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Programmed Cell Death Ligand 1 Expression in Primary Central Nervous System Lymphomas: A Clinicopathological Study

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Abstract. *Background/Aim: Programmed cell death ligand 1 (PD-L1)/programmed cell death 1 (PD-1) have been shown to predict response to PD-L1/PD-1-targeted therapy. We analyzed PD-L1 expression in primary central nervous system lymphomas (PCNSLs). Materials and Methods: PD-L1 protein and mRNA expression were evaluated in 64 PCNSL tissue samples. IFN-γ, IL-10, CD4, and CD8 mRNA expression was also evaluated. Results: PD-L1 protein was detected in tumor cells in 2 (4.1%) cases and in tumor microenvironments in 25 (52%) cases. PD-L1 mRNA positively correlated with IFN-γ (p=0.0024) and CD4 (p=0.0005) mRNA expression. IFN-γ mRNA positively correlated with CD8 mRNA expression (p=0.0001). Furthermore, tumor cell PD-L1 expression correlated positively with overall survival (p=0.0177), whereas microenvironmental PD-L1 expression exhibited an insignificant negative trend with overall survival (p=0.188). Conclusion: PD-L1 was expressed on both tumor and/or tumor-infiltrating immune cells in PCNSL. The biological roles of this marker warrant further investigation.*

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Primary central nervous system lymphomas (PCNSLs) are extranodal non-Hodgkin's lymphomas (NHLs) that develop in the central nervous system (CNS), including the brain, eye, spinal cord, or leptomeninges (1). Most PCNSLs are high-grade, B-cell-type NHLs with less favorable prognoses relative to systemic NHLs (1). Although the incidence of PCNSLs has reportedly increased, this may be attributable in part to a better understanding of the disease (2). The median overall survival (OS) has improved from 10 to 79 months with the administration of high-dose methotrexate (HD-MTX)-based chemotherapy (3-5). However, 10-35% of patients with refractory disease and 30-60% of those who develop a relapse require salvage treatment (6). Moreover, patients who develop refractory or relapsed disease after initial therapy have a poor prognosis (6). However, salvage treatment may improve a patient's outcomes and quality of life even if the optimal standard salvage regimen is unknown (6). The use of novel therapeutic agents with clear mechanisms to target recurrent PCNSL is warranted.

Cancer immunotherapy entered a new era following the discovery of novel agents that can interfere with specific immune checkpoints, such as the programmed death-1 receptor (PD-1, CD279)/programmed death-1 ligand (PD-L1, CD274) axis or cytotoxic T lymphocyte antigen-4 (CTLA-4) (7, 8). PD-1 is a cell surface-expressed, immunoglobulin superfamily co-inhibitory receptor with a 288-amino acid sequence. PD-L1 and PD-L2, the ligands of PD-1, are members of the B7 family. PD-L1 has a 290-amino acid sequence and is expressed on cancer cells, T cells, dendritic cells, macrophages, and other cells present in the tumor microenvironment (9). The PD-L1/PD-1 axis

has been shown to inhibit effector T-cell responses, thus allowing tumor cells to escape immune surveillance within tumor microenvironments (10, 11). By contrast, PD-1 is expressed on T cells, B-cells, and natural killer cells, and the presence of a large population of PD-1-positive tumor-infiltrating lymphocytes (TILs) is associated with favorable outcomes in patients with diffuse large B-cell lymphoma (DLBCL) (12). Immune checkpoint inhibitors can activate T cell responses to cancer cells by blocking ligand–receptor interactions. Ongoing clinical trials are evaluating immune checkpoint inhibitors that target the PD-L1/PD-1 axis in several advanced cancers. Several malignant tumors have been shown to express PD-L1 to escape T cell-mediated cytotoxicity (13, 14). Several reports have described an association of PD-L1 expression with poor survival in patients with various cancers, including melanoma; lung, renal, and ovarian cancers; and hematopoietic system malignancies (14-18). By contrast, other studies have revealed an association of PD-L1 expression with improved survival in patients with melanoma, lung, and colorectal cancer (19-21) or a lack of association with survival in patients with lung and kidney cancer (13, 22, 23). Furthermore, immunohistochemically-detected PD-L1 expression on both cancer cells and/or infiltrating immune cells was found to correlate with responses to PD-L1/PD-1 inhibitors (7, 24, 25), and has therefore been investigated as a potential predictive biomarker of responsiveness to these checkpoint inhibitors (26). PCNSLs originate in immune-privileged sites (27). However, the clinicopathological role of PD-L1 in patients with PCNSL remains unclear. In the present study, we investigated PD-L1 mRNA and protein expression in tumor cells and in the tumor microenvironment, and their associations with clinical variables such as *CD4, CD8, IFN-γ*, and *IL-10* mRNA in patients with PCNSL as a foundation for future clinical trials.

Materials and Methods

Samples and study population. Patients were diagnosed and treated at the Toyama Prefectural Central Hospital, Wakayama Medical University Hospital, Chiba University Hospital, and Yamaguchi University Hospital between 2000 and 2017. The inclusion criteria were histologically-proven PCNSL and no evidence of Human immunodeficiency virus (HIV)-1 infection or other immunodeficiencies. Computed tomography (CT) or magnetic resonance imaging (MRI) of the brain, thorax, abdomen, and pelvis were performed for all patients. During neurosurgery, tissues were frozen within 5 min in liquid nitrogen and stored at –80˚C. All specimens were reviewed by board-certified pathologists (YK, SI) who observed sections of paraffin-embedded tissues that were obtained from near the frozen sample. The normal serum lactate dehydrogenase (LDH) and cerebrospinal fluid (CSF) protein cut-off values were 216 IU/L and 45 mg/dl, respectively. Informed consent was obtained from all patients for the use of their samples in accordance with the guidelines of the respective Ethical Committees on Human Research.

Immunohistochemistry. Five-micrometer sections of the formalin-fixed, paraffin-embedded tissue specimens were evaluated. Primary antibodies against PD-L1 (clone E1L3N, Cell Signaling Technology, Danvers, MA, USA), CD163 (clone 10D6, Novocastra, Danvers, MA, USA), CD19 (clone LE-CD19, DAKO, Tokyo, Japan), B-cell CLL/lymphoma (BCL6) (DAKO), CD10 (Nichirei, Tokyo, Japan), and melanoma associated antigen (MUM) 1 (DAKO) were used for immunostaining. Anti-goat, -mouse, and -rabbit secondary antibodies (Nichirei, Tokyo, Japan) were subsequently applied. The antibodies were diluted and the immunoreaction to PD-L1 was enhanced by Can Get Signal Solution (TOYOBO, Tokyo, Japan). We also immunophenotyped the germinal center B-cell-like (GCB) and non-GCB subtype cases using antibodies specific for CD10, BCL-6, and MUM1, as described by Camilleri-Broet *et al*. (28). The staining intensity was classified as none (0 points), weakly positive (1 point), or moderately/strongly positive (2 points). The averages of three independent measurements were calculated to the first decimal place. For double immunostaining, secondary staining was performed using a goat anti-mouse antibody labeled with Alexa 488 and a goat anti-rabbit antibody labeled with Alexa 546 (Thermo Fisher, Waltham, MA).

Epstein–Barr virus genotyping. Genomic DNA was extracted using the AllPrep DNA/RNA/miRNA Universal Kit or AllPrep DNA/RNA FFPE Kit (QIAGEN, Hilden, Germany). Epstein–Barr virus (EBV) genotyping was performed by PCR assays for both type-1 and -2 in the common region of EBV nuclear antigen (EBNA) 2 with specific primers (29). The thermal cycle conditions were as follows: 94˚C for 2 min, 35 cycles of 94˚C for 30 s, 60˚C for 30 s, and 72˚C for 30 s, followed by 72˚C for 7 min. The EBV-positive Raji NHL (Riken, Tsukuba, Japan) and the EBV-negative A4/Fuk NHL cell lines (JCRB, Osaka, Japan) were used as positive and negative controls, respectively. EBNA-2C/G (estimated size: 250 bp) and -2C/B (300 bp) primers were used for the detection of EBV type-1 and -2, respectively. The PCR assay for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (260 bp) was performed as a sample quality control. Gel electrophoresis with 2% agarose was performed and stained with GelRed Nucleic Acid Stain (Biotium, Hayward, CA), following the manufacturer's instructions. The following primers were used for PCR: EBNA-2C (5'-AGGGATGCCTGGACACAAGA-3'), EBNA-2G (5'-GCCTCGGTTGTGACAGAG-3'), EBNA-2B (5'-TTGAAGAGTATGTCCTAAGG-3'); and *GAPDH* forward (5'-GCA CCGTCAAGGCTGAGAAC-3'), *GAPDH* reverse (5'-TGGTGAAG ACGCCAGTGGA-3').

RNA extraction and real-time quantitative PCR. Total RNA was extracted using the AllPrep DNA/RNA/miRNA Universal Kit (QIAGEN) and reverse-transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed using Platinum SYBR Green qPCR SuperMix (Invitrogen) and an ABI StepOne Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). The results were normalized to the value detected for *GAPDH*. The thermal cycling conditions were as follows: initial incubation at 50˚C for 2 min, denaturation at 95˚C for 2 min and 40 cycles of denaturation at 95˚C for 5 s, annealing at 55˚C for 10 s, and extension at 72˚C for 10 s. The following primers were used for real-time PCR:

PD-L1 forward (5'-TATGGTGGTGCCGACTACAA-3'), *PD-L1* reverse (5'-TGCTTGTCCAGATGACTTCG-3'); *IFN-γ* forward (5'-GACCAGAGCATCCAAAAGAGT-3'), *IFN-γ* reverse (5'-ATTGC TTTGCGTTGGACATTC-3'); *IL-10* forward (5'-GAGAACAGCT GCACCCACTT-3'), *IL-10* reverse (5'-CTCAGACAAGGCTTGGC AAC-3'); *CD4* forward (5'-GTCCCTTTTAGGCACTTGCTTCT-3'), *CD4* reverse (5'-TCTTTCCCTGAGTGGCTGCT-3'); *CD8* forward (5'-CCCTGAGCAACTCCATCATGT-3'), and *CD8* reverse (5'-GTGGGCTTCGCTGGCA-3'). The mRNA expression levels of target genes were determined as the fold-change relative to normal lymphocytes and calculated using the $2^{-\Delta\Delta CT}$ method (30).

Ethical approval. All procedures involving human participants were performed in accordance with the ethical standards of the respective institutional research committees and the Declaration of Helsinki.

Statistical analysis. The end-point measurements included progression-free survival (PFS) and OS, defined as the time interval from initial diagnosis to death or recurrence or to the last follow-up, respectively. Kaplan–Meier analysis was used to estimate survival, and a log-rank test was used to evaluate differences in survival between the groups. The associations of variables with OS were evaluated through a Cox proportional hazards regression model-based multivariate analysis. A p-value <0.05 was used to indicate statistical significance. All statistical analyses were performed using JMP version 10 software (SAS Institute Inc., Tokyo, Japan).

Results

Patient characteristics. The 64 patients' baseline characteristics are presented in Table I. The average age of the patients was 63.9 years (range=31-85 years). Thirty-eight patients (59.3%) were male and 26 (40.6%) were female. The Karnofsky performance scale (KPS) scores at first admission were ≥70 in 28 cases (43.7%) and <70 in 36 cases (56.2%). Ten (15.6%), 22 (34.3%), and 32 (50%) cases were classified into Memorial Sloan-Kettering Cancer Center (MSKCC) (31) prognostic risk group 1 (age <50 years), group 2 (age ≥50 years, KPS ≥70), and group 3 (age ≥50 years, KPS <70), respectively. All patients were diagnosed with histology-proven DLBCL. Forty patients (62.5%) presented with a single lesion, whereas 24 (37.5%) had multiple lesions. The deep structures of the brain (*e.g.*, periventricular lesion, basal ganglia, corpus callosum, brain stem, and/or cerebellum) were involved in 37 patients (57.8%). Elevated LDH serum levels were detected in 27 patients (45.0%). EBV test results were positive in 13 (20.3%) and negative in 48 (75%) cases. Immunophenotyping revealed the GCB subtype in 10 (18.1%) cases and the non-GCB subtype in 45 (81.8%) cases. Thirty-four (53.1%) patients were treated with three or more cycles of HD-MTX at 3 g/m^2 per course, and 24 (37.5%) patients received polychemotherapy containing HD-MTX. Forty patients experienced a relapse after responding to the first-line therapy (relapse rate, 65.5%). Second-line treatments were determined at the physician's discretion.

Table I. *Patient characteristics*.

	n (%)
Variable	Primary CNS lymphoma (n=64)
Age	
Average (range)	63.9 (31-85)
60≤	43 (67.1)
60>	21 (32.8)
Gender	
Male	38 (59.3)
Female	26 (40.6)
KPS	
70≤	28 (43.7)
70>	36 (56.2)
Involvement of deep region of the brain	
Yes	37 (57.8)
None	27 (42.1)
No. of lesion	
Solitary	40 (62.5)
Multiple	24 (37.5)
Immunophenotype	
GCB	10 (18.1)
Non-GCB	45 (81.8)
EBV	
Positive	13 (20.3)
Negative	48 (75.0)
LDH	
Elevated (216≤)	27 (45.0)
Not elevated (216>)	33 (55.0)
MSKCC score	
Group 1 (age<50)	10 (15.6)
Group 2 (age≥50, 70≤KPS)	22 (34.3)
Group 3 (age≥50, 70>KPS)	32 (50.0)
PD-L1 IHC tumor cell	
Positive	2 (4.1)
Negative	46 (95.8)
PD-L1 IHC stromal cell	
Positive	25 (52.0)
Negative	23 (47.9)
Chemotherapy	
HD-MTX	34 (53.1)
Polychemotherapy	24 (37.5)

EBV: Epstein-Barr virus; GCB: germinal center B cell; HD-MTX: high-dose methotrexate; IHC: immunohistochemistry; KPS: Karnofsky performance scale; MSKCC: Memorial Sloan-Kettering Cancer Center.

Detection of PD-L1 expression by immunohistochemistry and QPCR. As shown in Table I, we observed PD-L1 expression in tumor cells in 2 cases (4.1%) and in tumor-associated cells (*e.g.*, tumor-associated macrophage (TAM) and/or neutrophils) in 25 cases (52%). The anti-PD-L1 antibody revealed membrane staining in both lymphoma and tumor stromal cells. Representative PD-L1 immunostaining in a PCNSL is displayed in Figure 1. Positive PD-L1 staining in CD19-positive tumor cells and CD163-positive TAMs was

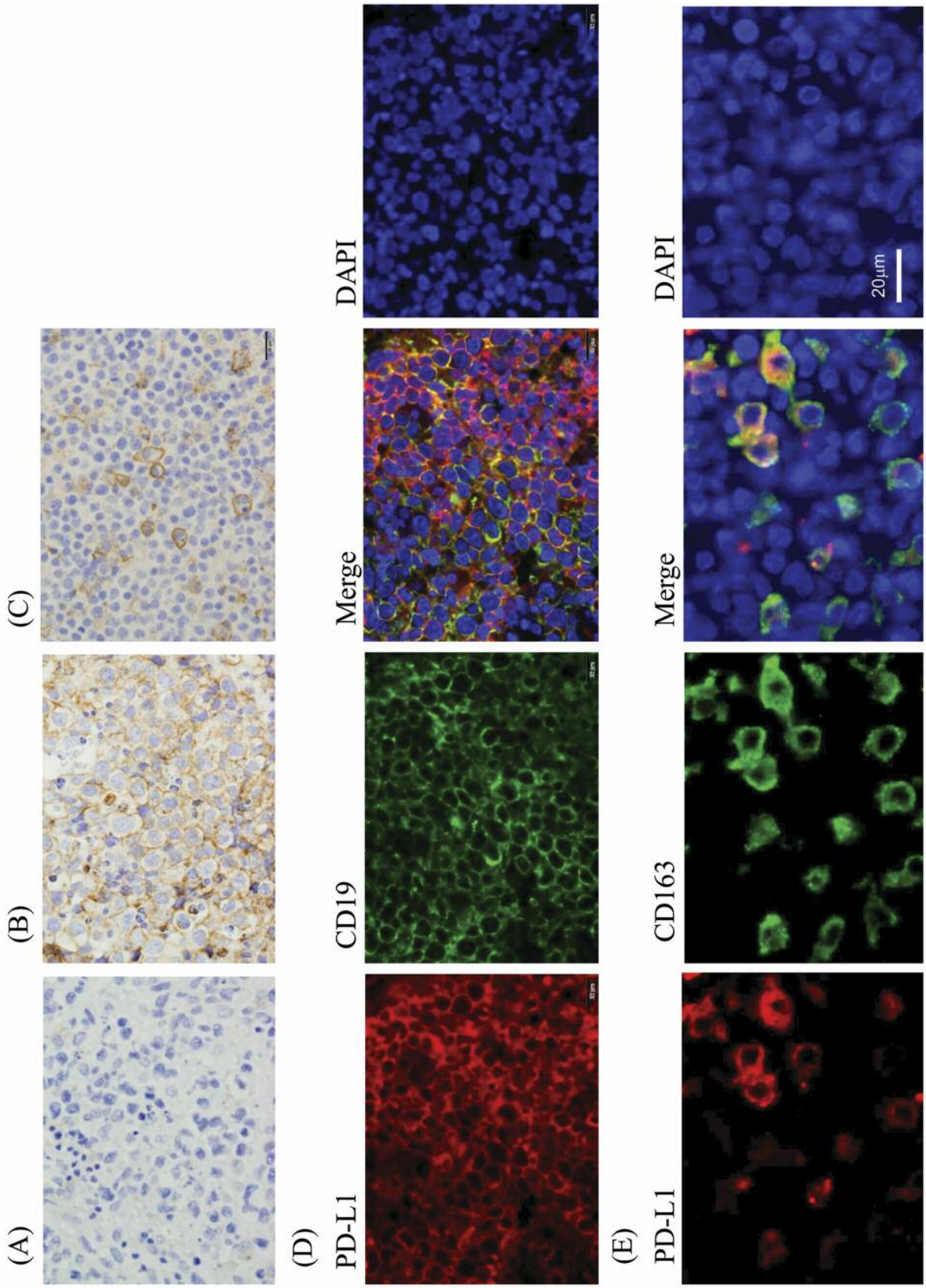

Figure 1. *Programmed cell death ligand (PD-L1) expression in lymphoma tissues. Representative images of PD-L1-immunostained primary central nervous system lymphomas (PCNSLs) are displayed. (A) Negative case, (B) PD-L1 positivity on lymphoma cells, (C) PD-L1 positivity on tumor-associated macrophages (TAMs), and (D) double-fluorescent immunostaining demonstrating positive PD-L1 staining of CD19-positive lymphoma cells. (E) Double-fluorescent immunostaining demonstrating positive PD-L1 staining of CD163-positive TAMs. Scale bar=20 μm.*

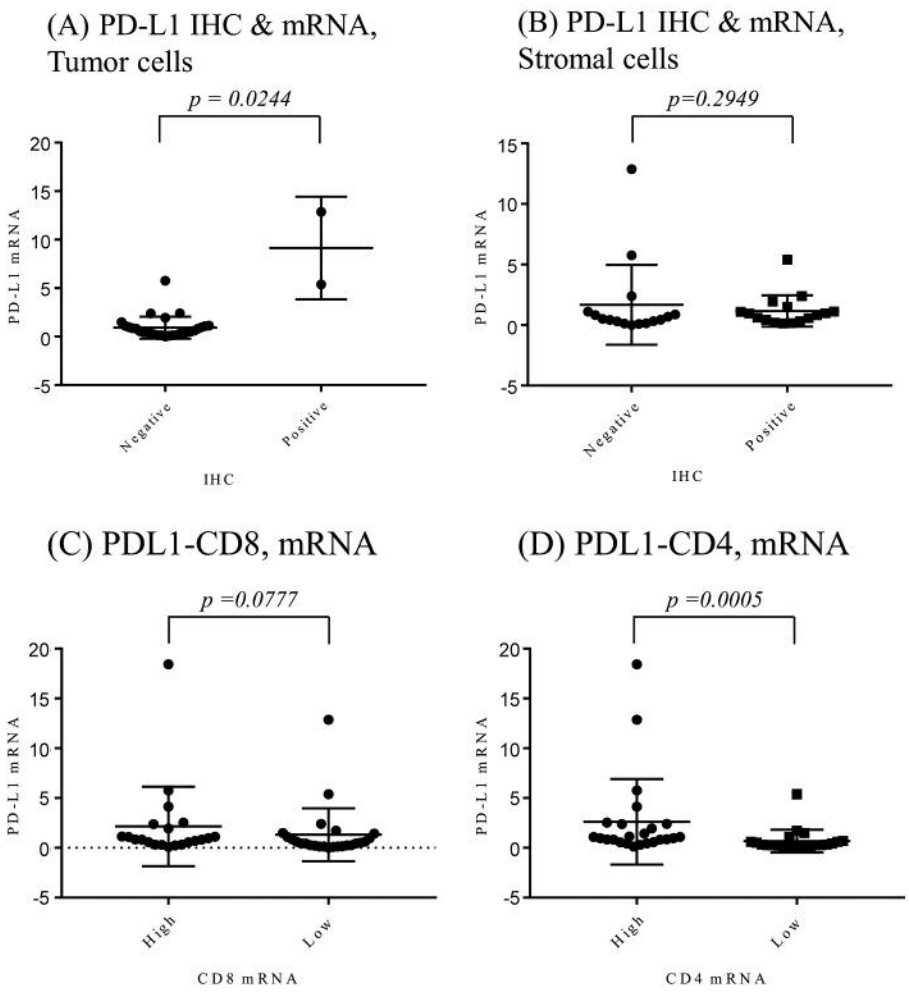

Figure 2. *Comparison between programmed cell death ligand (PD-L1) protein and CD4 and CD8 mRNA expression. (A) PD-L1 mRNA expression in cases with positive or negative tumor cells demonstrated with immunohistochemistry (IHC), and (B) PD-L1 mRNA expression in cases with positive or negative tumor microenvironments demonstrated with IHC. (C) PD-L1 mRNA expression in CD8 mRNA high or low groups. (D) PD-L1 mRNA expression in CD4 mRNA high or low groups.*

confirmed using double-fluorescent immunostaining. PD-L1 protein expression in tumor cells was found to correlate with *PD-L1* mRNA expression (p=0.0244) (Figure 2A). By contrast, PD-L1 protein expression in the tumor microenvironment did not correlate with *PD-L1* mRNA expression (p=0.2949) (Figure 2B).

PD-L1 expression correlates positively with IFN-γand CD8 expression. Potential links between *PD-L1* and *IFN-γ, IL-10, CD4*, and *CD8* mRNA expression were evaluated. *PD-L1* mRNA expression on tumor tissue was found to correlate with *CD8* and *CD4* mRNA expression (p=0.0777 and p=0.0005, respectively) (Figure 2C and D). Linear regression and Spearman's rank correlation analysis indicated that *PD-L1* mRNA expression correlated positively with *IFN-γmRNA* expression (r=0.3241, p=0.028) (Figure 3A), but not with *IL-10* mRNA expression in PCNSL tissue samples (r=–0.0429, p=0.7771) (Figure 3B). *IFN-γ* mRNA expression positively correlated with *CD8* mRNA (r=0.5332, p=0.0001) (Figure 3C), but not with *CD4* mRNA expression (r=0.1488, p=0.3238).

PD-L1 expression in tumor cells correlates with survival. The median OS of all patients was 32.3 months (95% confidence interval [CI]=25.4-66.7) with a 2-year survival rate of 66.4%. The median survival time and 2-year survival rate were not reached (NR) and 100% among patients with positive PD-L1 tumor cell

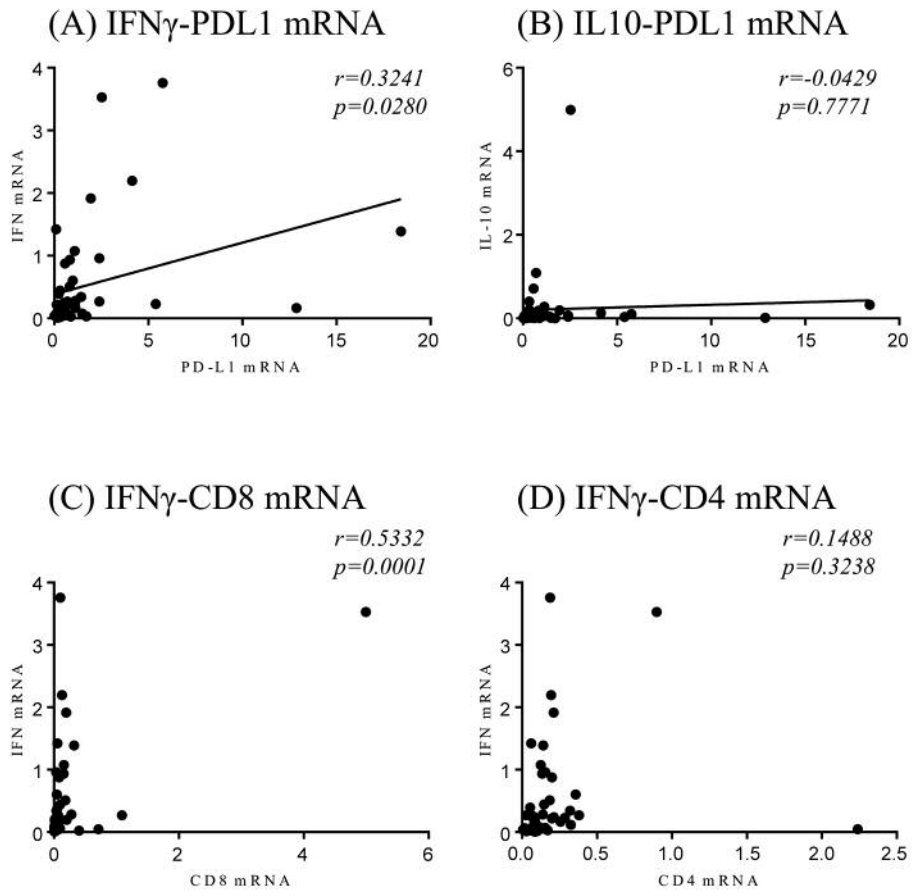

Figure 3. *Comparison between programmed cell death ligand (PD-L1) and interferon (IFN)-γ, interleukin (IL)-10, CD4, and CD8 mRNA expression. (A) Comparison between PD-L1 and IFN-γ mRNA expression. (B) Comparison between PD-L1 and IL-10 mRNA expression. (C) Comparison between CD8 and IFN-γ mRNA expression. (D) Comparison between CD4 and IFN-γ mRNA expression.*

immunohistochemistry, respectively, and 31.7 months (95%CI=22.5-66.7) and 66.3% among those with negative PD-L1 tumor cell immunohistochemistry (p=0.0177, Figure 4A), respectively. The median survival time and 2-year survival rate were 28.4 months (95%CI=13.6-66.7) and 64.4% among patients with positive PD-L1 tumor stromal cell immunohistochemistry, respectively, and 74.6 months (95%CI=22.5-115.5) and 71.4% among patients with negative PD-L1 tumor stromal cell immunohistochemistry (p=0.1880, Figure 4B), respectively. The median PFS was 30.7 months (95% CI, 6-NR) among patients in the *CD4* mRNA high expression group (*CD4* mRNA≥0.11839), and 10 months (95%CI=6-28.5) among patients in the CD4 mRNA low expression group (*CD4* mRNA<0.11839) (p=0.1943, Figure 4C). The median PFS was 28.5 months (95% CI, 4-NR) among patients in the *CD8* mRNA high expression group (*CD8* mRNA≥0.0573), and 10 months (95%CI=6-41) among patients in the *CD8* mRNA low expression group (*CD8* mRNA<0.0573) (p=0.5665, Figure 4D). Table II presents the results of multivariate analyses. KPS, number of lesions, and PD-L1 tumor cell immunohistochemistry were found to significantly associate with OS in the multivariate analyses. Table III also presents the results of the multivariate analyses. Gender, immunophenotype, MSKCC score, chemotherapy, and *CD8* mRNA expression were found to significantly associate with PFS in the multivariate analyses.

Discussion

The interaction of PD-1 on immune cells with PD-L1 on tumor cells leads to inhibition of anti-tumor immune cell effector functions (32). Immunotherapeutic approaches that target immune checkpoints are promising as treatment modalities for several types of cancer, and accordingly, recent clinical trials have investigated a large number of antibodies specific for the PD-1/PD-L1 interaction (7, 33). The anti-PD-1 receptor antibody nivolumab was particularly

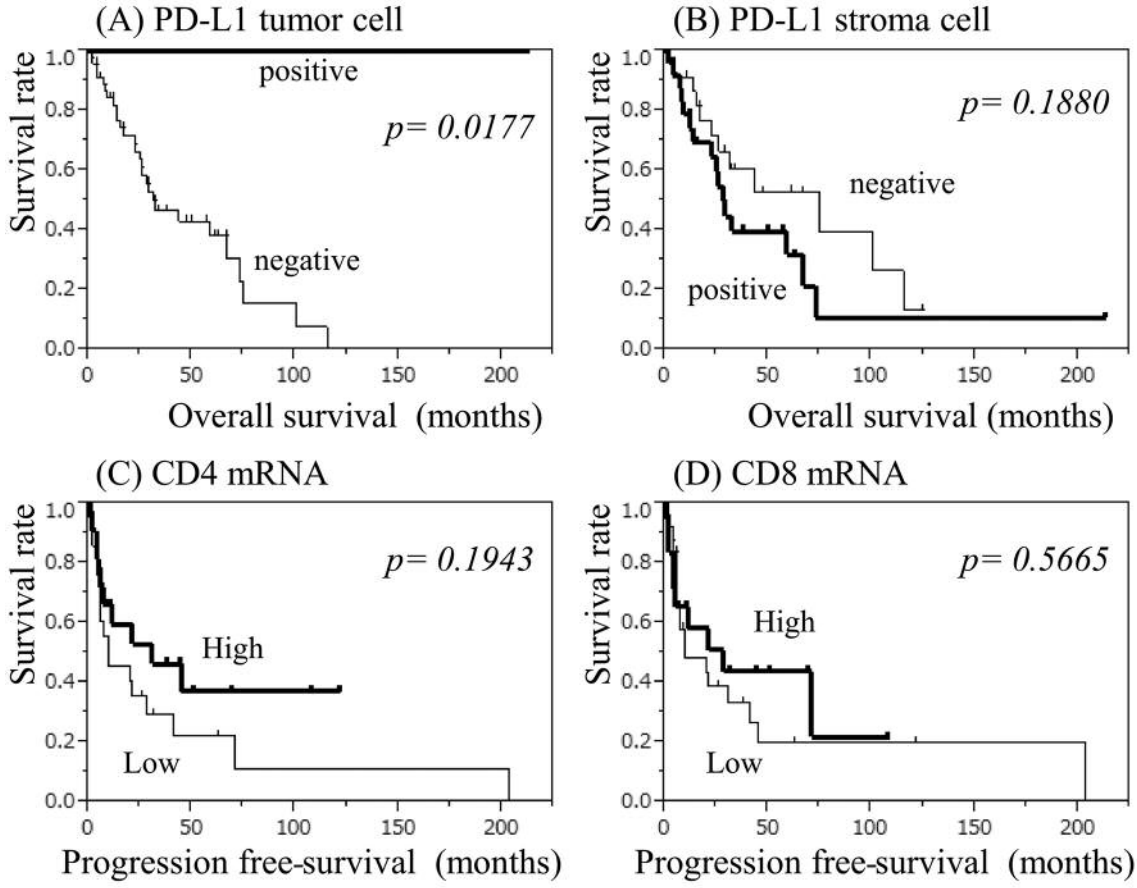

Figure 4. *Kaplan–Meier survival analysis of patients with central nervous system (CNS) lymphomas. (A) Overall survival of patients with tumor cell programmed cell death ligand 1 (PD-L1) positive or negative immunohistochemistry. (B) Overall survival of patients with tumor stromal cell PD-L1 positive or negative immunohistochemistry staining. (C) Progression-free survival of patients in CD4 mRNA high- or low-expression groups. (D) Progression-free survival of patients in CD8 mRNA high or low expression groups.*

effective in patients with Hodgkin's lymphoma, follicular lymphoma, and NHL (34-36). Several reports have also discussed PD-L1 expression on systemic DLBCL. Immunohistochemically, PD-L1-positive tumor cells were found in 3.7–20% of patients (37-40), whereas PD-L1-positive tumor-infiltrating immune cells were detected in 15.3% of patients (38). Both PD-L1-positive tumor cells (38, 41, 42) and tumor-infiltrating immune cells are associated with non-GCB subtype disease (38). In DLBCL, tumor cell PD-L1 positivity has been associated with inferior OS (38, 39), whereas tumor-associated cell PD-L1 positivity did not correlate significantly with survival (38, 42).

Several cell types, including macrophages, neutrophils, fibroblasts, lymphocytes, and endothelial cells, comprise the cancer microenvironment. Tumor progression is supported by interactions between tumor and stromal cells. Macrophages and neutrophils play key roles in the induction of apoptosis and exhaustion of tumor-reactive T cell immune responses *via* checkpoint molecules (25); notably, most TAMs suppress T helper type 1 responses (43). Several reports have described PD-L1 expression on macrophages and neutrophils in tumor tissues (25, 44). Furthermore, increased numbers of TAMs and neutrophils have been associated with poor outcomes in patients with Hodgkin's lymphoma, bladder cancer, and gastric cancer (44-46), but better outcomes in patients with breast cancer (47).

The CNS is an immune-privileged site that is carefully regulated by astrocytes, microglia, and immune cells (48). Few studies have addressed the role of the PD-L1/PD-1 axis in PCNSL. Berghoff *et al*. (49) investigated PD-L1/PD-1 expression in 20 cases of non-GCB PCNSL, and detected tumor cell PD-L1 expression in two cases (10%) and TAM expression in four cases (20%). No correlation was observed between PD-L1 expression and the number of CD8-positive TILs. Our report is the largest series to address PD-L1 expression in PCNSL. Despite the small patient cohort, PD-L1

Table II. *Correlations between the overall survival and the clinicopathological factors.*

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age	1.03 (1.00-1.06)	0.0407		
Gender	1.29 (0.68-8.51)	0.4311		
KPS	0.98 (0.95-1.00)	0.0931	0.14 (0.0084-1.55)	0.00816
Involvement of deep region of the brain	1.11 (0.58-2.13)	0.7451		
No. of lesion	0.64 (0.33-1.27)	0.2006	2.44 (0.52-15.1)	0.01907
Immunophenotype (GCB *vs*. non-GCB)	1.45 (0.63-3.91)	0.392		
EBV	1.49 (0.70-2.96)	0.4866		
LDH	0.86 (0.19-3.39)	0.847		
MSKCC score	4.92 (1.62-21.3)	0.0128		
Chemotherapy	0.79 (0.39-1.58)	0.5182		
PD-L1 IHC tumor cell	2.69E+9 (2.94-2.94)	0.0019	8.57e-11 (2.07e-86-0.32)	0.00015
PD-L1 IHC stromal cell	0.60 (0.27-1.28)	0.19	2.60 (0.79-9.26)	0.17957
IFNγmRNA	1.01 (0.15-4.05)	0.9894		
IL-10 mRNA	0.40 (0.02-2.54)	0.3817	0.99 (0.98-1.00)	0.15986
CD4 mRNA	0.21 (0.0006-11.26)	0.4954		
CD8 mRNA	0.73 (0.06-3.17)	0.7224		

CI: Confidence interval; EBV: Epstein-Barr virus; GCB: germinal center B cell; IHC: immunohistochemistry; HR: hazard ratio; KPS: Karnofsky performance scale; MSKCC: Memorial Sloan-Kettering Cancer Center; NR: not reached.

Table III. *Correlations between the progression-free survival and the clinicopathological factors.*

Variable	Univariate		Multivariate	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age	1.01 (0.98-1.04)	0.2223		
Gender	1.41 (0.74-2.76)	0.28	2.90 (0.84-11.41)	0.00795
KPS	0.78 (0.23-2.48)	0.6861		
Involvement of deep region of the brain	0.97 (0.51-1.85)	0.9362		
No. of lesion	0.89 (0.46-1.80)	0.7455		
Immunophenotype (GCB *vs*. non-GCB)	1.20 (0.45-2.72)	0.6782	0.39 (0.07-1.57)	0.03744
EBV	1.17 (0.56-2.75)	0.4951		
LDH	2.55 (0.64-8.92)	0.1736		
MSKCC score	3.53 (1.19-15.12)	0.0537	3.13 (0.38-66.68)	0.00387
Chemotherapy	0.76 (0.38-1.51)	0.4311	0.91 (0.28-2.97)	0.01672
PD-L1 IHC tumor cell	1.78E+9 (1.83-1.83)	0.0094		
PD-L1 IHC stromal cell	0.83 (0.39-1.72)	0.6362	1.61 (0.51-5.30)	0.16034
IFNγmRNA	0.89 (0.10-4.14)	0.9005	2.48 (0.89-6.51)	0.05653
IL-10 mRNA	0.92 (0.10-4.53)	0.9312		
CD4 mRNA	0.007 (1.65e-6-130.00)	0.0985		
CD8 mRNA	0.14 (3.48e-5-1.90)	0.1962	4.99 (0.00033-1640.05)	0.04983

CI: Confidence interval; EBV: Epstein-Barr virus; GCB: germinal center B cell; IHC: immunohistochemistry; HR: hazard ratio; KPS: Karnofsky performance scale; MSKCC: Memorial Sloan-Kettering Cancer Center; NR: not reached.

expression on lymphoma cells correlated with a superior OS. We further investigated the relationship between *PD-L1* mRNA and protein expression and observed a strong correlation of these parameters when PD-L1 was expressed on tumor cells. Furthermore, a multivariate analysis identified PD-L1 expression and *CD8* mRNA as favorable prognostic markers in our cohort. In our series, most PD-L1-positive stromal cells were CD163-positive macrophages. We observed a tendency toward a negative association between microenvironmental PD-L1 expression and longer OS. Moreover, *PD-L1* and

IFN-γ gene expression were found to correlate significantly in PCNSL. The mRNA levels of *CD4*-positive TILs reflected the number of TILs correlated with *PD-L1* mRNA levels. CD8 mRNA and *IFN-γ* gene expression were found to correlate significantly in PCNSL. Considering these issues, increased levels of TILs and TAMs enhance *IFN-γ* production through TAMs, resulting in increased expression of PD-L1 upregulation on tumor and tumor environmental immune cells.

Two previous reports have addressed checkpoint inhibitor immunotherapy for PCNSL. Nayak *et al*. treated four cases of refractory or recurrent PCNSL with nivolumab (50). All patients exhibited radiographic responses, with a median PFS of 17 months (range=13-17 months). However, the authors did not report PD-L1 expression in these cases. Furuse *et al*. treated one case of recurrent PCNSL with nivolumab (51). This patient achieved a complete remission for 10 months. However, it is difficult to estimate the efficacy of nivolumab in that case, as dendritic cell vaccination and bevacizumab were administered concurrently. However, the authors used immunohistochemistry to demonstrate positive PD-L1 expression on TAMs and negative PD-L1 expression on tumor cells.

High PD-L1 expression was thought to derive from responses to extrinsic factors, such as IFN-γ, or intrinsic genetic aberrations (52-55). Frequent 9p24.1/PD-L1/PD-L2 copy number alterations and increased PD-L1/PD-L2 expression on PCNSLs have been reported (56). Positive PD-L1 immunohistochemistry has been reported as a predictive biomarker of responsiveness to PD-L1/PD-1 inhibitors (7); however, tumors that do not express PD-L1 might still respond to these agents (7, 57). As reports of the correlation between PD-L1 expression and the prognoses of several tumor types have yielded varying results, the predictive role of PD-L1 expression remains controversial (13, 15-19, 22, 58). Notably, these reports describe several immunohistochemical antibodies for PD-L1 detection, various definitions of PD-L1 positivity and expression heterogeneity, and differences in expression thresholds, assay reproducibility, and cut-off values. Furthermore, few reports have discussed the precise locations of PD-L1-positive cells. A standardized immunochemical definition of PD-L1 positivity that would allow for the selection of populations that might benefit from these targeted agents is urgently needed.

This study was limited by the retrospective design. Additionally, our study involved a small number of patients, and the results should be further validated in a larger series. However, our study presented valuable information about PD-L1 expression on PCNSL.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

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References

1 Panageas KS, Elkin EB, DeAngelis LM, Ben-Porat L and Abrey LE: Trends in survival from primary central nervous system lymphoma, 1975-1999: a population-based analysis. Cancer *104*: 2466-2472, 2005.

2 Olson JE, Janney CA, Rao RD, Cerhan JR, Kurtin PJ, Schiff D, Kaplan RS and O'Neill BP: The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. Cancer *95*: 1504-1510, 2002.

3 Ferreri AJ, Reni M, Foppoli M, Martelli M, Pangalis GA, Frezzato M, Cabras MG, Fabbri A, Corazzelli G, Ilariucci F, Rossi G, Soffietti R, Stelitano C, Vallisa D, Zaja F, Zoppegno L, Aondio GM, Avvisati G, Balzarotti M, Brandes AA, Fajardo J, Gomez H, Guarini A, Pinotti G, Rigacci L, Uhlmann C, Picozzi P, Vezzulli P, Ponzoni M, Zucca E, Caligaris-Cappio F and Cavalli F: High-dose cytarabine plus high-dose methotrexate versus high-dose methotrexate alone in patients with primary CNS lymphoma: a randomised phase 2 trial. Lancet *374*: 1512-1520, 2009.

4 Thiel E, Korfel A, Martus P, Kanz L, Griesinger F, Rauch M, Roth A, Hertenstein B, von Toll T, Hundsberger T, Mergenthaler HG, Leithauser M, Birnbaum T, Fischer L, Jahnke K, Herrlinger U, Plasswilm L, Nagele T, Pietsch T, Bamberg M and Weller M: High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol *11*: 1036-1047, 2010.

5 Morris PG, Correa DD, Yahalom J, Raizer JJ, Schiff D, Grant B, Grimm S, Lai RK, Reiner AS, Panageas K, Karimi S, Curry R, Shah G, Abrey LE, DeAngelis LM and Omuro A: Rituximab, methotrexate, procarbazine, and vincristine followed by consolidation reduced-dose whole-brain radiotherapy and cytarabine in newly diagnosed primary CNS lymphoma: final results and long-term outcome. J Clin Oncol *31*: 3971-3979, 2013.

6 Reni M, Ferreri AJ and Villa E: Second-line treatment for primary central nervous system lymphoma. Br J Cancer *79*: 530-534, 1999.

7 Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM and Sznol M: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med *366*: 2443-2454, 2012.

8 Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton JM, Gupta A and Sznol M: Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med *369*: 122-133, 2013.

9 Keir ME, Butte MJ, Freeman GJ and Sharpe AH: PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol *26*: 677-704, 2008.

10 Drake CG, Jaffee E and Pardoll DM: Mechanisms of immune evasion by tumors. Adv Immunol *90*: 51-81, 2006.

11 Pardoll DM: The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer *12*: 252-264, 2012.

12 Ahearne MJ, Bhuller K, Hew R, Ibrahim H, Naresh K and Wagner SD: Expression of PD-1 (CD279) and FoxP3 in diffuse large B-cell lymphoma. Virchows Arch *465*: 351-358, 2014.

13 Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H and Nishimura M: B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res *10*: 5094-5100,2004.

14 Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, Honjo T and Fujii S: Programmed cell death 1 ligand 1 and tumor-infiltrating $CD8^+$ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA *104*: 3360-3365, 2007.

15 Thompson RH, Dong H, and Kwon ED: Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res *13*: 709s-715s, 2007.

16 Rizvi NA, Mazieres J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, Horn L, Lena H, Minenza E, Mennecier B, Otterson GA, Campos LT, Gandara DR, Levy BP, Nair SG, Zalcman G, Wolf J, Souquet PJ, Baldini E, Cappuzzo F, Chouaid C, Dowlati A, Sanborn R, Lopez-Chavez A, Grohe C, Huber RM, Harbison CT, Baudelet C, Lestini BJ and Ramalingam SS: Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol *16*: 257-265, 2015.

17 Gettinger SN, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, Powderly JD, Heist RS, Carvajal RD, Jackman DM, Sequist LV, Smith DC, Leming P, Carbone DP, Pinder-Schenck MC, Topalian SL, Hodi FS, Sosman JA, Sznol M, McDermott DF, Pardoll DM, Sankar V, Ahlers CM, Salvati M, Wigginton JM, Hellmann MD, Kollia GD, Gupta AK and Brahmer JR: Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. J Clin Oncol *33*: 2004-2012, 2015.

18 Zou W, Wolchok JD and Chen L: PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med *8*: 328rv4, 2016.

19 Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, Chen S, Klein AP, Pardoll DM, Topalian SL and Chen L: Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med *4*: 127ra37, 2012.

20 Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, Zlobec I, Eppenberger-Castori S, Tzankov A, Rosso R, Zuber M, Muraro MG, Amicarella F, Cremonesi E, Heberer M, Iezzi G, Lugli A, Terracciano L, Sconocchia G, Oertli D, Spagnoli GC and Tornillo L: Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer *49*: 2233-2242, 2013.

21 Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M, Herbst RS, Gettinger SN, Chen L and Rimm DL: Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest *94*: 107-116, 2014.

22 Mischinger J, Comperat E, Schwentner C, Stenzl A and Gakis G: Inflammation and Cancer: What Can We Therapeutically Expect from Checkpoint Inhibitors? Curr Urol Rep 16: 59, 2015.

23 Grigg C and Rizvi NA: PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? J Immunother Cancer *4*: 48, 2016.

24 Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, Chen L, Pardoll DM, Topalian SL and Anders RA: 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 *20*: 5064-5074, 2014.

25 Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS and Hodi FS: Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature *515*: 563-567, 2014.

26 Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, Koren-Michowitz M, Shimoni A and Nagler A: Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res *14*: 3044-3051, 2008.

27 Shechter R, London A and Schwartz M: Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educational gates. Nat Rev Immunol *13*: 206-218, 2013.

28 Camilleri-Broet S, Criniere E, Broet P, Delwail V, Mokhtari K, Moreau A, Kujas M, Raphael M, Iraqi W, Sautes-Fridman C, Colombat P, Hoang-Xuan K and Martin A: A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood *107*: 190-196, 2006.

29 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25*: 402-408,2001.

30 Hassan R, White LR, Stefanoff CG, de Oliveira DE, Felisbino FE, Klumb CE, Bacchi CE, Seuanez HN and Zalcberg IR: Epstein-Barr virus (EBV) detection and typing by PCR: a contribution to diagnostic screening of EBV-positive Burkitt's lymphoma. Diagn Pathol *1*: 17, 2006.

31 Abrey LE, Ben-Porat L, Panageas KS, Yahalom J, Berkey B, Curran W, Schultz C, Leibel S, Nelson D, Mehta M and DeAngelis LM: Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. J Clin Oncol *24*: 5711-5715, 2006.

32 Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR and Honjo T: Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med *192*: 1027-1034, 2000.

33 Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy

S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A and Wigginton JM: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med *366*: 2455-2465, 2012.

34 Armand P, Nagler A, Weller EA, Devine SM, Avigan DE, Chen YB, Kaminski MS, Holland HK, Winter JN, Mason JR, Fay JW, Rizzieri DA, Hosing CM, Ball ED, Uberti JP, Lazarus HM, Mapara MY, Gregory SA, Timmerman JM, Andorsky D, Or R, Waller EK, Rotem-Yehudar R and Gordon LI: Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. J Clin Oncol *31*: 4199-4206, 2013.

35 Westin JR, Chu F, Zhang M, Fayad LE, Kwak LW, Fowler N, Romaguera J, Hagemeister F, Fanale M, Samaniego F, Feng L, Baladandayuthapani V, Wang Z, Ma W, Gao Y, Wallace M, Vence LM, Radvanyi L, Muzzafar T, Rotem-Yehudar R, Davis RE and Neelapu SS: Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. Lancet Oncol *15*: 69-77, 2014.

36 Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA and Armand P: PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med *372*: 311-319, 2015.

37 Chen BJ, Chapuy B, Ouyang J, Sun HH, Roemer MG, Xu ML, Yu H, Fletcher CD, Freeman GJ, Shipp MA and Rodig SJ: PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res *19*: 3462-3473, 2013.

38 Kiyasu J, Miyoshi H, Hirata A, Arakawa F, Ichikawa A, Niino D, Sugita Y, Yufu Y, Choi I, Abe Y, Uike N, Nagafuji K, Okamura T, Akashi K, Takayanagi R, Shiratsuchi M and Ohshima K: Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. Blood *126*: 2193-2201, 2015.

39 Xing W, Dresser K, Zhang R, Evens AM, Yu H, Woda BA and Chen BJ: PD-L1 expression in EBV-negative diffuse large B-cell lymphoma: clinicopathologic features and prognostic implications. Oncotarget *7*: 59976-59986, 2016.

40 Horlad H, Ma C, Yano H, Pan C, Ohnishi K, Fujiwara Y, Endo S, Kikukawa Y, Okuno Y, Matsuoka M, Takeya M and Komohara Y: An IL-27/Stat3 axis induces expression of programmed cell death 1 ligands (PD-L1/2) on infiltrating macrophages in lymphoma. Cancer Sci *107*: 1696-1704, 2016.

41 Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ and Timmerman JM: Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. Clin Cancer Res *17*: 4232-4244, 2011.

42 Kwon D, Kim S, Kim PJ, Go H, Nam SJ, Paik JH, Kim YA, Kim TM, Heo DS, Kim CW and Jeon YK: Clinicopathological analysis of programmed cell death 1 and programmed cell death ligand 1 expression in the tumour microenvironments of diffuse large B cell lymphomas. Histopathology *68*: 1079-1089, 2016.

43 Biswas SK and Mantovani A: Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol *11*: 889-896, 2010.

44 Wang TT, Zhao YL, Peng LS, Chen N, Chen W, Lv YP, Mao FY, Zhang JY, Cheng P, Teng YS, Fu XL, Yu PW, Guo G, Luo P, Zhuang Y and Zou QM: Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. Gut, 2017. doi: 10.1136/gutjnl-2016-313075. [Epub ahead of print]

45 Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, Delaney A, Jones SJ, Iqbal J, Weisenburger DD, Bast MA, Rosenwald A, Muller-Hermelink HK, Rimsza LM, Campo E, Delabie J, Braziel RM, Cook JR, Tubbs RR, Jaffe ES, Lenz G, Connors JM, Staudt LM, Chan WC and Gascoyne RD: Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med *362*: 875-885, 2010.

46 Bostrom MM, Irjala H, Mirtti T, Taimen P, Kauko T, Algars A, Jalkanen S and Bostrom PJ: Tumor-Associated Macrophages Provide Significant Prognostic Information in Urothelial Bladder Cancer. PloS one *10*: e0133552, 2015.

47 Duchnowska R, Peksa R, Radecka B, Mandat T, Trojanowski T, Jarosz B, Czartoryska-Arlukowicz B, Olszewski WP, Och W, Kalinka-Warzocha E, Kozlowski W, Kowalczyk A, Loi S, Biernat W and Jassem J: Immune response in breast cancer brain metastases and their microenvironment: the role of the PD-1/PD-L axis. Breast Cancer Res *18*: 43, 2016.

48 Hudson LC, Bragg DC, Tompkins MB and Meeker RB: Astrocytes and microglia differentially regulate trafficking of lymphocyte subsets across brain endothelial cells. Brain Res *1058*: 148-160, 2005.

49 Berghoff AS, Ricken G, Widhalm G, Rajky O, Hainfellner JA, Birner P, Raderer M and Preusser M: PD1 (CD279) and PD-L1 (CD274, B7H1) expression in primary central nervous system lymphomas (PCNSL). Clin Neuropathol *33*: 42-49, 2014.

50 Nayak L, Iwamoto FM, LaCasce A, Mukundan S, Roemer MG, Chapuy B, Armand P, Rodig SJ and Shipp MA: PD-1 blockade with nivolumab in relapsed/refractory primary central nervous system and testicular lymphoma. Blood *129*: 3071-3073, 2017.

51 Furuse M, Nonoguchi N, Omura N, Shirahata M, Iwasaki K, Inui T, Kuroiwa T, Kuwabara H and Miyatake SI: Immunotherapy of Nivolumab with Dendritic Cell Vaccination Is Effective against Intractable Recurrent Primary Central Nervous System Lymphoma: A Case Report. Neurol Med Chir (Tokyo) *57*: 191-197, 2017.

52 Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, Chapuy B, Takeyama K, Neuberg D, Golub TR, Kutok JL and Shipp MA: Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction *via* JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. Blood *116*: 3268-3277, 2010.

53 Twa DD, Chan FC, Ben-Neriah S, Woolcock BW, Mottok A, Tan KL, Slack GW, Gunawardana J, Lim RS, McPherson AW, Kridel R, Telenius A, Scott DW, Savage KJ, Shah SP, Gascoyne RD and Steidl C: Genomic rearrangements involving programmed death ligands are recurrent in primary mediastinal large B-cell lymphoma. Blood *123*: 2062-2065, 2014.

54 Sanmamed MF and Chen L: Inducible expression of B7-H1 (PD-L1) and its selective role in tumor site immune modulation. Cancer J *20*: 256-261, 2014.

55 Green MR, Rodig S, Juszczynski P, Ouyang J, Sinha P, O'Donnell E, Neuberg D and Shipp MA: Constitutive AP-1 activity and EBV infection induce PD-L1 in Hodgkin

lymphomas and posttransplant lymphoproliferative disorders: implications for targeted therapy. Clin Cancer Res *18*: 1611-1618, 2012.

56 Chapuy B, Roemer MG, Stewart C, Tan Y, Abo RP, Zhang L, Dunford AJ, Meredith DM, Thorner AR, Jordanova ES, Liu G, Feuerhake F, Ducar MD, Illerhaus G, Gusenleitner D, Linden EA, Sun HH, Homer H, Aono M, Pinkus GS, Ligon AH, Ligon KL, Ferry JA, Freeman GJ, van Hummelen P, Golub TR, Getz G, Rodig SJ, de Jong D, Monti S and Shipp MA: Targetable genetic features of primary testicular and primary central nervous system lymphomas. Blood *127*: 869-881, 2016.

57. Ascierto PA, Kalos M, Schaer DA, Callahan MK and Wolchok JD: Biomarkers for immunostimulatory monoclonal antibodies in combination strategies for melanoma and other tumor types. Clin Cancer Res *19*: 1009-1020, 2013.

58 Ghebeh H, Mohammed S, Al-Omair A, Qattan A, Lehe C, Al-Qudaihi G, Elkum N, Alshabanah M, Bin Amer S, Tulbah A, Ajarim D, Al-Tweigeri T and Dermime S: The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia *8*: 190-198, 2006.

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