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Primary tumor resection for initially staged IV breast cancer

An emphasis on programmed death-ligand 1 expression, promoter methylation status, and survival

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Abstract

Conventional therapy modalities for advanced breast cancer are problematic, whereas checkpoint blockade immunotherapy has been considered as a promising approach. This study aims to determine programmed death-ligand 1 (PD-L1) expression and methylation status of PD-L1 promoter in primary tumor tissue and metastatic foci of patients with stage IV breast cancer.

Clinicopathological data and survival rates of 57 breast cancer patients, who were initially staged IV, and operated for intact tumors, were retrospectively analyzed. Immunohistochemical analysis of PD-L1 using 57 primary tumors, 33 paired metastatic lymph nodes, and 14 paired distant metastases was performed. Additionally, the methylation rate of the PD-L1 gene promoter region was determined with real-time polymerase chain reaction (PCR) analysis in 38 samples.

Overall PD-L1 expression in primary tumors was 23.1% (12/52). PD-L1 positivity was reduced in lymph nodes by 15.2% (5/33) and in distant metastases by 21.4% (3/14). PD-L1 expression diverged between primary and metastatic foci in a subset of cases (18.2% for lymph node and 33.3% for distant metastasis). In general, the PD-L1 promoter was not methylated, and mean methylation rates were low (min. 0%–max. 21%). We observed no correlation between PD-L1 expression, promoter methylation, and survival.

Neither the expression nor the methylation status of PD-L1 in patients, who were presented with stage IV breast cancer and operated for an intact primary tumor, had a statistically significant relation with survival. Discordance in PD-L1 expression between primary tumor and metastasis should be considered during pathological and clinical management of patients who would undergo checkpoint blockade therapy.

Abbreviations: ER = estrogen receptor, Her2 = human epithelial growth factor receptor 2, PCR = polymerase chain reaction, PD-L 1 = programmed death-ligand 1.

Keywords: breast, cancer, checkpoint blockade, immunotherapy, PD-L1

1. Introduction

Breast cancer treatment has evolved over the last few decades, and localized disease is now potentially curable with conventional treatment modalities. However, the treatment of metastatic disease is much more complicated. Some patients (6%–10%) present with metastatic disease at the time of diagnosis and some in whom metastasis cannot be detected during the initial diagnosis, might advance to a metastatic disease shortly after. There is a need for new treatment strategies that prolong overall survival and improve patient quality of life.[1] Chemotherapy, radiotherapy, hormonotherapy, and biologic agents are used in various combinations for the treatment of breast cancer, with a 30% overall survival increment, with regard to all stages, over the last 30 years. Nevertheless, this increase in the survival rate is only 3% at stage IV, and breast cancer remains one of the leading causes of death among women worldwide.[2,3] For initial stage IV patients and the patients who have progressed under standard care, immune therapy can be a new and promising treatment option.[4] The immune system plays a critical role in both the development and metastasis of cancer and checkpoint molecules are a crucial part of the equilibrium between stimulant and inhibitory mechanisms. Checkpoint molecules, such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1) molecules, expressed mainly on T cells, B cells, and natural killer cells, have a negative effect on T cell-mediated immune response.[5,6] PD-1 is a transmembrane protein, and its binding to the ligand programmed cell death-ligand 1 (PD-L1), and to a lesser extent, to the ligand programmed cell death-ligand 2 (PD-L2), activates the PD-1 pathway, attenuates T cell activity, and increases the function of the immune suppressive subtype of T cells, the regulatory T (T regs) cells.[7,8] PD-1 also plays an inhibitory role in the response of B cells, the activation of which

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causes susceptibility to apoptosis; an immune evasion mechanism used by the tumor cells.[6] Increased PD-L1 expression is often associated with negative prognostic factors and decreased survival in many cancers.[5,7] The anti-PD-1 or anti-PD-L1 monoclonal antibodies-mediated blockade of the PD-1 pathway provides an early positive response, with acceptable side effects.[9] Even though breast cancer is less immunogenic, there exist subtypes of breast cancer that show different immunologic properties, and successful clinical applications of these drugs in metastatic melanoma, non-small cell lung cancer, and renal cell cancer encourage its use for other common malignancies, including breast cancer. Studies on breast cancer PD-L1 expression in relation to survival have reported heterogeneous results, but PD-L1 expression is generally associated with poor prognostic factors, such as high grade, estrogen receptor (ER) negativity, and human epithelial growth factor receptor 2 (Her2) positivity.[10–13]

Historically the treatment of stage IV breast cancer consists of systemic therapy and radiotherapy to relieve symptoms. Surgery is only reserved for palliative treatment (such as those of bleeding, infection, or tumor necrosis). Demonstration of the survival benefit after resection of the primary tumor in a few stage IV cancers, including breast cancer, has justified primary tumor resection in stage IV cancer.[14–17] However, this approach is still a matter of debate and is not accepted as a part of standard treatment. Recently, some studies have shown improved survival after resection of the primary tumor in stage IV disease.[16–18] Even though it is not yet a standard treatment, a subgroup of patients might probably benefit from surgical excision of the intact primary tumor in stage IV breast cancer.

This study aims to investigate the PD-L1 expression pattern, and the promoter methylation rate in primary tumors, paired lymph nodes, and distant metastases, as well as the relationships with survival in initially staged IV breast cancer patients operated for the intact primary tumor.

2. Materials and methods

2.1. Selection of the cases

This study was approved by the institutional ethical committee (Go.18/318). Among 76 females diagnosed with stage IV disease at admission and operated for intact primary tumor or metastasis diagnosed within 2 months after the operation, between January 2001 and March 2008, 57 cases with available paraffin blocks of the primary tumor were included in the study. Thirty-three cases had available tissue representing lymph nodes, whereas 14 cases had a biopsy from metastasis. Slides of all cases were reviewed by 2 pathologists (NEI and KK), tumor representative areas were selected, and 4-mm diameter tissue microarrays were constructed from paraffin blocks.

2.2. Immunohistochemistry for PD-L1

Four-micron thick slides were obtained from 3 to 4 mm diameter tissue microarray paraffin blocks and stained for PD-L1 antibody (Cell Signaling, E1L3N, 1/400, Denver, MA) using Leica Bond-Max Autostainer, according to the manufacturer's instructions. Antigen retrieval was performed with EDTA pretreatment for 20 minutes. Membranous staining of over 5% of the cells was regarded as positive. PD-L1 expression in tumor-infiltrating inflammatory cells was also noted. In 5 cases, PD-L1 expression in the primary tumor could not be evaluated due to technical problems. PD-L1 expression in the lymph nodes and metastatic sites were available in 33 and 14 cases, respectively.

2.3. PD-L1 methylation analysis

Sections (5×10 microns-thick) were allocated for methylation analysis from 15 primary tumors, 18 lymph node metastases, and 5 distant metastases. DNA isolation from formalin fixed paraffin embedded (FFPE) samples was done by QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, Holland) according to the manufacturer's protocol. Bisulfite conversion of FFPE DNA from each sample (500 ng) was performed using an EZ DNA Methylation-Gold kit according to the manufacturer's protocol (Zymo Research, Irvine, CA). The DNA methylation of PD-L1 was determined using a qMSP assay.[19] Quantitative real-time PCR experiments were performed using the Light Cycler 480 (Roche, Basel, Switzerland).

2.4. Statistical analysis

Numerical data were presented with mean and standard deviation, and categorical data were presented using frequency and percent. The comparisons between independent groups were made using Fisher exact test or chi-square test for categorical data. Survival analyses were performed by Kaplan-Meier survival analysis. Cox proportional hazard regression was used to determine independent factors on survival probability. Comparisons of survival between independent prognostic groups were made using the log-rank test. SPSS software v23.0 (IBM Inc., Armonk, NY) was used. A P value of less than .05 was considered statistically significant.

3. Results

3.1. Clinical features

The clinicopathological characteristics of 57 patients are given in Table 1. All patients were female with a mean age of 52.7 ± 11.7 years. Eighteen patients (31%) were pre-menopausal. Histologically, the most common type was infiltrative ductal carcinoma (68%) followed by mixed infiltrative (ductal and lobular) carcinoma (16%) and infiltrative lobular carcinoma (9%). All tumors were graded as grade 2 or 3, except one. The mean tumor size was 4.9 ± 2.7 cm. Three patients were still alive at the time of the study, while 31 patients died, and 23 patients are lost to follow up. Overall median survival was 33 ± 38.9 months, ranging from 5 to 185 months. Patients whose metastasis was limited to bone lived significantly longer than those with visceral metastasis (126 ± 35.6 months vs 38 ± 6.2 months, $P = .013$). Mean survival of ER (−) and (+) cases were 33.0 ± 4.5 and 94.0 ± 12.7 respectively ($P = .0004$). Mean survival of triple negative cases was significantly low (21.8 ± 2.8 vs 83.3 ± 11.1, $P = .0004$). In a multivariate analysis including age, nodal status, and PD-L1, ER, and Her2 status only the ER status remained an independent prognostic factor (HR 4.5 CI 1.3–15.0, $P = .013$)

3.2. PD-L1 Immuno-expression

The overall PD-L1 expression in the primary tumor was 23.1% (12/52) (Fig. 1). A proportion [15.2% (5/33)] of metastatic tumors in the lymph nodes were positive for PD-L1, while 21.4% (3/14) of distant metastases expressed PD-L1. Tumor-infiltrating inflammatory cells also expressed PD-L1 in half of the primary

Table 1

Clinicopathological characteristics of 57 patients.

Clinicopathological parameter		N (%)
Type of surgery (n=57)	Modified radical mastectomy	29 (50.8)
	Radical mastectomy	14 (24.5)
	Simple mastectomy	8 (14.0)
	Lumpectomy±Axillary dissection	6 (10.5)
Histological type (n=57)	Ductal	38 (66.6)
	Mixed (ductal & lobular)	9 (15.7)
	Lobular	5 (8.7)
	Other	5 (8.7)
Tumor grade (n=51)	1	1 (1.9)
	2	26 (50.9)
	3	24 (47.0)
T status (n=55)	X	5 (9.0)
	1	1 (1.8)
	2	27 (49.0)
	3	12 (21.8)
	4	10 (18.1)
N Status (n=57)	X	11 (19.2)
	0	3 (5.2)
	1	12 (21.0)
	2	13 (22.8)
	3	18 (31.5)
Receptor status (n=56)	ER+/PR+/Her2-	21 (37.5)
	ER+/PR-/Her2-	7 (12.5)
	ER-/PR-/Her2+	14 (25)
	ER-/PR-/Her2-	9 (16.0)
	ER+/PR+/Her2+	5 (8.9)
Site of metastasis (n=57)	Bone only	14 (24.5)
	Solid organ	43 (75.5)

ER=estrogen receptor, Her 2=human ephitelial growth factor receptor 2, PR=progesterone recptor.

tumors and 43% of distant metastatic foci. PD-L1 expression was less frequent (around 20%) in ER-positive tumors (ER+PR+, ER +PR-, ER+PR+HER2+) compared to triple negative (25%) and HER2 positive groups (31%) (Table 2).

The differential expression of PD-L1 among primary tumor, lymph node, and distant metastatic foci are given in Table 3. The PD-L1 statuses of the primary tumor, lymph node, and distant metastatic foci were consistent in 81.8% and 66.7% of the cases, respectively. However, in a subset of cases, tumor cells in both regional and distant metastatic foci either started to express or lost PD-L1 regardless of PD-L1 status in the primary tumor (Fig. 2). In metastatic foci, 11.3% of the cases became PD-L1 positive, while 40.2% lost PD-L1 expression. Discordance in PD-L1 expression was also observed between lymph node metastasis and distant metastasis in some cases.

3.3. PD-L1 Methylation

PD-L1 methylation status data were available for 15 primary tumors, 18 lymph nodes, and 5 distant metastases. The mean PD-L1 methylation ratios were 6.0% (0–18.5), 5.2% (0–21), and 0% for the primary tumor, lymph node metastasis, and distant metastasis, respectively. PD-L1 immunoexpression and DNA methylation did not show a significant correlation.

3.4. PD-L1 expression and survival

The estimated median survival time of the PD-L1 negative cases after diagnosis was 48.0±6.6 months, in contrast to 33.0±5.6 months in PD-L1 positive patients (P=.181) (Fig. 3). The estimated median survival time after operation in the PD-L1 negative and PD-L1 positive cases were 47±8.5 and 29±4.8 months, respectively (P=.247). Estimated survivals were not statistically different between the PD-L1 positive and PD-L1 negative cases.

4. Discussion

Because of the heterogeneity of presentation, breast cancer treatment becomes more difficult as the disease advances. All

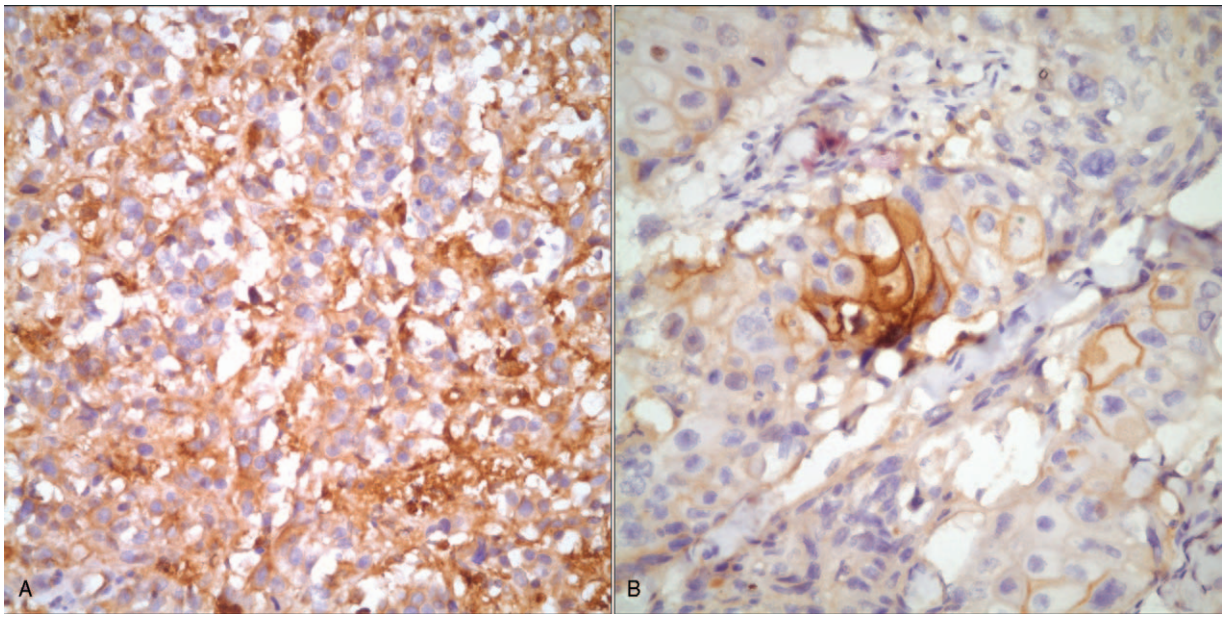
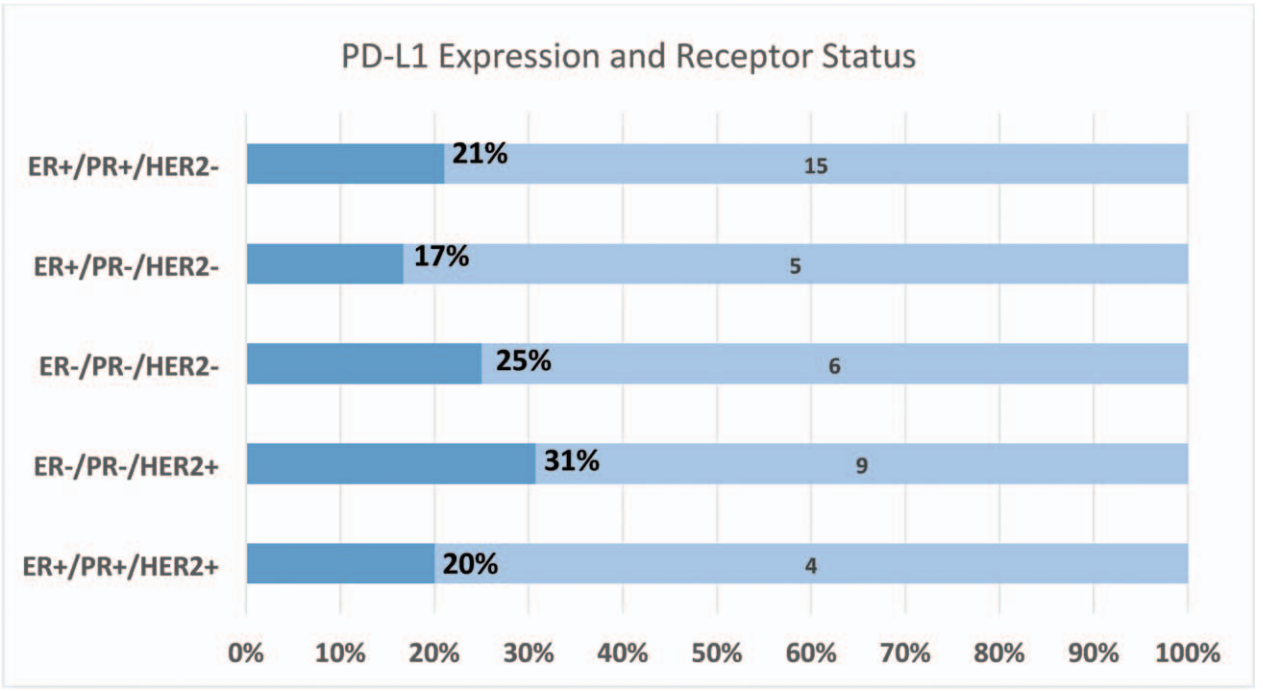


Figure 1. Immunohistochemical staining of PD-L1 in 2 high-grade primary tumors. A) Diffuse PD-L1 staining in a HER2 (+) breast carcinoma (400x). B) Focal but strong PD-L1 positivity in triple negative breast carcinoma (×400).

Table 2

PD-L1 expression and hormonal/HER2 status of tumors.



ER = estrogen receptor, Her 2 = human ephitelial growth factor receptor 2, PR = progesterone recptor, PD-L 1 = programmed death-ligand 1.

treatments must be individualized, and the selection of patients who will benefit from different treatment modalities is crucial.

Surgery for intact primary stage IV breast cancer is controversial. patients with good prognostic factors, such as young age, limited metastatic disease burden, or metastasis limited to bone, are reported to benefit from surgery.[20] We observed similar results in our study; patients whose metastasis is limited to bone showed prolonged survival compared to patients with solid organ metastasis. Several mechanisms have been postulated to explain this survival advantage in the surgically treated group. One is the decreasing tumor burden, thereby increasing the effectiveness of chemotherapy. Another possible mechanism is the prevention of systemic tumor spread by eliminating the source of circulating tumor cells, related to the disease progression.[21,22] Modulation of tumor-induced immune suppression is another postulated mechanism. Even though surgical excision can eliminate immune suppressive factors produced by tumor and helps with immune system recovery, surgery for intact primary stage IV breast cancer is controversial.[23]

A significant number of triple-negative and Her2+ breast cancers expressed PD-L1 in this study. These results are consistent with those of previous studies and support the notion that aggressive subtypes of breast cancer frequently express PD-L1.[24] Interestingly, contrary to previous reports,[25,26] our results showed a high PD-L1 expression in ER+ tumors, which could be due to bias resulting from the inclusion of only stage IV patients, regardless of hormonal status. Even though we observed no statistically significant difference between PD-L1 expression and survival (33.0 ± 5.6 vs 48.0 ± 6.6 months, $P = .181$), it can be postulated that PD-L1 (+) cases tend to have a poorer clinical prognosis. Small sample size and high loss to follow up rates might also have affected our results. Much as clinical trials continue anti-PD-1 and anti- PD-L1 therapies for breast cancer, especially the triple negative subtype, the patients who would benefit from the treatment is hard to predict, as clinical response rates are limited and often with adverse effects.[27] Using only PD-L1 as a predictive marker might be unsuitable as no universal approach to evaluate PD-L1 expression has been developed. The

Table 3

PD-L1 status of the primary tumor and paired metastatic lymph node/distant metastasis.

		Lymph Node Metastasis (%)		Distant Metastasis (%)	
		PD-L1 +	PD-L1 -	PD-L1 +	PD-L1 -
Primary Tumor	PD-L1 +	4 (50)	2 (25)	3 (25)	2 (17)
	PD-L1 -	1 (12.5)	1 (12.5)	6 (50)	1 (8)
Distant Metastasis	PD-L1 +	1 (11)	1 (11)		
	PD-L1 -	1 (11)	6 (67)		

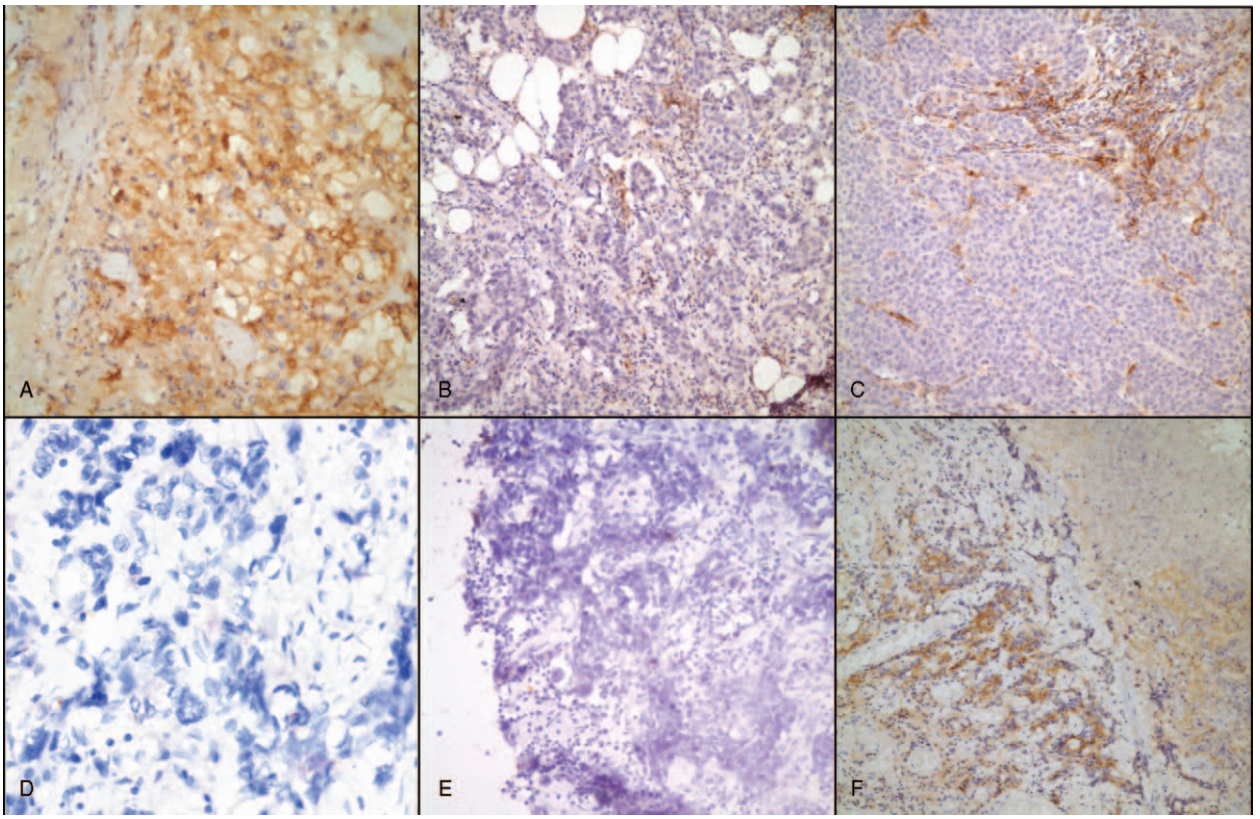


Figure 2. PD-L1 expression variability of 2 selected cases in the primary tumor, lymph node, and distant metastasis. The first row represents a high-grade HER2 positive breast carcinoma diffusely positive for PD-L1 in the primary tumor (A), whereas the lymph node (B) and liver metastasis (C) were negative for PD-L1. Please note the inflammatory cells surrounding metastatic focus express PD-L1. The second row represents triple-negative high-grade breast carcinoma and shows a reverse PD-L1 expression pattern; primary tumor (D) and its lymph node metastasis (E) were negative for PD-L1, while the distant metastatic focus in the brain (F) showed diffuse and strong PD-L1 expression.

differences between techniques, antibodies, and cut-off points affect comprehensive evaluation.[9].

What is the gold standard for the evaluation of PD-L1 expression: immunohistochemistry for protein expression or molecular studies on PD-L1 gene? The expression of PD-L1 is controlled at multiple levels, and not only the gene expressions, but also promoter methylation, post-transcriptional, translational, and post-translational modifications, can be regulated by specific DNA modifications, actively contributing to the overall levels of the PD-L1 protein.[28] PD-L1 can be induced in many cell types, and inflammatory factors are the major driver that upregulates its expression.[29] In our study since the methylation rate of the PD-L1 promoter was very low, the lack of PD-L1 expression might not be due to the epigenetic silencing of this gene. Nevertheless, inflammatory infiltrate (and the mediators derived thereof) might act as principal factors that modulate the presence of this inhibitory ligand in breast cancer cells.[30] However, as the samples included in this study were obtained from stage IV breast cancer patients who had not undergone systemic adjuvant, conventional chemotherapy, or radiotherapy, the gene expression profile or methylation status was not influenced by the treatment modalities.

Alteration in PD-L1 expression during the disease course between primary and metastatic tumors might also have clinical importance. This discordance is similar to the discrepancy in the ER, PR, and Her-2 statuses of the primary breast tumor and its distant metastatic foci, a well-documented phenomenon in breast cancer, which might also have an impact on treatment strategies.[3,31–33] Even though our study is limited by its small sample size and use of tissue microarrays instead of whole surface sections, we observed similar discordance in PD-L1 expressions of the primary tumor, lymph node, and distant metastasis, and our results were comparable to previous studies that reported the conversion of PD-L1 status in triple negative breast cancer patients.[34] Manson et al. reported a PD-L1 (on tumor) discordance between primary tumors and matched distant metastases in 28.5% of the patients with breast cancer.[35] Our study also showed that discordance could be present either way (PD-L1 might become positive or negative) in the primary tumor, lymph node, and distant metastasis. A few studies on other cancers have reported that there are responders who are PD-L1 (−), though PD-L1 (+) tumor response rates are higher.[36] It can be speculated that excision of a PD-L1 (−) primary tumor might facilitate the immune response, which can have a different PD-L1 status to the metastasis. Another possible explanation for this discrepancy is tumor heterogeneity frequently seen in breast cancer.[37,38] Also, many breast tumors show mixed morphology (16% of our cases are diagnosed as mixed ductal and lobular carcinoma) which may also reflect a genotypical and/or immunophenotypical diversity of a given tumor. Furthermore, it is known that breast tumor primaries can show a mosaic c-erbB2 expression and, in particular, may show a discrepancy between primary and metastatic foci in up to 26% of the cases, similar to the discrepancy in PD-L1 expression demonstrated in

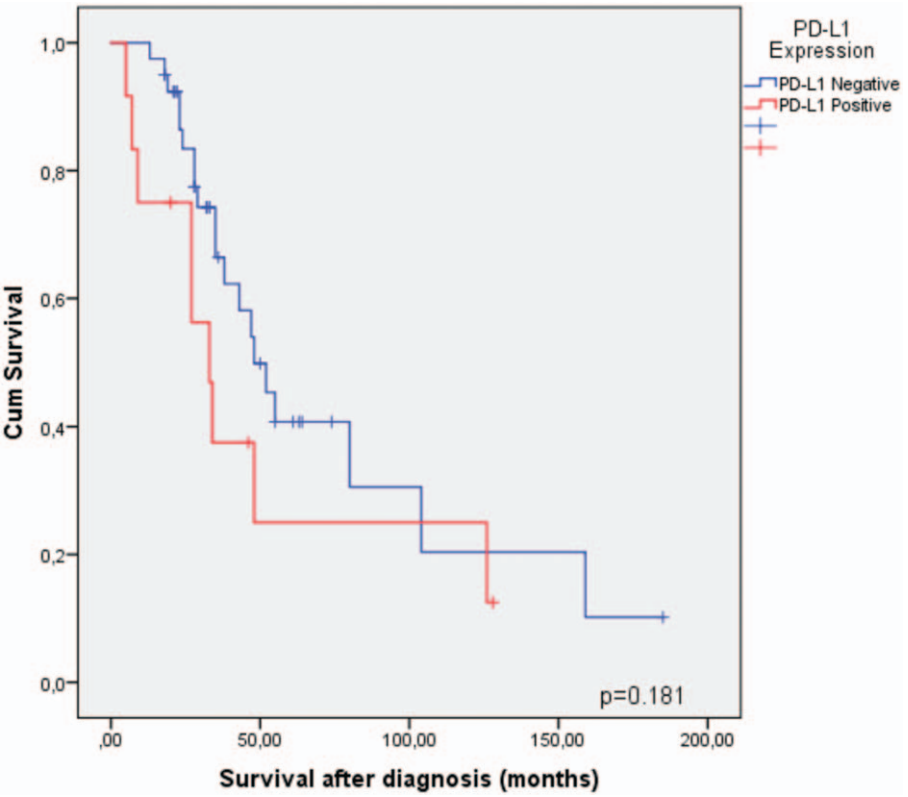


Figure 3. The relationship between tumor PD-L1 expression and survival.

our study.[39] As a result of primary tumor heterogeneity, the discrepancy in PD-L1 expression may also be simply due to the use of a relatively small sample (4-mm thick microarrays) as a representative of either primary or metastatic tumors. Lastly but more importantly, our results indicate that primary tumor evaluation alone might not be sufficient to make decisions about checkpoint blockade immunotherapy.

The main limitations of our study are its retrospective nature, small sample size, use of 4-mm microarrays, and high loss to follow up rates, but as primary tumor resection is not the standard care for initially metastatic breast cancer, finding cases and randomization was not feasible.

In conclusion, PD-L1 positivity might be associated with all (ER-positive, triple negative or Her2+) subtypes of stage IV breast cancer in patients who have been operated for the intact primary tumor. Evaluation of PD-L1 status of all tissues would likely aid decisions about possible anti-PD-L1 treatment, as there can be a discordance between primary and metastatic foci. PD-L1 methylation ratios were low, and DNA methylation did not show correlation with PD-L1 expression in this study. PD-L1 expression did not significantly correlate with survival, but a tendency of PD-L1 (−) patients towards a better prognosis compared to PD-L1 (+) patients was observed, which could be clinically significant.

Author contributions

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References

[1] O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist 2005;10(Suppl 3):20–9.
[2] Lim B, Hortobagyi GN. Current challenges of metastatic breast cancer. Cancer Metastasis Rev 2016;35:495–514.
[3] Harbeck N, Gnant M. Breast cancer. Lancet 2017;389:1134–50.
[4] Bertucci F, Finetti P, Birnbaum D, et al. The PD1/PDL1 axis, a promising therapeutic target in aggressive breast cancers. Oncoimmunology 2016; 5:e1085148.
[5] Wang X, Teng F, Kong L, et al. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther 2016; 9:5023–39.

[6] Uhercik M, Sanders AJ, Owen S, et al. Clinical significance of PD1 and PDL1 in human breast cancer. Anticancer Res 2017;37:4249–54.
[7] Ohaegbulam KC, Assal A, Lazar-Molnar E, et al. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med 2015;21:24–33.
[8] Schütz F, Stefanovic S, Mayer L, et al. PD-1/PD-L1 pathway in breast cancer. Oncol Res Treat 2017;40:294–7.
[9] Bertucci F, Goncalves A. Immunotherapy in breast cancer: the emerging role of PD-1 and PD-L1. Curr Oncol Rep 2017;19:64.
[10] Baptista MZ, Sarian LO, Derchain SF, et al. Prognostic significance of PD-L1 and PD-L2 in breast cancer. Hum Pathol 2016;47:78–84.
[11] 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;146:15–24.
[12] Zhang M, Sun H, Zhao S, et al. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. Oncotarget 2017;8:31347–54.
[13] Wang Q, Liu F, Liu L. Prognostic significance of PD-L1 in solid tumor: an updated meta-analysis. Medicine 2017;96:e6369.
[14] Gulack BC, Nussbaum DP, Keenan JE, et al. Surgical resection of the primary tumor in stage IV colorectal cancer without metastasectomy is associated with improved overall survival compared with chemotherapy/radiation therapy alone. Dis Colon Rectum 2016;59:299–305.
[15] Sun J, Song Y, Wang Z, et al. Clinical significance of palliative gastrectomy on the survival of patients with incurable advanced gastric cancer: a systematic review and meta-analysis. BMC Cancer 2013;13:577–1577.
[16] Khan SA, Stewart AK, Morrow M. Does aggressive local therapy improve survival in metastatic breast cancer. Surgery 2002;132:620–6.
[17] Harris E, Barry M, Kell MR. Meta-analysis to determine if surgical resection of the primary tumour in the setting of stage IV breast cancer impacts on survival. Ann Surg Oncol 2013;20:2828–34.
[18] Samiee S, Berardi P, Bouganim N, et al. Excision of the primary tumour in patients with metastatic breast cancer: a clinical dilemma. Curr Oncol (Toronto, Ont) 2012;19:e270–9.
[19] Gevensleben H, Holmes EE, Goltz D, et al. PD-L1 promoter methylation is a prognostic biomarker for biochemical recurrence-free survival in prostate cancer patients following radical prostatectomy. Oncotarget 2016;7:79943–55.
[20] Shien T, Iwata H. Significance of primary lesion resection in Stage IV breast cancer. Jpn J Clin Oncol 2017;47:381–4.
[21] Khoo BL, Lee SC, Kumar P, et al. Short-term expansion of breast circulating cancer cells predicts response to anti-cancer therapy. Oncotarget 2015;6:15578–93.
[22] Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–91.
[23] Danna EA, Sinha P, Gilbert M, et al. Surgical removal of primary tumor reverses tumor-induced immunosuppression despite the presence of metastatic disease. Cancer Res 2004;64:2205–11.
[24] 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;6:13506–19.
[25] Qin T, Zeng YD, Qin G, et al. High PD-L1 expression was associated with poor prognosis in 870 Chinese patients with breast cancer. Oncotarget 2015;6:33972–81.
[26] Dill EA, Gru AA, Atkins KA, et al. PD-L1 expression and intratumoral heterogeneity across breast cancer subtypes and stages: an assessment of 245 primary and 40 metastatic tumors. Am J Surg Pathol 2017;41:334–42.
[27] Nanda R, Chow LQ, Dees EC, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. J Clin Oncol 2016;34:2460–7.
[28] Zerdes I, Matikas A, Bergh J, et al. Genetic, transcriptional and post-translational regulation of the programmed death protein ligand 1 in cancer: biology and clinical correlations. Oncogene 2018;37:4639–61.
[29] Alsaab HO, Sau S, Alzhrani R, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. Front Pharmacol 2017;8:561.
[30] Karasar P, Esendagli G. T helper responses are maintained by basal-like breast cancer cells and confer to immune modulation via upregulation of PD-1 ligands. Breast Cancer Res Treat 2014;145:605–14.
[31] Regitnig P, Schippinger W, Lindbauer M, et al. Change of HER-2/neu status in a subset of distant metastases from breast carcinomas. J Pathol 2004;203:918–26.
[32] Zhu Y-Y, Si W, Ji T-F, et al. The variation and clinical significance of hormone receptors and Her-2 status from primary to metastatic lesions in breast cancer patients. Tumor Biol 2016;37:7675–84.
[33] Cao Y, Yue Y, Zhou X, et al. Change of HER2 status during disease recurrence predicts good prognosis for primary squamous cell carcinoma of the breast: A case report. Medicine 2019;98:e14654.
[34] 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.
[35] 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.
[36] 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;20:5064–74.
[37] Teixeira MR, Pandis N, Bardi G, et al. Clonal heterogeneity in breast cancer: karyotypic comparisons of multiple intra- and extra-tumorous samples from 3 patients. Int J Cancer 1995;63:63–8.
[38] Heim S, Teixeira MR, Dietrich CU, et al. Cytogenetic polyclonality in tumors of the breast. Cancer Genet Cytogenet 1997;95:16–9.
[39] Zidan J, Dashkovsky I, Stayerman C, et al. Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. Br J Cancer 2005;93:552–6.