be used to predict RR to neoadjuvant chemotherapy. According to the results of follow-up study of patients with breast cancer by Aas *et al*, the survival rate of patients with low Ki-67 expression was higher.[20] However, Jones *et al* showed no value in predicting the efficacy of neoadjuvant chemotherapy and pCR.[21] As a proliferation marker, those cancer cells that have higher KI-67 proliferate better and are more sensitive to chemotherapy drugs. This is not only true for operable breast cancer but also triple-negative breast cancer.[22,23] Although patients with higher KI-67 levels before the treatment generally have better RR to the treatment, it is also worth noting that some of the patients will change from KI-67 positive to negative and resistant to the treatment, thus evade or even metastasis to distal site, while will lead to worse outcome accompanied with reduced KI-67 levels.[24]

It is reported previously that luminal A and luminal B subtypes have better 5-year survival rate than ERBB2$^+$ and Basal-like subtypes, including DFS and OS.[3,11] Jones *et al* believed that Ki-67 had no significant predictive value for the efficacy of NAC and the evaluation of pCR.[21] Our study of 3-year survival

did not show statistical significant differences among all these 4 subtypes. Larger data set and more patients are needed to confirm our findings and for the further evaluation whether this is due to the better treatment strategies and patient care. It has been reported that neoadjuvant chemotherapy could extend OS and DFS for those patients with pCR, and patients with triple-negative breast cancer (TNBC) will have comparable survival rate as none of the patients with TNBC.[7] Our study showed similar result that DFS rate has statistical significant difference for cCR in comparison with PR. However, the number of pCR patients is relative low, and the follow-up time is 3 years instead of 5 years, which increases variables. However, according to the increased levels of cCR, this will lead to DFS.

Our study showed that breast cancer as a highly heterogeneous cancer with different molecular portrait and clinical pathological features has different responses to the treatment and survival rate. Among them, the molecular features can be used to predict response to the neoadjuvant chemotherapy treatment of doxorubicin with docetaxel combinational treatment. Our study showed that ERBB2^{+} and basal-like subtypes are more responsive to the neoadjuvant chemotherapy, and ER and KI-67 levels before the treatment correlate with the RR to the treatment. Furthermore, KI-67 can be used to predict the sensitivity to the treatment. There is no difference in OS and DFS among different subtypes of the diseases. How to further improve the pCR for late-stage luminal subtypes breast cancer and how to choose treatment strategy for those ER-negative patients with breast cancer after neoadjuvant therapy remain to be explored.

Authors' Note

Ling-Cheng Wang and Ling-Sheng Wang are co-first authors.

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Ling-Cheng Wang collected and wrote the data. Ling-Sheng Wang, Ai-Xia Li, and Shi-Man Chen analyzed and interpreted the data. Zhen-Zong Shi, Ya-Qiong Li, and Fei Han concepted and checked the data. Wei Huang and De-Qiang Zhu checked and revised the data. Ling-Cheng Wang is the guarantor.

Declaration of Conflicting Interests

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