ANTICANCER RESEARCH *40*: 1487-1494 (2020)
doi:10.21873/anticanres.14093

Clinicopathological and Prognostic Significance of Programmed Death Ligand 1 Expression in Korean Patients With Triple-negative Breast Carcinoma

HYUN-SOO KIM[1], SUNG-IM DO[2,3], DONG-HOON KIM[2] and SOPHIA APPLE[3]

[1]*Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea;*
[2]*Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea;*
[3]*Department of Pathology, City of Hope National Cancer Center, Duarte, CA, U.S.A.*

Abstract. *Background/Aim: The clinicopathological and prognostic significances of programmed death ligand 1 (PD-L1) expression in triple-negative breast carcinoma (TNBC) are still unclear. We investigated whether PD-L1 expression is associated with clinicopathological characteristics and outcomes of TNBC patients. Materials and Methods: We performed immunostaining for PD-L1 (SP142) in 83 TNBCs. Staining proportion of ≥1% was regarded as positive PD-L1 expression. Results: Positive intratumoral (IT) PD-L1 expression (19/83; 22.9%) was inversely associated with lymphovascular invasion (LVI) and distant metastasis, and was significantly associated with better disease-free survival for TNBC patients. Positive stromal PD-L1 expression (44/83; 53.0%) also correlated inversely with LVI. Conclusion: Positive IT PD-L1 expression was associated with favorable outcomes in TNBC. In addition, positive IT and stromal PD-L1 were inversely associated with LVI and distant metastasis of TNBC.*

Triple-negative breast carcinoma (TNBC) is a subtype of breast carcinoma that lacks expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) (1, 2). TNBC accounts for approximately 15% of all breast carcinomas (3). Compared to other subtypes, TNBC tends to have worse prognosis, and is often associated with a higher histological grade and more frequent rates of relapse and distant metastasis (4-6). There is no approved targeted therapy for TNBC. Therapeutic options for TNBC patients are limited to cytotoxic chemotherapy.

Although breast carcinoma is not traditionally considered strongly antigenic, some TNBCs show increased tumor-infiltrating lymphocytes (TILs) (7, 8). Several ongoing clinical trials are using programmed death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) immunotherapy in combination with chemotherapy. Thus far, these studies have shown mixed overall responses (9-12). PD-1 is expressed in activated T-lymphocytes, and PD-L1 is expressed on both the tumor cells and immune cells of the tumor microenvironment (13). PD-L1, which belongs to the B7 family, binds PD-1 and CD80 as counter receptors to offer negative signals that control and suppress cytotoxic T-lymphocyte responses in both autoimmune responses and evasion of tumor immunity (14-17). When PD-1 binds to PD-L1, PD-1 suppresses the immune functions of T-lymphocytes by inhibiting expression of their transcription factors, which leads to apoptosis of T-lymphocytes and potentiates tumor progression.

The prognostic implications of PD-L1 expression vary according to primary sites and histological subtypes of malignancies. Positive PD-L1 expression has been reported to be associated with worse prognosis in patients with non-small cell lung carcinoma, esophageal carcinoma, gastric carcinoma, renal cell carcinoma, and malignant melanoma (18-21). In contrast, significant associations have also been reported between PD-L1 positivity and better outcomes in patients with breast carcinoma (22-27). Antibodies to PD-L1, including atezolizumab and durvalumab, have been approved by the United States (US) Food and Drug Administration (FDA) to treat certain solid tumors, such as advanced urothelial carcinoma and non-small cell lung carcinoma, and are under development to treat other malignancies (28).

Correspondence to: Sung-Im Do, Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 29, Saemunan-ro, Jongno-gu, Seoul 03181, Republic of Korea. Tel: +82 220012393, Fax: +82 220012398, e-mail: sungim.do@samsung.com

Key Words: Breast, triple-negative breast carcinoma, programmed death ligand 1, intratumoral immune cells.

Studies that have investigated whether PD-L1 expression has favorable or adverse prognostic significance in breast carcinoma have reported conflicting findings. Some studies have shown a positive correlation between PD-L1 expression and favorable prognosis (29-33). Others have found a negative or non-significant correlation between PD-L1 expression and prognosis (34-38). The reason for these controversies may be that many studies interpreted cell types differently (tumor cells or immune cells), used different cut-offs to determine positivity, or used different clones. Currently, the College of American Pathologists (CAP) recommends the use of FDA-approved PD-L1 SP142 antibody (Ventana Medical Systems, Oro Valley, AZ, USA) and regards ≥1% expression as positive in TILs and not in tumor cells in TNBC, unlike other tumors (39).

The purpose of this study was to determine the prevalence of PD-L1 positivity in TNBC and examine the relationship between clinicopathological characteristics and outcomes in a Korean population. In addition, we reviewed the previously published literature regarding the prognostic significance of PD-L1 expression in TNBC.

Patients and Methods

Patient selection. Following approval (2020-02-007) by the Institutional Review Board of Kangbuk Samsung Hospital (Seoul, Republic of Korea), the records of 83 cases of TNBC diagnosed from 2008 to 2013 were retrieved from the pathology archives. All patients underwent segmental mastectomies with axillary lymph node dissection. None of the patients underwent neoadjuvant systemic chemotherapy. All available hematoxylin and eosin (H&E)-stained slides were reviewed by two board-certified pathologists specialized in breast pathology to evaluate nuclear grade, histological grade, presence of lymphovascular invasion (LVI), pathological tumor (pT) stage, and pathological node stage (pN). The development of locoregional recurrence and distant metastasis as well as survival data were retrieved from the electronic medical records. Overall survival (OS) was defined as the period from the date of initial diagnosis to the date of last contact. Disease-free survival (DFS) was defined as the period from the date of initial diagnosis to the date of first disease recurrence, which include locoregional recurrence and metastasis to distant organs.

Tissue acquisition. The surgically resected specimens were examined macroscopically, fixed in 10% neutral buffered formalin, processed, and embedded in paraffin using a standard protocol. After the review of all available H&E-stained slides, the most representative tumor areas were carefully selected and marked on individual formalin-fixed, paraffin-embedded tissue blocks. The tumor tissue cores (2 mm in diameter) were obtained from each specimen and manually arrayed in recipient tissue microarray (TMA) paraffin blocks (40-42).

Immunohistochemical evaluation. From the TMA blocks, serial 3-μm sections were cut consecutively and adhered to charged glass slides for subsequent H&E and immunohistochemical staining. PD-L1 immunostaining was performed using the FDA-approved kit (prediluted, clone SP142, Ventana Medical Systems). Immunostaining for ER (1:200, clone SP1, LabVision Corporation, Fremont, CA, USA), PR (1:200, clone PgR 636, Dako, Glostrup, Denmark), and HER2 (1:200, clone SP3, LabVision Corporation) was also performed and confirmed that all examined cases were TNBC. To meet the definition of TNBC, tumors had to be negative for ER and PR (<1% staining) and negative for HER2 (a score of 0 or 1+ by immunostaining or 2+ with HER2/centromere 17 reference probe ratio <2.0 by silver-enhanced in situ hybridization) (42). PD-L1 expression was evaluated separately in the intratumoral and stromal TILs, according to the interpretation guidelines recommended by the CAP (39), in which ≥1% of staining proportion with any intensity was regarded as positive expression. Negative PD-L1 expression was defined as <1% staining (Figure 1).

Statistical analysis. The Pearson's chi-square test, Fisher's exact test, or linear-by-linear association test was used to examine whether PD-L1 expression status is significantly associated with clinicopathological characteristics. Univariate survival analysis was performed to examine the prognostic significance of PD-L1 expression status with respect to DFS and OS. Kaplan–Meier plot was used to display survival curves. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as $p<0.05$.

Literature review. The Medline database was thoroughly searched using the PubMed retrieval service. Searches were performed in October 2019, using the terms "PD-L1", "breast carcinoma", and "TNBC". The resulting 118 publications were reviewed and 29 studies were identified that examined PD-L1 expression status in TNBC. Of them, 12 studies reporting PD-L1 expression in TILs and its association with survival data were selected.

Results

Patient characteristics. Patients' ages ranged from 25-79 years (mean: 47 years). The majority (75.9%; 63/83) of the patients were diagnosed with ≥ pT2 tumors. More than half (65.1%; 54/83) of the cases were diagnosed as pT2, and 9 (10.8%) cases were pT3 (9.6%; 8/83) or pT4 (1.2%; 1/83). The remaining 20 (24.1%) cases were pT1. Approximately three-fourths (74.7%; 62/83) of the cases showed high nuclear grade. Histological grades were 2 and 3 in 27 (32.5%) and 53 (63.9%) cases, respectively. Lymph node metastases were detected in 37 (44.6%) patients. During the median follow-up time of 89 months, 20 (24.1%) patients developed distant metastases.

Clinicopathological significance of PD-L1 Expression in TNBC. Intratumoral PD-L1 expression was positive in 19 (22.9%) cases (Figure 1). Table I summarizes the associations between intratumoral PD-L1 expression status and the clinicopathological characteristics of TNBC. The intratumoral PD-L1 positivity was inversely associated with lymphovascular invasion and distant metastasis. In the majority of cases with positive intratumoral PD-L1 expression, neither lymphovascular invasion nor distant metastasis were

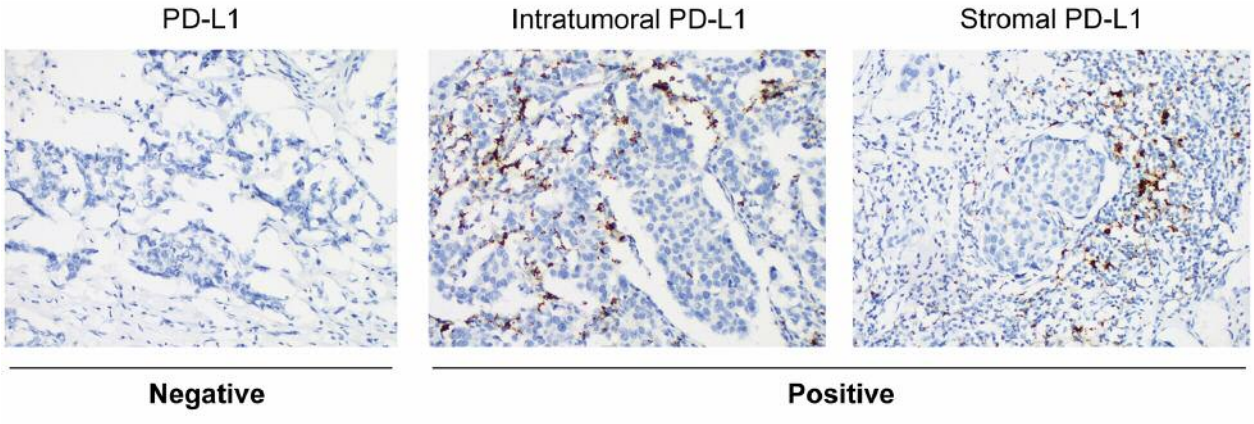

Figure 1. *Expression of programmed death ligand 1 (PD-L1) in triple-negative breast carcinoma.*

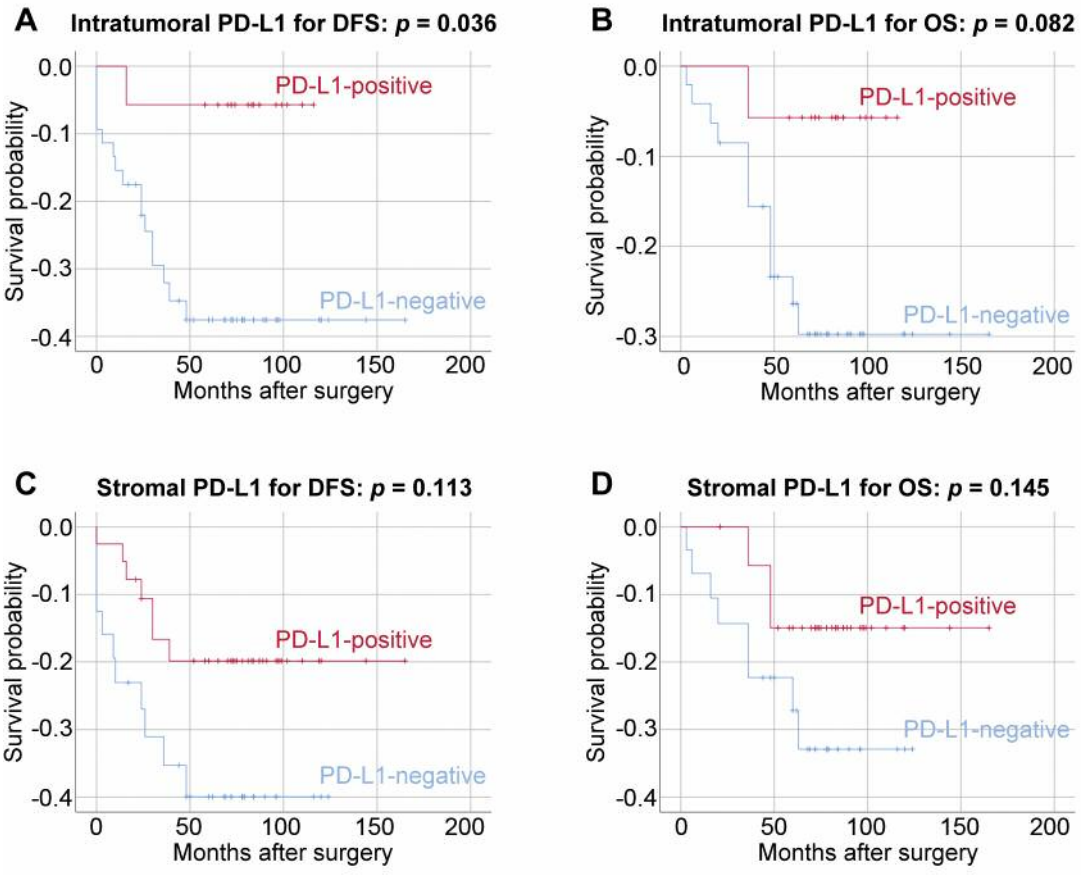

Figure 2. *Kaplan–Meier plots for disease-free survival (DFS) and overall survival (OS) according to intratumoral (A and B) and stromal (C and D) programmed cell death ligand 1 (PD-L1) expression in patients with triple-negative breast carcinoma.*

identified. Only two of the 19 (10.5%) patients whose tumors showed intratumoral PD-L1 positivity had lymphovascular invasion (p=0.001). Similarly, only one (5.3%) patient with positive intratumoral PD-L1 expression developed distant metastasis (p=0.033). No significant association was identified between the intratumoral PD-L1 expression status and other clinicopathological characteristics, including pT, histological and nuclear grades, and lymph node metastasis.

Table I. *Clinicopathological significance of programmed cell death ligand 1 (PD-L1) expression in the intratumoral tumor-infiltrating lymphocytes.*

Characteristic	PD-L1 expression		*p*-Value
	Positive	Negative	
Pathological T stage (pT)			
pT1	4 (20.0)	16 (80.0)	0.845
pT2	13 (24.1)	41 (75.9)	
pT3	2 (25.0)	6 (75.0)	
pT4	0 (0.0)	1 (100.0)	
Pathological N stage (pN)			
pN0	12 (26.1)	34 (73.9)	0.372
pN1	3 (23.1)	10 (76.9)	
pN2	4 (33.3)	8 (66.7)	
pN3	0 (0.0)	12 (100.0)	
Lymphovascular invasion			
Present	2 (5.7)	33 (94.3)	0.001*
Absent	17 (35.4)	31 (64.6)	
Histological grade			
1	0 (0.0)	3 (100.0)	0.243
2	5 (18.5)	22 (81.5)	
3	14 (35.9)	39 (64.1)	
Nuclear grade			
1	0 (0.0)	4 (100.0)	0.462
2	4 (23.5)	13 (76.5)	
3	15 (24.2)	47 (75.8)	
Lymph node metastasis			
Present	8 (20.5)	31 (79.5)	0.627
Absent	12 (26.1)	34 (73.9)	
Distant metastasis			
Present	1 (5.0)	19 (95.0)	0.033*
Absent	18 (28.6)	45 (71.4)	

*Statistically significant.

Table II. *Clinicopathological significance of programmed cell death ligand 1 (PD-L1) expression in the stromal tumor-infiltrating lymphocytes.*

Characteristic	PD-L1 expression		*p*-Value
	Positive	Negative	
Pathological T stage (pT)			
pT1	9 (45.0)	11 (55.0)	0.752
pT2	31 (57.4)	23 (42.6)	
pT3	4 (50.0)	4 (50.0)	
pT4	0 (0.0)	1 (100.0)	
Pathological N stage (pN)			
pN0	27 (58.7)	19 (41.3)	0.369
pN1	7 (53.8)	6 (46.2)	
pN2	6 (50.0)	6 (50.0)	
pN3	4 (33.3)	8 (66.7)	
Lymphovascular invasion			
Present	13 (37.1)	22 (62.9)	0.013*
Absent	31 (64.6)	17 (35.4)	
Histological grade			
1	0 (0.0)	3 (100.0)	0.002*
2	10 (37.0)	17 (63.0)	
3	34 (64.2)	19 (35.8)	
Nuclear grade	1	1 (25.0)	3 (75.0)
0.019*			
2	5 (29.4)	12 (70.6)	
3	38 (61.3)	24 (38.7)	
Lymph node metastasis			
Present	18 (46.2)	21 (53.8)	0.239
Absent	27 (58.7)	19 (41.3)	
Distant metastasis			
Present	8 (40.0)	12 (60.0)	0.181
Absent	36 (58.7)	27 (41.3)	

*Statistically significant.

Forty-four (53.0%) cases exhibited positive stromal PD-L1 expression (Figure 1). The associations of stromal PD-L1 expression status with the clinicopathological characteristics of TNBC are shown in Table II. The stromal PD-L1 showed a positive inverse correlation with lymphovascular invasion (p=0.013) and a positive correlation with both histological grade (p=0.002) and nuclear grade (p=0.019). Consistent with the results of the intratumoral PD-L1 expression, more than two-thirds (70.5%; 31/44) of the cases showing stromal PD-L1 positivity were free of lymphovascular invasion. In contrast, most of the cases (86.4%; 38/44) with positive stromal PD-L1 expression displayed high nuclear grade, and all cases showing stromal PD-L1 positivity showed histological grade 2 (22.7%; 10/4) or 3 (77.3%; 34/44). We observed no significant relationship between stromal PD-L1 expression status and other clinicopathological characteristics, including pT, lymph node metastasis, and distant metastasis.

Prognostic significance of PD-L1 expression in TNBC. In our TNBC study cohort, positive intratumoral PD-L1 expression was significantly associated with better DFS (p=0.036; Figure 2A). DFS for patients with intratumoral PD-L1-positive TNBC was higher than 90% during the entire observation period, whereas DFS for patients with intratumoral PD-L1-negative TNBC declined steadily (up to approximately 65%) during the first four years postoperatively. OS for patients with TNBC whose tumors were positive for PD-L1 was also higher than that for patients with intratumoral PD-L1-negative TNBC, but the difference was not statistically significant (p=0.083; Figure 2B). Similarly, there were no statistical differences in survival according to the stromal PD-L1 expression status, even though both DFS (Figure 2C) and OS (Figure 2D) for patients with stromal PD-L1-positive TNBC were higher than those for patients with negative stromal PD-L1 expression.

Table III. *Previously published results regarding prognostic significance of programmed cell death ligand 1 (PD-L1) expression in triple-negative breast carcinoma patients.*

Association with survival	Reference	No of cases	Definition of PD-L1 positivity	PD-L1 positivity	PD-L1 antibody (company; clone)	*p*-Value
Better	(22)	43	≥1%	72%	Abcam; polyclonal	0.03 (pCR); 0.01 (OS)
	(23)	218	Staining in one or more immune cells	36.7%	Abcam; 28-8	0.043 (DFS); 0.021 (OS)
	(24)	147	≥5%	84.4%	Cell Signaling Technology; E1L3N	Not applicable
	(25)	358	Staining percentage × intensity (H score) >5	61.8%	Ventana; SP263	0.001 (tumor) and 0.002 (stromal) (DFS)
	(26)	117	>70%	31.6%	EMD Millipore; polyclonal	0.025 (DFS); 0.006 (OS)
	(27)	55	Single very large dense cluster, multiple large clusters, or dense diffuse infiltration	37%	Cell Marque; NAT105	0.0095 (DFS); 0.0024 (OS)
Worse	(43)	108	≥1%	22%	Cell Signaling Technology; E1L3N	0.001 (DFS)
	(44)	22	≥1%	22.7%	Spring Bioscience; SP142	0.0032 (DFS); 0.0002 (OS)
	(45)	183	>10%	36.1%	Cell Signaling Technology; E1L3N	1.0e-5 (OS)
Insignificant	(46)	101	>5%	38.6%	Cell Signaling Technology; E1L3N	0.424 (DFS); 0.01 (DFS for cases with LN metastasis)
	(47)	103	Staining in one or more immune cells	67.9%	Abcam; 28-8	0.14 (DFS); 0.31 (OS)

pCR: Pathological complete response; OS: overall survival; DFS: disease-free survival; LN: lymph node.

Review of the literature. Table III summarizes previously published data regarding the prognostic significance of PD-L1 expression in patients with TNBC. A systematic review of the literature revealed that PD-L1 positivity can be associated with better (22-27) or worse (43-45) prognosis, or not significantly associated with patient outcome (46, 47). The most recent study (22) has documented that positive PD-L1 expression was associated with pathological complete response and higher OS. In contrast, a study by Zhu *et al*. (43) has reported that positive PD-L1 expression overrode the favorable prognosis associated with high TILs. In addition, Tomioka *et al*. (44) have suggested that positive PD-L1 expression exacerbated the worse survival associated with low TILs. Taken together, the findings of previously published studies offer no consensus on the prognostic significance of PD-L1 expression.

Discussion

A number of previous studies have reported an association between PD-L1 expression status and the clinicopathological characteristics of human malignancies (8, 48-56). However, there is no standard system for evaluating PD-L1 expression, and the authors of previous studies have applied different cut-off values for positive PD-L1 expression, and used different clones. Although definitions of PD-L1 positivity vary from ≥1% to >70% (Table III), recent guidelines have recommended that a specimen should be considered as having PD-L1 expression if it exhibits ≥1% of immune cells. In terms of PD-L1 antibodies for TNBC, SP142 (Ventana Medical Systems) is the only antibody that has been approved by the US FDA. Therefore, in this study we used the SP142 antibody with a cut-off value of ≥1% for positive PD-L1 expression.

We demonstrated that the intratumoral PD-L1 positivity was significantly associated with better DFS in TNBC. Based on the available literature, the prognostic implication of PD-L1 in TNBC is controversial, especially in terms of patient survival. Such conflicting results might be attributable to differences in sample size and stage, differences between tissue microarrays and whole sections, differences in antibodies and/or antigen retrieval, and staining procedures with varying degrees of sensitivity coupled with the lack of a standard evaluation method for immunostaining. However, recent literature suggests positive PD-L1 expression in TNBC as a favorable factor when it was evaluated in the immune cells and not in the tumor cells (22-27).

In this study, the stromal PD-L1 expression (53.0%) was higher than the intratumoral PD-L1 expression (22.8%). Interestingly, we showed that positive stromal PD-L1 expression was associated with higher nuclear and histological grades, both of which are known to be potentially worse prognostic factors. In contrast, we also observed a significant correlation between stromal PD-L1

positivity and less frequent lymphovascular invasion and distant metastasis, which is consistent with the inverse relationship between intratumoral PD-L1 positivity and lymphovascular invasion and distant metastasis. This inconsistency may reflect the intratumoral heterogeneity and complex functions of PD-L1 (57, 58). Further investigations are necessary to confirm or refute our results.

In conclusion, this study demonstrated that positive intratumoral PD-L1 expression is associated with favorable outcome in patients with TNBC. In addition, both the intratumoral and stromal PD-L1 positivities were inversely associated with lymphovascular invasion and distant metastasis of TNBC. This is the first study to examine PD-L1 expression using both the FDA-approved antibody for TNBC and the CAP guidelines for defining positivity (*i.e.*, ≥1% of immune cells rather than tumor cells). It remains unknown whether the use of anti-PD-L1 treatment can further increase the survival benefit for patients with PD-L1-positive TNBC, and this should be investigated in future studies.

Conflicts of Interest

None of the Authors has any conflicts of interest to declare regarding this study.

Authors' Contributions

All Authors made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of the data, as well as to drafting the manuscript, revising the article critically for important intellectual content, and providing final approval of the version to be published.

Acknowledgements

This research was supported by a grant from the National Research Foundation of Korea (NRF) funded by the Korean government (MSIT) (2018R1C1B5043725).

References

1 Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P and Narod SA: Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res *13*: 4429-4434, 2007. PMID: 17671126. DOI: 10.1158/1078-0432.CCR-06-3045

2 Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y and Pietenpol JA: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest *121*: 2750-2767, 2011. PMID: 21633166. DOI: 10.1172/JCI45014

3 Hunakova L, Horvathova E, Gronesova P, Bobal P, Otevrel J and Brtko J: Triorganotin isothiocyanates affect migration and immune check-point receptors in human triple-negative breast carcinoma mda-mb-231 cells. Anticancer Res *39*: 4845-4851, 2019. PMID: 31519587. DOI: 10.21873/anticanres.13670

4 Voduc KD, Cheang MC, Tyldesley S, Gelmon K, Nielsen TO and Kennecke H: Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol *28*: 1684-1691, 2010. PMID: 20194857. DOI: 10.1200/JCO.2009.24.9284

5 Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Rakha EA, Richardson AL, Schmitt FC, Tan PH, Tse GM, Weigelt B, Ellis IO and Reis-Filho JS: Basal-like and triple-negative breast cancers: A critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol *24*: 157-167, 2011. PMID: 21076464. DOI: 10.1038/modpathol.2010.200

6 Millar EK, Graham PH, O'Toole SA, McNeil CM, Browne L, Morey AL, Eggleton S, Beretov J, Theocharous C, Capp A, Nasser E, Kearsley JH, Delaney G, Papadatos G, Fox C and Sutherland RL: Prediction of local recurrence, distant metastases, and death after breast-conserving therapy in early-stage invasive breast cancer using a five-biomarker panel. J Clin Oncol *27*: 4701-4708, 2009. PMID: 19720911. DOI: 10.1200/JCO.2008.21.7075

7 Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, Kellokumpu-Lehtinen PL, Bono P, Kataja V, Desmedt C, Piccart MJ, Loibl S, Denkert C, Smyth MJ, Joensuu H and Sotiriou C: Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: Results from the finher trial. Ann Oncol *25*: 1544-1550, 2014. PMID: 24608200. DOI: 10.1093/annonc/mdu112

8 Gadducci A and Guerrieri ME: Immune checkpoint inhibitors in gynecological cancers: Update of literature and perspectives of clinical research. Anticancer Res *37*: 5955-5965, 2017. PMID: 29061774. DOI: 10.21873/anticanres.12042

9 Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, Su X, Wang Y, Gonzalez-Angulo AM, Akcakanat A, Chawla A, Curran M, Hwu P, Sharma P, Litton JK, Molldrem JJ and Alatrash G: Pd-l1 expression in triple-negative breast cancer. Cancer Immunol Res *2*: 361-370, 2014. PMID: 24764583. DOI: 10.1158/2326-6066.CIR-13-0127

10 Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD and Nielsen TO: Cd8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res *14*: R48, 2012. PMID: 22420471. DOI: 10.1186/bcr3148

11 Planes-Laine G, Rochigneux P, Bertucci F, Chretien AS, Viens P, Sabatier R and Goncalves A: Pd-1/pd-l1 targeting in breast cancer: The first clinical evidences are emerging. A literature review. Cancers (Basel) *11*, 2019. PMID: 31336685. DOI: 10.3390/cancers11071033

12 Katz H and Alsharedi M: Immunotherapy in triple-negative breast cancer. Med Oncol *35*: 13, 2017. PMID: 29255938. DOI: 10.1007/s12032-017-1071-6

13 Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, Overberg P, Rose I, Basu GD, Vranic S, Lynch HT, Von Hoff DD and Hamid O: Programmed cell death 1 (pd-1) and its ligand (pd-l1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev *23*: 2965-2970, 2014. PMID: 25392179. DOI: 10.1158/1055-9965.EPI-14-0654

14 Butte MJ, Keir ME, Phamduy TB, Sharpe AH and Freeman GJ: Programmed death-1 ligand 1 interacts specifically with the b7-1 costimulatory molecule to inhibit t cell responses. Immunity *27*: 111-122, 2007. PMID: 17629517. DOI: 10.1016/j.immuni.2007.05.016

15 Dong H, Zhu G, Tamada K and Chen L: B7-h1, a third member of the b7 family, co-stimulates t-cell proliferation and interleukin-10 secretion. Nat Med *5*: 1365-1369, 1999. PMID: 10581077. DOI: 10.1038/70932

16 Abe Y, Kobayashi H, Akizawa Y, Ishitani K, Hashimoto K and Matsui H: Possible application of ascites-infiltrating gamma-delta t cells for adoptive immunotherapy. Anticancer Res *38*: 4327-4331, 2018. PMID: 29970569. DOI: 10.21873/anticanres.12732

17 Pento JT: Monoclonal antibodies for the treatment of cancer. Anticancer Res *37*: 5935-5939, 2017. PMID: 29061772. DOI: 10.21873/anticanres.12040

18 Massi D, Brusa D, Merelli B, Ciano M, Audrito V, Serra S, Buonincontri R, Baroni G, Nassini R, Minocci D, Cattaneo L, Tamborini E, Carobbio A, Rulli E, Deaglio S and Mandala M: Pd-l1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. Ann Oncol *25*: 2433-2442, 2014. PMID: 25223485. DOI: 10.1093/annonc/mdu452

19 Wang A, Wang HY, Liu Y, Zhao MC, Zhang HJ, Lu ZY, Fang YC, Chen XF and Liu GT: The prognostic value of pd-l1 expression for non-small cell lung cancer patients: A meta-analysis. Eur J Surg Oncol *41*: 450-456, 2015. PMID: 25682184. DOI: 10.1016/j.ejso.2015.01.020

20 Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG and Xu N: Immunohistochemical localization of programmed death-1 ligand-1 (pd-l1) in gastric carcinoma and its clinical significance. Acta Histochem *108*: 19-24, 2006. PMID: 16530813. DOI: 10.1016/j.acthis.2006.01.003

21 Iacovelli R, Nole F, Verri E, Renne G, Paglino C, Santoni M, Cossu Rocca M, Giglione P, Aurilio G, Cullura D, Cascinu S and Porta C: Prognostic role of pd-l1 expression in renal cell carcinoma. A systematic review and meta-analysis. Target Oncol *11*: 143-148, 2016. PMID: 26429561. DOI: 10.1007/s11523-015-0392-7

22 Zhang L, Wang XI, Ding J, Sun Q and Zhang S: The predictive and prognostic value of foxp3+/cd25+ regulatory t cells and pd-l1 expression in triple negative breast cancer. Ann Diagn Pathol *40*: 143-151, 2019. PMID: 31096176. DOI: 10.1016/j.anndiagpath.2019.04.004

23 Sun WY, Lee YK and Koo JS: Expression of pd-l1 in triple-negative breast cancer based on different immunohistochemical antibodies. J Transl Med *14*: 173, 2016. PMID: 27286842. DOI: 10.1186/s12967-016-0925-6

24 Sobral-Leite M, Van de Vijver K, Michaut M, van der Linden R, Hooijer GKJ, Horlings HM, Severson TM, Mulligan AM, Weerasooriya N, Sanders J, Glas AM, Wehkamp D, Mittempergher L, Kersten K, Cimino-Mathews A, Peters D, Hooijberg E, Broeks A, van de Vijver MJ, Bernards R, Andrulis IL, Kok M, de Visser KE and Schmidt MK: Assessment of pd-l1 expression across breast cancer molecular subtypes, in relation to mutation rate, brca1-like status, tumor-infiltrating immune cells and survival. Oncoimmunology *7*: e1509820, 2018. PMID: 30524905. DOI: 10.1080/2162402X.2018.1509820

25 Lee J, Kim DM and Lee A: Prognostic role and clinical association of tumor-infiltrating lymphocyte, programmed death ligand-1 expression with neutrophil-lymphocyte ratio in locally advanced triple-negative breast cancer. Cancer Res Treat *51*: 649-663, 2019. PMID: 30064200. DOI: 10.4143/crt.2018.270

26 Choi SH, Chang JS, Koo JS, Park JW, Sohn JH, Keum KC, Suh CO and Kim YB: Differential prognostic impact of strong pd-l1 expression and 18f-fdg uptake in triple-negative breast cancer. Am J Clin Oncol, 2018. PMID: 29419531. DOI: 10.1097/COC.0000000000000426

27 Barrett MT, Lenkiewicz E, Malasi S, Basu A, Yearley JH, Annamalai L, McCullough AE, Kosiorek HE, Narang P, Wilson Sayres MA, Chen M, Anderson KS and Pockaj BA: The association of genomic lesions and pd-1/pd-l1 expression in resected triple-negative breast cancers. Breast Cancer Res *20*: 71, 2018. PMID: 29996881. DOI: 10.1186/s13058-018-1004-0

28 Guan J, Lim KS, Mekhail T and Chang CC: Programmed death ligand-1 (pd-l1) expression in the programmed death receptor-1 (pd-1)/pd-l1 blockade: A key player against various cancers. Arch Pathol Lab Med *141*: 851-861, 2017. PMID: 28418281. DOI: 10.5858/arpa.2016-0361-RA

29 Baptista MZ, Sarian LO, Derchain SF, Pinto GA and Vassallo J: Prognostic significance of pd-l1 and pd-l2 in breast cancer. Hum Pathol *47*: 78-84, 2016. PMID: 26541326. DOI: 10.1016/j.humpath.2015.09.006

30 Beckers RK, Selinger CI, Vilain R, Madore J, Wilmott JS, Harvey K, Holliday A, Cooper CL, Robbins E, Gillett D, Kennedy CW, Gluch L, Carmalt H, Mak C, Warrier S, Gee HE, Chan C, McLean A, Walker E, McNeil CM, Beith JM, Swarbrick A, Scolyer RA and O'Toole SA: Programmed death ligand 1 expression in triple-negative breast cancer is associated with tumour-infiltrating lymphocytes and improved outcome. Histopathology *69*: 25-34, 2016. PMID: 26588661. DOI: 10.1111/his.12904

31 Bertucci F, Finetti P, Colpaert C, Mamessier E, Parizel M, Dirix L, Viens P, Birnbaum D and van Laere S: Pdl1 expression in inflammatory breast cancer is frequent and predicts for the pathological response to chemotherapy. Oncotarget *6*: 13506-13519, 2015. PMID: 25940795. DOI: 10.18632/oncotarget.3642

32 Hou Y, Nitta H, Wei L, Banks PM, Lustberg M, Wesolowski R, Ramaswamy B, Parwani AV and Li Z: Pd-l1 expression and cd8-positive t cells are associated with favorable survival in her2-positive invasive breast cancer. Breast J *24*: 911-919, 2018. PMID: 30230111. DOI: 10.1111/tbj.13112

33 Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR, Viens P, Caldas C, Birnbaum D and Bertucci F: Prognostic and predictive value of pdl1 expression in breast cancer. Oncotarget *6*: 5449-5464, 2015. PMID: 25669979. DOI: 10.18632/oncotarget.3216

34 Tsang JY, Au WL, Lo KY, Ni YB, Hlaing T, Hu J, Chan SK, Chan KF, Cheung SY and Tse GM: Pd-l1 expression and tumor infiltrating pd-1+ lymphocytes associated with outcome in her2+ breast cancer patients. Breast Cancer Res Treat *162*: 19-30, 2017. PMID: 28058578. DOI: 10.1007/s10549-016-4095-2

35 Qin T, Zeng YD, Qin G, Xu F, Lu JB, Fang WF, Xue C, Zhan JH, Zhang XK, Zheng QF, Peng RJ, Yuan ZY, Zhang L and Wang SS: High pd-l1 expression was associated with poor prognosis in 870 chinese patients with breast cancer. Oncotarget *6*: 33972-33981, 2015. PMID: 26378017. DOI: 10.18632/oncotarget.5583

36 Mori H, Kubo M, Yamaguchi R, Nishimura R, Osako T, Arima N, Okumura Y, Okido M, Yamada M, Kai M, Kishimoto J, Oda Y and Nakamura M: The combination of pd-l1 expression and decreased tumor-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget *8*: 15584-15592, 2017. PMID: 28107186. DOI: 10.18632/oncotarget.14698

37 Park IH, Kong SY, Ro JY, Kwon Y, Kang JH, Mo HJ, Jung SY, Lee S, Lee KS, Kang HS, Lee E, Joo J and Ro J: Prognostic implications of tumor-infiltrating lymphocytes in association with programmed death ligand 1 expression in early-stage breast cancer. Clin Breast Cancer *16*: 51-58, 2016. PMID: 26364145. DOI: 10.1016/j.clbc.2015.07.006

38 Guo L, Li W, Zhu X, Ling Y, Qiu T, Dong L, Fang Y, Yang H and Ying J: Pd-l1 expression and cd274 gene alteration in triple-negative breast cancer: Implication for prognostic biomarker. Springerplus *5*: 805, 2016. PMID: 27390646. DOI: 10.1186/s40064-016-2513-x

39 Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Dieras V, Hegg R, Im SA, Shaw Wright G, Henschel V, Molinero L, Chui SY, Funke R, Husain A, Winer EP, Loi S, Emens LA and Investigators IMT: Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med *379*: 2108-2121, 2018. PMID: 30345906. DOI: 10.1056/NEJMoa1809615

40 Bae GE, Do SI, Kim K, Park JH, Cho S and Kim HS: Increased sphingosine kinase 1 expression predicts distant metastasis and poor outcome in patients with colorectal cancer. Anticancer Res *39*: 663-670, 2019. PMID: 30711943. DOI: 10.21873/anticanres.13161

41 Do SI, Kim HS, Kim K, Lee H, Do IG, Kim DH, Chae SW and Sohn JH: Predictive value of sphingosine kinase 1 expression in papillary thyroid carcinoma. Anticancer Res *37*: 5399-5405, 2017. PMID: 28982849. DOI: 10.21873/anticanres.11967

42 Do SI, Yoon G, Kim HS, Kim K, Lee H, Do IG, Kim DH, Chae SW and Sohn JH: Increased brahma-related gene 1 expression predicts distant metastasis and shorter survival in patients with invasive ductal carcinoma of the breast. Anticancer Res *36*: 4873-4882, 2016. PMID: 27630343. DOI: 10.21873/anticanres.11051

43 Zhu X, Zhang Q, Wang D, Liu C, Han B and Yang JM: Expression of pd-l1 attenuates the positive impacts of high-level tumor-infiltrating lymphocytes on prognosis of triple-negative breast cancer. Cancer Biol Ther *20*: 1105-1112, 2019. PMID: 30929569. DOI: 10.1080/15384047.2019.1595282

44 Tomioka N, Azuma M, Ikarashi M, Yamamoto M, Sato M, Watanabe KI, Yamashiro K and Takahashi M: The therapeutic candidate for immune checkpoint inhibitors elucidated by the status of tumor-infiltrating lymphocytes (tils) and programmed death ligand 1 (pd-l1) expression in triple negative breast cancer (tnbc). Breast Cancer *25*: 34-42, 2018. PMID: 28488168. DOI: 10.1007/s12282-017-0781-0

45 Adams TA, Vail PJ, Ruiz A, Mollaee M, McCue PA, Knudsen ES and Witkiewicz AK: Composite analysis of immunological and metabolic markers defines novel subtypes of triple negative breast cancer. Mod Pathol *31*: 288-298, 2018. PMID: 28984302. DOI: 10.1038/modpathol.2017.126

46 Li M, Li A, Zhou S, Xu Y, Xiao Y, Bi R and Yang W: Heterogeneity of pd-l1 expression in primary tumors and paired lymph node metastases of triple negative breast cancer. BMC Cancer *18*: 4, 2018. PMID: 29291717. DOI: 10.1186/s12885-017-3916-y

47 Brockhoff G, Seitz S, Weber F, Zeman F, Klinkhammer-Schalke M, Ortmann O and Wege AK: The presence of pd-1 positive tumor infiltrating lymphocytes in triple negative breast cancers is associated with a favorable outcome of disease. Oncotarget *9*: 6201-6212, 2018. PMID: 29464065. DOI: 10.18632/oncotarget.23717

48 Kaira K, Shimizu K, Endoh H, Imaizumi K, Kamiyoshihara M, Sugano M, Kawashima O, Tanaka S, Fujita A, Imai H, Kogure Y, Oyama T, Asao T and Shirabe K: Prognostic significance of tumor immunity in surgically resected pulmonary pleomorphic carcinoma. Anticancer Res *40*: 261-269, 2020. PMID: 31892575. DOI: 10.21873/anticanres.13948

49 Nakanishi K, Sakakura N, Matsui T, Ueno H, Nakada T, Oya Y, Shimizu J, Hida T, Hosoda W and Kuroda H: Clinicopathological features, surgical outcomes, oncogenic status and pd-l1 expression of pulmonary pleomorphic carcinoma. Anticancer Res *39*: 5789-5795, 2019. PMID: 31570483. DOI: 10.21873/anticanres.13782

50 Kang SH, Hwang HJ, Yoo JW, Kim H, Choi ES, Hwang SH, Cho YU, Jang S, Park CJ, Im HJ, Seo JJ, Kim N and Koh KN: Expression of immune checkpoint receptors on t-cells and their ligands on leukemia blasts in childhood acute leukemia. Anticancer Res *39*: 5531-5539, 2019. PMID: 31570447. DOI: 10.21873/anticanres.13746

51 Ando K, Hamada K, Watanabe M, Ohkuma R, Shida M, Onoue R, Kubota Y, Matsui H, Ishiguro T, Hirasawa Y, Ariizumi H, Tsurutani J, Yoshimura K, Tsunoda T, Kobayashi S and Wada S: Plasma levels of soluble pd-l1 correlate with tumor regression in patients with lung and gastric cancer treated with immune checkpoint inhibitors. Anticancer Res *39*: 5195-5201, 2019. PMID: 31519633. DOI: 10.21873/anticanres.13716

52 Takahashi H, Sakakura K, Arisaka Y, Tokue A, Kaira K, Tada H, Higuchi T, Okamoto A, Tsushima Y and Chikamatsu K: Clinical and biological significance of pd-l1 expression within the tumor microenvironment of oral squamous cell carcinoma. Anticancer Res *39*: 3039-3046, 2019. PMID: 31177146. DOI: 10.21873/anticanres.13437

53 Enkhbat T, Nishi M, Takasu C, Yoshikawa K, Jun H, Tokunaga T, Kashihara H, Ishikawa D and Shimada M: Programmed cell death ligand 1 expression is an independent prognostic factor in colorectal cancer. Anticancer Res *38*: 3367-3373, 2018. PMID: 29848685. DOI: 10.21873/anticanres.12603

54 Chiu YM, Tsai CL, Kao JT, Hsieh CT, Shieh DC, Lee YJ, Tsay GJ, Cheng KS and Wu YY: Pd-1 and pd-l1 up-regulation promotes t-cell apoptosis in gastric adenocarcinoma. Anticancer Res *38*: 2069-2078, 2018. PMID: 29599324. DOI: 10.21873/anticanres.12446

55 Park R, Williamson S, Kasi A and Saeed A: Immune therapeutics in the treatment of advanced gastric and esophageal cancer. Anticancer Res *38*: 5569-5580, 2018. PMID: 30275174. DOI: 10.21873/anticanres.12891

56 Kobayashi K, Seike M, Zou F, Noro R, Chiba M, Ishikawa A, Kunugi S, Kubota K and Gemma A: Prognostic significance of nsclc and response to egfr-tkis of egfr-mutated nsclc based on pd-l1 expression. Anticancer Res *38*: 753-762, 2018. PMID: 29374699. DOI: 10.21873/anticanres.12281

57 Bassanelli M, Sioletic S, Martini M, Giacinti S, Viterbo A, Staddon A, Liberati F and Ceribelli A: Heterogeneity of pd-l1 expression and relationship with biology of nsclc. Anticancer Res *38*: 3789-3796, 2018. PMID: 29970498. DOI: 10.21873/anticanres.12662

58 Saito Y, Horiuchi S, Morooka H, Ibi T, Takahashi N, Ikeya T, Shimizu Y and Hoshi E: Inter-tumor heterogeneity of pd-l1 expression in non-small cell lung cancer. J Thorac Dis *11*: 4982-4991, 2019. PMID: 32030214. DOI: 10.21037/jtd.2019.12.24

Received February 5, 2020
Revised February 14, 2020
Accepted February 14, 2020