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Evaluation of Immune Reaction and PD-L1 Expression Using Multiplex Immunohistochemistry in HER2-Positive Breast Cancer: The Association With Response to Anti-HER2 Neoadjuvant Therapy

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Abstract

We examined PD-L1 expression together with immune markers in HER2⁺ breast cancers and its association with the response to anti-HER2 therapy. Our results have demonstrated intratumoral CD8⁺ lymphocytes were positively associated with pathologic complete response (pCR) in multivariate analysis, but PD-L1 was not. PD-L1's effect on pCR was probably dependant on coexistence of intratumoral CD8⁺ lymphocytes. Our results suggest examination of intratumoral CD8⁺ cells together with PD-L1 is useful in predicting response to anti-HER2 therapy in HER2⁺ breast cancer patients.

Background: Immune reaction with tumor-infiltrating lymphocytes (TILs) has been extensively investigated in breast cancer. Programmed cell death 1 and its ligand (PD-L1) are key physiologic suppressors of cytotoxic immune reaction. However, the combination of TILs with PD-L1 expression has not been well studied in breast cancer.

Patients and Methods: A multi-color immunohistochemical multiplex assay simultaneously detecting PD-L1, CD8, and CD163 was performed on biopsy whole sections from 123 HER2-positive (HER2⁺) breast cancers, including 64 treated with anti-HER2 neoadjuvant therapy and subsequent resection.

Results: PD-L1 expression was identified in 88 cases (72%) including 21 (17%) in tumor cells and 67 (55%) in immune cells. PD-L1 expression was positively associated with high Nottingham grade, high nuclear grade, and a high level of CD8⁺ and CD163⁺ cells. Among the 64 patients who received neoadjuvant therapy, 39 had pathologic complete remission (pCR) and 25 had incomplete

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Disclosure

H. Nitta and P.M. Banks are employees of Ventana Medical Systems, Inc. The remaining authors have stated that they have no conflicts of interest.

response. Multivariate analysis showed progesterone receptor negativity, *HER2/chromosome 17 centromere (CEN17)* ratio and intratumoral CD8⁺ cells were significantly associated with pCR. Furthermore, all patients with intratumoral CD8⁺ cells but no PD-L1 expression achieved pCR.

Conclusion: Our data have shown that examination of intratumoral CD8⁺ cells together with PD-L1 expression proves useful in predicting response to anti-HER2 targeted therapy in patients with HER2⁺ breast cancer.

Keywords

Breast carcinoma; Cytotoxic T-cells; Immune checkpoint system; Pathologic complete response; Tumor-associated lymphocytes

Introduction

Fifteen to twenty percent of breast cancers harbor HER2 gene amplification and/or protein overexpression.¹⁻⁵ Anti-HER2 therapy, such as trastuzumab, has shown efficacy for HER2-positive (HER2⁺) breast cancer patients, and this modality has been increasingly used in the management of HER2⁺ breast cancer.^{6,7} Pathologic complete response (pCR) is observed in 30% to 50% of HER2⁺ patients.⁸⁻¹³

Recent reports have shown that the presence of tumor-infiltrating lymphocytes (TILs) predicts response to neoadjuvant chemotherapy suggesting that the immune defense system might play a role in augmenting chemotherapy-induced tumor cell death.¹⁴⁻²³ One key immune modulatory pathway is mediated by the programmed cell death 1 (PD-1)-programmed cell death 1 ligand (PD-L1) axis, which plays an integral physiologic role in limiting the primary cytotoxic immune response. PD-L1 expression by tumor cells or in the tumor microenvironment has been reported in a variety of tumors. Results from preclinical studies support the idea that inhibition of the interaction between PD-L1 and PD-1 in the tumor microenvironment might promote tumor regression, and various agents targeting PD-1 or PD-L1 in clinical trials have shown robust response rates in a variety of tumor types.²⁴⁻²⁹ Limited data have been reported on the expression of PD-L1 in tumor cells and/or immune cells in breast cancer, but preliminary reports are divergent, with PD-L1 expression reported to be prognostically favorable in some but unfavorable in other studies.³⁰⁻³⁴ One recent study that investigated PD-L1 expression in breast cancer showed PD-L1 expression to be correlated with TILs, nearing statistical significance in predicting pCR.³⁵ However, the cohort in this study included all subtypes of breast cancer being treated with neoadjuvant therapy, such as estrogen receptor (ER)-positive, HER2⁺, and triple-negative breast cancers.³⁵ To our knowledge, PD-L1 expression and its correlation with response to neoadjuvant chemotherapy with anti-HER2 therapy in HER2⁺ breast cancer has been studied in only a single published report.³⁶ Because of the promising results of clinical trials of PD-1/PD-L1 antibodies in other cancer types, together with the recent preliminary results of TIL application to breast cancer, we undertook an evaluation of TILs and PD-L1 expression in HER2⁺ breast cancer and their association with pCR after anti-HER2 neoadjuvant therapy.

Patients and Methods

Patients, Specimens, and Pathological Assessment of the Response to Anti-HER2 Neoadjuvant Therapy

This study was approved by The Ohio State University institutional research board. Informed consent was obtained from all individual patients included in the study. Initially, the study cohort included 123 consecutive HER2⁺ breast cancer cases with archival biopsy specimens available from 2012 to 2015. Immunostains were performed on the original biopsy specimens from all 123 cases. Among these cases, 64 had anti-HER2 neoadjuvant therapy followed by surgical resection (36 lumpectomy and 28 mastectomy). For neoadjuvant chemotherapy, all patients received 4 cycles of adjuvant chemotherapy (AC; doxorubicin/cyclophosphamide) together with paclitaxel/docetaxel and trastuzumab except 4 patients with pCR and 3 patients with residual tumors who received 4 cycles of AC together with PTD (pertuzumab, trastuzumab, and docetaxel).

At our institution, HER2 immunohistochemistry (IHC) as well as fluorescence in situ hybridization (FISH) were performed on the initial core needle biopsies to confirm HER2 positivity in accordance with the 2013 HER2 American Society of Clinical Oncology/College of American Pathologists updated guidelines.³⁷

Resection specimens were evaluated by careful gross examination and exhaustive microscopic examination to search any residual invasive tumor. pCR was defined as no detectable residual invasive tumor in breast tissue and no metastasis in lymph node. For any specimens with pCR, the entire tumor bed(s) was submitted for histological examination.

Multi-Color Multiplex IHC With PD-L1, CD8, CD163, and Microscopic Assessment of Checkpoint Immune System Activity

Multicolor multiplex IHC capable of showing colocalization of PD-L1 (clone SP263, rabbit; Ventana Medical Systems, Inc, Tucson, AZ) with other immune markers (CD8: clone MRQ26, mouse; Ventana Medical Systems, Inc, and CD163: clone SP57, rabbit; Ventana Medical Systems, Inc) was performed on freshly cut whole sections from pretreatment biopsies. The IHC was evaluated in consensus viewing by 2 pathologists (P.M.B., a specialized hematopathologist, and Z.L., a specialized breast pathologist). A membranous PD-L1 staining in tumor cells or immune cells was considered as specific staining. A positive PD-L1 expression among tumor cells was defined as any membranous staining in 1% of tumor cells to maximize the assay sensitivity for PD-L1⁺ cases. For immune cells, a minimum of an estimated 10% of the respective population of cells with PD-L1 colocalization was considered as positive. The following parameters were assessed: PD-L1 expression in tumor cells (PD-L1 TC), PD-L1 expression in peritumoral immune cells (PD-L1 PTIC), PD-L1 expression in intratumoral immune cells (PD-L1 ITIC), intratumoral (IT) CD8⁺ immune cells (IT-CD8⁺), peritumoral CD8⁺ immune cells (PT-CD8⁺), intratumoral CD163⁺ macrophages (IT-CD163⁺), and peritumoral CD163⁺ macrophages (PT-CD163⁺). The term “macrophage” was used as a surrogate for CD163⁺ cells, with the acknowledgement that this marker is specific for all antigenpresenting cells, including

specialized accessory cells as well as macrophages. Some representative images with different combinations of CD8, CD163, and PD-L1 expression are illustrated in Figure 1.

Statistical Analyses

Statistical analysis was performed using SAS version 9.4 for Windows (SAS Institute, Inc, Cary, NC). Descriptive statistics were used to summarize patient clinical and pathologic characteristics. Categorical data were expressed as frequency and percentage, and continuous variables as medians and ranges. For the univariate analysis, Fisher exact or χ^2 test was used to examine the associations between responses to anti-HER2 neoadjuvant chemotherapy and the categorical clinical and pathologic characteristics. Wilcoxon rank sum test was used to compare the continuous variables between the pCR group and incomplete response group. For multivariate analysis, variables with $P > .05$ were removed sequentially using backward selection method and odds ratio with 95% confidence interval were calculated. P values $< .05$ were considered statistically significant.

Results

Assessment of the Checkpoint Immune System in 123 HER2⁺ Breast Carcinomas

Among all 123 cases, PD-L1 expression was identified in 88 cases (72%) including 21 cases (17%) with PD-L1 TC and 67 cases (55%) with PD-L1 PTIC/ITIC only. Almost all cases with PD—L1-expressing tumor cells had PD-L1 expression in immune cells except 2 cases. The overall PD-L1 expression was positively associated with high Nottingham grade and high nuclear grade, but not with ER/progesterone receptor (PR) status or lymph node metastasis. Furthermore, PD-L1 expression was positively associated with a high level of intratumoral/peritumoral CD8⁺ and CD163⁺ cells (Table 1). Moreover, PD-L1 TC⁺ cases had significantly higher levels of intratumoral CD8⁺ and CD163⁺ cells than PD-L1 ITIC/PTIC⁺ cases (Table 1).

Clinical and Pathologic Characteristics of 64 Cases With Anti-HER2 Neoadjuvant Chemotherapy and Subsequent Resection

Among 123 cases, 64 had been treated with anti-HER2 neoadjuvant chemotherapy and subsequent resection. The clinical and pathologic findings were tallied for these 64 cases: the median age at diagnosis was 56 years (range, 30-76 years); 98% cases were Nottingham grade 3 or 2; 32% (16 of 50) had lymph node metastasis; 48% (31 of 64) were ER⁺; 31% (20 of 64) were PR⁺; all cases were *HER2* FISH-amplified; 55 cases were HER2 IHC 3⁺; 2 cases were HER2 IHC 1⁺, and 7 cases were HER2 IHC 2⁺. The average *HER2* counts per cell was 17.8 (range, 3.2-40.3) and the average *HER2/chromosome 17 centromere (CEN17)* ratio was 2.75 (1.2-23.0).

The Association of PD-L1 Expression and Checkpoint Immune Response With pCR Among HER2⁺ Breast Carcinomas

Among the 64 cases with neoadjuvant therapy and subsequent resection, PD-L1 TC was identified in 6 of 64 cases (9.4%), PD-L1 PTIC was identified in 43 of 64 cases (67.2%), and PD-L1 ITIC was identified in 11 of 64 cases (17.2%). In total, 43 cases showed PD-L1 expression in any of those 3 types of cell populations (67.2%).

Thirty-nine patients (61%) had pCR and 25 (39%) had incomplete response. First, univariate analysis was performed to examine the associations between the response and clinical and pathologic characteristics, including age, grades, ER and PR positivity, HER2 signals, *HER2/CEN17* ratio, and checkpoint immune system parameters. The difference was not significant in relation to age, Nottingham grade or nuclear grade, comparing the group with pCR with the group with incomplete response. There were significantly less ER⁺ (36% vs. 68%; $P = .012$) and PR⁺ (18% vs. 52%; $P = .004$) cases in the pCR group than in the incomplete response group. The pCR group showed significantly greater median *HER2* signal (20.1 vs. 12.8; $P = .002$) and *HER2/CEN17* ratio (7.6 vs. 4.6; $P < .001$) than the incomplete response group. The pCR was significantly positively associated with PD-L1 PTIC and PD-L1 expression in any cells, but not PD-L1 TC or PD-L1 ITIC. For other checkpoint immune system parameters, pCR was positively associated with PT-CD163⁺ cells, PT-CD8⁺ cells, and IT-CD8⁺ cells, but not IT-CD163⁺ cells (Table 2).

A multivariable logistic regression model was then performed, including all the variables with $P < .1$ in the univariate analysis. Variables with $P > .05$ were removed sequentially from the multivariate model using the backward selection method. In this model only PR positivity ($P = .014$), *HER2/CEN17* ratio ($P = .007$), and IT CD8⁺ cells ($P = .021$) were significantly associated with incomplete response to anti-HER2 targeted therapy, but neither PD-L1 PTIC nor PD-L1 in any cells was associated with incomplete response (Table 3).

Next, all cases were stratified on the basis of the presence of CD8⁺ TILs and PD-L1 TC; including: (1) cases with no/minimal immune response (immune desert); (2) cases with only PT-CD8⁺ cells without PD-L1 TCs; (3) cases with only PT-CD8⁺ cells with PD-L1 TCs; (4) all cases with only PT-CD8⁺ cells regardless of PD-L1 expression; (5) cases with IT-CD8⁺ cells with PD-L1 TCs; (6) cases with IT-CD8⁺ cells, but no PD-L1 TCs (primary immune response, pattern 2); and (7) all cases with IT-CD8⁺ cells regardless of PD-L1 expression. Consistent with the multivariate analysis, the percentage of cases with residual tumors was significantly lower in cases with IT-CD8⁺ cells regardless of PD-L1 expression (8%) than cases with no/minimal immune response (63%) or cases with only PT-CD8⁺ cells regardless of PD-L1 expression (41%). Furthermore, all 8 cases with IT-CD8⁺ cells and negative PD-L1 TC showed pCR, but 1 of 5 cases (20%) with IT-CD8⁺ cells and positive PD-L1 TC showed residual tumor, although the difference was not statically significant because of the small sample size (Figure 2).

Discussion

To our knowledge, the current study is the first to investigate PD-L1 expression together with CD8⁺ lymphocytes and their association with response to anti-HER2 neoadjuvant therapy in pure HER2⁺ breast cancers. First, our data have shown that PD-L1 expression was associated with high Nottingham grade, high nuclear grade, and intratumoral/peritumoral immune reaction. Second, our data confirmed the previous findings of the positive association of PR negativity and CD8⁺ lymphocytes with pCR in HER2⁺ breast cancers. Finally, although PD-L1 expression was associated with pCR as shown in univariate analysis, it was not an independent factor in multivariate analysis, and probably dependent on coexistence of CD8⁺ lymphocytes.

Pathologic complete response to anti-HER2 neoadjuvant therapy is a presumptive surrogate for disease-free survival in patients with HER2⁺ breast cancer. In our cohort, the pCR rate was 61%, which was consistent with the previous reported pCR rates of approximately 40% to 50% from several large clinical studies.⁸⁻¹³ Potential factors for achieving pCR have been extensively investigated, such as hormone receptor (HR) status and Ki-67 index. Higher pCR rates were found in HR negative tumors in the GeparQuattro, Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (Neo-ALTTO) and Chemotherapy, Herceptin and Lapatinib in Operable Breast cancer (CHER-LOB) trials,³⁸⁻⁴⁰ but not in the Taxol Epirubicin Cyclophosphamide Herceptin Neoadjuvant (TECHNO) study.⁴¹ One study also reported 52% of HER2⁺/HR⁻ tumors to have achieved pCR, which was significantly higher than the pCR rate in luminal B-HER2 hybrid (33%; weak to moderate ER⁺/HER2⁺) and Luminal A-HER2 hybrid (8%; strong ER⁺/HER2⁺) tumors.⁴² Another study observed a high Ki-67 index (> 50%) to be an independent predictive factor for pCR in HER2⁺ patients.⁴³ Besides these factors, other studies have reported a positive association between TILs and a pCR in HER2⁺ breast cancer patients treated with anti-HER2 neoadjuvant therapy.¹⁴⁻²³ One recent study reported PD-L1 expression to be correlated with TILs and near significance in predicting pCR.³⁵ However, the cohort in this study included all subtypes of breast cancers, such as ER⁺, HER2⁺, and triple-negative breast cancers.³⁵ We are aware of only a single previously reported study addressing the issues approached by the current study.³⁶ This study involved a large number of cases and concluded that there was no significant association of either the presence of TILs or PD-L1 expression with pCR. Limitations of that study included the use of only routine hematoxylin and eosin stained slides for assessment of TILs, as well as only a single antibody assay for PD-L1, and this assay used an antibody (SP142) recently reported to be relatively insensitive to the targeted protein.^{44,45} A recent study has shown that the anti-PD-L1 antibody used in current study, clone SP263 (Ventana Medical Systems, Inc), performs similarly to clone E1L3N (Cell Signaling Technology, Danvers, MA).^{46,47}

In the current study, PD-L1 expression was examined in relation to markers for cytotoxic T-cells and antigen presenting cells by using a multicolor multiplex IHC capable of showing colocalization of PD-L1 with these other cell type markers (CD8 and CD163). This novel technique has allowed us to accurately localize PD-L1 TC and/or immune cells, accurately assess CD8⁺ cells, and CD163⁺ macrophages. Multiple checkpoint immune parameters were assessed in our study as described in the Patients and Methods section. This allowed resolution of several complex immune response patterns, each potentially corresponding to a distinct functional response to tumor: “immune desert” to nonrecognition, intratumoral CD8⁺ cells to intact primary cytotoxic immune response, and peritumoral immune response with PD-L1 production possibly mediation by other checkpoint players, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or CD47.

Because PD-L1 mediates an immune evasion mechanism to suppress the immune response to tumor cells, it has been speculated that PD-L1 expression would be expected to be associated with a poorer prognosis. However, studies of breast cancer and other cancers, including lung cancer and melanoma, have shown that PD-L1 expression can be associated with more favorable prognosis.^{35,48-51} In keeping with the findings of our current study, previous studies have described PD-L1 expression as being associated with prominent TILs.

^{35,48-51} Therefore, PD-L1 expression might just reflect an association with increased TILs during antitumor immune response, rather than an association with tumor immune evasion in this setting. One likely explanation for such findings is that, in these studied tumors PD-L1 expression by tumor as well as immune cells represents an adaptive response to an intensive primary cytotoxic immune attack on tumor neoantigens, rather than a primary, constitutive tumor cell production of PD-L1 on the basis of genetically determined activation pathways.⁴⁸ This would render these neoplasms all the more sensitive to either driver pathway (eg, HER2)-blocking therapy or to checkpoint immune pathway blocking therapy. The extreme contrast to such cases would be those exhibiting the “immune desert” pattern, in which there is no evidence of immune recognition of or reaction against tumor cells. This idea was at least partially supported by our present data showing that all cases with intratumoral CD8⁺ cells, but no PD—L1-expressing tumor cells had a pCR.

The greatest limitation of this study is perhaps its small cohort size. Despite this limitation, the presence of intratumoral CD8⁺ cells as a positive predictor for pCR was confirmed, in agreement with previous reports.¹⁴⁻²³ Other limitations include the retrospective nature of the study, lack of long-term outcome follow-up of the patients, and this in particular in relation to the association of survival with PD-L1 expression and checkpoint immune response.

Conclusion

Our data suggest that examination of CD8⁺ cells together with PD-L1 expression proves useful in predicting response of HER2⁺ breast cancer to anti-HER2 targeted therapy and might play a role in selecting HER2⁺ breast cancer patients for future immune checkpoint blockade therapy.

Clinical Practice Points

- Studies of PD-L1 expression in breast cancer are limited and divergent, with PD-L1 expression found to be prognostically favorable in some but unfavorable in the others.
- Most of previous studies included all subtypes of breast cancers in their cohorts and the clinical impact of PD-L1 expression in specific subtypes of breast cancers, especially HER2-positive breast cancers, is largely unknown.
- In the current study, we examined PD-L1 expression together with immune markers in HER2-positive breast cancers and its correlation with the response to anti-HER2 neoadjuvant therapy.
- New findings include: 1) PD-L1 expression was associated with high Nottingham grade, high nuclear grade and intratumoral/peritumoral immune reaction in HER2-positive breast cancers; 2) intratumoral CD8⁺ lymphocytes were positively associated with pathologic complete response (pCR) to anti-HER2 neoadjuvant therapy; 3) PD-L1 expression was not an independent factor in predicting pCR, and probably dependant on coexistence of intratumoral CD8⁺ lymphocytes.

- Our data have demonstrated that examination of intratumoral CD8+ cells together with PD-L1 expression proves useful in predicting response to anti-HER2 targeted therapy in patients with HER2-positive breast cancer and may play a role in selecting HER2-positive breast cancer patients for future immune checkpoint blockade therapy.

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Figure 1.

Representative Images of Different Immune Reactions and Programmed Cell Death 1 Ligand (PD-L1) Expression in HER2-Positive Breast Carcinoma, Detected Using Anti-PD-L1 Multiplex Immunohistochemistry (Anti-CD8 in Green, Anti-CD163 in Red, and Anti-PD-L1 in Brown). (A) “Immune Desert” (No Immune Reaction) With Only Scattered CD163⁺ Cells and Very Rare CD8⁺ Cytotoxic T-Cells. Note the Absence of Brown PD-L1 Staining. (B) Intratumoral Immune Reaction With Abundant CD8⁺ and CD163⁺ Cells, But No PD-L1 Staining. (C) Peritumoral Immune Reaction With CD8⁺ and CD163⁺ Cells at the Periphery and Weak PD-L1 Expression in Tumor Cells. (D) Peritumoral Immune Reaction With CD8⁺ and CD163⁺ Cells at the Periphery and Strong PD-L1 Expression in Tumor Cells. (E) Peritumoral Immune Response With Variable PD-L1 Expression in Immune Cells. (F) Intratumoral and Peritumoral Immune Response With Variable PD-L1 Expression in Tumor Cells as Well as Immune Cells (Magnification, 200×)

Figure 2.

Percentages of Cases With Residual Tumor in Groups With Different Checkpoint Immune Response and Programmed Cell Death 1 Ligand (PD-L1) Expression. *Statistically Significant

Abbreviations: IT = intratumoral; PD-L1 TC⁻ = PD-L1 expressing tumor cells negative; PD-L1 TC⁺ = PD-L1 expressing tumor cells positive; PT = peritumoral.

Table 1
Clinical and Pathologic Features Associated With PD-L1 Expression in 123 HER2⁺ Cases

	Total		PD-L1 ⁻		PD-L1 ⁺		P (PD-L1 ⁻ / PD-L1 ⁺)		PD-L1 TC ⁺		PD-L1 IC ⁺		P (PD-L1 TC ⁺ / PD-L1 IC ⁺)	
Total Cases	123	100%	35	28.5%	88	71.5%			21	17.1%	67	54.5%		
Age	57.1	30-90	56.9	34-89	57.21	30-90	NS		55.72	30-90	57.68	30-80	NS	
Histology														
Ductal	122	99.2%	35	100.0%	87	98.9%	NS		21	100.0%	66	98.5%		
Lobular	1	0.8%	0	0.0%	1	1.1%			0	0.0%	1	1.5%	NS	
NG														
<3	55	44.7%	22	62.9%	33	37.5%	.0107		6	28.6%	27	40.3%	NS	
3	68	55.3%	13	37.1%	55	62.5%			15	71.4%	40	59.7%		
Nuclear														
<3	30	24.4%	13	37.1%	17	19.3%	<.001		5	23.8%	12	17.9%	NS	
3	93	75.6%	22	62.9%	71	80.7%			16	76.2%	55	82.1%		
ER⁺	72	58.5%	24	68.6%	48	54.5%	NS		11	52.4%	37	55.2%	NS	
PR⁺	52	42.3%	17	48.6%	35	39.8%	NS		10	47.6%	25	37.3%	NS	
Lymph Node														
Negative	68	68.0%	18	72.0%	50	66.7%	NS		12	70.6%	38	65.5%	NS	
Positive	32	32.0%	7	28.0%	25	33.3%			5	29.4%	20	34.5%		
PT-CD163⁺	110	89.4%	23	65.7%	87	98.9%	<.0001		21	100.0%	66	98.5%	NS	
PT-CD8⁺	90	73.2%	15	42.9%	75	85.2%	<.0001		20	95.2%	55	82.1%	NS	
IT-CD163⁺	43	35.0%	3	8.6%	40	45.5%	.0001		18	85.7%	22	32.8%	<.0001	
IT-CD8⁺	32	26.0%	1	2.9%	31	35.2%	.0002		13	61.9%	18	26.9%	.0034	

Abbreviations: ER = estrogen receptor; IT = intratumoral; NG = Nottingham grade; NS = not significant; PD-L1 = programmed cell death 1 ligand; PD-L1 IC = programmed cell death 1 ligand-expressing immune cells; PD-L1 TC = programmed cell death 1 ligand-expressing tumor cells; PR = progesterone receptor; PT = peritumoral.

Univariate Analysis of Factors Associated With pCR and Incomplete Response to Anti-HER2 Targeted Therapy

Table 2

	pCR		Incomplete Response		<i>P</i>
	Median or n	Range or %	Median or n	Range or %	
Case	39		25		
Age, y	54	30-70	57	34-76	.142
Nottingham Grade	3	2-3	3	1-3	.642
Nuclear Grade	3	2-3	3	2-3	.645
ER ⁺	14	36%	17	68%	.012
PR ⁺	7	18%	13	52%	.004
HER2 Signals Per Cell	20.1	3.6-35.4	12.8	3.2-40.3	.002
HER2/CEN17 Ratio	7.6	2.3-23.0	4.6	1.2-15.0	<.001
PD-L1 TC	4	10%	2	8%	1
PD-L1 PTIC	32	82%	11	44%	.002
PD-L1 ITIC	9	23%	2	8%	.178
PD-L1 (in Any Cells)	32	82%	11	44%	.002
PT CD163 ⁺	37	95%	19	76%	.048
PT CD8 ⁺	32	82%	13	52%	.010
IT CD163 ⁺	12	31%	5	20%	.341
IT CD8 ⁺	12	31%	1	4%	.011

P < .05 was highlighted in bold.

Abbreviations: ER = estrogen receptor; IT = intratumoral; pCR = pathologic complete response; PD-L1 PTIC = programmed cell death 1 ligand-expressing peritumoral immune cells; PD-L1 ITIC = programmed cell death 1 ligand-expressing intratumoral immune cells; PD-L1 TC = programmed cell death 1 ligand-expressing tumor cells; PR = progesterone receptor; PT = peritumoral.

Table 3
Multivariate Analysis of Factors Associated With pCR and Incomplete Response to Anti-HER2 Targeted Therapy

	pCR		Incomplete Response		P
	Median or n	Range or %	Median or n	Range or %	
Total Cases	39		25		
ER ⁺	14	36%	17	68%	.739
PR ⁺	7	18%	13	52%	.014
HER2 Signals Per Cell	20.1	3.6-35.4	12.8	3.2-40.3	.598
HER2/CEN17 Ratio	7.6	2.3-23.0	4.6	1.2-15.0	.007
PD-L1 PTIC	32	82%	11	44%	.205
PD-L1 (in any cells)	32	82%	11	44%	.205
PT CD163 ⁺	37	95%	19	76%	.712
PT CD8 ⁺	32	82%	13	52%	.328
IT CD8 ⁺	12	31%	1	4%	.021

P < .05 was highlighted in bold.

Abbreviations: ER = estrogen receptor; IT = intratumoral; pCR = pathologic complete response; PD-L1 = programmed cell death 1 ligand; PD-L1 PTIC = programmed cell death 1 ligand-expressing peritumoral immune cells; PR = progesterone receptor; PT = peritumoral.