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Comprehensive immunohistochemical study of programmed cell death ligand 1 (PD-L1). Analysis in 5536 cases revealed consistent expression in trophoblastic tumors

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Abstract

Programmed cell death 1/programmed cell death ligand (PD-1/PD-Ls) axis is crucial for the modulation of immune responses and self-tolerance. Also, aberrant PD-L1 expression on the tumor cells or tumor-associated inflammatory cells accelerates immune evasion of tumor cells. In the past decade, PD-1/PD-Ls immune checkpoint inhibitors were introduced to cancer treatment trials and, in some cases, showed significant anti-cancer effects. PD-L1 immunohistochemical staining is considered a potential predictor of clinical response to PD-1/PD-Ls immune checkpoint inhibitors treatment. However, immunohistochemical data on PD-L1 expression in different types of cancer especially rare entities remains incomplete. In this study, PD-L1 expression was immunohistochemically analyzed in 5536 tumors including germ cell, epithelial, mesenchymal, melanocytic/neuroectodermal, and lymphohematopoietic tumors as well as in a set of human normal tissues including a fetus. Immunohistochemistry was performed with E1L3N rabbit monoclonal antibody and Leica Bond Max automation using multitumor blocks containing up to 70 tumor samples. PD-L1 was constitutively and strongly expressed in placental trophoblasts as well as choriocarcinomas and trophoblastic components of germ cell tumors. Also, the neoplastic cells of classical Hodgkin's lymphoma, anaplastic large cell lymphoma, schwannoma, thymoma, and squamous cell carcinoma of various sites frequently expressed PD-L1. In gastrointestinal adenocarcinomas, PD-L1-expression was associated with *EBER*-positivity and mismatch repair-deficiency. In addition, PD-L1 was variably expressed in non-neoplastic macrophages and dendritic cells. PD-L1 immunohistochemistry may have some role in immunophenotypic differential diagnosis of tumors and pinpointing potential candidates for anti-PD-1/PD-Ls immune checkpoint therapy.

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Keywords

programmed cell death ligand 1 (PD-L1); immunohistochemistry; choriocarcinoma; classical Hodgkin's lymphoma; anaplastic large cell lymphoma (ALCL); schwannoma; mismatch repair deficiency; Epstein-Barr virus

INTRODUCTION

Programmed cell death 1/programmed cell death ligand (PD-1/PD-Ls) axis is crucial for the modulation of the immune system to reduce collateral tissue damage from the inflammatory response to infectious microorganisms in peripheral tissues. In addition, direct evidence that PD-1-deficient mice develop spontaneous autoimmune diseases suggests an inhibitory and regulatory role for PD-1/PD-Ls interaction in T-cell responses and the maintenance of self-tolerance. (1, 2)

PD-1 (CD279) is a cell surface receptor that belongs to the immunoglobulin superfamily and also a member of the extended CD28/CTLA-4 family. The extracellular region of PD-1 is 28% identical to CTLA-4, another immune checkpoint molecule. (1, 3) PD-1 is mainly expressed on activated T cells whereas other non-T lymphocytes such as B cells and natural killer (NK) cells express PD-1 only upon induction. (4, 5) PD-1 is a co-stimulatory molecule and, once engaged by its ligands PD-Ls, PD-1 (probably along with the other cooperative pathways) inhibits kinases that are involved in T cell activation through the phosphatase SHP2. (6) When T cells were exposed to chronic antigen stimulation such as chronic viral infection or cancer, high levels of persistent PD-1 expression is induced and leads to T cell exhaustion or anergy.

PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), two physiological ligands for PD-1, have been identified as cell-surface glycoproteins belonging to the B7 family. (6–9) These B7 family members share 37% sequence homology and are located within 100kb of the genomic locus at 9p24, probably evolving through gene duplication. (9) In peripheral tissues, PD-L1 is primarily induced by interferon-γ (IFNγ), from T helper 1 (TH1) cells under inflammatory conditions.

In early studies, melanoma, ovarian, colon and lung cancer tissues were reported to express high levels of PD-L1. (10) Subsequently, other human cancers such as esophageal, gastric, and hepatocellular carcinoma were reported to express PD-L1. Moreover, PD-L1 expression was linked to tumor aggressiveness and a poor clinical prognosis. (11–13) It was reported that aberrantly activated oncogenic signals induce PD-L1 expression on tumor cells and allow them to evade the host immune system by dampening antitumor immunity through anergy or apoptosis of antigen-specific T-cells. (14, 15) Furthermore, PD-L1-expressing tumor-associated inflammatory cells (TAIs, those includes macrophages and lymphocytes infiltrating and/or surrounding neoplastic tissue) are also reported to induce tumor immune evasion. (16–18)

In the past decade, several immune checkpoint inhibitors (nivolumab and pembrolizumab for PD-1/PD-Ls inhibition; tremelimumab and ipilimumab for CTLA-4/B7 inhibition) were

introduced for the treatment of several cancer types to conquer tumor immune evasion. Recently, significant anti-cancer effects of nivolumab were demonstrated in phase 3 clinical trials for the treatment of non-small cell lung cancer (NSCLC) as well as other malignancies. (19–22) PD-L1 expression, as detected by immunohistochemistry, is a potential biomarker to predict clinical response to PD-1/PD-Ls checkpoint inhibitors in the clinical setting. (19–22) Several types of cancer have been analyzed for PD-L1 expression, but a comprehensive analyses, including rare germ cell and mesenchymal tumors has not been performed. (10–13, 23–25)

The aim of this study was to evaluate potential utility of PD-L1 immunohistochemistry in diagnostic pathology and identify additional tumor types for PD-1/PD-Ls axis inhibitor treatment.

MATERIAL and METHODS

5536 anonymized tumors, including germ cell, epithelial, mesenchymal, melanocytic/neuroectodermal, and lymphohematopoietic tumors, and normal tissues derived from surgical specimens were assembled to multitumor blocks containing 30 to 70 rectangular tissue samples as previously described. (26) The size of tumor tissue samples was estimated to exceed the size of a single 0.6mm^2 core by a factor of 10–15. All tumors, selected for this study, were extensively characterized histologically and immunohistochemically. In addition, a 10-week-old fetus was immunohistochemically analyzed.

The rabbit monoclonal antibodies clone E1L3N (#13684) and 28-8 (ab205921) against PD-L1 were obtained from Cell Signaling Technology, Inc. (Danvers, MA) and Abcam Inc. (Cambridge, MA) , respectively. The antibodies were applied at a dilution of 1:200 (E1L3N) and 1:500 (28–8) to selected normal and tumor tissue arrays. Both of the antibodies showed almost similar staining patterns in placental trophoblast, choriocarcinoma, anaplastic large cell lymphoma (ALCL), and squamous cell carcinoma of lung. However, we selected clone E1L3N because of its lower background signal.

Immunostaining was performed with the Leica Bond-Max automation and Leica Refine detection kit. (Leica Biosystems, Bannockburn, IL) The protocol included *in situ* deparaffinization and high-pH epitope retrieval for 25 minutes, incubation with primary antibody for 30 minutes, polymer for 15 minutes, postpolymer for 15 minutes, and DAB as the chromogen for 10 minutes, followed by 5-minute hematoxylin counterstaining. MLH1, MSH2, MSH6, and PMS3 immunohistochemistry was performed to analyze mismatch repair (MMR) system status as previously reported. (27) For the detection of Epstein-Barr virus (EBV) infection, Bond™ Ready-to-Use ISH EBER Probe was used in Leica Bond-Max automation system according to the manufacturer instructions. (Leica Biosystems, Bannockburn, IL)

The stained sections were independently evaluated by two pathologists (SI and MM). PD-L1 immunoreactivity in placental trophoblasts and peripheral nerves were used as external and internal positive controls, respectively. PD-L1 has been reported to be expressed on not only

tumor cells but also dendritic cells and TAIs, therefore, we evaluated PD-L1 expression in both neoplastic cells and TAIs with a detection cut-off of 5%.

Chi-square test or Fisher's exact test were performed by SPSS software (IBM, Armonk, NY) to analyze the statistical correlation between PD-L1-expression and other tumor status such as MMR-deficiency, *EBER*-positivity, p16-, and ALK-expression.

RESULTS

Normal tissues and fetus

Among the normal adult tissue components, highest PD-L1-expression was detected in placental trophoblasts. (Fig. 1A) Macrophages such as alveolar ones, dendritic cells of lymphoid tissue, peripheral nerves probably schwann cells, smooth, and skeletal muscle cells showed variable PD-L1 positivity. (Fig. 1B) Organs such as adrenal gland, brain, genitourinary-tract, kidney, liver, lung, mammary gland, pancreas, salivary gland, and thyroid gland showed no PD-L1 expression. Fetal tissues with the exception of placental trophoblasts were also negative for PD-L1 expression.

Germ cell tumors

The results of PD-L1 immunostaining in germ cell tumors have been summarized in Table 1. Choriocarcinomas were nearly uniformly positive for PD-L1 with a membrane pattern (Fig. 2A). Similarly, 1 placental site trophoblastic tumor and 6 mixed germ cell tumors of testis showed PD-L1 positivity in the trophoblastic cellular elements. Other germ cell tumors, such as seminoma, embryonal carcinoma, and yolk sac tumor showed PD-L1 expression (57–81%), only in TAIs, especially macrophages. (Figure 2B, Supplementary Table S1) Teratoma cells showed no PD-L1 expression.

Other epithelial neoplasms

Expression status of PD-L1 in epithelial non germ-cell tumors is shown in Table 2. Thymic tumors showed frequent PD-L1 expression. In thymomas, 79% of cases showed PD-L1 positivity in neoplastic epithelial cells, whereas, tumor-associated thymocytes were negative for PD-L1. (Fig. 3A, Table 2) Thymic carcinoma cells showed lower PD-L1 positivity than thymomas (38% of cases). (Table 2)

Squamous cell carcinoma cells of the tongue and tonsils (Fig. 3B) showed higher PD-L1 labeling (61–67% of cases), while those of esophagus showed lowest PD-L1 expression (39% of cases). No statistical correlation was detected between p16- and PD-L1-expression in any of the oral squamous cell carcinomas. (Supplementary Table S2) The former was most often detected in tonsillar carcinoma.

PD-L1 was only rarely expressed in colorectal adenocarcinoma cells (13% of cases, with 5–100% of positive cells, median 40%). (Figure 3C and Table 2) 17% of colorectal adenocarcinomas showed MMR-deficiency. (Figure 3D) Statistically positive correlation was detected between the MMR-deficiency and PD-L1-expression in colorectal adenocarcinoma. (Table 3)

Gastric adenocarcinoma cells also showed rare PD-L1 expression (14% of cases, 5–100% of positive cells, median 40%, Fig. 3E and Table 2). In this study, 8% (15 of 180 cases) and 6% (11 of 190 cases) of gastric adenocarcinomas were determined as MMR-deficienct and *EBER*-positive tumors, respectively. (Fig. 3F) MMR-deficiency and *EBER*-positivity were detected in a mutually exclusive manner. Statistically positive correlation was detected between the PD-L1-expression and MMR-deficienct or *EBER*-positive status. (Table 4)

Neoplastic cells of endometrioido adenocarcinoma and serous carcinoma of the ovary, invasive ductal and lobular carcinomas of the breast, hepatocellular carcinoma, and prostate adenocarcinoma only rarely expressed PD-L1 (2–6% of cases). (Table 2)

In other epithelial tumors, PD-L1 expression was detected in neoplastic cells (5–36% of cases) or TAIs (1–59%). TAIs of Merkel cell carcinoma showed a high frequency of PD-L1 expression (59% of cases). (Supplementary Table S1)

Hematopoietic and lymphoid tumors

PD-L1 expression in lymphohematopoietic tumors has been summarized in Table 5. 96% of classical Hodgkin's lymphoma cases showed PD-L1 expression in neoplastic Hodgkin's and Reed-Sternberg cells (10–100% of positive cells, median 80%, Fig. 4A). In this study, 30% (14 of 47 cases) of classical Hodgkin's lymphoma showed *EBER*-positivity. Although all of the *EBER*-positive cases showed PD-L1 expression, no statistical correlation was detected between *EBER*- and PD-L1-expression. (Supplementary Table S3)

ALCL cells also showed frequent and strong PD-L1 expression on the cell membrane (87% of cases, 10–100% of positive cells, median 100%, Fig. 4B). No statistical correlation was detected between ALK- and PD-L1-expression. (Supplementary Table S4)

A minor populations of diffuse large B-cell lymphoma (DLBCL) and NK- and T-cell lymphoma cells showed positivity for PD-L1 (18% and 16% of cases, respectively). Although only 2 cases of DLBCL showed *EBER*-positivity, statistical correlation was detected between *EBER*- and PD-L1-expression. (Table 6) Neoplastic cells of mantle cell lymphoma, nodal marginal zone lymphoma, T- and B-lymphoblastic lymphoma, follicular lymphoma, and small cell lymphoma showed no PD-L1 expression. Conversely, lymphoma-associated inflammatory cells showed frequent PD-L1 expression (29–75% of cases), except for small cell lymphoma. (Supplementary Table S1)

Mesenchymal and neuroectodermal tumors

PD-L1 expression in mesenchymal tumors has been summarized in Table 7. Among mesenchymal tumors, the highest PD-L1 positivity was detected in peripheral schwannomas (89% of cases, 5–100% of positive cells, median 90%, Fig. 5A). Gastric shwannomas showed less consistent and weaker PD-L1 expression than peripheral schwannomas. (67% of cases, 5–100% of positive cells, median 90%) TAIs in schwannoma were negative for PD-L1. (Supplementary Table S1) Neurofibroma showed less frequent positivity (44% of cases, 5–100% of positive cells, median 70%) while granular cell tumors were negative for PD-L1. Potential schwannoma mimics such as solitary fibrous tumor, fibrous histiocytoma, and perineurioma were all negative for PD-L1. In both malignant peripheral nerve sheath tumor

(MPNST, Fig. 5B) and neuroblastoma, 21% of cases showed PD-L1 expression. In neuroblastomas, PD-L1 expression was limited to the schwannian stroma.

Minor populations of embryonal rhabdomyosarcoma cells showed weak PD-L1 expression (17% of cases, 10–80% of positive cells, median 40%, Fig. 5C). An isolated case of alveolar soft part sarcoma (9% of cases) showed membranous PD-L1 expression. (Fig. 5D) Rare malignant melanoma 17% of cases) and SDH-deficient GIST (13% of cases) expressed PD-L1. Other soft tissue tumors such as glomus tumor, and synovial sarcoma were negative for PD-L1. In mesenchymal tumors, TAIs also showed much lower PD-L1 expression compared to carcinomas and lymphomas. (Supplementary Table S1)

DISCUSSION

In physiological conditions, the PD-1/PD-Ls axis is crucial for the regulation of immune responses to minimize collateral tissue damage as well as maintaining self-tolerance. However, in neoplastic disease, once PD-Ls are aberrantly expressed on tumor cells and/or TAIs, they help tumor cells evade the host immune system and promote tumor growth. In the past decade, PD-1/PD-Ls immune checkpoint inhibitors have been introduced in anti-cancer therapy. (19–22) To predict a clinical response to these drugs, PD-L1 immunohistochemical staining has been used as a potential biomarker. (19–22) In this study, PD-L1 expression was immunohistochemically analyzed in normal and neoplastic tissue to assess potential diagnostic utility of PD-L1 immunohistochemistry and to identify specific neoplasms that may be amenable to anti-PD-1/PD-Ls immune checkpoint therapy.

In normal tissues, the most striking PD-L1-expression was detected in placental trophoblast (Fig. 1A). Interestingly, the fetus showed no PD-L1 expression. These results reflect the nature of the trophoblast as a maternal-fetal barrier protecting the fetus from the maternal immune system. (28, 29) Indeed, critical roles for the PD-L1 in fetomaternal tolerance have been demonstrated in a mouse model. PD-L1-deficient females exhibited decreased allogeneic fetal survival rates compared to littermates and heterozygote controls. (30) It is not surprising that choriocarcinoma, a malignant trophoblastic tumor, showed strong PD-L1 expression among germ cell tumors. These observations indicate that PD-L1 could be a supplementary marker to identify choriocarcinoma or trophoblastic elements in other germ cell tumors.

PD-L1 was consistently expressed in normal peripheral nerves. The physiological roles of PD-L1 in nervous tissue have not fully explored. However, PD-L1 was indicated as a suppressor for the inflammatory response and neuropathic pain occurring after peripheral nerve injury in PD-L1 knock out mice. (31) Among neoplasms, schwannomas showed consistent PD-L1 expression, whereas neurofibroma and MPNST showed rarer and weaker expression. Schwannoma mimics such as gastrointestinal stromal tumor (GIST), fibrous histiocytoma, perineurioma, solitary fibrous tumor, and meningioma were almost always negative. These results indicate that PD-L1 could have a role in the differential diagnosis of schwannoma. However, it should be considered that minority of melanotic and desmoplastic melanomas are also positive for PD-L1.

In analyzed thymic tumors, 79% and 38% of thymoma and thymic carcinoma cells showed PD-L1 expression, respectively. (Table 2) Recently, two groups reported frequent PD-L1 expression in thymic tumors. (24, 25) One study showed that 68% of thymoma and 75% of thymic carcinoma neoplastic cells were positive for PD-L1 using rabbit monoclonal antibody 15 from Sino Biological (Beijing, China). This study also showed that PD-L1 high-expressing thymic tumors had a significantly worse overall survival in an adjusted (age/sex) analysis. (25) However, other study using E1L3N rabbit monoclonal antibody showed that that 23% of thymomas and 70% of thymic carcinomas were positive for PD-L1 and PD-L1-positivity was not a significantly negative factor for overall survival in a multivariate analysis. (24). Thus, additional studies are needed to explain these divergent results.

Recently, gastric cancers were classified into 4 categories such as EBV-infected, microsatellite unstable, genomically stable, and chromosomally unstable tumors, by the comprehensive molecular analysis by the Cancer Genome Atlas (TCGA) project. (33) It was also found that EBV-infected tumors carry PIK3CA mutations, extreme DNA hypermethylation, and amplification of 9p24 region including *JAK2, PD-L1* and *PD-L2*. This study confirmed the positive correlation of *EBER*- and PD-L1-expression by typical methods such as *in situ* hybridization and immunohistochemistry. (Table 4) Our study also showed a positive correlation between MMR-deficiency and PD-L1-expression (Table 4) and that only 11% of other two types (genomically stable and chromosomally unstable tumors) were positive for PD-L1.

Aberrantly activated oncogenic signals due to PTEN-loss, EGFR-mutation, or ALK-translocation were reported to induce PD-L1 overexpression in neoplastic cells. (14, 15, 32) It was also reported that ALCLs, carrying nucleophosmin (NPM)/anaplastic lymphoma kinase (ALK) translocation, were induced to PD-L1 overexpression via the NPM/ALK-STAT3 axis activation. (14) However, no correlation between PD-L1- and ALK-expression statuses was demonstrated in this study. (Supplementary Table S4) Moreover, 9 of 10 ALK-negative ALCLs also showed strong PD-L1 expression. These results strongly indicated that there could be alternative pathway(s) regulating PD-L1-expression in ALCLs.

EBV is significantly associated with classical Hodgkin's lymphoma. (34) It was reported that the induction of the EBV latent membrane proteins, latent membrane protein 1 (LMP1) or LMP2a, in normal germinal center B cells is sufficient to mimic a Hodgkin's Reed-Sternberg cell-like phenotype. (35, 36) Furthermore, LMP1 was reported to increase *PD-L1* expression by up-regulating its promoter activity via a JAK3-dependent manner. (37) Thus, *EBER*-positive classical Hodgkin's lymphomas would be induced into PD-L1 expression via this pathway. (Supplementary Table S3) On the other hand, *EBER*-negative classical Hodgkin's lymphoma cases also showed frequent PD-L1 expression (94%, 31 of 33 cases, Supplementary Table S3). It was reported that genomic amplification of 9p24 region including *PD-L1* leads to PD-L1 expression in Hodgkin's lymphoma cells. (38) These *EBER*-negative classical Hodgkin's lymphoma cases might carry genomic amplification of 9p24 region.

In other viral infections, HPV-infection was reported to correlate with PD-L1-expression in squamous cell carcinomas of tonsil. (39) In this study, 90% and 93% of tonsil squamous cell

carcinoma showed PD-L1 and p-16-expression, respectively. However, no statistical correlation was detected between PD-L1- and p16-expression. (Table 2 and Supplementary Table S2)

It has been reported that PD-L1-expressing dendritic cells or TAIs are able to induce tumor immune evasion. (16–18) In current study, seminoma and various carcinomas often showed such PD-L1-positive cells whereas mesenchymal tumors were less frequently associated with PD-L1-expressing inflammatory cells. (Supplementary Table S1) Clinical or experimental investigation is needed to determine whether tumors with PD-L1-positve TAIs could be targets for immune check point inhibition therapy.

In clinical trials, PD-1/PD-Ls inhibitors were introduced to the treatment of the patients with PD-L1 expressing tumors, such as melanoma, NSCLC, renal cell cancer, and Hodgkin's Lymphoma. (19–22) Recently, advanced squamous-cell and other non-squamous-cell NSCLC patients were treated with nivolumab or docetaxel to compare their antitumor activity. (20, 21) Both squamous-cell and non-squamous-cell NSCLC patients treated by nivolumab showed significantly better overall survival, response rate, and progression-free survival than docetaxel treated patients. However, the hazard ratio for death was lower in squamous-cell carcinoma patients [0.59 (95% CI, 0.44–0.79), P<0.001] than non-squamous cell NSCLC patient [0.73 (96% CI, 0.59–0.89), P=0.002] indicating better treatment success for squamous cell NSCLC patients. In addition, refractory Hodgkin's lymphoma patients showed a response to nivolumab treatment. (19) These results indicate that PD-L1-expressing tumors, such as germ cell tumors with trophoblastic differentiation and MPNSTs, might be successfully treated by PD-1/PD-Ls checkpoint inhibitors.

In conclusion, PD-L1 immunohistochemistry using rabbit monoclonal antibody clone E1L3N might have a role in diagnostic immunophenotyping of germ cell tumors with trophoblastic differentiation, schwannoma, thymoma, classical Hodgkin's and ALCL because of their consistent PD-L1 positivity. Our data presented here may also be useful in identifying potential targets for anti-PD-1/PD-Ls immune checkpoint inhibition therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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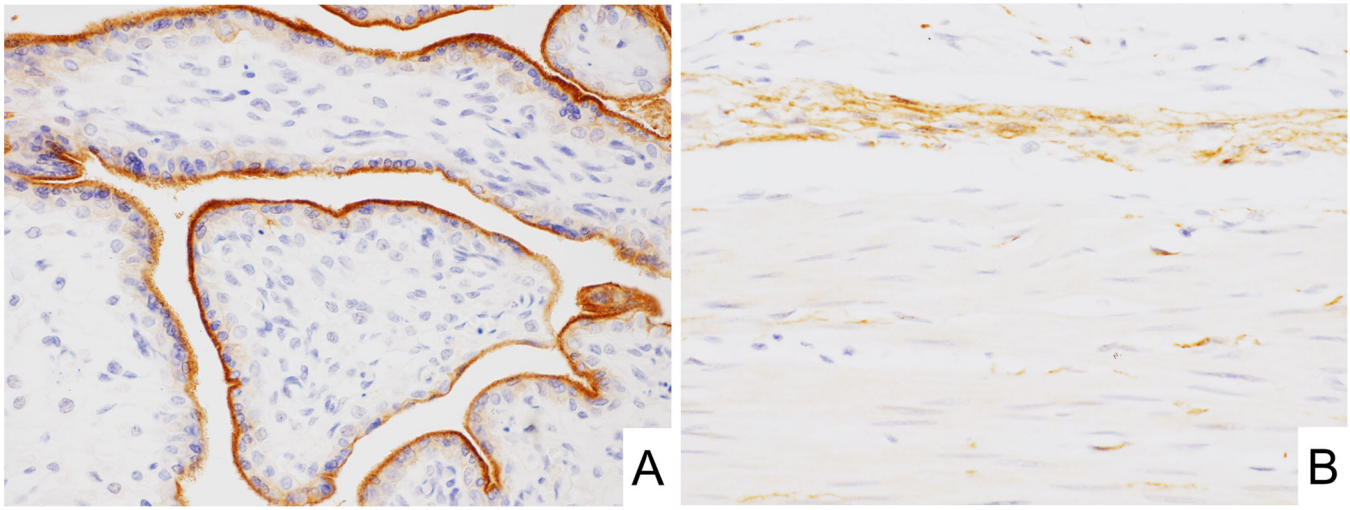

FIGURE 1. CD171 expression in normal tissue

A, Placental trophoblast cells strongly expressed PD-L1 on the cell membrane. B, Peripheral nerve bundles and smooth muscle cells of the stomach showed weak PD-L1 expression.

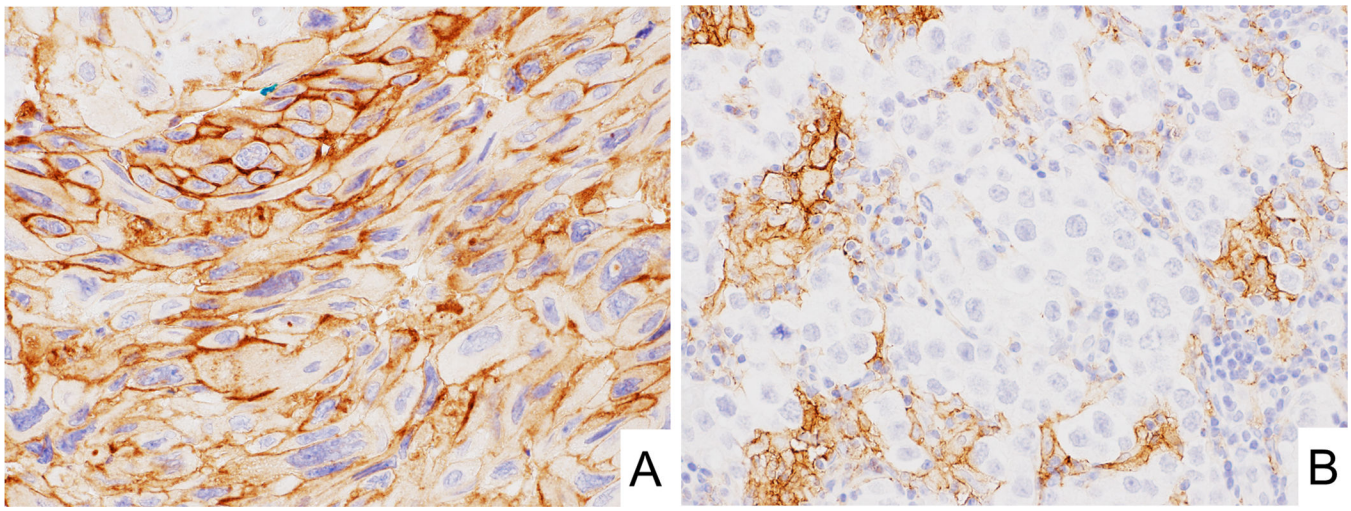

FIGURE 2. PD-L1 expression in germ cell tumors

A, Choriocarcinomas showed strong PD-L1 expression on the cell membrane. B, In seminomas, neoplastic cells expressed no PD-L1 whereas tumor-associated inflammatory cells showed strong PD-L1 expression.

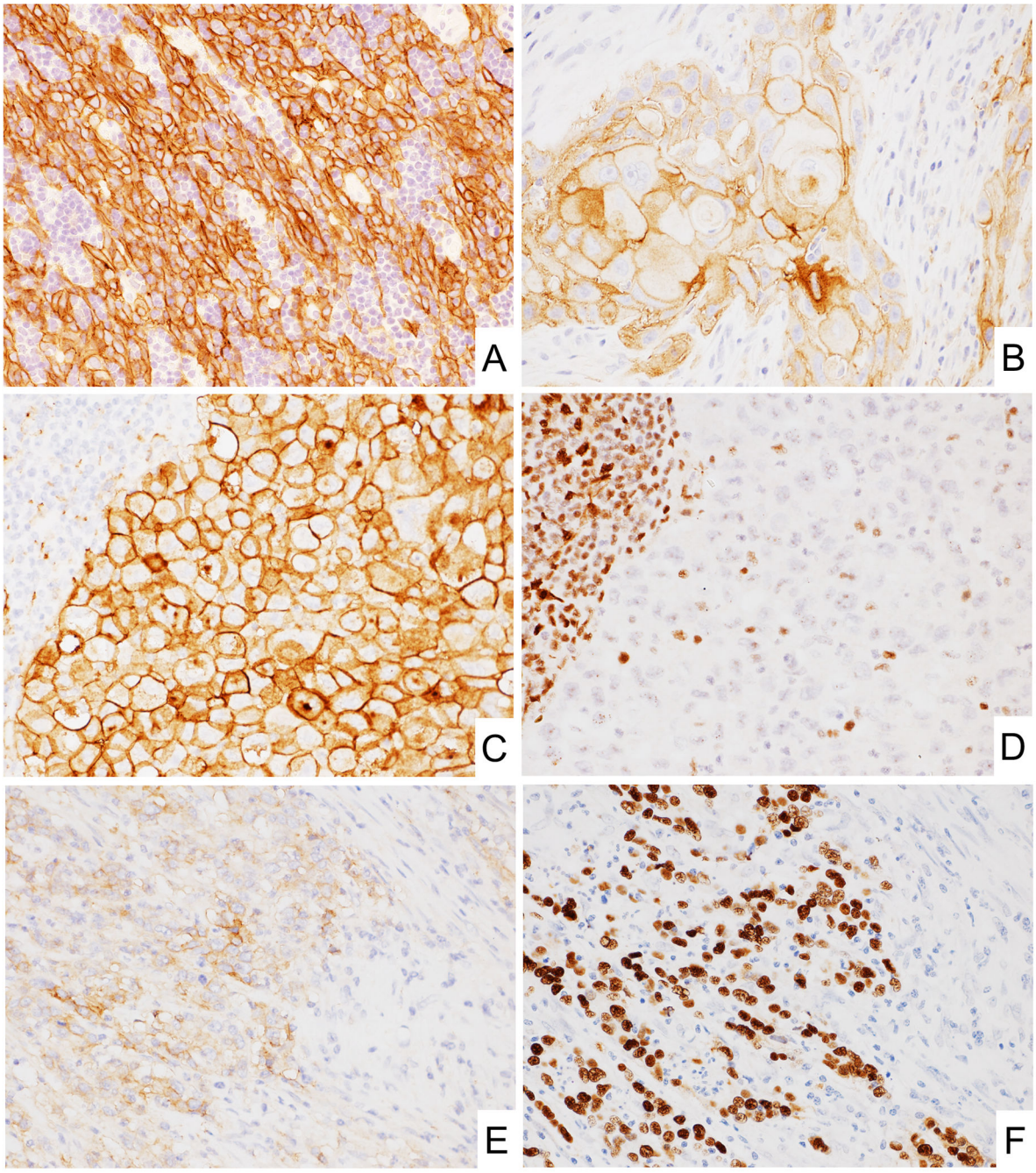

FIGURE 3. PD-L1 expression in epithelial tumors

A, Neoplastic epithelial cells of B2 thymoma showed strong PD-L1 expression on the cell membrane. B, Membranous PD-L1 expression in squamous cell carcinoma of tonsil. C and D, MMR-deficient colorectal adenocarcinoma showed strong PD-L1 expression. (C) PD-L1 staining. (D) MLH1 staining. E and F, *EBER*-positive gastric adenocarcinoma showed PD-L1 expression. (E) PD-L1 staining. (F) *EBER in situ* hybridization.

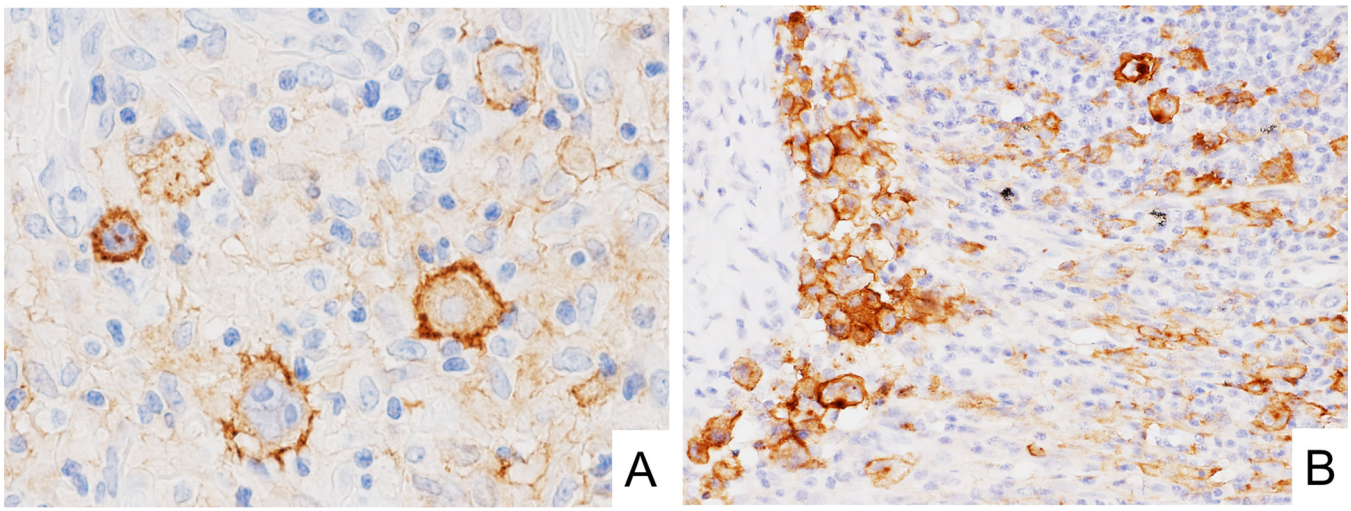

FIGURE 4. PD-L1 expression in lymphomas

A, PD-L1 expression in classical Hodgkin's lymphoma. B, Anaplastic large cell lymphoma showed strong and diffuse PD-L1 expression.

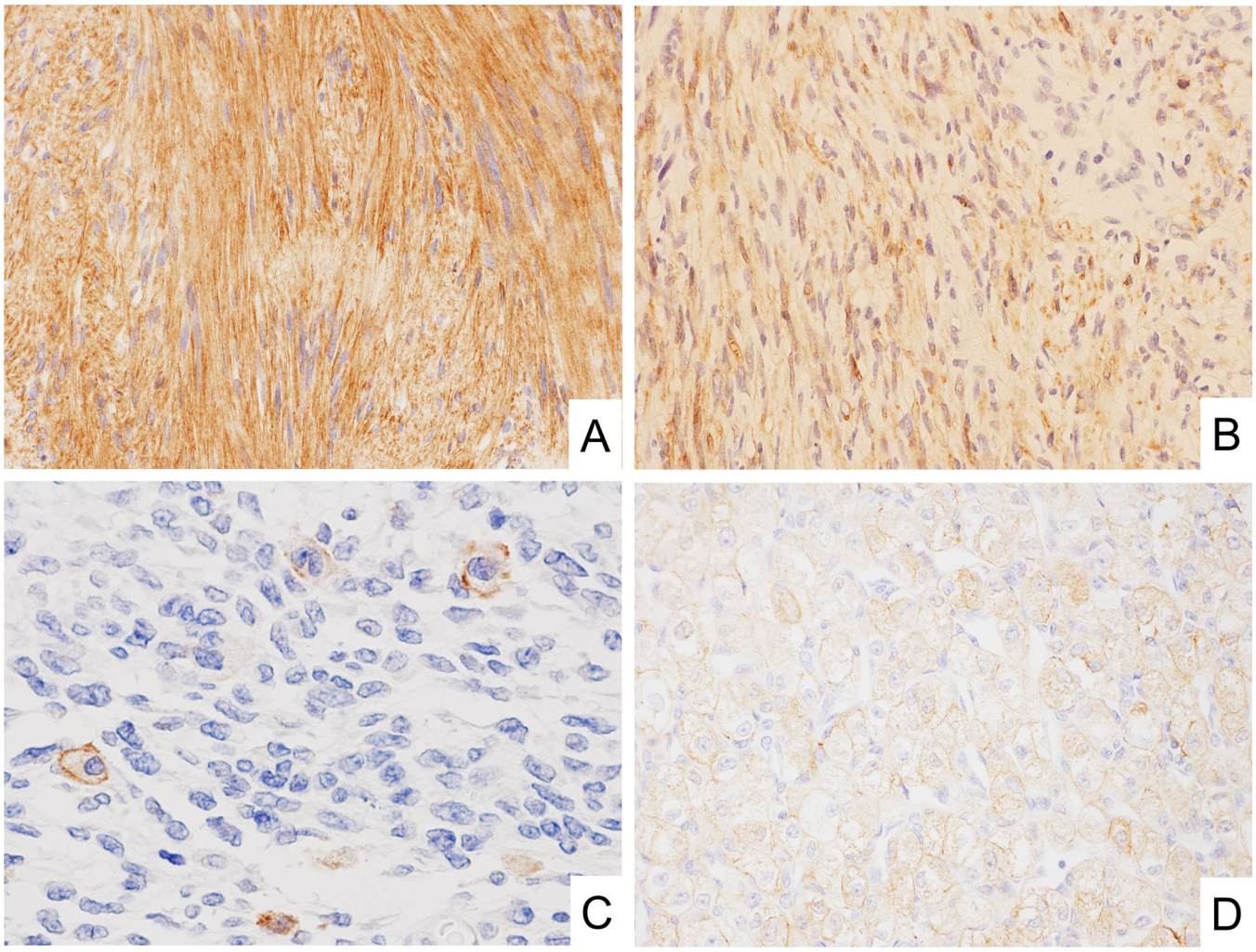

FIGURE 5. PD-L1 expression in mesenchymal tumors

A, Schwannoma showed diffuse cytoplasmic PD-L1 expression. B, Occasional cases of malignant peripheral nerve sheath tumors were weakly positive for PD-L1. C, A subpopulation of embryonal rhabdomyosarcoma cells showed faint PD-L1 expression. D, A case of alveolar soft part sarcoma showed weak but diffuse membranous PD-L1 expression.

Table 1

PD-L1 expression in germ cell tumors

	Cases	/	All	(%)	Range (Median)
Uterus, choriocarcinoma	8	/	8	100.0	90–100 (100)
Germ cell tumor with trophoblastic elements *	8	/	8	100.0	10–100 (50)
Testis, embryonal carcinoma	1	/	34	2.9	5
Testis, seminoma	0	/	91	0.0	-
Testis, teratoma	0	/	17	0.0	-
Testis, Yolk sac tumor	0	/	7	0.0	-

* These include 1 placental site trophoblastic tumor and 6 testicular germ cell tumors with trophoblastic differentiation.

Table 2

PD-L1 expression in epithelial tumors

	Cases	/	All	(%)	Range (Median)
Tumors of squamous cell differentiation					
Thymoma	26	/	33	78.8	5–100 (100)
Tongue, squamous cell carcinoma	26	/	39	66.7	5–100 (70)
Tonsil, squamous cell carcinoma	25	/	41	61.0	5–100 (50)
Skin, squamous cell carcinoma	20	/	33	60.6	5–100 (50)
Lung, squamous cell carcinoma	33	/	56	58.9	5–100 (80)
Gingiva, squamous cell carcinoma	25	/	43	58.1	5–100 (80)
Esophagus, squamous cell carcinoma	38	/	98	38.8	5–100 (20)
Thymic carcinoma	6	/	16	37.5	60–100 (100)
Tumors of adenocarcinoma-like differentiation					
Lung, adenocarcinoma	30	/	137	21.9	5–100 (75)
Pancreas, invasive ductal carcinoma	35	/	224	15.6	5–100 (60)
Cholangiocellular carcinoma	6	/	41	14.6	20–100 (90)
Stomach, adenocarcinoma	27	/	190	14.2	5–100 (40)
Colorectum, adenocarcinoma	61	/	478	12.8	5–100 (40)
Uterus, endometrioid adenocarcinoma	25	/	192	13.0	5–100 (10)
Breast, invasive ductal carcinoma	20	/	366	5.5	5–100 (60)
Ovary, endometrioid adenocarcinoma	2	/	45	4.4	10–40 (25)
Ovary, serous carcinoma	2	/	50	4.0	10–90 (50)
Prostate, adenocarcinoma	1	/	28	3.6	100
Hepatocellular carcinoma	4	/	128	3.1	10–90 (40)
Breast, invasive lobular carcinoma	1	/	56	1.8	5
Other epithelial tumors					
Thyroid gland, papillary carcinoma	16	/	44	36.4	10–100 (30)
Malignant mesothelioma	56	/	186	30.1	5–100 (80)
Uterus, carcinosarcoma	14	/	52	26.9	5–100 (35)
Urothelial Carcinoma	47	/	185	25.4	5–100 (80)
Kidney, chromophobe renal cell carcinoma	15	/	86	17.4	10–100 (100)

	Cases	/	All	(%)	Range (Median)
Kidney, papillary renal cell carcinoma	10	/	96	10.4	10–100 (60)
Lung, small cell carcinoma	4	/	48	8.3	40–100 (90)
Skin, Merkel cell carcinoma	2	/	27	7.4	100 (100)
Kidney, clear cell renal cell carcinoma	15	/	287	5.2	5–100 (85)

Table 3

PD-L1 expression in colorectal adenocarcinomas

Colorectal adenocarcinoma subtypes (n=506)	PD-L1 (tumor cells; %)
MMR-preserved (n=424)	6.8
MMR-deficient (n=82)	44.7**

Chi-square test,

** p<0.01

Table 4

PD-L1 expression in gastric adenocarcinomas

Gastric adenocarcinoma subtypes (n=180 in MMR analysis; n=190 in EBV analysis)	**PD-L1 (tumor cells; %)**
MMR-preserved (n=165)	12.1
MMR-deficient (n=15)	46.7**
EBER-negative (n=179)	12.3
EBER-positive (n=11)	45.5*

Fisher's exact test,

** $p<0.01$,

* $p<0.05$

Table 5

PD-L1 expression in tumors of hematopoietic system

	Cases	/	All	(%)	Range (Median)
Classical Hodgkin's lymphoma	45	/	47	95.7	10–100 (80)
Anaplastic large cell lymphoma	13	/	15	86.7	10–100 (100)
Myeloid sarcoma	3	/	13	23.1	10–30 (20)
Diffuse large B-cell lymphoma	15	/	84	17.9	5–100 (60)
T, NK-cell lymphoma	5	/	31	16.1	40–100 (100)
Follicular lymphoma	0	/	51	0.0	
Lymphoblastic lymphoma	0	/	8	0.0	
Mantle cell lymphoma	0	/	32	0.0	
Nodal marginal zone lymphoma	0	/	6	0.0	
Small cell lymphoma	0	/	19	0.0	
Spleen, chronic myeloid leukemia	0	/	6	0.0	

Table 6

PD-L1 expression in diffuse large B-cell lymphoma (DLBCL)

DLBCL subtypes (n=84)	PD-L1 (tumor cells; %)
EBV-negative (n=82)	15.9
EBV-infected (n=2)	100.0 *

Fisher's exact test,

* $p<0.05$

Table 7

PD-L1 expression in mesenchymal tumors

	Cases	/	All	(%)	Range (Median)
Peripheral, schwannoma	58	/	65	89.2	5–100 (90)
Stomach, schwannoma	4	/	6	66.7	5–100 (90)
Neurofibroma	17	/	39	43.6	5–100 (70)
MPNST	12	/	57	21.1	5–100 (50)
Neuroblastoma	5	/	29	17.2	10–95 (95)
Embryonal rhabdomyosarcoma	9	/	54	16.7	10–80 (40)
Uterus, leiomyoma	18	/	125	14.4	5–80(20)
Meningioma	18	/	192	9.4	5–100 (50)
Stomach, SDH-difficient GIST	5	/	55	9.1	30–100 (90)
Alveolar soft part sarcoma	1	/	11	9.1	100
Desmoplastic melanoma	3	/	33	9.1	20–80 (40)
Nephroblastoma	2	/	24	8.3	30–50 (40)
Kaposi’s sarcoma	3	/	37	8.1	30–90 (40)
Angiosarcoma	7	/	100	7.0	10–100 (40)
Malignant melanoma	5	/	78	6.4	20–100 (40)
Small intestine, GIST	7	/	111	6.3	5–100 (50)
Leiomyosarcoma	4	/	69	5.8	40–100 (80)
Stomach, conventional GIST	6	/	218	2.8	40–100 (100)
PEComa	2	/	75	2.7	10–30 (20)
Ewing’s sarcoma/PNET	1	/	38	2.6	20
Alveolar rhabdomyosarcoma	1	/	42	2.4	20
Dermatofibrosarcoma protuberans	1	/	44	2.3	100
Benign fibrous histiocytoma	0	/	59	0.0	-
Desmoplastic small round cell tumor	0	/	14	0.0	-
Glomus tumor	0	/	58	0.0	-
Granular cell tumor	0	/	28	0.0	-
Ovary, fibroma and thecoma	0	/	31	0.0	-
Perineurioma	0	/	6	0.0	-

	Cases		All	(%)	Range (Median)
Solitary fibrous tumor	0	/	27	0.0	-
Synovial sarcoma	0	/	34	0.0	-

MPNST: malignant peripheral nerve sheath tumor, GIST: gastrointestinal stromal tumor, PNET: primitive neuroectodermal tumor.