A Potential Predictive Biomarker for Miller/Payne Grading: PD-L1 Expression before Neoadjuvant Chemotherapy in Breast Cancer

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Keywords

Breast cancer · Neoadjuvant chemotherapy · Immunohistochemical analysis · Tissue microarrays · Programmed death ligand 1 · Miller/Payne system · Predictive biomarker

Abstract

Background and Objective: The aim of this study was to investigate the value of programmed death ligand 1 (PD-L1) expression as a predictive biomarker for Miller/Payne grading before neoadjuvant chemotherapy (NACT) in breast cancer. **Patients and Methods:** The expression of PD-L1 in pretreatment biopsies of breast cancer was assessed by immunohistochemistry in tissue microarrays. The results were analyzed using SPSS 22.0 statistical software. **Results:** Of 53 female patients, 10 (18.9%) patients had a grade 5 (G5) response, and 12 (22.6%) patients showed PD-L1 expression, including 7 (13.2%) patients with staining in tumor cells (TCs) and 8 (15.1%) patients with staining in peritumoral lymphocytes (PTLCs). Logistic regression analysis revealed that G5 response to NACT was significantly associated with TCs or PTLCs PD-L1 positivity, whether with univariate analysis (TCs PD-L1: $p = 0.00$, OR 20.50, 95% CI 3.11–134.94; PTLCs PD-L1: $p = 0.02$, OR 6.50, 95% CI 1.27–33.20) or with multivariate analysis (TCs PD-L1: $p = 0.00$, OR 42.23, 95% CI 3.36–530.90; PTLCs PD-L1: $p = 0.02$, OR 9.07, 95% CI 1.37–

60.02). The same trend was found in the luminal subgroup analysis (TCs PD-L1: $p = 0.02$, OR 23.43, 95% CI 1.66–331.58; PTLCs PD-L1: $p = 0.01$, OR 47.89, 95% CI 2.47–927.41). **Conclusion:** G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1-positive expression in pretreatment biopsies; it can be expected that PD-L1 will become a new independent biomarker of response to NACT in breast cancer.

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Introduction

Breast cancer is the most prevalent malignancy in women that seriously endangers women's health. According to the Global Cancer Statistics 2018 report, breast cancer is the most commonly diagnosed cancer (24.2%) and the leading cause of cancer death (15.0%) among females [1]. Currently, the main treatments for breast cancer include surgery, chemotherapy, radiation, endocrine therapy, and targeted therapies. Neoadjuvant chemotherapy (NACT) involves patients receiving systemic cytotoxic therapy before surgical removal of the tumor, also termed induction or preoperative chemotherapy. NACT is increasingly being used to treat patients with locally advanced breast cancer; it is especially preferred for operable patients with proven lymph node metastasis or which

Table 1. Neoadjuvant chemotherapy regimens of 53 patients with breast cancer

NACT	Median cycle	Cases, <i>n</i> (%)
CE	4 (2–4)	6 (11.3)
CE→T	8 (6–8)	36 (67.9)
TE	6 (6)	4 (7.5)
TEC	6 (6)	6 (11.3)
TB	4 (4)	1 (1.9)

B, carboplatin; C, cyclophosphamide; E, epirubicin; T, docetaxel.

are not suitable for breast-conserving surgery [2, 3]. Recent research and clinical trials have shown a significant correlation of breast cancer responses to NACT with survival and prognosis [4, 5]. Patients who achieve a pathologic complete response (pCR) to NACT tend to have improved disease-free survival (DFS) and overall survival (OS) compared with patients with residual invasive disease [4, 6–8]. In a multivariate analysis of known prognostic factors, the Miller/Payne (MP) grading system was an independent predictor of OS or DFS [9]. Therefore, effectively predicting which patients can achieve grade 5 response/pCR before NACT is of great clinical significance.

At present, programmed death ligand 1 (PD-L1) and tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment have become a research focus in many tumors and suggest immune evasion or immune resistance to endogenous antitumor activity; the positive expression of PD-L1 is also a common event in breast cancer [10–15]. However, the expression of PD-L1 in breast cancer cells or peritumoral lymphocytes (PTLCs) in pretreatment biopsies and its correlation with the response to NACT has not been extensively investigated. Here, we aim to investigate the correlation of MP grading with PD-L1 expression before NACT and explore the predictive biomarker value of PD-L1 in pretreatment biopsies in the response to NACT in breast cancer.

Materials and Methods

Patients and Clinicopathological Characteristics

Female patients with primary breast cancer treated with NACT from January 1, 2016, to October 31, 2018, at Weifang People's Hospital were analyzed. The institution ethics review board approved this study. The criteria for inclusion were as follows: (1) histologically confirmed invasive carcinoma at core needle biopsy in our hospital; (2) both NACT and surgical procedures were performed in our hospital; (3) the MP grade was available after surgery; and (4) never received chemotherapy, radiation, endocrine therapy, or targeted therapies before NACT. A total of 75 patients were enrolled. The exclusion criteria were as follows: (1) the status

of estrogen receptor (ER), progesterone receptor (PR), and HER2 expression and the Ki-67 score were not completely available for biopsy diagnoses; (2) breast cancer recurred for a second time after being treated; and (3) the biopsy sample is insufficient or severe artifacts present. Thus, 21 patients were excluded from the cohort only due to the last exclusion criterion. In addition, 1 case was excluded from the cohort due to deficient staining in the tissue. Finally, a total of 53 patients were included in the analysis of the results. The median age was 51.4 years (range 26.8–69.4).

Treatment Methods

All patients received 2–8 cycles of NACT, and the median cycle of NACT was 8 (Table 1). A total of 32.1% (17/53) had HER2 expression, and 22.6% (12/53) received HER2 targeted therapies in addition to NACT.

Reagents

PD-L1 (CD274) Mouse Monoclonal Antibody (Clone ID: UMAB228) from OriGene Technologies (Beijing, China).

Tissue Microarrays and Immunohistochemistry

Briefly, representative tumor areas were selected in hematoxylin/eosin-stained slides, which served as a useful guide for selecting the area to be sampled, and 3-mm cores were obtained using a needle and seated into a recipient paraffin block with a spacing of 2 mm between the tissue microarrays (TMAs) samples. Once the TMAs were constructed, the samples were sectioned into 5- μ m continuous slides. The cases were evaluated on 3 previously constructed TMAs containing formalin-fixed, paraffin-embedded tissues from 53 invasive primary breast carcinomas. Next, the TMAs were stained using an immunohistochemistry (IHC) staining system, the Dako Autostainer Link 48 (NYSE:A, Palo Alto, CA, USA), according to their manual.

Serial pretreatment biopsies sections (5 μ m) were cut from all TMAs, mounted on slides, and baked for 1 h at 65 °C. The slides were de-paraffinized and rehydrated as follows: xylene I and II, 15 min, respectively; 100% ethanol, 3 min; 95% ethanol, 3min; 75% ethanol, 3 min; distilled water, 3 min. The sections were antigen-retrieved at 95 °C for 20 min in the PT Link. This was followed by incubation with primary antibody for 30 min, secondary antibody for 20 min, and stained in diaminobenzidine. The slides were counterstained for 1–2 min with hematoxylin, differentiated for 10 s with 0.1% HCl, washed with running water, blued for 30 s with ammonia water, dehydrated for 2 min using gradient ethanol washes (75, 95% I, 95% II, and 100%) and in xylene I and II for 3 min, respectively. Finally, these slides were sealed with neutral gum. The adjacent normal breast tissue in each TMA block served as the internal control on the slides. Immunohistochemical staining for PD-L1 was performed on all TMAs.

Assessment Criteria

The expression of PD-L1 in pretreatment biopsies was assayed with an immunohistochemical method and evaluated under a high-resolution optical microscope to observe the staining of cells. PD-L1 staining was scored in both the tumor cells (TCs) and the PTLCs [16]. PTLCs and TCs staining was identified as positive when clear membranous staining was present in $\geq 1\%$ of cells [17, 18]. Analysis was conducted by 1 intermediate and 1 senior pathologist, disagreement was resolved by discussion and consensus and, where required, through discussion with a pathologist with content expertise.

Breast cancer intrinsic molecular subtypes were determined by ER, PR, Ki-67, and HER2 status following the 2011 St. Gallen International Expert Consensus recommendations [19]. The pathological response to NACT was assessed by the MP grading system

Fig. 1. Representative photographs of PD-L1 expression in pretreatment biopsies (all images $\times 400$ magnification). **a** Positive PD-L1 staining on tumor cells. **b** Negative PD-L1 staining on tumor cells. **c** Positive PD-L1 staining on peritumoral lymphocytes. **d** Negative PD-L1 staining on peritumoral lymphocytes.

[9], and pCR was defined as MP grade 5. TNM staging was assessed according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. The clinical staging of the breast tumors was performed by breast ultrasound, mammography, or MRI. The node status was determined by ultrasound or needle biopsy.

Statistical Analysis

All data were analyzed using SPSS statistical software (version 22.0; IBM, Armonk, NY, USA). The χ^2 and Fisher's exact probability tests were used to analyze differences between qualitative data. The Spearman test was used to analyze correlations between TCs PD-L1 expression and PTLCs PD-L1 expression. Univariate and multivariate logistic regression were used to analyze the associations between PD-L1 expression and MP grades. Statistical results were considered significant with a p value < 0.05 .

Results

MP Grades and the Response to NACT

The pathological responses to NACT of 53 patients were assessed by the MP grading system as follows: 10 (18.9%) patients were appraised as having a grade 5 (G5) response (pCR), 7 (13.2%) patients as having a grade 4 (G4) response, 10 (18.9%) patients as having a grade 3 (G3) response, 19 (35.8%) patients as having a grade 2

Table 2. PD-L1 expression before NACT of 53 patients with breast cancer

	TCs PD-L1		χ^2 <i>p</i> value	<i>r</i> <i>p</i> value
	negative	positive		
PTLCs PD-L1				
Negative	41 (77.4)	4 (7.5)	2.68	0.30
Positive	5 (9.4)	3 (5.7)	0.10	0.03

Values are *n* (%). TCs PD-L1, PD-L1 expression in tumor cells; PTLCs PD-L1, PD-L1 expression in peritumoral lymphocytes.

(G2) response, and 7 (13.2%) patients as having a grade 1 (G1) response.

PD-L1 Protein Expression

Of 53 patients, 12 (22.6%) patients showed PD-L1 expression, including 4 (7.5%) patients with staining in TCs only, 5 (9.4%) patients with staining in PTLCs only, and 3 (5.7%) patients with staining in both TCs and PTLCs. There was no significant difference in PD-L1 expression in pretreatment biopsies between the TCs and PTLCs ($p > 0.05$), but there was a positive correlation ($r = 0.30$, $p = 0.03$; Fig. 1; Table 2).

Table 3. Baseline parameters and distribution of Miller/Payne grades and univariate logistic analysis of predictive factors for Miller/Payne grades in breast cancer patients treated with NACT

	Miller/Payne grades, <i>n</i> (%)		χ^2	<i>p</i> value	Univariate logistic analysis		
	N pCR/G1–4	pCR/G5			OR	95% CI	<i>p</i> value
Age			0.22	0.64			
<50 years	18 (78.3)	5 (21.7)			1		
≥50 years	25 (83.3)	5 (16.7)			0.72	0.18–2.86	0.64
Menstrual status			0.02	0.88			
Premenopausal	21 (84.0)	4 (16.0)			1		
Postmenopausal	22 (78.6)	6 (21.4)			1.43	0.35–5.80	0.62
Tumor stage			0.00	1.00			
cT1–2	35 (81.4)	8 (18.6)			1		
cT3–4	8 (80.0)	2 (20.0)			1.09	0.19–6.17	0.92
Nodal status			0.04	0.85			
cN–	5 (71.4)	2 (28.6)			1		
cN+	38 (82.6)	8 (17.4)			0.53	0.09–3.21	0.48
ER			1.57	0.21			
Negative	18 (72.0)	7 (28.0)			1		
Positive	25 (89.3)	3 (10.7)			0.31	0.07–1.36	0.12
PR			0.47	0.49			
Negative	18 (75.0)	6 (25.0)			1		
Positive	25 (86.2)	4 (13.8)			0.48	0.12–1.95	0.31
HER2			1.82	0.18			
Negative	31 (86.1)	5 (13.9)			1		
Positive	12 (70.6)	5 (29.4)			2.58	0.63–10.55	0.19
Ki-67			0.00	1.00			
<14%	4 (80.0)	1 (20.0)			1		
≥14%	39 (81.2)	9 (18.8)			0.92	0.09–9.28	0.95
Subtypes			0.62	0.88			0.87
Triple negative	6 (75.0)	2 (25.0)			1		
Luminal	29 (82.9)	6 (17.1)			0.62	0.10–3.85	0.61
HER2	8 (80.0)	2 (20.0)			0.75	0.08–6.96	0.80
TCs PD-L1			10.87	0.00			
Negative	41 (89.1)	5 (10.9)			1		
Positive	2 (28.6)	5 (71.4)			20.50	3.11–134.94	0.00
PTLCs PD-L1			3.81	0.05			
Negative	39 (86.7)	6 (13.3)			1		
Positive	4 (50.0)	4 (50.0)			6.50	1.27–33.20	0.02

TCs PD-L1, PD-L1 expression in tumor cells before NACT; PTLCs PD-L1, PD-L1 expression in peritumoral lymphocytes before NACT.

Baseline Parameters and Distribution of MP Grades

There was a significant difference in TCs PD-L1 expression before NACT between the G5 and G1–4 groups ($p = 0.00$) and a marginal difference in PTLCs PD-L1 expression ($p = 0.05$). There was no significant difference in age, menstrual status, clinical tumor stage, nodal status, ER, PR, HER2, Ki-67, and molecular subtypes between the G5 and G1–4 groups ($p > 0.05$; Table 3).

Univariate Analysis of MP Grades

Univariate logistic regression analysis revealed that G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1 positivity (TCs PD-L1: $p = 0.00$, OR 20.50, 95% CI 3.11–134.94; PTLCs PD-

L1: $p = 0.02$, OR 6.50, 95% CI 1.27–33.20), while it was unrelated to age, menstrual status, clinical tumor stage, nodal status, ER, PR, HER2, Ki-67, and molecular subtypes (Table 3).

Multivariate Logistic Analysis of MP Grades

The factors brought into multivariate logistic regression analyzes included age, clinical tumor stage, nodal status, molecular subtypes (luminal, HER2, or triple negative), and PD-L1 expression before NACT (TCs or PTLCs PD-L1 expression was analyzed, respectively). The results showed that G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1-positive expression (TCs PD-L1: $p = 0.00$, OR

Table 4. Multivariate logistic analysis of predictive factors for Miller/Payne grades in breast cancer patients treated with NACT

Variable	<i>n</i>	TCs PD-L1			PTLCs PD-L1		
		OR	95% CI	<i>p</i> value	OR	95% CI	<i>p</i> value
Age							
<50 years	23	1			1		
≥50 years	30	0.74	0.13–4.04	0.72	0.46	0.09–2.35	0.35
Tumor stage							
cT1–2	43	1			1		
cT3–4	10	1.62	0.19–13.94	0.66	1.30	0.17–9.91	0.80
Nodal status							
cN–	7	1			1		
cN+	46	2.40	0.13–44.68	0.56	0.36	0.05–2.80	0.36
Subtypes				0.62			0.92
Triple negative	8	1			1		
Luminal	35	1.03	0.08–12.80	0.98	1.02	0.11–9.42	0.99
HER2	10	2.81	0.14–55.61	0.50	1.53	0.09–25.17	0.77
TCs PD-L1							
Negative	46	1					
Positive	7	42.23	3.36–530.90	0.00			
PTLCs PD-L1							
Negative	45				1		
Positive	8				9.07	1.37–60.02	0.02

TCs PD-L1, PD-L1 expression in tumor cells before NACT; PTLCs PD-L1, PD-L1 expression in peritumoral lymphocytes before NACT.

Table 5. Multivariate logistic analysis of predictive factors for Miller/Payne grades in luminal subgroup of breast cancer patients treated with NACT (*n* = 35)

Variable	<i>n</i>	TCs PD-L1			PTLCs PD-L1		
		OR	95% CI	<i>p</i> value	OR	95% CI	<i>p</i> value
Age							
<50 years	16	1			1		
≥50 years	19	0.95	0.10–8.69	0.96	0.39	0.03–4.71	0.46
Tumor stage							
cT1–2	29	1			1		
cT3–4	6	1.92	0.14–26.74	0.63	1.89	0.13–27.36	0.64
Nodal status							
cN–	6	1			1		
cN+	29	3.06	0.11–86.90	0.56	1.08	0.04–27.50	0.97
TCs PD-L1							
Negative	30	1					
Positive	5	23.43	1.66–331.58	0.02			
PTLCs PD-L1							
Negative	31				1		
Positive	4				47.89	2.47–927.41	0.01

TCs PD-L1, PD-L1 expression in tumor cells before NACT; PTLCs PD-L1, PD-L1 expression in peritumoral lymphocytes before NACT.

42.23, 95% CI 3.36–530.90; PTLCs PD-L1: *p* = 0.02, OR 9.07, 95% CI 1.37–60.02). The other factors were not related to G5 response, including age, clinical tumor stage, nodal status, and molecular subtypes (Table 4).

The same result was found in the luminal subgroup analysis (TCs PD-L1: *p* = 0.02, OR 23.43, 95% CI 1.66–331.58; PTLCs PD-L1: *p* = 0.01, OR 47.89, 95% CI 2.47–927.41; Table 5).

Discussion

PD-L1 is expressed by a wide range of cancer cell types including breast cancer. PD-L1 interacts with the immune cell surface protein PD-1 to convey an inhibitory signal to TILs to reduce cytotoxic T-cell activity and stimulate regulatory T-cell development, thereby extinguishing the immune response, impeding immune responses, and achieving immune evasion or immune resistance to endogenous antitumor activity [10, 15]. Recent reports have found that the presence of TILs, including T cells and dendritic cells, predicts the response to NACT [20–23]; PD-L1 expression in breast cancer correlated with TILs [24]. Reports have shown that patients who achieve pCR from neoadjuvant therapy tend to have improved DFS and OS [4, 6–8]. As patients with PD-L1 positivity would benefit from NACT in breast cancer, we researched on it preliminarily; it is very important to effectively predict response to NACT.

PD-L1 Expression in TCs and MP Grades in Breast Cancer

In a total of 245 primary and 40 metastatic breast carcinomas evaluated with TMAs using IHC, the positive rates of TCs PD-L1 staining were 12% [25], which is consistent with the 13.2% found in the present study. Wimberly et al. [12] found that the immunofluorescence expression of PD-L1 in 80 breast cancer patients treated with NACT was associated with pCR and was nearing significance in predicting pCR by multivariate analysis. Using DNA microarray technology, Sabatier et al. [26] found that a high expression of PD-L1 mRNA was associated with pCR in all 265 breast cancer patients treated with NACT by univariate analysis, whereas none of the tested clinicopathological features (including age, ER or PR or HER2 status, pathological type) was. There was a predictive value of PD-L1 expression in basal and HER2-enriched cases, but not in luminal A, luminal B, or normal-like cases. In our study, G5 response to NACT in breast cancer was significantly associated with TCs PD-L1 positivity in pretreatment biopsies both in the total breast cancer population and in luminal subtype. Similar to other studies that used IHC technology, PD-L1 protein expression in TCs was found to be a predictive biomarker of pCR both in hormone receptor-positive patients and all 94 locally advanced breast cancer patients treated with NACT (with paclitaxel plus cisplatin weekly regimens, which was not the preferred therapy) by both univariate and multivariate logistic regression analysis [27]. However, the predictive value of PTLCs PD-L1 expression for pCR was not analyzed, and the molecular subtypes and nodal status were not included in the analysis, which were included in our study.

PD-L1 Expression in PTLCs and MP Grades in Breast Cancer

Recent reports have found that the presence of TILs, including T cells and dendritic cells, predicts the response to NACT [20–22]; PD-L1 expression in breast cancer correlated with TILs [24] and predicted pCR to NACT [28, 29]. Our research confirmed that there was a positive correlation between TCs PD-L1 expression and PTLCs PD-L1 expression before NACT in breast cancer ($r = 0.30$, $p = 0.03$; Table 2), while G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1-positive expression before NACT both in the total breast cancer population and in luminal subtype.

Predictive Biomarkers for MP Grade 5 (pCR) in Breast Cancer

NACT is the preferred treatment for patients with locally advanced breast cancer. According to previous studies on breast cancer NACT, the clinical response rates ranged from 50 to 90%, and the pCR rates were between 4 and 60%. The pCR rate is associated with time from start of NACT to surgery, NACT cycles, ER or PR or HER2 status, breast cancer subtype, Nottingham grade, mitotic count, Ki-67 score, TILs, stromal infiltration lymphocytes, and/or chemotherapy strategy (specially HER2-directed therapy with trastuzumab). In addition, the definition of pCR (applied to axilla and breast, or to breast only), breast cancer subtype (e.g., HER2+ type regardless of ER or PR status, luminal subtype was defined as ER+ and/or PR+ and HER2–), or other variables included varied in different reports, so that results also varied by univariate or multivariate analysis; sometimes, because variables are strongly associated, predictive factors cannot both be included in a significant multivariate model [2, 6, 9, 23, 30–33]. Generally, tumor subtype was associated with pCR rate; the HER2 (regardless of ER or PR status) and triple-negative subtypes had higher pCR rates than the luminal subtype (ER+ or PR+ and HER2–) [23, 33]. An independent association between pCR and breast cancer subtype was found by multivariate analysis in a meta-analysis [33], while another report showed that clinical stage, HER2 positivity, and mitotic count were significant predictors of pCR in multivariate analysis [23]. In our study, the pCR (G5) rate was 18.9%, applying subtypes recommended by the 2011 St. Gallen International Expert Consensus; G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1 positivity before NACT both in the total breast cancer population and in luminal subtype by multivariate logistic regression. So, more rigorous studies to predict MP G5 or pCR need to be designed.

Conclusion

G5 response to NACT in breast cancer was significantly associated with TCs or PTLCs PD-L1-positive expression before NACT, and PD-L1 promises to be a potential predictive biomarker for response to NACT in breast cancer.

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Statement of Ethics

The patients gave their written informed consent, and the study protocol was approved by the ethics committee of Weifang People's Hospital.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed to the study conception and design. Guarantors of the integrity of the entire study were F.-R.H., Y.-X.Z., and R.-Z.M. Study concept and design were performed by F.-R.H. Literature research was performed by C.L., F.-R.H., R.-Z.M., and Y.-H.G. Clinical studies were performed by C.L., F.-R.H., Y.-H.G., Y.-N.Z., Y.-T.Z., F.-M.C., and S.-G.Z. Experimental studies and data analysis were performed by C.L., Y.-X.Z., G.-Y.H., H.W., and Y.-P.Z. Statistical analysis was performed by C.L., Y.-H.G., M.-C.W., and F.-R.H. Manuscript preparation was performed by C.L. Manuscript editing was performed by C.L. and F.-R.H.

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