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RESEARCH PAPER

Predictive and prognostic value of PDL1 protein expression in breast cancer patients in neoadjuvant setting

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ABSTRACT

Objective: Programmed death-ligand-1 (PDL1) is a molecule involved in immune evasion in various kinds of tumors. Here, we aim to determine whether the expression of PDL1 protein is related to the response of patients to neoadjuvant therapy and survival outcome.

Methods: Immunohistochemistry (IHC) was performed on paraffin-embedded tumor samples from core needle biopsy before neoadjuvant therapy (NAT). Univariate and multivariate logistic regression were used to analyze the associations between PDL1 protein expression and pathological complete response (pCR) outcome. Kaplan-Meier plot and log-rank test were used to compare disease-free survival (DFS) between groups. A cox proportional hazards model was used to calculate the adjusted hazard ratio (HR) with 95% confidential interval (95%CI).

Results: A total of 94 patients were included for IHC testing. PDL1 protein expression on tumor cells was associated with better pCR rate in both univariate (OR = 2.621, p = 0.043) and multivariate (OR = 3.595, p = 0.029) logistic regression analysis. It was also associated with shorter DFS both by log-rank test (p = 0.015) and cox hazard model (HR = 22.824, 95%CI 1.621–321.284, p = 0.020). In hormone receptor (HR)-positive patients, PDL1 protein expression was also associated with better pCR (OR = 2.362, p = 0.022). It was also associated with poor DFS (HR = 18.821, 95%CI 1.645–215.330, p = 0.018).

Conclusions: Our results show that PDL1 protein expression is a predictive biomarker of pCR and a prognostic factor of DFS in breast cancer patients and HR-positive subgroups.

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Introduction

Breast cancer is one of the most common malignancies in women worldwide. Over the years, neoadjuvant chemotherapy has become a regular procedure of breast cancer treatment. Neoadjuvant chemotherapy (NAT) was not only used for the locally advanced patients with large tumor burden, providing surgery opportunities and breast-conserving opportunities but also used to early predict the patients' responsiveness to the treatment. Pathological complete response (pCR) refers to the status when no tumor cell residues or only ductal carcinoma in situ remains in the surgical specimens after preoperative treatment. Besides the fact that a great number of large-scale clinical trials have confirmed better survival outcomes in patients reached pCR in NAT[1-4], there were still patients not achieving pCR results in the same NAT treatment. Therefore, how to predict the patient's response to NAT in the early stage and find out the predictive factor of pCR seem particularly important.

Programmed death-ligand-1 (PDL1), expressed on tumor cells, T cells, natural killer cells (NKs) as well as dendritic cells (DCs), is a trans-membrane glycoprotein mostly known for its critical role in tumor immune evasion. When combined with its ligand PD1, which is mainly expressed on the surface of the T cell membrane, PDL1 could induce T cell apoptosis and promote T cell differentiation towards regulatory T cells[5]. Innate absence of PDL1 expression is associated with autoimmune diseases such as lupus[6]. The subsequent change of PDL1 expression is often related to tumor immune evasion.

Clinically, PDL1 is associated with poorer prognosis in a variety of solid tumors, such as melanoma, renal cancer, and lung cancer[7-12]. As for treatment value, antibodies of PD1/PDL1 have now been approved by the U.S. Food and Drug Administration for the clinical management of melanoma and renal cancer. However, the role of PDL1 in breast cancer oncogenesis and treatment is still quite obscure currently. The objective response rates for clinical trials of PDL1 in the treatment of breast cancer are much lower than those for melanoma[7]. Several observational studies focused on the clinical and prognostic value of PDL1 have led to different conclusions. Many studies have reported a relatively consistent result that PDL1 represents a good survival in triple negative breast cancer (TNBC)[13–16], whereas the role is of PDL1 is not clear in HR-positive patients.

Two neoadjuvant clinical trials (SHPD001 and SHPD002) were conducted in our department with paclitaxel plus cisplatin weekly treatment, demonstrating a high pCR rate[17]. Among all patients, the pCR rate was 34.4%. In human epidermal growth factor 2 (HER2) positive breast cancer and the triple negative

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breast cancer (TNBC), the pCR rate was 52.4% and 64.7%, respectively. This excellent effect is generally believed to be associated with metronomic chemotherapy and immune regulation[18,19]. However, in HR-positive patients, the pCR rate is less than ideal, so we would like to find out the predictive biomarker of pCR in this part of patients to improve the therapeutic effect. Therefore, we examined the expression of PDL1 in patients from the clinical trial and explored the predictive and prognostic value of PDL1 in neoadjuvant chemotherapy in breast cancer. We hypnotized that the expression of PDL1 protein is a predictor of pCR result and survival outcome in all patients and HR-positive subgroup.

Results

Basic clinical and pathological features of patients

A total of 94 patients were provided with paraffin-embedded specimens for immunohistochemistry. Of all patients, 39% had a tumor greater than 5 cm, 41% had HER2 gene overexpression, and 50% of the patients had a ki67 expression level of more than 30%, which was consistent with the locally advanced nature of the included patients.

PDL1 was expressed on tumor cells on 50% of the breast cancer patients. Twelve percent of the patients had a positive TILS staining. Representative tissue staining was presented in Figure 1. PDL1 was expressed on 66.7% of the HER2 overexpressing breast cancer and 66.7% of the TNBC, and somehow less detected in luminal-like breast cancer (47.6%). However, no significant correlations between PDL1 expression and ER, PR, HER2 or other clinicopathological factors were found (Table 1).

Predictive value of PDL1 protein expression

Both univariate (OR = 2.621, p = 0.043) and multivariate (OR = 3.595, p = 0.029) logistic regression tests showed that positive PDL1 expression was associated with better pCR rate. At the same time, patients with high ki67 level (OR = 5.071, p = 0.008) or those with ER-negative tumors (OR = 0.110, p = 0.004) also tended to reach pCR after NAT (Table 2).

In subgroup analysis, a similar trend was observed in HR-positive patients and HER2 positive patients. In HR-positive subgroup, univariate (OR = 2.089, p = 0.026) and multivariate (OR = 2.362, p = 0.022) logistic regression test showed that positive PDL1 expression was associated with better pCR rate (Table 3). At the same time, HER2 status (OR = 4.667, P = 0.032) and ki67 status (univariate OR = 3.694, P = 0.018) was also an independent predictor of pCR. In HER2 positive subgroup, univariate (OR = 4.667, p = 0.032) and multivariate (OR = 7.979, p = 0.024) logistic regression test showed that positive PDL1 expression was associated with better pCR rate (Table 4). We failed to perform an effective subgroup analysis due to the small number of patients with TNBC breast cancer.

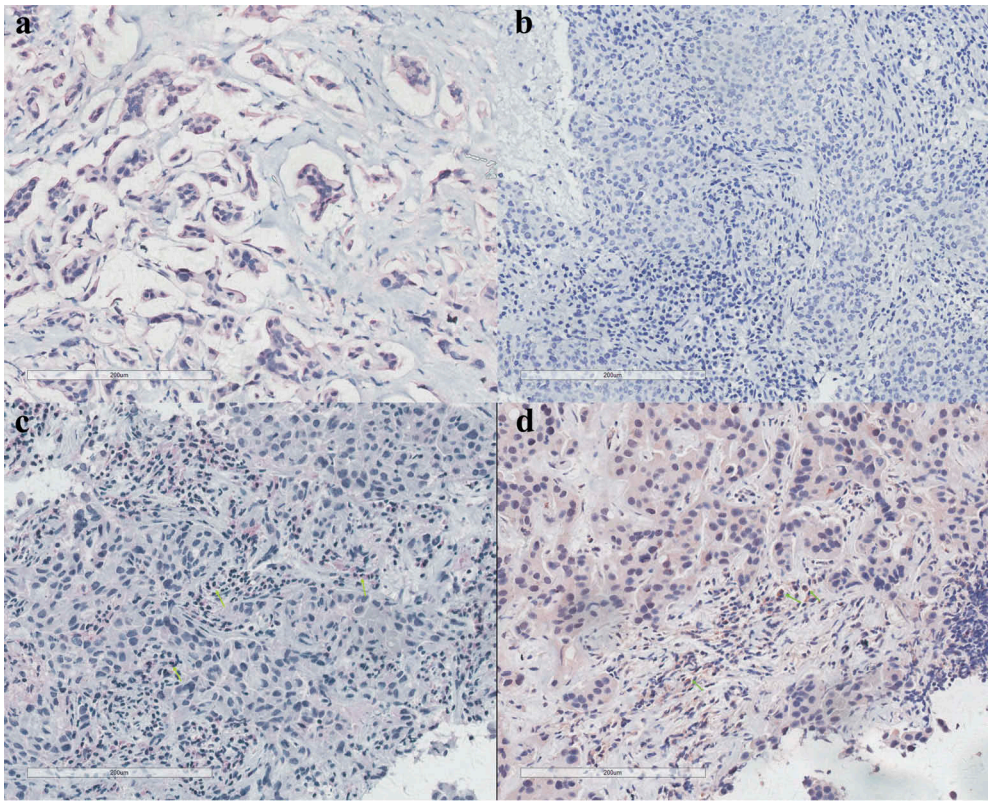

Figure 1. Different PDL1 immunochemistry staining levels.

A. Positive PDL1 staining on tumor cells; B. Negative PDL1 staining; C. Positive PDL1 staining on TILS (green arrow marking TILS); D. Positive PDL1 staining on tumor cells and TILS (green arrow marking TILS).

Table 1. Correlations between PDL1 expression and clinicopathological factors.

Characteristics	PDL1 +	PDL1-	*P*-value
Age			
<50	23	26	0.536
≥50	24	21	
Tumor size			
≤5 cm	27	28	0.641
>5 cm	20	17	
ER status			
Negative	13	11	0.636
Positive	34	36	
PR status			
Negative	10	4	0.082
Positive	37	43	
ki67 status			
≤30	23	24	0.837
>30	24	23	
HER2 status			
Negative	26	29	0.530
Positive	21	18	

Table 2. Univariate and multivariate analysis for PDL1 protein expression and pCR outcome.

	Univariate			Multivariate		
	N	OR	P	N	OR	P
Age						
<50	49	1		49	1	
≥50	45	1.015	0.973	45	0.616	0.398
Tumor size						
≤5 cm	55	1		55	1	
>5 cm	37	1.280	0.594	37	0.675	0.501
ER status						
Negative	24	1		24	1	
Positive	70	0.163	**0.000**[a]	70	0.110	**0.004**[a]
HER2						
Negative	55	1		55	1	
Positive	39	3.324	**0.014**[a]	39	3.002	0.053
ki67 status						
≤30	47	1		47	1	
>30	47	4.233	**0.004**[a]	47	5.071	**0.008**[a]
PR status						
Negative	14	1		14	1	
Positive	80	0.333	0.064	80	2.626	0.279
PD-L1						
Negative	47	1		47	1	
Positive	47	2.621	**0.043**[a]	47	3.595	**0.029**[a]

a: $p < 0.05$ considering statistical significant.

Table 3. Predictive value of PDL1 expression in HR-positive BC.

	Univariate		Multivariate	
Clinicopathological factors	OR	P	OR	P
Age				
<50	1		1	
≥50	0.873	0.792	0.965	0.951
Tumor size				
≤5 cm	1		1	
>5 cm	1.227	0.694	0.976	0.967
HER2				
Negative	1		1	
Positive	2.929	**0.042**[a]	3.226	**0.045**[a]
ki67 status				
≤30	1		1	
>30	3.694	**0.018**[a]	4.669	**0.015**[a]
PD-L1				
Negative	1		1	
Positive	2.089	**0.026**[a]	2.365	**0.022**[a]

Although only 12.7% of the patients had a positive TILS staining, it showed a strong predictive value of pCR. Both univariate (OR = 4.34, p = 0.022) and multivariate (OR = 4.119, p = 0.044) logistic regression tests showed that positive PDL1 expression on TILS was associated with better pCR rate.

Table 4. Predictive value of PDL1 expression in HER2 positive BC.

	Univariate		Multivariate	
Clinicopathological factors	OR	P	OR	P
Age				
<50	1		1	
≥50	2.86	0.124	1.388	0.699
Tumor size				
≤5 cm	1		1	
>5 cm	1.750	0.402	0.726	0.713
ER				
Negative	1		1	
Positive	0.278	0.072	0.210	0.089
ki67 status				
≤30	1		1	
>30	3.422	0.074	5.776	0.052
PD-L1				
Negative	1		1	
Positive	4.667	**0.032**[a]	7.979	**0.024**[a]

a: $p < 0.05$ considering statistical significant.

Prognostic value of PDL1 protein expression

With the median follow up time of 27 months, six events occurred in PDL1 positive patients and one event occurred in PDL1 negative patients. Kaplan-Meier plot showed that PDL1-negative patients yielded better survival than PDL1-positive counterparts (Log-rank p = 0.015; Figure 2A). Cox hazard model also showed that patients without PDL1 expression (HR = 22.824, p = 0.020 , 95%CI 1.621–321.284) survived better (Table 5). The patient's age (HR = 0.123, p = 0.050 , 95%CI 0.015–0.999), and postoperative lymph node status (HR = 37.897, p = 0.003 , 95%CI 3.391–423.536) were also independent prognostic factors of DFS.

In subgroup analysis, a similar trend was observed in HR-positive patients. Kaplan-Meier plot showed that PDL1-positive patients had poorer survival than the PDL1-negative patients (Log-rank p = 0.020; Figure 2B). Cox hazard model also suggested a higher risk of recurrence in the PDL1-positive group (HR = 18.821, p = 0.018; Table 6). The prediction value of PDL1 was not observed in HER2-positive BC (Log-rank p = 0.056; Figure 2C). We failed to perform an effective subgroup analysis due to the small number of patients with TNBC breast cancer.

Discussion

PDL1 gene is well known in cancer immunology. Compared to other types of cancer, the role of PDL1 is relatively ambiguous in breast cancer, especially in HR-positive breast cancer. Our study showed that PDL1 is a predictive factor of response to NAT and long-term survival in breast cancer patients and HR-positive subtype as well. As far as we know, this is the first time that the predictive and prognostic value of PDL1 protein was reported in HR-positive breast cancer patients.

According to our results, patients with PDL1 protein expression are more likely to reach pCR in all populations and in HR-positive BC populations, but at the same time are more susceptible to recurrence.

Regarding the predictive value of PDL1 in neoadjuvant chemotherapy, our results were basically consistent with other researchers. Wimberly[20] found in a small sample study that the immunofluorescence expression of PDL1 in breast cancer was associated with pCR in patients; Bertucci[21] had

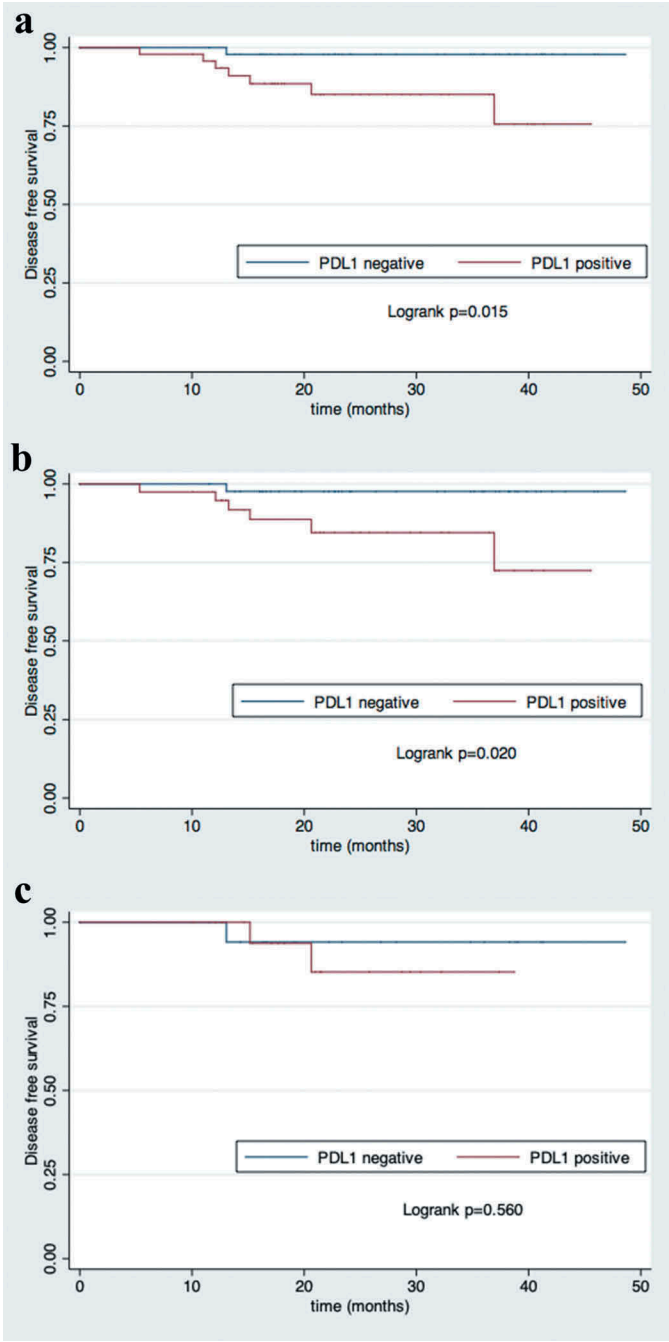

Figure 2. Kaplan-Meier plot for different PDL1 protein expressions. A. All patients; B. HR positive patients; C. HER2 positive patients.

Table 5. Multivariable analyses of associations between clinicopathological factors and disease-free survival in all patients.

	Disease-free survival		
Clinicopathological factors	HR	95%CI	P value
PDL1			
Negative	1		
Positive	22.824	1.621–321.284	**0.020**[a]
Age			
≤50	1		
>50	0.123	0.015–0.999	**0.050**[a]
Tumor size			
≤5	1		
>5	5.602	0.967–32.449	0.055
ER			
Negative	1		
Positive	0.496	0.043–5.784	0.576
HER2 status			
Negative	1		
Positive	2.875	0.428–19.325	0.277
ki67 status			
≤30	1		
>30	2.653	0.478–14.706	0.264
PR			
Negative	1		
Positive	0.266	0.018–3.933	0.335
ypLN			
Negative	1		
Positive	37.897	3.391–423.536	**0.003**[a]

a: $p < 0.05$ considering statistical significant.

Table 6. Prognostic value of PDL1 expression in HR-positive BC.

	Disease-free survival		
Clinicopathological factors	HR	95%CI	P value
PDL1			
Negative	1		
Positive	18.821	1.645–215.330	**0.018**[a]
Age			
≤50	1		
>50	0. 256	0.041–1.606	0.146
Tumor size			
≤5	1		
>5	10.047	1.115–90.555	**0.040**[a]
HER2			
Negative	1		
Positive	2.654	0.703–42.968	0.284
ki67			
≤30	1		
>30	4.140	0.575–29.841	0.159
ypLN			
Negative	1		
Positive	48.649	2.973–796.003	**0.006**[a]

a: $p < 0.05$ considering statistical significant.

detected PDL1 mRNA expression in inflammatory breast cancer patients and found that PDL1 mRNA up-regulation was associated with pCR; Sabatier[22] used DNA microarray technology to analyze breast cancer tissue and found that patients with high expression of PDL1 mRNA in the general population were likely to achieve pCR, but the study did not find the predictive value of PDL1 mRNA expression in ER-positive breast cancer.

For the first time, our study observed the predictive effect of PDL1 expression on pCR in HR-positive breast cancer subpopulations. Hormone receptor-positive breast cancer are a bunch of breast cancer characterized by slow disease progression and relatively good prognosis. However, this subtype of breast cancer is also known for its insensitivity to chemotherapy. Thus, PDL1 could be used to predict pCR in HR-positive breast cancer to avoid unnecessary NAT.

The prognostic value of PDL1 is quite different in each study. Studies from LI[13], Beckers[14], and Sabatier[22] showed that PDL1 protein expression or mRNA up-regulation in TNBC or Basal-like breast cancer represented a good prognosis. But in Chen's study, residual PDL1 expression in patients after neoadjuvant chemotherapy was associated with poor long-term survival. One explanation is that the residual tumor tissues tended to be luminal subtype because TNBC is known more sensitive to NAT. This is consistent with our findings that HR-positive breast cancer with PDL1 protein

expression is prone to disease recurrence. Our findings are also supported by Muenst's results, in which luminal-B patients had a poorer survival with PDL1 expression. Thus, we hypothesize that the prognostic value of PDL1 in breast cancer patients is depended on different breast cancer subtypes. Different constituent ratios of breast cancer subtypes in each study are likely to affect the final conclusion.

Of note, PDL1—as a molecule that involved in tumor immune escape—is thought to indicate a poor over all survival in many tumors[7-12]. The mechanism is generally thought to be related to a PDL1-mediated T-killer cell apoptosis and T-regulatory cell differentiation[5,23]. Nevertheless, laboratory experiments showed that chemotherapy could induce the expression of PDL1 and other immune escape-associated molecules (CD47, CD73, etc.) in breast cancer cells[23-25], leading to inactivation of T effector cells. Therefore, these tumor cells that survived chemotherapy will have a higher immune escape capacity, leading to long-term recurrence and metastasis of the disease[23]. This might explain the result from our study that patients with PDL1 protein expression tended to receive pCR result but were more likely to suffer disease relapse.

Relatively small sample size is clearly one main deficit of our study, resulting in the failure to perform subgroup analyzes in TNBC and HER2 positive breast cancer. And due to the short follow-up period, the OS analysis cannot be carried out yet, pending further follow up.

In conclusion, our research demonstrates that PDL1 protein expression is a predictive factor of pCR result from neoadjuvant therapy and DFS in breast cancer patients and HR-positive subtypes.

Methods & materials

Patients and specimen

BC patients from two paclitaxel- and cisplatin-based neoadjuvant clinical trials were included. The two trials were separately registered in ClinicalTrials.gov as SHPD001 (NCT02199418) and SHPD002 (NCT02221999).

Women aged ≥18 years old with histologically confirmed locally advanced invasive breast cancer were included. For all patients, Paclitaxel 80 mg/m^2 was given weekly starting on day 1 for 16 weeks; Cisplatin 25 mg/m^2 was given weekly on days 1, 8, and 15 every 28 days for four cycles. For HER2 positive patients in SHPD001, trastuzumab was recommended concurrently. All HER2 positive patients in SHPD002 received concurrent trastuzumab. For hormone receptor positive patients in SHPD002, endocrine therapy of aromatase inhibitor or gonadotropin-releasing hormone agonist was randomized together with chemotherapy according to their menstrual status. Planned surgery was given sequentially after neoadjuvant chemotherapy.

The tissue sample was collected at core needle biopsy before any treatment. Patients' information was collected at core needle biopsy, including patient's age, menstrual status, family history, size of the preoperative primary tumor, estrogen and progesterone receptor status of the puncture specimen, HER-2 receptor status of the puncture specimen, and PCR status after NAT. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Both clinical trials have been approved by the Ethics Committee of Renji hospital. All patients provided with informed consents.

Immunohistochemistry (IHC)

Estrogen receptor (ER), progesterone receptor (PR), ki67, HER2, and PDL1 were performed on paraffin-embedded tumor samples from biopsy. ER, PR, HER2, ki67 was detected using rabbit monoclonal antibodies SP1, EE2, 4B5 (F. Hoffmann-La Roche Ltd.), MIBI (Leica Biosystems Newcastle Ltd,). PDL1 was detected using the rabbit anti-PDL1 monoclonal antibody E1L3N (Cell Signaling Technology, INC.).

ER and PR positive was defined as more than 1% of positive nuclear staining, ki67 level was recorded as a continuous value. HER2 assessment was conducted according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) recommendations[26]. PDL1 expression was assessed according to clinical trial criteria[27] with minor modulation: membranous and cytoplasmic staining of tumor cell was counted for PDL1 tumor assessment; cytoplasmic staining of the tumor infiltrating lymphocytes (TILS) was counted for PDL1 stromal assessment. PDL1 positive was specified as more than 1% of positive staining on the tumor cell. TILS positive was defined as more than 1% of the positive staining on TILS.

Statistical analysis

Correlations between PDL1 protein expression and other clinicopathological characteristics were tested using the chi-squared test. Univariate and multivariate logistic regression tests were used to analyze the associations between PDL1 expression and pCR outcome. Disease-free survival (DFS) was used for survival analysis. DFS was defined as the time from surgery to the first disease relapse including one of the following events: a distant disease metastasis, recurrence of ipsilateral locoregional invasive disease, contralateral breast cancer or death. Survival curve was derived from Kaplan–Meier method; the log-rank test was used to compare survival difference. Cox proportional hazards model was used to calculate the adjusted hazard ratio (HR) with 95% confidential interval (CI). Patient age, tumor size, ER, PR, HER2, and ki67 were adjusted. Statistical results were considered significant with a P value <0.05. All statistical analysis was carried out using STATA statistics SE 14 (Stata Corp LP, College Station, TX). Kaplan-Meier plot was drawn in SPSS statistics version 23 (SPSS, Inc., Chicago, IL, USA).

Disclosure of potential conflicts of interests

No potential conflicts of interest were disclosed.

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