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ORIGINAL RESEARCH

Non-Luminal Disease Score for Everolimus in Patients with Hormone Receptor-positive and Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: A Multicenter and Retrospective Study

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Purpose: This study aims to explore the role of the non-luminal disease score (NOLUS) for everolimus in patients with hormone receptor-positive and human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced breast cancer (ABC).

Methods: NOLUS has previously been established as an algorithm: NOLUS (0–100) = − 0.45 × ER(%) − 0.28 × PR(%) + 0.27 × Ki67(%) + 73. Information of cancer patients was retrospectively collected from three cancer centers in China.

Results: Totally, 198 HR+/HER2- ABC patients with complete records in expression rates (%) of ER, PR and Ki67 were enrolled in the study. The expression rates (%) of ER, PR, and Ki67 were 38.8 ± 27.9 *versus* 80.9 ± 14.2 ($p < 0.001$), 13.9 ± 14.3 *versus* 50.2 ± 30.4 ($p < 0.001$), and 37.8 ± 23.6 *versus* 28.7 ± 19.9 ($p = 0.04$), respectively, for NOLUS-positive patients and NOLUS-negative patients. For the overall population, the median PFS was 5.8 months *versus* 5.1 months in NOLUS-positive and NOLUS-negative patients ($p = 0.16$, HR = 0.75, 95% CI = 0.50, 1.12). The median 1L-, 2L, and 3L-PFS was 13.9 months *versus* 11.8 months ($p = 0.22$, HR = 1.63, 95% CI = 0.74, 3.62), 6.7 months *versus* 3.6 months ($p = 0.08$, HR = 0.34, 95% CI = 0.10, 1.18), and 4.6 months *versus* 4.0 months ($p = 0.81$, HR = 1.07, 95% CI = 0.63, 1.79) respectively, for NOLUS-positive patients and NOLUS-negative patients.

Conclusion: NOLUS-positive patients have a lower percentage of ER and PR, but a higher percentage of Ki67 index. The correlation between the benefits of everolimus and NOLUS failed to develop significance, suggesting that NOLUS may not be applicable in predicting everolimus efficacy in patients with HR+/HER2- ABC. Further research is expected.

Keywords: NOLUS, advanced breast cancer, everolimus, HR+/HER2- breast cancer, efficacy

Introduction

Hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2-) (HR+/HER2-) breast cancer represents the most common type of breast cancer, accounting for approximately 60% of all cases.[1,2] Owing to the positive expression of estrogen receptor (ER) and progesterone receptor (PR), HR+/HER2- breast tumor cells are sensitive to endocrine therapy (ET).[3] Patients with HR+/HER2- breast cancer exhibit superior survival outcomes in comparison to other subtypes of breast cancer.[2] A challenge that arises in clinical practice is that such a population of patients inevitably develops endocrine resistance to ET.[4] The combination of ET and targeted therapy has emerged as a promising option for patients with HR+/HER2- breast cancer, particularly those who have developed metastases.

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Currently, the most favored therapeutic approach of first-line (1L) treatment for patients with HR+/HER2- advanced breast cancer (ABC) is ET in conjunction with cyclin-dependent kinase 4 (CDK4) and 6 (CDK4/6) inhibitors.[5–8] Following progressive disease to CDK4/6 inhibitors, patients may opt for other CDK4/6 inhibitors, targeted drugs, chemotherapy, or other treatment modalities.[9,10]

The mammalian target of rapamycin (mTOR) exists as two distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTORC2.[11] Everolimus, a classical type of mTORC1 inhibitor, is proven to effectively overcome endocrine resistance and serve as an alternative for patients with HR+/HER2- ABC post 1L-CDK4/6 inhibitor therapy.[9–11] The addition of everolimus to ET has been demonstrated to enhance drug responses and clinical outcomes of patients with HR+/HER2- ABC, while maintaining a tolerable toxicity profile.[12–14] Nevertheless, a subset of patients was not responsive to everolimus therapy, underscoring the necessity of developing predictive markers to assess individual sensitivity prior to initiating everolimus treatment.

Gene expression profiling, such as the 50-gene classifier (PAM50), has the potential to distinguish gene expression-based intrinsic subtypes and predict drug efficacy.[15–17] Previously, a pathology-based predictive model was constructed to predict PAM50 non-luminal disease within HR+/HER2- breast cancer, namely the non-luminal disease score (NOLUS).[18] The model incorporates three commonly used pathological factors, involving the percentage of estrogen receptor (ER), progesterone receptor (PR), and Ki67 index in immunohistochemistry (IHC). It has been shown to inform clinical medication in patients with HR+/HER2- ABC.[19] However, the association between NOLUS and everolimus efficacy remained unclear. We previously reported that there is an association between the expression of Ki67 and the regulatory associated protein of mTORC1 (RPTOR), and that the expression levels of the Ki67 index impact the clinical efficacy of everolimus in patients with HR+/HER2- ABC in a real-world setting. These findings indicated that NOLUS, composed of the percentage of ER, PR, and Ki67 index, may possess an impression on everolimus efficacy and be used to predict survival benefits from everolimus. Consequently, we aim to investigate whether NOLUS could be employed for the management of everolimus in a substantial proportion of patients with HR+/HER2- ABC, based on multicenter and real-world data.

Methods and Patients

Patients and Data Collection

Patient information was collected from three cancer hospitals in China, including National Cancer Center (NCC), Chinese PLA General Hospital, and Peking University Cancer Hospital and Institute. The inclusion criteria for eligible patients were as listed. (1) Female and breast cancer samples. (2) Patients received everolimus as salvage therapy, not as neoadjuvant therapy or adjuvant therapy. (3) Patients exhibited a positive status of HR (ER or PR) and a negative status of HER2. The status of ER, PR, and HER2 was determined by IHC staining according to the guidelines established by the American Society of Clinical Oncology.[20] The ER- or PR-positive status is defined as tumors with an IHC score of greater than 1% for ER or PR. Tumors with IHC scores of 0, 1+, and 2+ but negative fluorescence in situ hybridization (FISH) were regarded as HER2-negative tumors. (4) Complete medical records for everolimus treatment were required. (5) The expression rates (%) of ER, PR, and Ki67 index were available.

NOLUS Calculation and Clinical Outcomes

NOLUS was calculated using the following formula: NOLUS (0–100) = − 0.45 × ER(%) − 0.28 × PR(%) + 0.27 × Ki67 (%) + 73, which was derived from a published literature.[18] According to the literature, patients with NOLUS greater than or equal to 51.38 were classified as NOLUS-positive patients (non-luminal disease), while patients with NOLUS less than 51.38 were classified as NOLUS-negative patients (luminal disease).[18]

The efficacy of everolimus was evaluated based on three indicators, including progression-free survival (PFS), objective response rate (ORR), and clinical benefit rate (CBR). PFS was described as the interval between the initiation of everolimus treatment and the confirmation of disease progression or death. ORR was defined as the proportion of patients who achieved a complete response (CR) or partial response (PR). CBR measured the percentage of patients who attained a CR, PR, or stable disease (SD) for a minimum of six months.

Statistical Analyses

A chi-squared test was employed to assess the comparability of patient characteristics between the NOLUS-positive and NOLUS-negative groups. The Pearson correlative method was used to analyze correlations of different variables. The variables were subjected to a rank-sum test for comparison. Genomic analyses were based on data from the Cancer Genome Atlas (TCGA) platform. The Log rank test was utilized to evaluate prognostic differences between distinct groups via the survfit function of the Survival package from the R software. To vividly show survival outcomes between NOLUS-positive and NOLUS-negative groups, the Kaplan–Meier survival curve was drawn. A P value of less than 0.05 was regarded as statistically significant, with the false discovery rate (FDR) considered in appropriate contexts. All statistical analyses were conducted using SPSS (version 29.0) and R software (version 4.3.2).

Results

Identification of Eligible Patients

From January 2014 to November 2022, 2518 patients with malignant tumors who were treated with everolimus in three cancer hospitals in China were included (NCC, 2281 cancer patients; Chinese PLA General Hospital, 128 breast cancer patients; Peking University Cancer Hospital & Institute, 109 breast cancer patients). Among them, 652 female patients with ABC were selected. One hundred and ninety-seven patients were excluded due to the unavailability of Ki67 data in their electronic medical records. Furthermore, 21 patients with a negative status of HR and 38 patients with a positive status of HER2 were excluded, resulting in 396 patients with HR+/HER2- ABC. Particularly, a patient who had a HER2 IHC score of 3+ but negative FISH was enrolled in the study. The patient obtained poor drug responses to anti-HER2 therapy but achieved favorable drug responses to ET. Considering the clinicopathological features and drug responses to different therapeutical strategies, the patient was defined as HR+/HER2- and included in the subsequent analysis of this study.

To analyze disease-free survival (DFS), 39 patients were ruled out due to no radical surgery or an unknown time for surgery. Fifty-two patients with irregular medication on everolimus were precluded because it might influence the analytic results on the clinical outcomes for everolimus. Subsequently, the expression rates (%) of PR and ER for NOLUS calculation were used as inclusion criteria. Of these patients, 90 patients lacked the expression rates of ER and 103 patients lacked the expression rates of PR. A total of 198 patients with adequate information on the expression rates of both PR and ER were qualified for ensuing analyses. Figure S1 provides an overview of patient selection in the study.

Baseline Features of Eligible Patients

Among eligible patients, the median age was 52 years, with 88.9% of patients being younger than 65 years of age. The majority of patients (95.0%) had a positive status of both ER and PR. 73.7% of patients had a pathological type of invasive ductal carcinoma. Over 90% of patients received ET in adjuvant setting following surgery. With regard to everolimus therapy, 31.8%, 13.1%, and 55.1% of patients received everolimus as 1L-, 2L-, and 3L-treatment, respectively. According to NOLUS scores, 167 patients (84.3%) were categorized as NOLUS-negative, and 31 patients (15.7%) were categorized as NOLUS-positive. The median age of NOLUS-negative patients was 51 years, while that of NOLUS-positive patients was 55 years. Table 1 shows the baseline characteristics of patients in the two subgroups. To see, the features between patients in the NOLUS-negative subgroup and the NOLUS-positive subgroup were generally similar.

Association Between NOLUS Score and mTOR Pathway

Based on data from TCGA database, we explored the correlation between the gene expression of estrogen receptor 1 (ESR1), which encodes ER, and the gene expression of RPTOR. The results from the GEPIA2 platform[21] showed a statistical, positive correlation between RPTOR expression and ESR1 expression ($p = 0$, $R = 0.33$) (Figure 1A). In addition, pathway analysis based on GSCA web server[21] revealed that RPTOR expression positively activated the ER pathway (FDR = 0.03) (Figure 1B and Table S1). These results suggest that the activity of the ER pathway may have several effects on the activity of the mTORC1 which serves as the drug target of everolimus.[11] Therefore, we further evaluate the association between NOLUS and everolimus efficacy, intending to identify the predictive merits of NOLUS for everolimus therapy based on real-world and multicenter data.

Table 1 Baseline Characteristics of NOLUS-Negative and NOLUS-Positive Patients

Characteristics	NOLUS-Negative (N=167)	NOLUS-Positive (N=31)	P Value
Age (years)			
Mean±SD	51.6±10.4	53.0±9.9	
Median[min-max]	51.0[30.0, 75.0]	55.0[32.0, 69.0]	
Age			1
< 65 years	148(88.6%)	28(90.3%)	
≥65 years	19(11.4%)	3(9.7%)	
Pathological type [a]			0.66
Invasive ductal carcinoma	122(73.1%)	24(87.1%)	
Mix	30(18.0%)	5(16.1%)	
Others	2(1.2%)	1(3.2%)	
Surgery [b]			0.53
Modified radical mastectomy	147(88.8%)	25(80.6%)	
Breast-conserving	17(10.2%)	5(16.1%)	
Adjuvant therapy			
Endocrine therapy	165(98.8%)	29(93.5%)	0.22
Radiotherapy	98(58.7%)	16(51.6%)	0.54
Chemotherapy	156(93.4%)	30(96.8%)	0.76
Bone metastasis			0.07
No bone metastasis	83(49.7%)	21(67.7%)	
Only bone metastasis	48(28.7%)	3(9.6%)	
Accompanied with other metastases	36(21.6%)	7(22.6%)	
Visceral metastasis			0.7
No visceral metastasis	86(51.5%)	18(58.1%)	
Only visceral metastasis	37(22.2%)	7(22.6%)	
Accompanied with other metastases	44(26.3%)	6(19.4%)	
Liver metastasis			0.98
No	122(73.1%)	22(71.0%)	
Yes	45(26.9%)	9(29.0%)	
Lung metastasis			0.29
No	122(73.1%)	26(83.9%)	
Yes	45(26.9%)	5(16.1%)	
Disease-free survival (months)			
Mean±SD	57.1±48.2	61.9±47.9	
Median[min-max]	45.6[1.0, 356.8]	46.7[0.8, 179.3]	
Disease-free survival			0.52
≥3 years	105(62.9%)	17(54.8%)	
< 3 years	62(37.1%)	14(45.2%)	
Disease-free survival			0.64
≥5 years	59(35.3%)	9(29.0%)	
< 5 years	108(64.7%)	22(71.0%)	
Treatment lines of everolimus therapy			0.69
First line	55(32.9%)	8(25.8%)	
Second line	21(12.6%)	5(16.1%)	
Third line	91(54.5%)	18(58.1%)	

Notes: a Information about histological types from 14 patients was missing. b Type of surgery from 4 patients at local hospitals were lost.

Abbreviation: NOLUS, non-luminal disease score; SD, standard deviation.

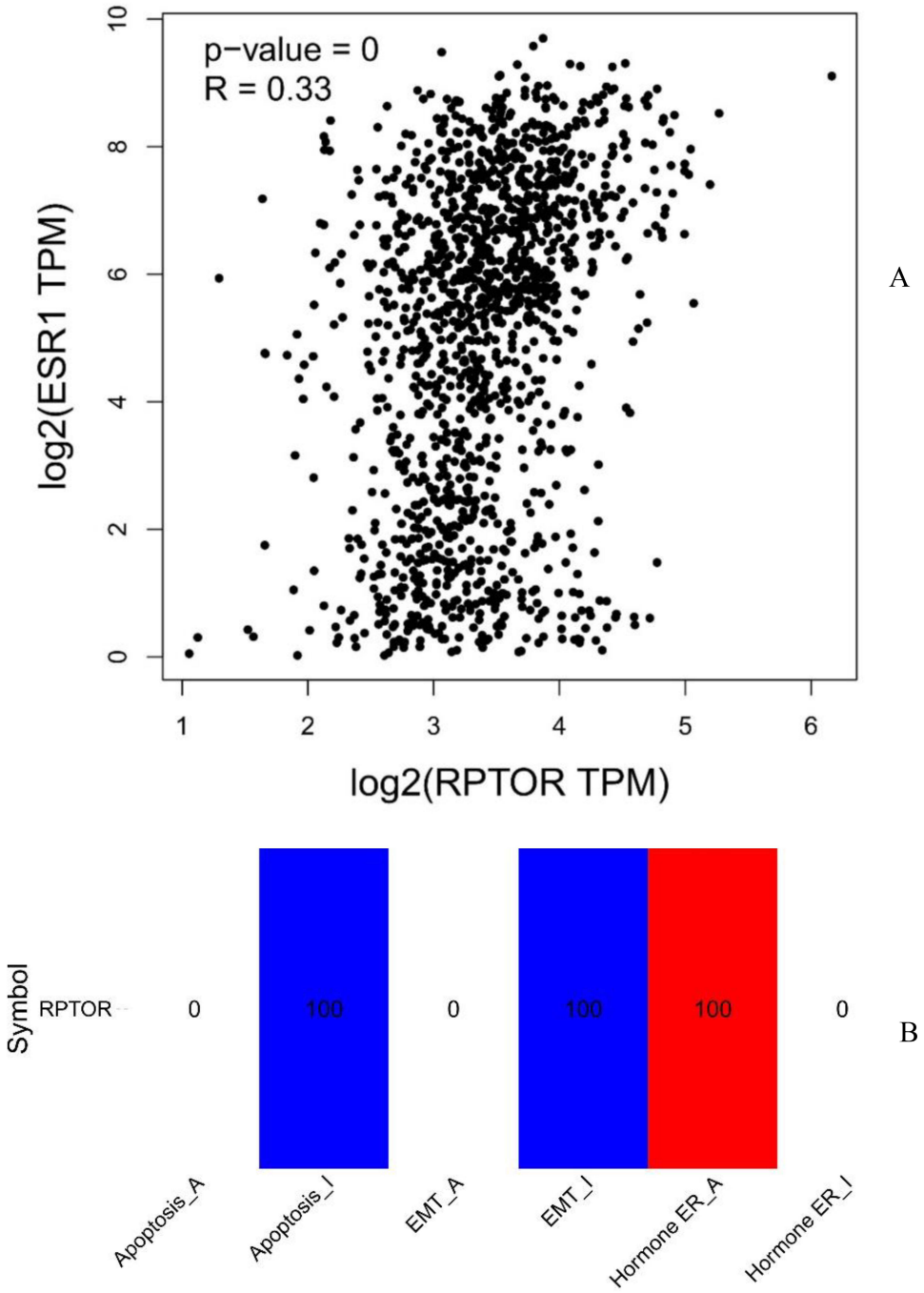

Figure 1 Online analyses for estrogen receptor (ER) and RPTOR. (**A**) The scatter diagram showing the correlation between ESR1 and RPTOR expression based on the TCGA database. P value and the correlation index (R value) are displayed. (**B**) The bar chart showing the role of RPTOR expression in the activity of associated pathways. Blue bar represents inhibition, red bar represents activation. The deeper color represents greater effects for inhibition or activation. The effect of inhibition or activation is annotated in the middle of each bar.

Abbreviations: (EMT), epithelial-mesenchymal transition; (mTOR), mammalian target of rapamycin; (RPTOR), regulatory associated protein of mTOR complex 1; (A), activate; (I), inhibit.

Expression Rates of Markers in NOLUS-Positive/Negative Patients

The expression rates (%) for all patients (N = 198) were 74.3 ± 22.9 for ER, 44.6 ± 31.4 for PR, and 30.1 ± 20.8 for Ki67. A significant difference was observed in the expression status of ER, PR, and Ki67 between NOLUS-positive

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patients (N = 31) and NOLUS-negative patients (N = 167). In particular, the expression rates for ER in NOLUS-positive patients were 38.8 ± 27.9, which was significantly lower than 80.9 ± 14.2 observed in NOLUS-negative patients ($p < 0.001$). Regarding the percentage of PR expression, NOLUS-positive patients had a decreased rate of 13.9 ± 14.3 *versus* 50.2 ± 30.4 for NOLUS-negative patients ($p < 0.001$). The expression rates (%) in NOLUS-positive patients and NOLUS-negative patients were respectively 37.8 ± 23.6 and 28.7 ± 19.9 for Ki67 ($p = 0.04$). The expression of HER2 was found to be largely comparable between NOLUS-positive patients and NOLUS-negative patients. Figure 2 or Table S2 shows details of expression rates of ER, PR, Ki67, and HER2 in NOLUS-positive and NOLUS-negative patients.

Survival Analyses

Table 2 presents the survival outcomes of NOLUS-negative and NOLUS-positive patients. The median PFS was 5.8 months in NOLUS-positive patients, which was similar to 5.1 months in NOLUS-negative patients ($p = 0.16$, HR = 0.75, 95% CI = 0.50, 1.12) (Figure 3a). The clinical benefits, including ORR and CBR, manifested comparable trends between the two subgroups. The ORR for NOLUS-positive patients was calculated to be 19.4%, slightly lower than 28.4% in NOLUS-negative patients ($p = 0.43$). The CBR for NOLUS-positive patients was 64.5%, which was higher than 52.1% observed in NOLUS-negative patients ($p = 0.28$). Stratified by the treatment lines of everolimus, a further analysis of the differences in PFS of NOLUS-positive and NOLUS-negative patients was performed.

A total of 63 patients received everolimus as 1L treatment, including 8 NOLUS-positive patients and 55 NOLUS-negative patients. The median PFS for NOLUS-positive patients was 13.9 months, which was numerically longer than 11.8 months for NOLUS-negative patients. Nevertheless, the discrepancy between the two groups showed no statistical significance ($p = 0.22$, HR = 1.63, 95% CI = 0.74, 3.62) (Figure 3B).

5 NOLUS-positive patients and 21 NOLUS-negative patients were treated with everolimus as 2L therapy. The median PFS for NOLUS-positive patients was 6.7 months, which tended to be longer than 3.6 months for NOLUS-negative patients ($p = 0.08$). However, no significant correlation was observed (HR = 0.34, 95% CI = 0.10, 1.18) (Figure 3C).

The remaining 109 patients received everolimus as 3L or > 3L therapy (18 NOLUS-positive patients and 91 NOLUS-negative patients). The median PFS for NOLUS-positive patients was 4.6 months *versus* 4.0 months for NOLUS-negative patients ($p = 0.81$, HR = 1.07, 95% CI = 0.63, 1.79) (Figure 3D).

Discussion

In the context of HR+/HER2- metastatic breast tumors, non-luminal diseases evolve an intrinsic molecular characterization that aligns with the profiles of HER2-enriched and basal-like breast tumors. This phenomenon is not uncommon, as evidenced by the prevalence of these profiles in the literature.[22–24] For example, a study analyzing over a hundred paired samples of primary and metastatic breast tumors showed that the incidence of the HER2-enriched phenotype within HR+/HER2- ABC was as high as 22%.[22] For HR+/HER2- patients who are resistant to ET, the percentage of non-luminal diseases within HR+/HER2- breast tumors is observed to increase. A retrospective analysis of tumor samples from patients with HR+/HER2- ABC who developed resistance to aromatase inhibitors in the BOLERO-2 study found that, a high proportion of 32% of HR+/HER2- tumors were identified as HER2-enriched breast tumors.[23] The intrinsic pathological phenotypes exert a pivotal influence on the biological behavior and drug responses of breast tumor cells. From a clinical perspective, these intrinsic phenotypes of non-luminal tumors within HR+/HER2- breast tumors are characterized by the following clinical features, including lower expression rates of ER, higher sensitivity to chemotherapy, and unfavorable survival outcomes for breast cancer at both early and metastatic settings.[15,24–26] Consistent with previous studies, we found that NOLUS-positive patients with non-luminal breast tumors have lower expression rates of ER and PR, and higher levels of Ki67 index in IHC. A high Ki67 expression rate represents a greater proportion of tumor cells in the proliferative phase, which is typically associated with enhanced sensitivity to chemotherapy and poor clinical outcomes.[27–29]

Based on online analyses, we found that the expression of RPTOR was significantly, and positively correlated with the expression of ESR1, and activated the ER pathway. This can be explained by the findings from previous studies. RPTOR is a principal gene for encoding mTORC1, whose upregulation enhances the activity of the ER pathway via

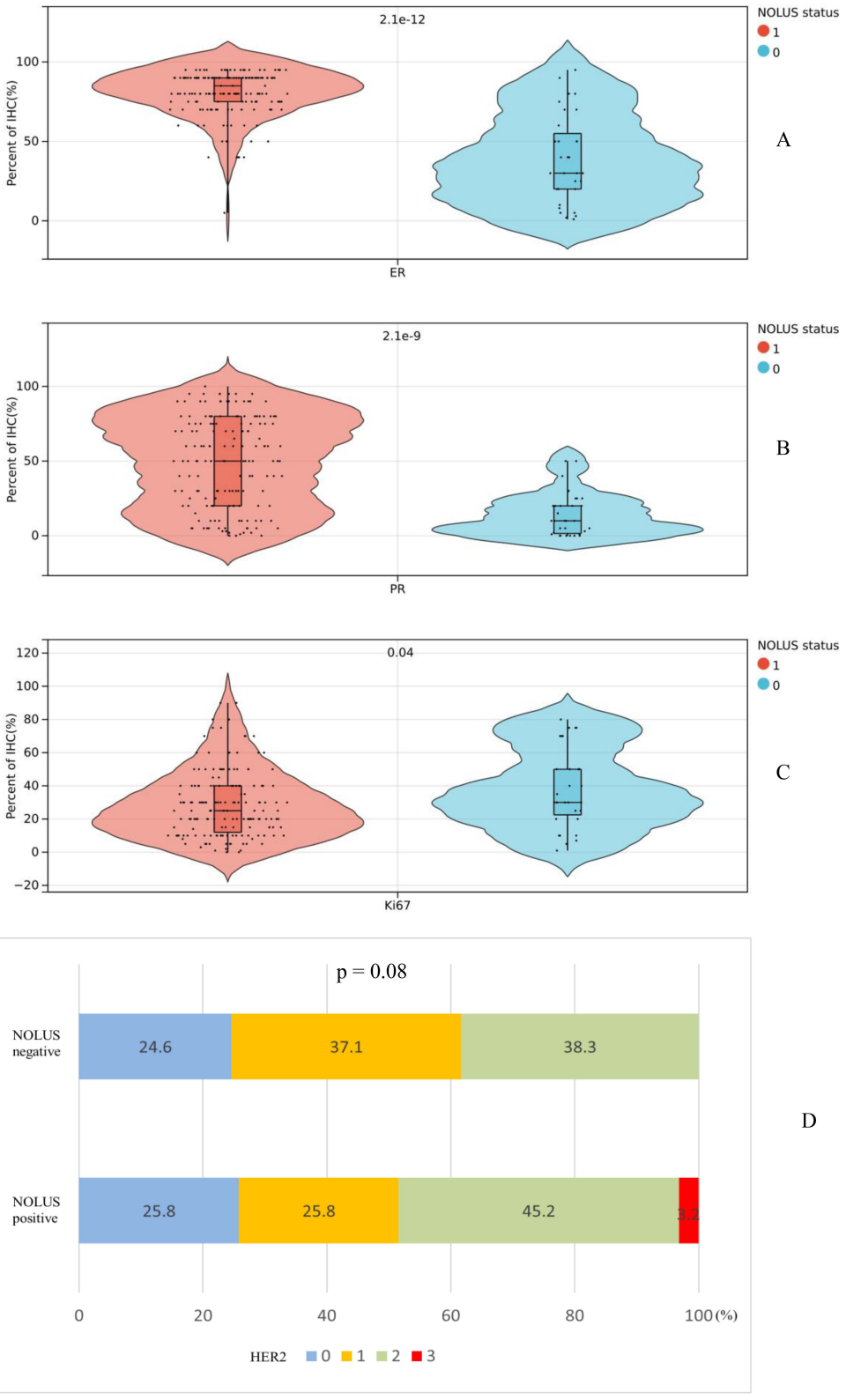

Figure 2 The expression rates (%) of IHC in NOLUS-positive and NOLUS-negative patients. The number of zero represents patients with NOLUS-positive status, and the number of one represents patients with NOLUS-negative status. (**A**) Estrogen receptor (ER), (**B**) progesterone receptor (PR), (**C**) Ki67, and (**D**) human epidermal growth factor (HER2). One NOLUS-positive patient was HER2 3+ in IHC, but without amplification in fluorescence in situ hybridization.

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Table 2 Clinical Outcomes of NOLUS-Negative and NOLUS-Positive Patients

Outcomes	NOLUS-Negative (N=167)	NOLUS-Positive (N=31)	P Value
Objective response rate	47(28.1%)	6(19.4%)	0.43
Clinical benefit rate	87(52.1%)	20(64.5%)	0.28
Progression-free survival (PFS)			
Median (95% CI, months)	5.4 (4.0, 6.9)	6.0 (4.6,7.4)	0.16
1L-PFS	11.8 (6.7, 16.8)	13.9 (2.7, 25.1)	0.22
2L-PFS	3.6 (1.4, 5.8)	6.7 (3.5, 10.0)	0.08
3L-PFS	4.0 (3.5, 4.5)	4.6 (2.1, 7.1)	0.81

Abbreviations: NOLUS, non-luminal disease score; PFS, progression-free survival; 1L, first line, 2L, second line; 3L, third line.

activating mTORC1 expression, and consequently promotes drug resistance of breast tumors to ET.[11,30] It contributes to one of the important mechanisms of endocrine resistance. Drugs blocking mTORC1, like everolimus, have been demonstrated to exert anti-cancer effects and to maintain sensitivity to endocrine drugs in breast tumor cells.[31] Integrating our previous findings of correlation with the expression of Ki67 and RPTOR, we reasonably postulate that NOLUS may serve as a potential predictor of the clinical benefits of everolimus in patients with HR+/HER2- ABC. Therefore, we evaluated the association of NOLUS and everolimus efficacy in a large population of patients with HR +/HER2- ABC in a real-world and multicenter setting to determine the predictive value of NOLUS for everolimus therapy.

NOLUS has been developed as a model for distinguishing intrinsic phenotypes within HR+/HER2- breast tumors.[18] Based on the pathological scores of ER, PR, and Ki67 in IHC, NOLUS can easily and roughly identify a patient as NOLUS-positive or NOLUS-negative patient,[18] which is capable of assessing the risk of resistance to CDK4/6 inhibitors in patients with HR+/HER2- ABC.[19] Unfortunately, we did not obtain positive results for NOLUS in clinical outcomes of everolimus therapy in terms of ORR, CBR, and PFS. Reasons for this phenomenon are analyzed as follows.

First and foremost, the mTOR inhibitor has a simultaneous and suppressive function on both the HER2 and ER pathways.[31] The anti-tumor role of everolimus in HR+/HER2- breast tumors has been extensively investigated and substantiated. One of the most prominent studies for everolimus in HR+/HER2- breast tumors is the BOLERO-2 study.[12,32] It showed that everolimus plus exemestane therapy significantly improved drug responses and survival outcomes of patients with HR+/HER2- ABC. Concurrently, the drug efficacy of everolimus in HER2-enriched breast tumors was displayed in the BOLERO-1 study, which showed that adding everolimus to paclitaxel and trastuzumab therapy extended PFS of patients with HR-/HER2+ ABC.[33] As previously reported, treatment regimens including everolimus yielded profound and comparable outcomes in disease control for patients with HR +/HER2- ABC, especially for those receiving everolimus as 1L- or 2L-therapy.[34] From this perspective, it may explain the negative results of this study. This indicates that everolimus is efficacious irrespective of the intrinsic phenotype within HR+/HER2- breast tumors in metastatic setting, whether they are HR+/HER2- or HER2-enriched tumors. Conceivably, NOLUS is not able to investigate the drug efficacy of everolimus in patients with luminal diseases or non-luminal diseases within HR+/HER2- breast cancer. However, the role of everolimus in HER2-enriched tumors is not certain. In the BOLERO-3 study, the addition of everolimus failed to result in clinical benefits for patients with HER2-enriched ABC that were resistant to trastuzumab.[35] More efforts are warranted.

Second, the data pertaining to the expression rates of ER, PR, and Ki67 were procured from multiple cancer centers. Although the criteria for determining ER and PR positivity have been established, the interpretation of their expression rates (expressed as a percentage) is subjective and dependent on the pathologist's assessment of the slides. Interpretations of the expression rates of ER, PR, and Ki67 in the same tumor specimen may vary between pathologists

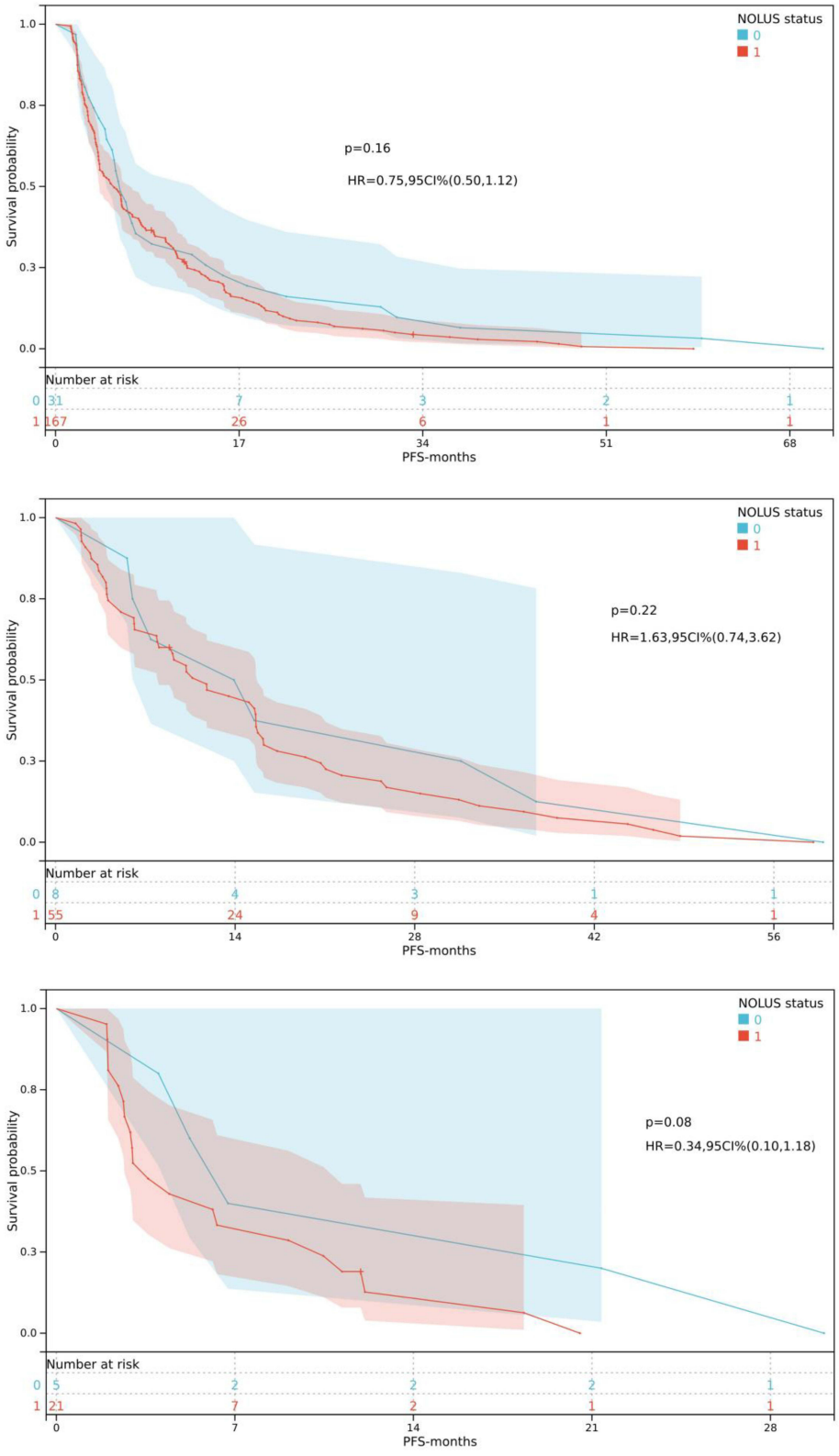

Figure 3 Continued.

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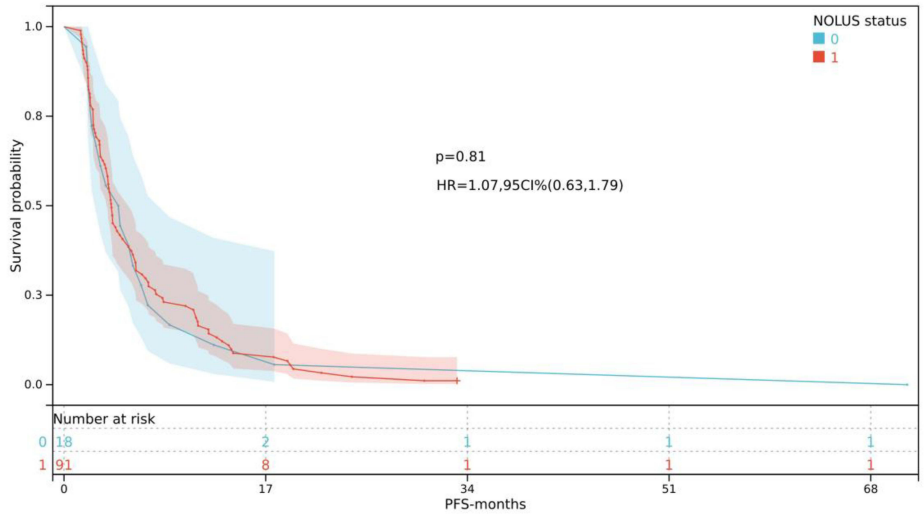

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Figure 3 Kaplan–Meier curves of progression-free survival (PFS) for patients with HR+/HER2- ABC stratified by treatment lines of everolimus. The green curve or the number of zero represents NOLUS-positive patients, and the red curve or the number of one represents NOLUS-negative patients. PFS for (**A**) overall population, (**B**) patients receiving everolimus as first-line therapy, (**C**) patients receiving everolimus as second-line therapy, and (**D**) patients receiving everolimus as third-line or more than third-line therapy are displayed, respectively. Median survival, risk tables, p value, HR and 95% CI are shown.

from different institutions or even within the same institution. This may burden the results as NOLUS calculation was entirely dependent on the percentage of ER, PR, and Ki67 in IHC. Other factors, including the retrospective nature of the study, and limited samples in each treatment line of everolimus, may also have an impact on the results of the study.

Conclusion

Tumor tissue from NOLUS-positive and NOLUS-negative patients exhibits notable discrepancies in ER, PR, and Ki67 levels as determined by IHC. NOLUS-positive patients have a lower percentage of ER/PR, and a higher expression of Ki67. NOLUS is not significantly correlated with survival outcomes of everolimus in patients with HR+/HER2- ABC, indicating that NOLUS may not be suitable for predicting the efficacy of everolimus in this patient population. Further studies are anticipated to elucidate the relationship between NOLUS and everolimus efficacy.

Data Sharing Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Ethical Statement

The study was conducted according to institutional guidelines of the Ethics Committee of Cancer Institute and Hospital, Chinese Academy of Medical Science (approval number 12-123/657). The informed consent was waived due to its retrospective and non-interventional nature.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst*. 2014;106(5). doi:10.1093/jnci/dju055.
2. Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast*. 2015;24:S26–35.
3. Waks AG, Winer EP. Breast cancer treatment: a review. *JAMA*. 2019;321(3):288–300. doi:10.1001/jama.2018.19323
4. Schettini F, Buono G, Cardalesi C, Desideri I, De Placido S, Del Mastro L. Hormone receptor/human epidermal growth factor receptor 2-positive breast cancer: where we are now and where we are going. *Cancer Treat Rev*. 2016;46:20–26. doi:10.1016/j.ctrv.2016.03.012
5. Hortobagyi GN, Stemmer SM, Burris HA, et al. Overall survival (OS) results from the Phase III MONALEESA-2 (ML-2) trial of postmenopausal patients (pts) with hormone receptor positive/human epidermal growth factor receptor 2 negative (HR+/HER2L) advanced breast cancer (ABC) treated with endocrine therapy (ET) ± ribociclib (RIB). *Ann Oncol*. 2021; 32:S1290–S1291.
6. Goetz MP, Toi M, Huober J, et al. MONARCH 3: interim overall survival (OS) results of abemaciclib plus a nonsteroidal aromatase inhibitor (NSAI) in patients (pts) with HR+, HER2-advanced breast cancer (ABC). *Ann Oncol*. 2022;33(7):S1384–S1384. doi:10.1016/j.annonc.2022.08.009
7. Xu B, Zhang Q, Zhang P, et al. Dalpiciclib or placebo plus fulvestrant in hormone receptor-positive and HER2-negative advanced breast cancer: a randomized, Phase 3 trial. *Nat Med*. 2021;27(11):1904–1909. doi:10.1038/s41591-021-01562-9
8. Gradishar WJ, Moran MS, Abraham J, et al. NCCN guidelines(R) insights: breast cancer, version 4.2023. *J Natl Compr Canc Netw*. 2023;21 (6):594–608. doi:10.6004/jnccn.2023.0031
9. Morrison L, Loibl S, Turner NC. The CDK4/6 inhibitor revolution - a game-changing era for breast cancer treatment. *Nat Rev Clin Oncol*. 2024;21 (2):89–105. doi:10.1038/s41571-023-00840-4
10. Ashai N, Swain SM. Post-CDK 4/6 inhibitor therapy: current agents and novel targets. *Cancers*. 2023;15(6):1855. doi:10.3390/cancers15061855
11. Miricescu D, Totan A, Stanescu S II, Badoiu SC, Stefani C, Greabu M. PI3K/AKT/mTOR signaling pathway in breast cancer: from molecular landscape to clinical aspects. *Int J mol Sci*. 2020;22(1):173. doi:10.3390/ijms22010173
12. Yardley DA, Noguchi S, Pritchard KI, et al. Everolimus plus exemestane in postmenopausal patients with HR(+) breast cancer: BOLERO-2 final progression-free survival analysis. *Adv Ther*. 2013;30(10):870–884. doi:10.1007/s12325-013-0060-1
13. Im YH, Karabulut B, Lee KS, et al. Safety and efficacy of everolimus (EVE) plus exemestane (EXE) in postmenopausal women with locally advanced or metastatic breast cancer: final results from EVEREXES. *Breast Cancer Res Treat*. 2021;188(1):77–89. doi:10.1007/s10549-021-06173-z
14. Tesch H, Stoetzer O, Decker T, et al. Efficacy and safety of everolimus plus exemestane in postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative locally advanced or metastatic breast cancer: results of the single-arm, phase IIIB 4EVER trial. *Int, J, Cancer*. 2019;144(4):877–885. doi:10.1002/ijc.31738
15. Lopez-Tarruella S, DEl Monte-Millan M, Roche-Molina M, et al. Correlation between breast cancer subtypes determined by immunohistochemistry and n-COUNTER PAM50 assay: a real-world study. *Breast Cancer Res Treat*. 2024;203(1):163–172. doi:10.1007/s10549-023-07094-9
16. Ming W, Li F, Zhu Y, et al. Predicting hormone receptors and PAM50 subtypes of breast cancer from multi-scale lesion images of DCE-MRI with transfer learning technique. *Comput Biol Med*. 2022;150:106147. doi:10.1016/j.compbiomed.2022.106147
17. Canino F, Piacentini F, Omarini C, et al. Role of intrinsic subtype analysis with pam50 in hormone receptors positive HER2 negative metastatic breast cancer: a systematic review. *Int J mol Sci*. 2022;23(13):7079. doi:10.3390/ijms23137079
18. Pascual T, Martin M, Fernández-Martínez A, et al. A pathology-based combined model to identify PAM50 non-luminal intrinsic disease in hormone receptor-positive HER2-negative breast cancer. *Front Oncol*. 2019;9. doi:10.3389/fonc.2019.00303
19. Futamura M, Nakayama T, Yoshinami T, et al. Detection of high-risk patients resistant to CDK4/6 inhibitors with hormone receptor-positive HER2-negative advanced and metastatic breast cancer in Japan (KBCSG-TR-1316). *Breast Cancer*. 2023;30(6):943–951. doi:10.1007/s12282-023-01485-y
20. Wolff AC, Somerfield MR, Dowsett M, et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO-college of American pathologists guideline update. *J Clin Oncol*. 2023;41(22):3867–3872. doi:10.1200/JCO.22.02864
21. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res*. 2019;47(W1):W556–W560. doi:10.1093/nar/gkz430
22. Cejalvo JM, Martinez de Duenas E, Galvan P, et al. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. *Cancer Res*. 2017;77(9):2213–2221. doi:10.1158/0008-5472.CAN-16-2717
23. Prat A, Brase JC, Cheng Y, et al. Everolimus plus exemestane for hormone receptor-positive advanced breast cancer: a PAM50 intrinsic subtype analysis of BOLERO-2. *Oncologist*. 2019;24(7):893–900. doi:10.1634/theoncologist.2018-0407
24. Cejalvo JM, Pascual T, Fernandez-Martinez A, et al. Clinical implications of the non-luminal intrinsic subtypes in hormone receptor-positive breast cancer. *Cancer Treat Rev*. 2018;67:63–70. doi:10.1016/j.ctrv.2018.04.015
25. Prat A, Cheang MC, Galvan P, et al. Prognostic value of intrinsic subtypes in hormone receptor-positive metastatic breast cancer treated with letrozole with or without lapatinib. *JAMA Oncol*. 2016;2(10):1287–1294. doi:10.1001/jamaoncol.2016.0922
26. Fujii T, Kogawa T, Dong W, et al. Revisiting the definition of estrogen receptor positivity in HER2-negative primary breast cancer. *Ann Oncol*. 2017;28(10):2420–2428. doi:10.1093/annonc/mdx397
27. Hu X, Chen W, Li F, et al. Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. *Front Oncol*. 2023;13:1053125. doi:10.3389/fonc.2023.1053125
28. Brown J, Scardo S, Method M, et al. A real-world retrospective study of the use of Ki-67 testing and treatment patterns in patients with HR+, HER2- early breast cancer in the United States. *BMC Cancer*. 2022;22(1):502. doi:10.1186/s12885-022-09557-6
29. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst*. 2009;101(10):736–750. doi:10.1093/jnci/djp082
30. Marques-Ramos A, Cervantes R. Expression of mTOR in normal and pathological conditions. *mol Cancer*. 2023;22(1). doi:10.1186/s12943-023-01820-z
31. Lee JJ, Loh K, Yap YS. PI3K/Akt/mTOR inhibitors in breast cancer. *Cancer Biol Med*. 2015;12(4):342–354. doi:10.7497/j.issn.2095-3941.2015.0089

32. Piccart M, Hortobagyi GN, Campone M, et al. Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: overall survival results from BOLERO-2dagger. *Ann Oncol*. 2014;25(12):2357–2362. doi:10.1093/annonc/mdu456
33. Toi M, Shao Z, Hurvitz S, et al. Efficacy and safety of everolimus in combination with trastuzumab and paclitaxel in Asian patients with HER2+ advanced breast cancer in BOLERO-1. *Breast Cancer Res*. 2017;19(1):47. doi:10.1186/s13058-017-0839-0
34. Tan Y, Jiang H, Ma F, et al. Efficacy of everolimus-based therapy in advanced triple-positive breast cancer: experience from three cancer centers in China. *Thorac Cancer*. 2023;14(34):3397–3405.
35. Andre F, O'Regan R, Ozguroglu M, et al. Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol*. 2014;15(6):580–591. doi:10.1016/S1470-2045(14)70138-X