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PD-L1 Expression in Triple-negative Breast Cancer—a Comparative Study of 3 Different Antibodies

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Background: Assessment of programmed death protein-ligand 1 (PD-L1) in triple-negative breast cancer (TNBC) has entered daily practice to identify patients eligible for treatment with immune checkpoint inhibitors. However, different antibodies and different cut-offs for PD-L1 positivity are used, and the interchangeability of these methods is not clear. The aim of our study was to analyze whether different PD-L1 antibodies can be used interchangeably to identify TNBC patients as PD-L1 positive.

Methods: A tissue microarray encompassing 147 TNBC cases was immunohistochemically analyzed using 3 different antibodies against PD-L1: SP142, SP263, and E1L3N. PD-L1 positivity was determined as $\geq 1\%$ of positive tumor-associated immune cells. The staining patterns of the 3 antibodies were compared and correlated with clinicopathological data.

Results: A total of 84 cases were evaluable for PD-L1 analysis with all 3 antibodies. PD-L1 was positive in 50/84 patients (59.5%) with SP263, in 44/84 (52.4%) with E1L3N, and in 29/84 (34.5%) with SP142. There was no statistical difference between the performance of SP263 and E1L3N, but both antibodies stained significantly more cases than the SP142 antibody.

Conclusions: Our results show that the 3 PD-L1 antibodies identify different TNBC patient subgroups as PD-L1 positive and, therefore cannot be used interchangeably. Additional studies are needed to further investigate the use and impact of different PD-L1 antibody clones for predictive selection of TNBC patients for treatment with immune checkpoint inhibitors.

Key Words: triple-negative breast cancer, TNBC, PD-L1, immune checkpoint inhibition, immune assays

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With over 2 million new cases and 600'000 deaths worldwide in 2018, breast cancer is the most commonly diagnosed type of cancer and accounts for the highest number of cancer deaths in women.[1] However, breast cancer is not a uniform disease but consists of different subtypes with diverse molecular profiles and biological behavior. The triple-negative molecular subtype, which lacks hormone receptor expression and amplification of *HER2* gene, accounts for about 15% of all breast cancer cases.[2] While the Her2 positive and hormone receptor-positive subtypes can be treated with targeted therapies against Her2 and endocrine therapy, the treatment of triple-negative breast cancer (TNBC) typically consists of neoadjuvant and adjuvant cytotoxic chemotherapy.[3,4] The limited effectiveness combined with a high number of toxic side effects of this treatment underlines the need for more targeted therapeutics in this disease.[5] Recently, treatment options for TNBC with immune checkpoint inhibitors, especially inhibitors of the programmed death protein 1 (PD-1)/PD-ligand 1 (PD-L1) pathway, have gained substantial interest.[3] Usually, the use of these types of treatments is recommended based on PD-L1 expression. Several studies have shown promising results with overall response rates (ORR) up to 18.5%, with a tendency for higher ORR if patients were preselected based on PD-L1 positivity.[6–9] Importantly, all these clinical trials used different antibodies and different cut-offs for PD-L1 positivity.

In 2019, the encouraging results of the Impassion130 study led to the approval of atezolizumab, a monoclonal antibody against PD-L1, for the treatment of advanced TNBC patients.[5,10,11] Selection of patients for treatment with atezolizumab was based on the SP142 assay (Ventana Medical Systems).[10] Together with the approval of atezolizumab, the US Food and Drug Administration (FDA) approved this assay as a companion diagnostic, and thus PD-L1 assessment in TNBC with the SP142 assay is increasingly requested by pathologists worldwide. At the

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moment, SP142 is the only validated assay for the selection of patients suitable for combinational treatment with atezolizumab and nab-paclitaxel in the adjuvant setting.[12] However, there are various commercially available and laboratory-developed assays for the assessment of PD-L1 using different antibodies and platforms. Moreover, contrary to the FDA, the European Medicine Agency (EMA) does not request an assessment of PD-L1 by the SP142 assay for the selection of TNBC patients eligible for atezolizumab treatment. Some of the most commonly used antibodies in daily clinical practice are the SP263 (Ventana Medical Systems) and the E1L3N (Cell Signaling Technology, Inc.) antibodies.

Multiple comparative studies have shown that SP142 identifies fewer PD-L1 positive cases than other PD-L1 antibody clones, not only in TNBC,[13–16] but also in NSCLC and urothelial carcinoma.[17–20] In this study, we compared the staining patterns of the 3 PD-L1 antibodies SP142, SP263, and E1L3N in TNBC using a tissue microarray encompassing 147 TNBC cases with associated clinicopathological data. The aim was to see whether these 3 antibodies can be used interchangeably or whether they identify different proportions and subgroups of TNBC patients as PD-L1 positive. The data are reported according to the reporting recommendations for tumor marker prognostic studies (REMARK).[21]

MATERIALS AND METHODS

Tissue Microarray and Patients Characteristics

A tissue microarray (TMA) containing 147 TNBC formalin-fixed-paraffin-embedded (FFPE) blocks collected between 1985 and 2015 at the Institute of Pathology, University Hospital Basel, was used for analysis. All samples consisted of primary TNBC, including invasive ductal (invasive carcinoma of no special type, including cases with medullary features), lobular, and adenoid-cystic subtypes. Before inclusion in the study, all cases were revised by an experienced breast pathologist (S.M.) to verify the diagnosis of TNBC. Where necessary, staining of estrogen and progesterone receptors as well as Her2 was repeated according to the ASCO/CAP guidelines.[22]

Clinicopathological information such as age at the time of diagnosis, survival, tumor localization, pT and pN stage, the diameter of the tumor, histologic diagnosis and BRE grade were collected and reported in Tables 1a and 1b

Immunohistochemistry

4 μm sections from the FFPE TMA block were cut for immunohistochemistry. The sections were pretreated with CC1. The sections were incubated with 3 different clones against PD-L1: E1L3N (dilution 1:50, Cell Signaling) for 12 minutes, SP142 (dilution 1:50) for 60 minutes, and SP263 (ready to use) for 32 minutes. DAB was used as chromogen, and counterstaining was performed with Hematoxylin (Roche). The immunostaining protocols for all antibodies were optimized and validated. All analyses were performed on the BenchMark XT automated immunostainer using the OptiView detection system (Ventana Medical System Inc.). PD-L1 expression was detectable in the cytoplasm and on the cell surface of immune cells and tumor cells.

The immunohistochemical evaluation was performed by 2 experienced breast pathologists (S.M. and T.V.). All cases were evaluated separately by the 2 pathologists. For each tissue punch, the percentage of positively stained tumor-associated immune cells per tumor area was estimated. A corresponding Hematoxylin and Eosin (H&E) section was available to ensure the scoring of immune cells and not tumor cells. According to the protocol of the Impassion130 trial,[10] a tumor was considered positive for PD-L1 expression if $\geq 1\%$ of tumor-associated immune cells were positive. Representative images of PD-L1 expression assessed with the different antibodies are shown in Fig. 1.

Statistical Analysis

To correlate the expression of all the markers tested with clinicopathological features, we used χ^2or Fisher exact test where appropriate. P values <0.05 were considered statistically significant. All analyses were performed using GraphPad Prism (v. 8).

RESULTS

Age at the time of diagnosis of the 147 female patients with TNBC ranged from 24 to 90 years (mean age 62 y).

First, we evaluated the positivity of the different antibody clones as described in the methods. PD-L1 staining was seen in 56/100 (56%) cases with SP263, in 49/100 (49%) with E1L3N, and 38/116 (32.8%) with SP142. A total of 84/147 (57%) cases were evaluable for PD-L1 analysis with all 3 antibodies. PD-L1 was positive in 50/84 TNBC (59.5%) with SP263 (mean 3.39, median 1, range 0 to 20, SD 5.5), in 44/84 (52.4%) with E1L3N (mean 2.87, median 1, range 0 to 20, SD 5.4), and in 29/84 (34.5%) with SP142 (mean 1.92, median 0, range 0 to 30, SD 4.8). We then compared these results between the different PD-L1 clones. While there was no statistical difference between the performance of SP263 and E1L3N ($P = 0.4372$), both antibodies stained significantly more cases than the SP142 antibody ($P = 0.0019$ and $P = 0.029$, respectively).

Taken together, these results suggest a higher score in staining with SP263 and E1L3N clones when compared with the SP142 clone.

PD-L1 and Tumor/Nodal Stages

We subsequently compared the different staining performances in relation with the clinicopathological data.

In the cases evaluable for the SP263 reaction, a total of 80 cases had either pT1 or pT2 stage, and 45 of these cases (56.25%) were PD-L1 positive. Nineteen cases were classified as pT3 or pT4, of which 10 (52.63%) were PD-L1 positive ($P = 0.8022$).

Within the group evaluable for the E1L3N staining, 40 (83.33%) of pT1/pT2 cases were PD-L1 positive. Fifty-one cases were pT3 or pT4, of which 40 (78.43%) were PD-L1 positive ($P = 0.6145$).

TABLE 1. Clinicopathological Data of the TNBC Patients: (a) All Cases; (b) Only Cases Evaluable With all Three Antibodies.

Clinicopathological Parameters	N. Patients	Percentage
(a) Clinicopathological data of the TNBC patients (all cases).		
mean age at diagnosis, years (±SD)	62 (±14.8)	—
mean tumor diameter, mm (±SD)	31 (±19.2)	—
histological diagnosis		
ductal	141	95.9
lobular	5	3.4
adenoid-cystic	1	0.7
pT		
1	42	28.6
2	79	53.7
3	13	8.8
4	11	7.4
na	2	1.4
pN		
0	75	51
1	42	28.6
2	14	9.5
3	10	6.8
na	6	4.1
BRE grade		
1	1	0.7
2	26	17.7
3	119	81
na	1	0.7
(b)Clinicopathological data of the TNBC patients (only cases evaluable with all 3 antibodies).		
mean age at diagnosis, years (±SD)	63 (±14.9)	
mean tumor diameter, mm (±SD)	32 (±20.7)	
histological diagnosis		
ductal	80	95.2
lobular	4	4.8
pT		
1	21	25
2	45	53.6
3	8	9.5
4	9	10.7
na	1	1.2
pN		
0	43	51.2
1	20	23.8
2	11	13.1
3	5	6.0
na	5	6.0
BRE grade		
1	0	0
2	14	16.7
3	70	83.3
na	0	0

Within the group evaluable for SP142 staining, 30 (31.91%) of 94 pT1/pT2 cases were PD-L1 positive, whereas only 7 (33.33%) of the pT3/pT4 tumors were PD-L1 positive ($P > 0.9999$).

Looking at the SP263 staining, no association between PD-L1 positivity and pN stage was seen ($P > 0.9999$), and the same was true for the E1L3N and the SP142 staining ($P = 0.5391$ and $P = 0.8406$, respectively).

Furthermore, no statistically significant association between BRE grading and PD-L1 expression for any of the different antibodies was detected. Table 2 summarizes the PD-L1 results.

DISCUSSION

In our comparative immunohistochemical study, using a cutoff of $\geq 1\%$ of tumor-associated immune cells, the SP263 and the E1L3N clones identified a significantly higher number of PD-L1 positive cases (up to 59.5%) compared with SP142 (34.5% of cases). In other words, if SP263 or E1L3N are used to screen for PD-L1 positivity in TNBC patients, more patients will be identified as having PD-L1 positive tumors and thus qualify for atezolizumab treatment, with unknown benefits and potentially unnecessary toxicities. The apparently peculiar differences between these antibodies against the same protein have already been reported.[12,16,23] Consistent with previously published studies, this illustrates that the SP263 and E1L3N antibodies are not interchangeable with SP142 due to their highly different positivity rates.[12,13,16]

Of note, ongoing clinical trials, such as the IMpassion031 phase 3 trial, are investigating the anti-PD-L1 treatment of TNBC also in the neoadjuvant setting.[24] In this study, the addition of atezolizumab resulted in improved pathologic complete response (PCR) rates, especially in the SP142-PD-L1 positive population (defined as $\geq 1\%$ PD-L1 expressing tumor-associated immune cells).[24] A phase 2 study testing the addition of durvalumab, an anti-PD-L1 antibody, to neoadjuvant chemotherapy in TNBC found higher PCR rates in patients with PD-L1 positive tumor cells who received durvalumab 2 weeks before the start of chemotherapy than patients receiving placebo.[25] PD-L1 expression was evaluated after randomization by SP263, and positivity was defined as $\geq 1\%$ of tumor cells with membranous staining or $\geq 1\%$ of tumor-infiltrating lymphocytes with membranous or cytoplasmic staining.[25] Similarly, an ongoing phase 2 trial has shown that a combination of the PD-1 inhibitor pembrolizumab and standard neoadjuvant chemotherapy more than doubles the estimated PCR not only in TNBC, but also in hormone receptor-positive/Her2 negative breast cancer.[26] However, PD-L1 expression was not investigated as a predictive marker in this trial.[26] The promising preliminary findings of these trials could lead to an increase in requests for PD-L1 testing in breast cancer, not only for advanced stage TNBC, but also for newly diagnosed and hormone receptor-positive breast cancer patients.

There are conflicting results concerning the role of PD-L1 in prognosis and survival in breast cancer. One systematic review and meta-analysis found a correlation between high PD-L1 expression with high-risk prognostic factors such as higher tumor grade, lymph node metastasis and a high number of tumor-infiltrating lymphocytes, as well as shortened overall and disease-free survival (DFS).[27] Similar results were found in another meta-analysis suggesting PD-L1 as a biomarker for poor prognosis in breast cancer.[28] However, there are also studies associating overexpression of PD-L1 with higher rates of pathologic response to chemotherapy and better survival in breast cancer.[29–31] These differences may be

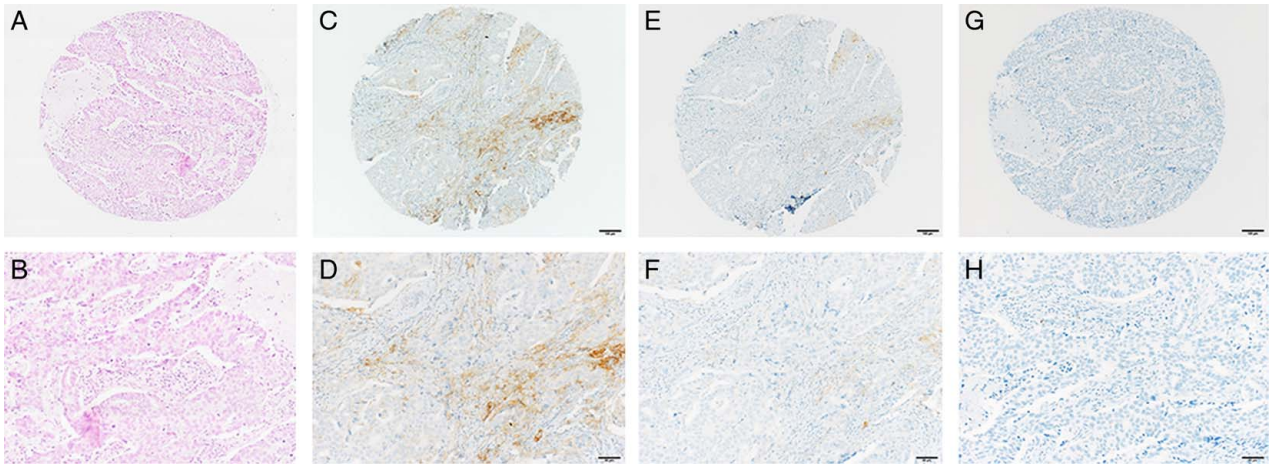

FIGURE 1. Comparison of 1 TNBC tissue punch stained with the 3 different antibodies. a + b: H&E staining, c + d: SP263, e + f: E1L3N, g + h: SP142. Upper row (A,C,E,G): magnification 100x, lower row (B,D,F,H): magnification 200x.

due to the selection of different antibodies and detection methods and heterogeneous patient collectives.[28] In our study, there was no association between PD-L1 positivity and pT or pN stage.

Currently, a harmonization of the PD-L1 status assessment in breast cancer is hampered not only by a variety of different PD-L1 clones, platforms, and scoring systems but also by the lack of robust data regarding the optimal timepoint of PD-L1 testing as well as the tissue most suitable for analysis, considering that PD-L1 expression is a dynamic process. One study found that PD-L1 expression on tumor and immune cells is concordant between the primary tumor and distant metastases in only half to two-thirds of breast cancer patients.[32] Another study in TNBC found higher and more frequent PD-L1 expression on tumor and immune cells in synchronous axillary lymph node metastases than in the corresponding primary tumor.[33] This emphasizes the need for larger standardized prospective studies to address these questions.

A limiting factor of our study is the TMA design. Since the tissue punches were chosen from the tumor center, assessment of immune cells at the border of the tumor was not possible. In addition, taking into account the irregular distribution of immune cells throughout the tumor, it is possible that centrally located intratumoral immune cells were equally missed due to the small tissue punch diameter.

As stated, due to folding or tissue loss in the TMA, we were only able to evaluate 84 out of 147 cases with all 3 antibodies. The resulting small sample size is certainly a limitation of TMA's technical circumstances and could explain the lack of statistical significance for some of our results.

TABLE 2. PD-L1 Results of Tumor-associated Immune Cells

	SP263	E1L3N	SP142
Total evaluable cases	84	84	84
PD-L1 positive (number)	50	44	29
PD-L1 positive (percentage)	59.5	52.4	34.5

CONCLUSIONS

Our data show that the 3 PD-L1 clones SP142, SP263, and E1L3N identify a different number of PD-L1 positive cases when using a cut-off of $\geq 1\%$ of tumor-associated immune cells and can therefore not be used interchangeably. Further studies are needed to investigate the role and impact of different PD-L1 antibody clones as predictive markers for treatment with immune checkpoint inhibitors in patients with breast cancer.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424.
2. Bauer KR, Brown M, Cress RD, et al. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007;109:1721–1728.
3. Planes-Laine G, Rochigneux P, Bertucci F, et al. PD-1/PD-L1 targeting in breast cancer: The first clinical evidences are emerging. A literature review. *Cancers*. 2019;11:1033.
4. Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 4)†. *Ann Oncol*. 2018;29:1634–1657.
5. Li CH, Karantza V, Aktan G, et al. Current treatment landscape for patients with locally recurrent inoperable or metastatic triple-negative breast cancer: a systematic literature review. *Breast Cancer Res*. 2019;21:143.
6. Lazarus G, Audrey J, Iskandar AWB. Efficacy and safety profiles of programmed cell death-1/programmed cell death ligand-1 inhibitors in the treatment of triple-negative breast cancer: A comprehensive systematic review. *Oncol Rev*. 2019;13:425.

7. Nanda R, Chow LQM, Dees EC, et al. Pembrolizumab in Patients with advanced triple-negative breast cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol*. 2016;34:2460–2467.
8. Adams S, Schmid P, Rugo HS, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. *Ann Oncol: official journal of the European Society for Medical Oncology*. 2019;30:397–404.
9. Adams S, Loi S, Toppmeyer D, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. *Ann Oncol: official journal of the European Society for Medical Oncology*. 2019;30:405–411.
10. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med*. 2018;379:2108–2121.
11. FDA. FDA approves atezolizumab for PD-L1 positive unresectable locally advanced or metastatic triple-negative breast cancer. Available at: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-atezolizumab-pd-l1-positive-unresectable-locally-advanced-or-metastatic-triple-negative. (Accessed January 27, 2021).
12. Rugo H, Loi S, Adams S, et al Exploratory analytical harmonization of PD-L1 immunohistochemistry assays in advanced triple-negative breast cancer: A retrospective substudy of IMpassion130 (Abstract). *San Antonio Breast Cancer Symposium*. San Antonio, Texas, USA,2019.
13. Scott M, Scorer P, Barker C, et al. Comparison of patient populations identified by different PD-L1 assays in triple-negative breast cancer (TNBC)(abstract). *Ann Oncol*. 2019;30:III4.
14. Noske A, Ammann J, Wagner D-C, et al. Reproducibility and concordance of 4 clinically developed programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) assays in triple negative breast cancer (TNBC)(abstract). *Ann Oncol*. 2019;30:v130–v131.
15. Rugo H, Loi S, Adams S, et al. Performance of PD-L1 immunohistochemistry (IHC) assays in unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC): Post-hoc analysis of IMpassion130 (abstract). *Ann Oncol*. 2019;30:v858–v859.
16. Reisenbichler ES, Han G, Bellizzi A, et al. Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. *Mod Pathol*. 2020;33:1746–1752.
17. Tretiakova M, Fulton R, Kocherginsky M, et al. Concordance study of PD-L1 expression in primary and metastatic bladder carcinomas: comparison of four commonly used antibodies and RNA expression. *Mod Pathol*. 2018;31:623–632.
18. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: Results of blueprint phase 2 project. *J Thorac Oncol*. 2018;13:1302–1311.
19. Schwamborn K, Ammann JU, Knüchel R, et al. Multicentric analytical comparability study of programmed death-ligand 1 expression on tumor-infiltrating immune cells and tumor cells in urothelial bladder cancer using four clinically developed immunohistochemistry assays. *Virchows Arch*. 2019;475:599–608.
20. Gonzalez-Ericsson PI, Stovgaard ES, Sua LF, et al. The path to a better biomarker: application of a risk management framework for the implementation of PD-L1 and TILs as immuno-oncology biomarkers into breast cancer clinical trials and daily practice. *J Pathol*. 2020:250;667–684.
21. Sauerbrei W, Taube SE, McShane LM, et al. Reporting recommendations for tumor marker prognostic studies (REMARK): An abridged explanation and elaboration. *J Natl Cancer Inst*. 2018;110:803–811.
22. Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med*. 2014;138:241–256.
23. Munari E, Zamboni G, Lunardi G, et al. PD-L1 expression in non-small cell lung cancer: evaluation of the diagnostic accuracy of a laboratory-developed test using clone E1L3N in comparison with 22C3 and SP263 assays. *Hum Pathol*. 2019;90:54–59.
24. Mittendorf EA, Zhang H, Barrios CH, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. *Lancet*. 2020;396:1090–1100.
25. Loibl S, Untch M, Burchardi N, et al. A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. *Ann Oncol*. 2019;30:1279–1288.
26. Nanda R, Liu MC, Yau C, et al. Effect of Pembrolizumab Plus Neoadjuvant Chemotherapy on Pathologic Complete Response in Women With Early-Stage Breast Cancer: An Analysis of the Ongoing Phase 2 Adaptively Randomized I-SPY2 Trial. *JAMA Oncol*. 2020;6:676–684.
27. Li S, Chen L, Jiang J. Role of programmed cell death ligand-1 expression on prognostic and overall survival of breast cancer: a systematic review and meta-analysis. *Medicine*. 2019;98:e15201.
28. Guo Y, Yu P, Liu Z, et al. Prognostic and Clinicopathological Value of Programmed Death Ligand-1 in Breast Cancer: A Meta-Analysis. *PLoS ONE*. 2016;11:e0156323.
29. Sabatier R, Finetti P, Mamessier E, et al. Prognostic and predictive value of PDL1 expression in breast cancer. *Oncotarget*. 2015;6:5449–5464.
30. Bertucci F, Finetti P, Colpaert C, et al. PDL1 expression in inflammatory breast cancer is frequent and predicts for the pathological response to chemotherapy. *Oncotarget*. 2015;6:13506–13519.
31. Schalper KA, Velcheti V, Carvajal D, et al. In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas. *Clin Cancer Res*. 2014;20:2773–2782.
32. Manson QF, Schrijver W, Ter Hoeve ND, et al. Frequent discordance in PD-1 and PD-L1 expression between primary breast tumors and their matched distant metastases. *Clin Exp Metastasis*. 2019;36:29–37.
33. Li M, Li A, Zhou S, et al. Heterogeneity of PD-L1 expression in primary tumors and paired lymph node metastases of triple negative breast cancer. *BMC Cancer*. 2018;18:4.