



ORIGINAL ARTICLE

Discrepancy of PD1/ PD-L1 status between primary and metastatic triple negative breast cancer in diagnostic biopsies



Manar Moustafa*, Basma Hamed Ibrahim

Pathology Department, Faculty of Medicine, Zagazig University, Egypt

Received 17 January 2025; accepted 24 February 2025

Available online 18 March 2025

KEYWORDS

PDL-1;
Surgical biopsy;
Primary TNBC;
Metastatic TNBC

Abstract

Introduction: The evaluation of programmed death protein-ligand 1 (PD-L1) in triple-negative breast cancer (TNBC) has become routine for accurate decisions regarding immune checkpoint therapy. The aim of our paper was to analyze whether the PD-L1 evaluated in biopsy specimens accurately reflects its expression in the whole tumor and paired metastases in TNBC.

Methods: Immunohistochemistry was applied on 66 biopsy and resection specimens from TNBC cases and matched metastasis to determine PD-L1 status. PD-L1 was evaluated using 4 scoring methods immune cell (IC) and tumor cell (TC) with a 1% as the cutoff value, and combined positive scores (CPSs) with a 1% and 10% as the cutoff value.

Results: A total of 66 TNBC patients show a statistically significant PDL1 IC positive expression with unfavorable prognostic factors. PD-L1 was positive in IC more than TC in biopsy specimen and in surgical specimen. The overall concordance between biopsies and surgical specimens at IC score was 55.2%, at CPS 1% it was 65.2%, and at CPS 10 it was 68.2%. The pooled discordance rate varied according to the cells that expressed PD-L1: A total of 37.9% of cases had discordant primary/metastatic PD-L1 status when it was assessed on immune cells, 31.8% when it was assessed on tumor cells, and 28.8% when it was assessed by CPS > 10%.

Conclusions: Misclassifying TNBC patients as PD-L1-negative at biopsy can lead to disqualification from anti-PD-L1 therapy. PD-L1 status conversion occurs frequently between primary tumor and metastases, emphasizing the need for multiple biopsies, adequate tissue sampling, additional tests, and standardization in immunotherapy selection.

© 2025 SESPM. Published by Elsevier España, S.L.U. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

* Corresponding author.

E-mail address: mmahmd@medicine.zu.edu.eg (M. Moustafa).

PALABRAS CLAVE

PDL-1;
Biopsia quirúrgica;
TNBC primario;
TNBC metastásico

Discrepancia del estado de PD1/PD-L1 entre el cáncer de mama triple negativo primario y metastásico en biopsias de diagnóstico**Resumen**

Introducción: La evaluación del ligando 1 de la proteína de muerte programada (PD-L1) en el cáncer de mama triple negativo (TNBC) se ha convertido en una rutina para tomar decisiones precisas con respecto a la terapia de puntos de control inmunológico. El objetivo de nuestro estudio fue analizar si el PD-L1 evaluado en muestras de biopsia refleja con precisión su expresión en todo el tumor y metástasis pareadas en TNBC.

Métodos: Se aplicó inmunohistoquímica en 66 muestras de biopsia y resección de casos de TNBC y metástasis coincidentes para determinar el estado de PD-L1. PD-L1 se evaluó utilizando 4 métodos de puntuación células inmunes (IC) y células tumorales (TC) con un 1% como valor de corte, y puntuaciones positivas combinadas (CPS) con un 1% y 10% como valor de corte.

Resultados: En 66 TNBC se observó una expresión positiva de PDL1 IC estadísticamente significativa con factores pronósticos desfavorables. PD-L1 fue positivo en IC más que en TC en la muestra de biopsia y en la muestra quirúrgica. La concordancia general entre biopsias y muestras quirúrgicas en la puntuación IC fue del 55,2%, en la CPS 1% fue del 65,2% y en la CPS 10 fue del 68,2%. La tasa de discordancia agrupada varió según las células que expresaban PD-L1 el 37,9% de los pacientes presentó discordancia en el estado de PD-L1 primario/metastásico cuando se evaluó en células inmunitarias, el 31,8% cuando se evaluó en células tumorales y el 28,8% cuando se evaluó mediante CPS > 10%.

Conclusiones: Clasificar erróneamente a los pacientes con TNBC como PD-L1 negativo en la biopsia puede llevar a la descalificación del tratamiento anti-PD-L1. La conversión del estado de PD-L1 ocurre con frecuencia entre el tumor primario y las metástasis, lo que enfatiza la necesidad de biopsias múltiples, muestreo de tejido adecuado, pruebas adicionales y estandarización en la selección de inmunoterapia.

© 2025 SESPM. Publicado por Elsevier España, S.L.U. Se reservan todos los derechos, incluidos los de minería de texto y datos, entrenamiento de IA y tecnologías similares.

Introduction

Triple-negative breast cancer is a very aggressive subtype of BC that makes up 15–20% of all breast carcinoma. It is linked to a particularly aggressive course of disease and a high death rate.¹

Recently, therapy possibilities for TNBC with immune checkpoint inhibitors, particularly inhibitors of PD-1/PD-L1 axis, have attracted great interest.² PD-L1 expressed in malignant cells interacts with T cells, inhibiting the host's anti-tumor immune response.³

PD-L1 inhibition for TNBC is presently being investigated in both the adjuvant and neoadjuvant settings. In the neoadjuvant instance, treatment decisions will be made based on PD-L1 expression in small needle biopsies as opposed to whole sections of tumor tissue. Furthermore, PD-L1 expression in TNBC is known to be varied and differently expressed across time and location.^{4,5} The phenomenon of receptor conversion as breast cancer progresses from early to metastatic is widely documented. This observation emphasizes the importance of getting metastatic biopsies, which lead to changes in treatment in around 15–20% of patients.^{6,7} There is currently insufficient understanding about how these various sample procedures affect the assessment of PD-L1 expression and how this influences treatment recommendations. This creates a unique set of diagnostic issues because PD-L1 expressions in this context must be determined preoperatively rather than on resection material.

Also, there is a difference in the evaluation of PD 1/PD L1 across primary TNBC tumors and paired metastases, as well as the positivity threshold for assessment. We will investigate whether PD-1/PD-L1 expression measured by core biopsy correctly represents its expression in TNBC when compared to postresection tissues and associated metastases.

Patients and methods

Between January 2020 and December 2023, 66 cases of selected female patients with triple-negative invasive breast carcinomas archive paraffin blocks were gathered at Zagazig University, Pathology Department.

The specimens were collected via large needle core biopsy before any systemic treatment (from breast tissue) and modified radical mastectomy after treatment with neoadjuvant chemotherapy (n = 66). The study also included lymph nodes with metastases from specific cases of TNBC. Even though some patients had access to several lymph nodes, each patient only had one representative node examined based on (certain criteria, such as the biggest tumor load and metastatic deposit).

The clinicopathological data were gathered from pathology reports that were accompanied by tissue specimens, as well as reports for metastatic BC that had matched primary breast cancer specimens.

Histopathology and immunohistochemistry procedure

Each case involved cutting 3–5 μm sections from formalin-fixed, paraffin-embedded tissue blocks. To confirm the diagnosis, sections were stained using hematoxylin and eosin (H&E) and examined using light microscopy. The Dako EnVision™ kit (Dako, Copenhagen, Denmark) was used for the immunohistochemistry assay. It is a polymer detection system. Pretreatment was carried out using the approved three-in-one specimen preparation method. Formalin-fixed paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval in a preheated working solution of EnVision Target Retrieval Solution, Low pH (code K8005, Agilent) in the PT Link Pretreatment module at 97 °C for 20 minute, then allowed to cool to 65 °C. The sections were rinsed for 5 minutes with EnVision TM Wash Buffer (DM 831, code K 8007, Agilent) in the PT Link Rinse Station (Agilent). Endogenous peroxidase activity was eliminated with EnVision Peroxidase-Blocking Reagent Ready to Use (SM801, Agilent) for 5 minutes at room temperature.

Then auto stainer was used as follows: Primary antibody PD-L1 (PD-L1Rb, isotope IgG, Clone CAL10 1:100 dilution, Biocare medical) The reaction was visualized by incubating the sections with diaminobenzidine (DAB) for 15 minute after that Mayer's hematoxylin was used. Positive control (tonsil) and negative controls were used.

Interpretation of immunostaining

PD-L1 immunoreactivity was evaluated by two pathologists using a binocular microscope (MC30, Micros, Austria) with a camera (MU1000A, Amscope, USA).

PD-L1 positivity was determined as an immune cell score < 1%, tumor cell < 1% components, and combined positive score:

- Tumor cells: partial or complete staining of membrane was included.
- Immune cells: Membrane and cytoplasmic staining of lymphocytes and macrophages were included.

The area containing necrosis or foreign material was excluded from the tumor. Only tissues with at least 50 live tumor cells and accompanying stroma were judged suitable for inclusion in this investigation. The final IC score was calculated based only on the biopsy cores harboring invasive tumor cells.

CPS was determined as the combined number of PD-L1 stained TCs, tumor-infiltrating lymphocyte cells and macrophages (intratumorally and in the surrounding stroma) divided by the total number of TCs, multiplied by 100. We evaluated CPS at a threshold of 1 and 10 according to PD-L1 evaluation.

Statistics

All data were collected, tabulated, and statistically analyzed using SPSS version 23 (Statistical Package for Social Science, Chicago, Illinois, USA). We determined the frequency and percentage, which were presented as qualitative

variables. When applicable, use the Chi-squared or Fisher's exact test. Spearman's correlation coefficient was used to determine the relationship between the variables. *P-values* < 0.05 were considered statistically significant.

Results

The clinicopathological and histopathological data of the studied cases

The age of patients at the time of initial diagnosis ranged from 25 to 72 years. Most of them are above 50 years old (56%)., Most of the tumor size is more than 2 cm (68%), grade 3 (74%). There was a statistical link between PDL1 IC positive expression and poor prognosis (large tumor size, high grade, TILS, necrosis, and fibrosis) (Table 1).

Prevalence of PD-L1 expression in TNBC biopsies and surgical specimens

PD-L1 was positive in immune cells more than tumor cells respectively in biopsy specimens (48.5%, 30.5%) respectively also in surgical specimens (65%, 39.4%) (Fig. 1). The overall concordance between biopsies and surgical specimens at IC was 65.2%, at CPS ≥ 1 was 65.2%, and at CPS ≥ 10 was 68.2%. There is a significant association between TNBC biopsies and surgical specimens (Table 2).

First, according to the IC ≥ 1 cut-off, 32 (48.5%) of the TNBC biopsy specimens were PD-L1-positive, and 34 (51.5%) were PD-L1-negative. Second, we assessed PD-L1 expressions in tissue specimens that had been surgically removed and matched the PD-L1 biopsies predominantly. During the postoperative inspection, 17 (25.8%) resection specimens that were first categorized as PD-L1-negative turned out to be positive. Ten specimens had no PD-L1 expressions at all, and seven of the PD-L1-negative specimens had PD-L1 expressions covering less than 1% of the tumor area. PD-L1-positive TNBCs were detected in 17 (25.8%) more surgical specimens than biopsy specimens, and the difference was statistically significant ($p = 0.0077$) (Table 2).

Second, PD-L1 positivity as determined by the CPS ≥ 10 cut-offs, is predictive in advanced TNBC. Of the TNBC biopsy specimens, 24 (36.4%) had PD-L1 positivity and 42 (63.6%) had PD-L1 negativity. Second, we assessed PD-L1 expressions in tissue specimens that had been surgically removed and matched the PD-L1 biopsies predominantly. Compared to biopsy specimens, we discovered 15 (22.7%) more PD-L1-positive TNBCs in surgical specimens; this difference was statistically significant ($p = 0.0021$) (Table 2). The PD-L1 status was verified in resection specimens for every instance in which the biopsy was deemed positive for PD-L1 staining, and six instances of false positives were discovered.

Prevalence of PD-L1 expression in primary TNBC specimens and metastatic

PD-L1 was positive in immune cells more than tumor cells respectively in metastatic biopsy (30.3%, 7.6%) respectively also in surgical specimens (65%, 39.4%) (Fig. 2).

Table 1 clinicopathological and histopathological parameters of primary TNBC (N = 66).

Variables		Number (66)	Percentage (100%)	PDL 1 IC		P value
				+ve	-ve	
Age	≤ 50 year	29	43.9%	14	15	0.0108 (S)
	> 50 year	37	56.1%	29	8	
Tumor size	≤ 2 cm	21	31.8%	7	14	0.0002 (S)
	< 2 cm	45	68.2%	36	9	
grades	Grade 1	0	0%	0%	0%	0.0004 (S)
	Grade 2	17	25.8%	8	9	
	Grade 3	49	74.2%	35	14	
Lymph nodes	pNO	0	0%	0	0	0.0486 (S)
	pN1	35	53%	19	16	
	pN2	14	21.2%	11	3	
	pN3	17	25.8%	13	4	
Distant metastasis	Present	20	30.3%	13	7	1.0 (NS)
	Absent	46	69.7%	30	16	
TILS	Mild	36	54.5%	17	19	0.0005 (S)
	Moderate	22	33.3%	18	4	
	Severe	8	12.1%	8	0	
Necrosis	Present	40	66.6%	34	6	0.0001 (HS)
	Absent	26	39.4%	7	19	
fibrosis	Mild	29	43.9%	21	8	0.0001 (HS)
	Moderate	15	22.7%	15	0	
	Severe	22	33.3%	7	15	

Tumor-infiltrating lymphocytes = TILs, χ^2 : Chi square test NS: non-significant ($P > 0.05$) Significant ($P < 0.05$). Highly significant < 0.001 positive (+ve) negative (-ve).

We evaluated PD-L1 positivity in surgically resected tissue specimens; 65% of cases were positive, while twenty cases (30%) of paired metastatic specimens were PD-L1-positive. The pooled discordance rate changed based on the cells that expressed PD-L1: 37.9% of cases had discordant primary/metastatic PD-L1 status when it was assessed on immune cells, 31.8% when it was assessed on tumor cells, and 28.8% when it was evaluated by CPS. As shown in Table 3, the direction of change was more frequently from PD-L1-positive primary tumor to PD-L1-negative metastasis than vice versa, regardless of assessed cell type, the

direction being positive to negative versus negative to positive, respectively.

Discussions

The results reported on PD-L1 positivity in TNBC, as well as its predictive and prognostic value, are inconsistent. The main causes are the diverse assays used (SP142, SP263, and 22C3 assays), the absence of a uniform scoring methodology, the geographic heterogeneity of the tumor itself, and the

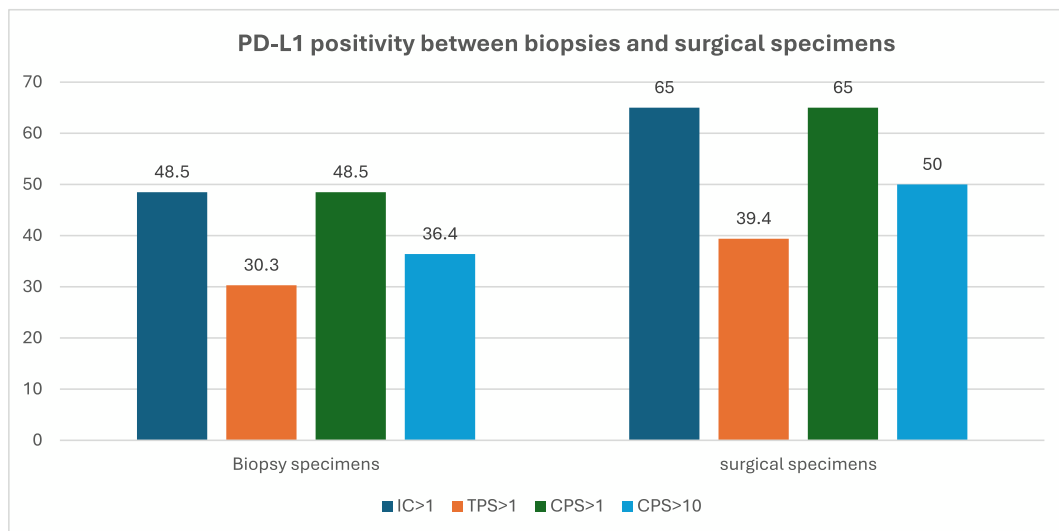


Fig. 1 PD-L1 positivity between biopsies and surgical specimens for each assay and scoring method.

Table 2 Association of PD-L1 positivity between biopsies and surgical specimens for each assay and scoring method using χ^2 tests.

Biopsy cores	Primary site		χ^2 value	P value
	Positive	Negative		
PD-L1 IC < 1%				
Positive	26	6	7.091	0.0077
Negative	17	17		
PD-L1 TPS < 1%				
Positive	17	3	24.999	0.00001
Negative	9	37		
PD-L1 cps < 1%				
Positive	26	6	7.091	0.0077
Negative	17	17		
PD-L1 cps < 10%				
Positive	18	6	9.428	0.0021
Negative	15	27		

CPS, Combined Positive Score; IC, immune cell; TPS, Tumor Proportion Score, χ^2 : Chi square test NS: non-significant ($P > 0.05$) Significant ($P < 0.05$). Highly significant < 0.001 .

reproducibility of IHC-based detection of PD-L1 positive.^{8,9} Because different PD-L1 scoring systems are used in the literature, we decided to utilize different cut-off values for PD-L1 expressions and examined whether the PD-L1 expression evaluated by core biopsy accurately reflects its expression in TNBC compared to postresection specimens and metastatic TNBC.

In our investigation, PD-L1 was more commonly found in immune cells than tumor cells in both specimens. These findings were consistent with previous studies demonstrating the preferential staining of PD-L1 on IC in malignancies from different tissues.¹⁰ In TNBC, PD-L1-IC expression by PD-L1 was reported from 28 to 56% while a range of 5–37% was reported for its TC expression.^{11–14}

In our research work, we identified a statistically significant PDL1 IC positive expression with negative prognostic variables, which is also consistent with a recent study.^{12,15} However, PD-L1 expression is considered a clinically important predictive indicator,¹⁶ Our study

Table 3 Comparison of PD-L1 positivity in primary versus metastatic sites.

Metastatic sites	Primary site		P value	
	Positive	Negative		
PD-L1 IC < 1%				
Positive	19	1	11.26	0.0008
Negative	24	22		
PD-L1 TPS < 1%				
Positive	5	0	8.32	0.0039
Negative	21	40		
PD-L1 cps < 1%				
Positive	19	1	11.26	0.0008
Negative	24	22		
PD-L1 cps < 10%				
Positive	14	0	17.77	0.0001
Negative	19	33		

CPS, Combined Positive Score; IC, immune cell; TPS, Tumor Proportion Score, χ^2 : Chi square test NS: non-significant ($P > 0.05$) Significant ($P < 0.05$). Highly significant < 0.001 .

observed no correlation between PDL1 IC positivity and distant metastasis, which is consistent with previous findings.¹⁷

Only a few researches have investigated the role of PD-L1 heterogeneity in tumor misdiagnosis. Noske et al. investigated PD-L1 expression in TNBC and discovered the concordance rates between biopsies and resection specimens were 78% for SP263 (which is more reliable and consistent across different samples), 72% for 22C3, and 54% for SP142¹⁸. Our comparison of PD-L1 expression in primary TNBC biopsies with matching surgical specimens demonstrated a lower prevalence of PD-L1 positivity 32 (48.5%) in biopsies as compared to surgical excisions 43 (65%), determined by the PDL 1(+) IC $\geq 1\%$ cut-off. Dobritoiu et al. tested PD-L1 status in TNBC. They found a 30% positive rate in TNBC core biopsies and 52% in corresponding resection tissues for IC $\geq 1\%$.¹⁹

Second, we assessed PD-L1 expressions in surgically removed tissue specimens that matched predominantly PD-L1 biopsies. We discovered 17 (25.8%) more PD-L1-positive TNBCs in surgical than biopsy specimens, and the difference

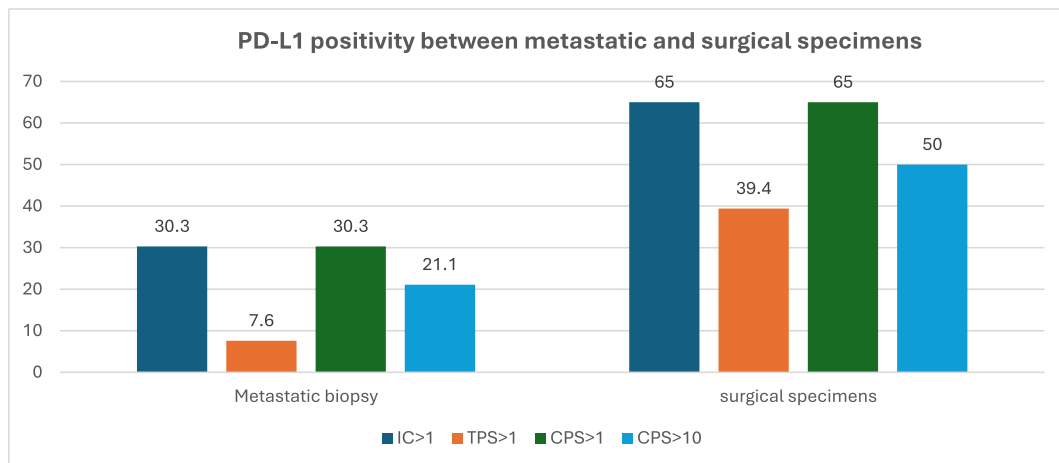


Fig. 2 PD-L1 positivity between biopsies and surgical specimens for each assay and scoring method.

was statistically significant. Similar to our study, Dobritoiu et al. discovered that after evaluating the resection tissues, another 16 tumors became PD-L1-positive, whereas none of the earlier PD-L1-positive tumors was reclassified as negative.¹⁹ Larger PD-L1 aggregates, smaller biopsy samples, and greater intra-tumor heterogeneity all increased the probability of misdiagnosis.^{18–21}

Our results do not support the notion that core biopsies should be preferred for preanalytical reasons. Also, Ho Baek et al. used SP142 assays twice on biopsy and surgical material from 77 early TNBC cases. After the second evaluation, 68.8% of the specimens were classified PD-L1-positive, as opposed to 37.6% after the original test.²² Khan et al. also reported in-tumor PD-L1 expression heterogeneity, as evidenced by the lack of agreement between the results of multiple biopsy samples from the same tumor.²³

We discovered that primary BC showed higher rates of PD-L1 expression than metastases. This outcome is consistent with findings from earlier studies.^{23–26}

In our investigation, we discovered that the difference in PD-L1 expression in immune cells between main and metastatic BC tissue was bidirectional. In 24 cases (36.4%), women with PD-L1 positive initial tumors had PD-L1 negative distant metastases, while one case of PD-L1 negative patients had PD-L1 positive metastases. Boman et al. discovered that half of female cases with PD-L1 positive initial tumors had PD-L1 negative distant metastases, while one-third of PD-L1 negative patients had PD-L1 positive metastases.²⁷

Overall, there are reasons behind these findings. Technical differences may also have a role. Metastatic core needle biopsies may be treated differently than surgical pathology specimens. Differences in lengths of warm and cold ischemia time, time of fixation, pH of formalin, temperature of paraffin at embedding time, and acid decalcification of bone can all influence IHC results.^{27–29} Tumor heterogeneity, variations in the tumor microenvironment, technical variations in antibody clones, staining procedures, and scoring criteria, as well as temporal shifts in PD-L1 expression brought on by previous treatments or immune modulation, could all contribute to variations in PD-L1 status in biopsies, surgical specimens, and metastases. Accurate therapy classification and the optimization of PD-L1 as a predictive biomarker depend on an understanding of these aspects.

In conclusion, our findings indicate that a considerable fraction of TNBC patients may be misdiagnosed as PD-L1-negative at biopsy and so excluded from anti-PD-L1 therapy. In addition, PD-L1 levels differ between the main tumor and the metastasis in TNBC. PD-L1 status conversion was common between primary tumor and paired metastasis, emphasizing the importance of multiple biopsies, adequate tissue sampling from metastatic biopsies, additional PD-L1 tests, and analytic standardization of PD-L1 assessment for selecting eligible patients for immunotherapy. We therefore propose that the investigation of PD-L1 status be added to the clinical routine of review of hormone receptor and HER2 status during the initial diagnostic approach to TNBC.

Limitations

The following limitations need to be addressed: (1) the cohort of 66 valid cases was small (2) it is unknown whether

the cores taken for the tissue sample were representative of the whole tumor, in highly heterogeneous cancers.

Funding

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for profit sectors.

Ethical considerations

This study was conducted in accordance with the amended Declaration of Helsinki and approved by the local ethical council, Zagazig University, Institutional Review Board (IRB) for human studies (reference number is ZU-IRB #:145–23-April-2023).

Patients consent

The patients' consent for participation and for publication have been obtained.

Conflict of interest

We have no conflict of interest to disclose.

References

1. Bando Y, Kobayashi T, Miyakami Y, Sumida S, Kakimoto T, Saijo Y, Uehara H. Triple-negative breast cancer and basal-like subtype: pathology and targeted therapy. *J Med Investig.* 2021;68(34):213–9. <https://doi.org/10.2152/jmi.68.213>.
2. Tan Q, Yin S, Zhou D, Chi Y, Man X, Li H. Potential predictive and prognostic value of biomarkers related to immune checkpoint inhibitor therapy of triple-negative breast cancer. *Front Oncol.* 2022;12:779786. Published 2022 Apr 29: <https://doi.org/10.3389/fonc.2022.779786>.
3. Yi M, Zheng X, Niu M, Zhu S, Ge H, Wu K. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol Cancer.* 2022;21(1):28. Published 2022 Jan 21: <https://doi.org/10.1186/s12943-021-01489-2>.
4. Yuan C, Liu Z, Yu Q, Wang X, Bian M, Yu Z, Yu J. Expression of PD-1/PD-L1 in primary breast tumours and metastatic axillary lymph nodes and its correlation with clinicopathological parameters. *Sci Rep.* 2019;9(1):14356. <https://doi.org/10.1038/s41598-019-50898-3>.
5. Manson QF, Schrijver WAME, ter Hoeve ND, Moelans CB, van Diest PJ, 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. <https://doi.org/10.1007/s10585-018-9950-6>.
6. Zou Y, Hu X, Zheng S, Yang A, Li X, Tang H, Kong Y, Xie X. Discordance of immunotherapy response predictive biomarkers between primary lesions and paired metastases in tumours: a systematic review and meta-analysis. *EBioMedicine.* 2021;63: 103137. <https://doi.org/10.1016/j.ebiom.2020.103137>.
7. Vranic S, Gatalica Z. PD-L1 testing by immunohistochemistry in immuno-oncology. *Biomol Biomed.* 2023;23(1):15–25. Published 2023 Feb 1: [10.1038/s41598-022-7953](https://doi.org/10.1038/s41598-022-7953).
8. Gonzalez-Ericsson PI, Stovgaard ES, Sua LF, Reisenbichler E, Kos Z, Carter JM, 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 in breast cancer

- clinical trials and daily practice. *J Pathol.* 2020;250(5):667–84. <https://doi.org/10.1002/path.5406>.
9. Ivanova M, Frascarelli C, Cerbelli B, Pignataro MG, Pernazza A, Venetis K, et al. PD-L1 testing in metastatic triple-negative breast cancer: interobserver and interplatform reproducibility of CE-IVD assays for CPS and IC scores. *Hum Pathol.* 2024;144:22–7. <https://doi.org/10.1016/j.humpath.2024.01.008>.
10. Downes MR, Slodkowska E, Katabi N, Jungbluth AA, Xu B. Inter- and intraobserver agreement of programmed death ligand 1 scoring in head and neck squamous cell carcinoma, urothelial carcinoma and breast carcinoma. *Histopathology.* 2020;76(2):191–200. <https://doi.org/10.1111/his.13946>.
11. Al-Jussani GN, Dabbagh TZ, Al-Rimawi D, Sughayer MA. Expression of PD-L1 using SP142 CDx in triple negative breast cancer. *Ann Diagn Pathol.* 2021;51:151703. <https://doi.org/10.1016/j.anndiagpath.2021.151703>.
12. Li Y, Vennapusa B, Chang CW, Tran D, Nakamura R, Sumiyoshi T. Prevalence study of PD-L1 SP142 assay in metastatic triple-negative breast cancer. *Appl Immunohistochem Mol Morphol.* 2021;29(4):258–64. <https://doi.org/10.1097/PAL.0000000000000857>.
13. Sui D, Wu Y, Chen H, Liu H, Zhang J, Resetkova E, Moulder SL, Wang WL, Huo L. Comparison of three FDA-approved diagnostic immunohistochemistry assays of PD-L1 in triple-negative breast carcinoma. *Hum Pathol.* 2021;108:42–50. <https://doi.org/10.1016/j.humpath.2020.11.004>.
14. Ni Y, Tsang JY, Shao Y, Poon IK, Tam F, Shea K-H, et al. Combining analysis of tumor-infiltrating lymphocytes (TIL) and PD-L1 refined the prognostication of breast cancer subtypes. *Oncologist.* 2022;27(4):e313–27. <https://doi.org/10.1093/oncolo/oyab063>.
15. Hoda RS, Brogi E, Dos Anjos CH, Grabenstetter A, Ventura K, Patil S, Selenica P, Weigelt B, Reis-Filho JS, Traina T, Robson M, Norton L, Wen HY. Clinical and pathologic features associated with PD-L1 (SP142) expression in stromal tumor-infiltrating immune cells of triple-negative breast carcinoma. *Mod Pathol.* 2020;33(11):2221–32. <https://doi.org/10.1038/s41379-020-0606-0>.
16. Gluz O, Kolberg-Liedtke C, Prat A, Christgen M, Gebauer D, Kates R, Paré L, Grischke EM, Forstbauer H, Braun M, Warm M, Hackmann J, Uleer C, Aktas B, Schumacher C, Kuemmel S, Wuerstlein R, Pelz E, Nitz U, Kreipe HH, Harbeck N. Efficacy of deescalated chemotherapy according to PAM50 subtypes, immune and proliferation genes in triple-negative early breast cancer: Primary translational analysis of the WSG-ADAPT-TN trial. *Int J Cancer.* 2020;146(1):262–71. <https://doi.org/10.1002/ijc.32488>.
17. Schmidt G, Guhl MM, Solomayer EF, Wagenpfeil G, Hammadeh ME, Juhasz-Boess I, Endrikat J, Kasoha M, Bohle RM. Immunohistochemical assessment of PD-L1 expression using three different monoclonal antibodies in triple negative breast cancer patients. *Arch Gynecol Obstet.* 2022;306(5):1689–95. <https://doi.org/10.1007/s00404-022-06529-w>.
18. Noske A, Steiger K, Ballke S, Kiechle M, Oettler D, Roth W, et al. Comparison of assessment of programmed death-ligand 1 (PD-L1) status in triple-negative breast cancer biopsies and surgical specimens. *J Clin Pathol.* 2023. <https://doi.org/10.1136/jcp-2022-208637>.
19. Dobritoiu F, Baltan A, Chefani A, Billingham K, Chenard MP, Vaziri R, et al. Tissue selection for PD-L1 testing in triple negative breast cancer (TNBC). *Appl Immunohistochem Mol Morphol.* 2022;30(8):549–56. <https://doi.org/10.1097/PAL.0000000000001053>.
20. Zdenka M, Kowalewski A, Borowczak J, Łysik-Miśkurka J, Andrusiewicz H, Nowikiewicz T, Szyberg Ł. Diagnostic biopsy does not accurately reflect the PD-L1 expression in triple-negative breast cancer. *Clin Exp Med.* 2023;23(8):5121–7. <https://doi.org/10.1007/s10238-023-01190-2>.
21. Ben Dori S, Aizic A, Zubkov A, Tsuriel S, Sabo E, HersHKovitz D. The risk of PD-L1 expression misclassification in triple-negative breast cancer. *Breast Cancer Res Treat.* 2022;194(2):297–305. <https://doi.org/10.1007/s10549-022-06630-3>.
22. Baek SH, Kim JH, Bae SJ, Ji JH, Lee Y, Jeong J, et al. SP142 PD-L1 assays in multiple samples from the same patients with early or advanced triple-negative breast cancer. *Cancers.* 2022. <https://doi.org/10.3390/cancers14133042>.
23. Kahn AM, Golestani R, Harigopal M, Puztai L. Intratumor spatial heterogeneity in programmed death-ligand 1 (PD-L1) protein expression in early-stage breast cancer. *Breast Cancer Res Treat.* 2023. <https://doi.org/10.1007/s10549-023-06977-1>.
24. Zhu L, Narloch JL, Onkar S, Joy M, Broadwater G, Luedke C, et al. Metastatic breast cancers have reduced immune cell recruitment but harbor increased macrophages relative to their matched primary tumors. *J Immunother Cancer.* 2019;7:265. <https://doi.org/10.1186/s40425-019-0755-1>.
25. Hutchinson KE, Yost SE, Chang CW, Johnson RM, Carr AR, McAdam PR, Halligan DL, Chang CC, Schmolze D, Liang J, et al. Comprehensive profiling of poor-risk paired primary and recurrent triple-negative breast cancers reveals immune phenotype shifts. *Clin Cancer Res.* 2020;26:657–68. <https://doi.org/10.1158/1078-0432.CCR-19-1773>.
26. Rozenblit M, Huang R, Danziger N, Hegde P, Alexander B, Ramkissoon S, Blenman K, Ross JS, Rimm DL, Puztai L. Comparison of PD-L1 protein expression between primary tumors and metastatic lesions in triple negative breast cancers. *J Immunother Cancer.* 2020;8(2):e001558. <https://doi.org/10.1136/jitc-2020-001558>.
27. Boman C, Zerdas I, Mårtensson K, Bergh J, Foukakis T, Valachis A, et al. Discordance of PD-L1 status between primary and metastatic breast cancer: a systematic review and meta-analysis. *Cancer Treat Rev.* 2021;99:102257. <https://doi.org/10.1016/j.ctrv.2021.102257>.
28. Reisenbichler ES, Han G, Bellizzi A, Bossuyt V, Brock J, Cole K, Fadare O, Hameed O, Hanley K, Harrison BT, Kuba MG, Ly A, Miller D, Podoll M, Roden AC, Singh K, Sanders MA, Wei S, Wen H, Pelekanou V, Rimm DL. 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. <https://doi.org/10.1038/s41379-020-0544-x>.
29. Han EK, Woo JW, Suh KJ, Kim SH, Kim JH, Park SY. PD-L1 (SP142) expression in primary and recurrent/metastatic triple-negative breast cancers and its clinicopathological significance. *Cancer Res Treat.* 2024;56(2):557–66. <https://doi.org/10.4143/crt.2023.1025>.