Consistent expression of PD-L1 in tumor microenvironment with peripheral PD-1/PD-L1 in circulating T lymphocytes of operable breast cancer: a diagnostic test

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Abstract

Background: The expression of PD-L1 in the immune microenvironment can guide the application of immunosuppressants. In order to monitor the immune status of the body, repeated biopsies have to be taken. Our research aims to find new and convenient means to evaluate this indicator.

Methods: Eighty-three cases of newly diagnosed operable breast cancer without receiving preoperative treatment, were recruited from Beijing Shijitan Hospital between November 2018 and November 2019. The expression of PD-1/PD-L1 on circulating T lymphocytes was detected by flow cytometry and the expression of PD-L1 on immune cells in tumor microenvironment was detected by immunohistochemistry.

Results: The median percentage of positive PD-1 and PD-L1 expression on circulating T lymphocytes was 15.2% and 0.7%, respectively. The peripheral PD-1 had no relationship with clinicopathological characteristics, but the peripheral PD-L1 expression had a correlation with lymph node metastasis ($p = 0.005$) and Her-2 expression ($p = 0.034$) ($p < 0.05$). The positive rate of PD-L1 expression was 32.9% in tumor microenvironment. PD-L1 expression in tumor microenvironment had a significant correlation with PD-1/PD-L1 expression on circulating T lymphocytes, the correlation coefficients being 0.24 ($p < 0.05$) and 0.26 ($p < 0.05$), respectively. To predict the PD-L1 expression in tumor microenvironment, the area under the receiver operating characteristic curve was 0.65 and 0.66 for peripheral PD-1 and PD-L1, respectively. High level of peripheral PD-1/PD-L1 expression was associated with the odds ratios of 5.42 and 4.76 for positive PD-L1 expression in tumor microenvironment.

Conclusion: Peripheral PD-1/PD-L1 expression had a significant consistency with PD-L1 expression in tumor microenvironment and could act as an alternative choice of tissue detection, for the patients intolerable of biopsy.

Keywords: Peripheral PD-1/PD-L1, Tumor microenvironment, Consistent expression, Breast cancer

Background

With the severe challenge from breast cancer (BC) worldwide, novel therapeutic options are emerging in recent years [1–3]. The immune checkpoint inhibitors (ICIs) were approved to improve the prognosis of non-small cell lung cancer, melanoma, and many solid tumors [4, 5]. Triple-negative breast cancer (TNBC) is a particular subtype

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of BC, characterized by negative expression of hormone receptors and Her-2. TNBC had a high number of tumor-infiltrated lymphocytes (TILs) in tumor environment (TME) and was approved for the immunotherapy of PD-1/PD-L1 inhibitors [6]. The PD-L1 positive rate was reported to range from 38 to 78% in TNBC, and the variation was related with the ethnicity of patients, previous treatment and metastasis [7–10]. TME expression of PD-1/PD-L1 related with the ethnicity of patients, previous treatment to range from 38 to 78% in TNBC, and the variation was reported to be correlated to the clinicopathological characteristics and the clinical responses to ICIs [7, 11, 12].

Immune checkpoint molecules on peripheral lymphocytes changed dramatically with treatment and reflected the clinical efficacy of anti-cancer treatment [13]. However, tissue biopsy is time-consuming, and traumatic. More than half patients were fearful and anxious to biopsy, and 5.2% of them had complications. Liquid biopsy testing in peripheral blood can avoid these limitations [14, 15], and provided an alternative choice for patients intolerable to biopsy. This study evaluated the feasibility of alternative PD-1/PD-L1 on circulating T lymphocytes to TME PD-L1 expression in BC patients.

Methods

Aim

The purpose of this study was to explore the correlation and consistency of PD-1/PD-L1 expression on circulating T lymphocytes and PD-L1 expression in TME.

Ethical approval

This study was approved by the Ethics Committee of Beijing Shijitan Hospital, Capital Medical University. Patients provided the written informed consent.

Patients

Eighty-three cases of operable BC patients were recruited at Beijing Shijitan Hospital between November 2018 and November 2019. Patients did not have any invasions to skin or chest wall. Patients did not have any diagnosis of inflammatory BC, autoimmune diseases, heart, brain, kidney, or other vital organs insufficiency. Patients should have the Eastern Cooperative Oncology Group (ECOG) > 2 and did not receive any preoperative treatments. Peripheral blood samples were collected preoperatively and tumor tissues were obtained through biopsy or surgery.

Tumor size was categorized by the diameter, ≤ 2 cm (T1), ≤ 5 cm (T2) and > 5 cm (T3). Clinical tumor node metastasis (cTNM) stage was classified as I (T1N0M0), II (T0–1 N1 M0, T2N0–1 M0, T3N0 M0) and III (T0–2 N2 M0, T3 N1–2 M0, T4 N0–3 M0, T0–4 N3 M0) stage. Histological grade was defined by the scores estimated according to the glandular duct formation, nuclear pleomorphism and mitotic ability. The score range of grade I II and III was 3 to 5, 6 to 7 and 8 to 9, respectively.

Statistical analysis

All data were analyzed by SPSS software (version 23.0). The Mann–Whitney U test was used to analyze the relationship between age, lymph node metastasis, ER, PR, HER-2, Ki-67 index and the positive levels of PD-1/PD-L1 on circulating T lymphocytes. Spearman correlation test was used to analyze the relationship between tumor size, cTNM stage, histological grade and the positive levels of
PD-1/PD-L1 on circulating T lymphocytes. Kruskal–Wallis test was used to analyze the relationship between the molecular subtype and the positive levels of PD-1/PD-L1 on circulating T lymphocytes. \( \chi^2 \) test was used to analyze the relationship between age, lymph node metastasis, ER, PR, HER-2, Ki-67 index, molecular subtype and the TME PD-L1 expression; Mann–Whitney U test was used to analyze the relationship between the tumor size, cTNM stage, histological grade and the TME PD-L1 expression. Spearman correlation test was used to analyze the relationship between the positive levels of PD-1/PD-L1 on circulating T lymphocytes and the positive levels of TME PD-L1.

The receiver operative character (ROC) curve was illustrated between the peripheral PD-1/PD-L1 and TME PD-L1 expression, and the validity was estimated by the area under the curve (AUC). The cut-off value of peripheral PD-1/PD-L1 versus TME PD-L1 expression was calculated and the percentage of PD-1/PD-L1 on circulating T cells was transformed to categorical variable by the cut-off value. The odds ratios of expression of peripheral PD-1/PD-L1 in the categorical variables to TME PD-L1 expression were estimated in Logistical Regression Model with age adjustment. All analyses were two-tailed and significant level was 0.05.

Results

The positive levels of PD-1/PD-L1 in peripheral blood samples and TME

In peripheral blood, CD3\(^+\) circulating T lymphocytes had positive expression of PD-1 (Fig. 1A) and PD-L1 (Fig. 1B). The median percentage of PD-1/PD-L1 positive T lymphocytes was 15.2% and 0.7%, respectively. TME PD-L1 expression in immune cells had a heterogeneity (Fig. 2A and B) and the median percentage was 32.9%.

Correlation with clinicopathological characteristics

The positive levels of PD-1/PD-L1 on circulating T lymphocytes did not have any correlation with age, tumor size,
**Fig. 2** The high and the low level of PD-L1 positive immune cells in TME, A Patients with low level of PD-L1 positive immune cells in TME (IHC × 400), B Patients with high level of PD-L1 positive immune cells in TME (IHC × 400)

**Table 1** The relationship between the percentage of PD-1/PD-L1 positive circulating T lymphocytes and clinicopathological characters

|                         | n   | Percentage of PD-1/PD-L1 positive circulating T lymphocytes (%) | PD-1, median (IQR) | P   | PD-L1, median (IQR) | P   |
|-------------------------|-----|-----------------------------------------------------------------|-------------------|-----|---------------------|-----|
| Age[^a^]                |     |                                                                  |                   |     |                     |     |
| ≤50                     | 25  | 14.40(8.30–37.90)                                               | 0.80(2.00–3.40)   |     |                     |     |
| >50                     | 52  | 15.25(3.60–41.20)                                               | 0.757             | 0.006| 0.70(0–6.50)        | 0.806|
| Tumor size[^b^]         |     |                                                                  |                   |     |                     |     |
| T1                      | 43  | 16.50(3.60–39.70)                                               | 0.90(0.20–6.50)   |     |                     |     |
| T2                      | 30  | 12.80(8.80–41.20)                                               | 0.70(0–3.40)      |     |                     |     |
| T3                      | 4   | 12.10(8.60–14.60)                                               | 0.227             | 0.352| 0.55(0.40–1.10)     | 0.352|
| Lymph node metastasis[^c^] |   |                                                                  |                   |     |                     |     |
| Yes                     | 21  | 15.20(3.60–39.70)                                               | 1.40(0.30–3.50)   | 0.005|                     |     |
| No                      | 55  | 14.60(6.80–41.20)                                               | 0.816             | 0.005| 0.60(0–3.40)        | 0.005|
| cTNM stage[^b^]         |     |                                                                  |                   |     |                     |     |
| I                       | 37  | 16.50(8.20–39.70)                                               | 0.60(0.20–6.50)   |     |                     |     |
| II                      | 24  | 17.15(3.60–41.20)                                               | 0.90(0–3.40)      |     |                     |     |
| III                     | 16  | 11.20(8.60–19.80)                                               | 0.066             | 0.265| 0.55(0.10–2.50)     | 0.265|
| Histological grade[^b^] |     |                                                                  |                   |     |                     |     |
| I                       | 6   | 14.20(12.80–18.50)                                              | 1.15(0.10–3.20)   |     |                     |     |
| II                      | 43  | 16.30(3.60–28.10)                                               | 0.70(0.20–3.40)   |     |                     |     |
| III                     | 8   | 25.40(8.30–41.20)                                               | 0.284             | 0.549| 1.30(0.40–3.50)     | 0.549|
| ER[^a^]                 |     |                                                                  |                   |     |                     |     |
| Positive                | 54  | 15.25(3.60–37.90)                                               | 0.70(0.10–3.40)   | 0.922|                     |     |
| Negative                | 22  | 15.55(8.30–41.20)                                               | 0.637             | 0.947| 0.70(0–6.50)        | 0.947|
| PR[^a^]                 |     |                                                                  |                   |     |                     |     |
| Positive                | 50  | 15.25(3.60–41.20)                                               | 0.65(0.10–3.40)   |     |                     |     |
| Negative                | 26  | 15.55(8.30–39.70)                                               | 0.996             | 0.034| 0.75(0–6.50)        | 0.034|
| HER-2[^a^]              |     |                                                                  |                   |     |                     |     |
| Positive                | 15  | 12.90(8.30–30.10)                                               | 0.50(0–3.20)      | 0.158|                     |     |
| Negative                | 58  | 15.55(3.60–41.20)                                               | 0.432             | 0.034| 0.90(0.10–3.50)     | 0.034|
| Ki-67 index[^a^]        |     |                                                                  |                   |     |                     |     |
| Low(≤ 14%)              | 22  | 15.10(8.20–28.10)                                               | 0.450             | 0.158| 0.70(0.20–6.50)     | 0.158|
| High (> 14%)            | 53  | 14.60(3.60–41.20)                                               | 0.807             |       |                     |       |
| Molecular subtype[^c^]  |     |                                                                  |                   |     |                     |     |
| Luminal A               | 18  | 14.25(8.20–26.00)                                               | 0.70(0.20–2.90)   |     |                     |     |
| Luminal B               | 36  | 16.50(3.60–37.90)                                               | 1.10(0.20–3.40)   |     |                     |     |
| TNBC                    | 13  | 20.79(8.60–41.20)                                               | 1.45(0.40–3.50)   |     |                     |     |
| HER-2 overexpression    | 8   | 11.05(8.30–28.10)                                               | 0.450             | 0.152| 0.40(0–6.50)        | 0.152|

[^a^] Mann-Whitney U test,[^b^] Spearman correlation test,[^c^] Kruskal-Wallis test
cTNM stage, histological grade, ER and PR status, Ki-67 index and molecular subtype (Table 1). The median percentage of PD-L1 positive circulating T lymphocytes was 1.4% in patients with lymph node metastasis, significantly higher than that in patients without lymph node metastasis (0.6%, Table 1); The median percentage of PD-L1 positive circulating T lymphocytes in HER-2 positive patients was 0.5%, significantly lower than that in HER-2 negative patients (0.9%, Table 1). The median percentage of PD-1 positive circulating T lymphocytes had no relationship with lymph node metastasis and HER-2 expression (Table 1).

The percentage of PD-L1 positive immune cells in TME had no relationship with age, tumor size, lymph node metastasis, cTNM stage, histological grade, ER/PR and HER-2, Ki-67 index and molecular subtype (Table 2).

The consistency between peripheral and TME PD-1/PD-L1 expression
The correlation coefficients between percentage of PD-1/ PD-L1 positive circulating T lymphocytes and percentage of PD-L1 positive immune cells in TME were 0.24 ($p=0.046$) and 0.26 ($p=0.034$), respectively.

Table 2 The relationship between PD-L1 expression in TME and clinicopathological characters

|                        | PD-L1 expression |        | P   |
|------------------------|------------------|--------|-----|
|                        | Positive (n = 24) | Negative (n = 49) |     |
| Age, n(%)$^a$          |                  |        |     |
| ≤ 50                   | 8(33.33%)        | 18(36.73%) | 0.776 |
| > 50                   | 16(66.67%)       | 31(63.27%) |        |
| Tumor size, n(%)$^b$   |                  |        |     |
| T1                     | 14(58.33%)       | 28(57.14%) | 0.930 |
| T2                     | 9(37.50%)        | 19(38.78%) |        |
| T3                     | 1(4.17%)         | 2(4.08%)  |        |
| Lymph node metastasis, n(%)$^a$ |            |        |     |
| Yes                    | 7(29.17%)        | 13(26.53%) | 0.812 |
| No                     | 17(70.83%)       | 36(73.47%) |        |
| cTNM stage, n(%)$^b$   |                  |        |     |
| I                      | 13(54.17%)       | 24(48.98%) |        |
| II                     | 6(25.00%)        | 15(30.61%) |        |
| III                    | 5(20.83%)        | 10(20.41%) |        |
| Histological grade, n(%)$^b$ |             |        |     |
| I                      | 15(62.50%)       | 4(10.26%)  | 0.131 |
| II                     | 14(70.00%)       | 31(79.49%) |        |
| III                    | 5(25.00%)        | 4(10.26%)  |        |
| ER, n(%)$^a$           |                  |        |     |
| Positive               | 16(66.67%)       | 38(77.55%) | 0.319 |
| Negative               | 8(33.33%)        | 11(22.45%) |        |
| PR, n(%)$^a$           |                  |        |     |
| Positive               | 15(62.50%)       | 33(67.35%) | 0.682 |
| Negative               | 9(37.50%)        | 16(32.65%) |        |
| HER-2, n(%)$^a$        |                  |        |     |
| Positive               | 7(29.17%)        | 8(16.67%)  | 0.218 |
| Negative               | 17(70.83%)       | 40(83.33%) |        |
| Ki-67 index, n(%)$^a$  |                  |        |     |
| Low(≤ 14%)             | 6(25.00%)        | 15(31.25%) | 0.582 |
| High (> 14%)           | 18(75.00%)       | 33(68.75%) |        |
| Molecular subtype, n(%)$^c$ |             |        |     |
| Luminal A              | 5(20.83%)        | 13(26.53%) |        |
| Luminal B              | 11(45.83%)       | 25(51.02%) |        |
| TRIPLE-NEGATIVE BREAST CANCER | 4(16.67%) | 7(14.29%)  |        |
| HER-2 overexpression   | 4(16.67%)        | 48(16.67%) | 0.317 |

$^a$ Chi-square test, $^b$ Mann-Whitney U test, $^c$ Kruskal-Wallis test
The AUC between the percentage of PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 expression was 0.65 (95%CI 0.53, 0.76) and 0.66 (95%CI 0.54, 0.77) (Fig. 3), with the cut-off values of 14.6% and 1.1%, respectively.

Higher percentage of PD-1/PD-L1 positive circulating T lymphocytes in peripheral blood was associated with a 5.42-fold ($p = 0.007$) and 4.76-fold high probability to be TME PD-L1 positive ($p = 0.005$, Table 3).

**Subgroup analysis in molecular subtypes**

In Luminal A subtype, the AUC value was 0.86 (95%CI = 0.62–0.98, $p < 0.001$) and 0.73 (95%CI = 0.47–0.91, $p = 0.12$) between PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 positive expression (Fig. 4A). In Luminal B subtype, the AUC value was 0.60 (95%CI = 0.42–0.77, $p = 0.317$) and 0.65 (95%CI = 0.47–0.81, $p = 0.209$) between PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 positive expression (Fig. 4B). In TNBC subtype, the AUC value was 0.71 (95%CI = 0.38–0.93, $p = 0.313$) and 0.75 (95%CI = 0.41–0.95, $p = 0.175$) between PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 positive expression (Fig. 4C). In HER-2 overexpression subtype, the AUC value was 1.00 (95%CI = 0.54–1.000, $p < 0.001$) and 0.50 (95%CI = 0.12–0.88, $p > 0.999$) between PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 positive expression (Fig. 4D).

**Discussion**

In this study, we observed a significant consistency of peripheral PD-1/PD-L1 expression with TME PD-L1 expression. BC patients with higher level of peripheral PD-1/PD-L1 were more likely to be PD-L1 positive in TME.

The Impassion 130 study revealed that PD-L1 inhibitor Atezolizumab combined with chemotherapy significantly prolonged the median PFS by 2.5 months in PD-L1 positive patients compared with chemotherapy alone [7, 8]. In our study, PD-1/PD-L1 expression on circulating T lymphocytes had a significant consistency with the PD-L1 expression in TME. Immune checkpoint proteins, including CTLA-4, CD28 and PD-1, are co-stimulatory or co-suppressive proteins expressed on the surface of antigen-presenting cells and T lymphocytes, and maintain the immune balance by up- or down-regulating T lymphocyte functions [18, 19]. Tumor cells express PD-L1 and bind to PD-1 on the immune cells to escape recognition and elimination from the immune system.
The link between PD-1 and PD-L1 down-regulated the cell counts and function of T lymphocytes, and induced occurrence, progression and drug resistance of malignant cells [20, 21].

Higher level of TILs indicated a better prognosis of BC [22–24]. PD-1/PD-L1 expression in TME was the indicator for PD-1 inhibitor therapy [25, 26], and more than 1% expression rate of PD-L1 was eligible for atezolizumab treatment for BC [22, 26]. But the expression of immune checkpoint proteins in peripheral blood and TME was dynamically changing during treatment [27–30]. It is necessary to repeat tissue biopsies for the optimistic treatments. For the inconvenient tissue biopsy, liquid

### Table 3

The correlation between percentage of PD-1/PD-L1 positive circulating T lymphocytes and TME PD-L1 expression in Logistic Regression Model

| Percentage of PD-1 positive circulating T lymphocytes | Crude Odds ratio (95%CI) | P | Adjusted Odds ratio (95%CI) | P |
|-----------------------------------------------------|--------------------------|---|-----------------------------|---|
| ≤ 14.6%                                             | 1.00                     | 1.00 |                            | 1.00 |
| > 14.6%                                             | 5.16 (1.61–16.51)        | 0.006 | 5.42 (1.66–17.67)          | 0.005 |

| Percentage of PD-L1 positive circulating T lymphocytes | ≤ 1.1% | 1.00 | 1.00 |
|-------------------------------------------------------|--------|-----|-----|
| > 1.1%                                                | 4.93 (1.64–14.99)        | 0.005 | 4.76 (1.54–14.72)          | 0.007 |

* adjusting age

### Fig. 4

ROC curve of peripheral PD-1/PD-L1 expression and TME PD-L1 expression in molecular subtypes. A Luminal A, B Luminal B, C TNBC, D HER-2 overexpression
biopsy of peripheral blood became the alternative, and circulating T lymphocytes were reported to reflect the condition of TILs in TME [27, 31]. PD-1/PD-L1 expression on circulating T lymphocytes had a significant consistency with the TME PD-L1 expression, especially in Luminal A and HER-2 overexpression subtypes.

PD-L1 expression on circulating T lymphocytes was related to lymph node metastasis and HER-2 expression, which were not consistent with other studies [32–34]. This might be caused by the application of different antibodies and scoring standards. Since Atezolizumab was the first approved checkpoint inhibitor of PD-1/PD-L1 for BC immunotherapy, we chose the recommended antibody SP142 and the results are more reliable [26, 35]. Previous treatments and clinical stage affected PD-1/ PD-L1 expression and immunotherapy efficacy [36–40]. The recruited patients were in early or mid-stage and never received any treatment preoperatively. Heterogeneity of research subjects between studies contributed to the variation in results. The positive rate of TME PD-L1 was 36%, in consistent with the Impassion130 trial in Japan [9].

The limited sample size was the first limitation in this study. The low percentage of PD-L1 positive circulating T lymphocytes was the second limitation. Lack of soluble PD-1/PD-L1 detection in peripheral blood was another limitation.

Conclusions
Peripheral PD-1/PD-L1 expression on circulating T lymphocytes had a certain correlation and consistency with TME PD-L1 expression, and was potential to be an alternative to the TME detection, especially for the patients intolerable to tissue biopsy. However, this result still needs to be verified with a larger sample size and we will definitely continue to conduct subsequent studies.
8. Schmid P, Rugo HS, Adams S, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2020;21(1):44–59.

9. Iwata H, Inoue K, Kaneko K, et al. Subgroup analysis of Japanese patients in a Phase 3 study of atezolizumab in advanced triple-negative breast cancer (IMpassion130). Jpn J Clin Oncol. 2019;49(12):1083–91.

10. Ho AY, Barker CA, Arnold BB, et al. A phase 2 clinical trial assessing the efficacy and safety of pembrolizumab and radiotherapy in patients with metastatic triple-negative breast cancer. Cancer. 2020;126(4):850–60.

11. Huang W, Ran R, Shao B, Li H. Prognostic and clinicopathological value of PD-L1 expression in primary breast cancer: a meta-analysis. Breast Cancer Res Treat. 2019;178(1):17–33.

12. Wyss J, Dislich B, Koeberl VH, et al. Stromal PD-1/PD-L1 Expression Predicts Outcome in Colon Cancer Patients. Clin Colorectal Cancer. 2019;18(1):e20–38.

13. Vilain RE, Mennes AM, Wilmott JS, et al. Dynamic Changes in PD-L1 Expression and Immune Infiltrates Early During Treatment Predict Response to PD-1 Blockade in Melanoma. Clin Cancer Res. 2017;23(7):5024–33.

14. Overman MJ, Modak J, Kopetz S, et al. Use of research biopsies in clinical trials: are risks and benefits adequately addressed? J Clin Oncol. 2015;33(11):17–22.

15. Aquilin M, Oza AM, Pond GR, Siu LL. Impact and perceptions of mandatory tumor biopsies for correlative studies in clinical trials of novel anticancer agents. J Clin Oncol. 2006;24(30):4801–7.

16. Taube JM, Klein A, Brahmer JR, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res. 2014;20(19):5064–74.

17. Vennapusa B, Baker K, Kowamez M, et al. Development of a PD-L1 Complementary Diagnostic Immunohistochemistry Assay (SP142) for Atezolizumab. Appl Immunohistochem Mol Morphol. 2019;27(2):92–100.

18. Lim S, Phillips JB, Madeira da Silva L, et al. Interplay between Immune Checkpoint Proteins and Cellular Metabolism. Cancer Res. 2017;77(6):1245–9.

19. Rowshanhavan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131(1):58–67.

20. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25(21):9543–53.

21. Kuzurozumi S, Fuji T, Matsumoto H, et al. Significance of evaluating tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) expression in breast cancer. Med Mol Morphol. 2017;50(4):185–94.

22. Du H, Yi Z, Wang L, Li Z, Niu B, Ren G. The co-expression characteristics of LAG3 and PD-1 on the T cells of patients with breast cancer reveal a new therapeutic strategy. Int Immunopharmacol. 2020;80:106113.

23. Syed Khaja AS, Toor SM, El Salhat H, et al. Preferential accumulation of regulatory T cells with highly immunosuppressive characteristics in breast tumor microenvironment. Oncotarget. 2017;8(20):33159–71.

24. Incorvaia L, Fanale D, Badalamenti G, et al. Programmed Death Ligand 1 (PD-L1) as a Predictive Biomarker for Pembrolizumab Therapy in Patients with Advanced Non-Small-Cell Lung Cancer (NSCLC). Adv Ther. 2019;36(10):2600–17.

25. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018;379(22):2108–21.

26. Pico de Coana Y, Wolodarski M, van der Haar Avila I, et al. PD-1 checkpoint blockade in advanced melanoma patients: NR cells, monocyteic subsets and host PD-L1 expression as predictive biomarker candidates. Oncoimmunology. 2020;9(11):1786888.

27. Kamada T, Togashi Y, Tay C, et al. PD-1 (+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. Proc Natl Acad Sci U S A. 2019;116(20):9999–10008.

28. Zhang L, Wang J, Wei F, et al. Profiling the dynamic expression of checkpoint molecules on cytokine-induced killer cells from non-small-cell lung cancer patients. Oncotarget. 2016;7(28):43604–15.

29. Han JJ, Kim DW, Koh J, et al. Change in PD-L1 Expression After Acquiring Resistance to Gefitinib in EGFR-Mutant Non-Small-Cell Lung Cancer. Clin Lung Cancer. 2016;17(4):265–70 (e262).

30. Callahan MK, Wolchok JD. Reconstruct or Reboot? How Does Anti-PD-1 Therapy Change Tumor-Infiltrating Lymphocytes? Cancer Cell. 2019;36(3):215–7.

31. 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(9):1746–52.

32. Muenst S, Schaerli AR, Gao F, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat. 2014;146(1):15–24.

33. Sun WY, Lee YK, Koo JS. Expression of PD-L1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med. 2016;14(1):173.

34. Kim MJ, Adams S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): Where to go from here? Cancer. 2018;124(10):2086–103.

35. Samanta D, Park Y, Ni X, et al. Chemotherapy induces enrichment of CD47(+)/CD73(+)/PDL1(+) immune evasive triple-negative breast cancer cells. Proc Natl Acad Sci U S A. 2018;115(6):E1239–48.

36. Takeuchi S, Toyokawa G, Takada K, Shoji F, Okamoto T, Maehara Y. Combination Therapy of Radiotherapy and Anti-PD-1/PD-L1 Treatment in Non-Small-Cell Lung Cancer: A Mini-review. Clin Lung Cancer. 2018;19(1):12–6.

37. Takamori S, Toyokawa G, Takada K, Shoji F, Okamoto T, Maehara Y. Combination Therapy of Radiotherapy and Anti-PD-1/PD-L1 Treatment in Non-Small-Cell Lung Cancer. Semin Oncol. 2018;45(1):61–70.

38. Rojko L, Reiniger L, Teglasi V, et al. Soluble PD-L1 Expression in Circulation as a Predictive Marker for Recurrence and Prognosis in Gastric Cancer: Direct Comparison of the Clinical Burden Between Tissue and Serum PD-L1 Expression. Ann Surg Oncol. 2019;26(3):876–83.

39. Rowshanravan B, Halliday N, Sansom DM. PD-L1 expression in primary breast cancer patients. Oncotarget. 2016;7(28):43604–15.

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