**TITLE:** Expression status of PD-L1 and B7-H3 in mesothelioma

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**ABBREVIATIONS:** MPM: malignant pleural mesothelioma, OS: overall survival, PD-L1: programmed death ligand 1, PD-1: human programmed death 1, PFS: progression free survival, B7-H3: B7 homolog 3, IHC: immunohistochemistry, ORR: overall response rate

**RUNNING TITLE:** B7-H3 expression in 90% of mesothelioma
ABSTRACT

Mesothelioma is a rare, aggressive malignancy with poor outcome, and has limited treatment options. The aim of this study was to perform a comprehensive analysis of PD-L1 and B7-H3 expression in mesothelioma. We investigated the protein expression of PD-L1 and B7-H3 and their potential correlation with histological subtype, which might help to develop new therapies targeting these immune-checkpoint molecules. Expression analysis of PD-L1 and B7-H3 was performed by immunohistochemistry using serial tissue sections of specimens obtained from 31 patients with mesothelioma. Tumors were classified into 22 epithelioid, 6 sarcomatoid, and 3 biphasic types. Of the 31 patients, 13 (41.9%) were positive for PD-L1 and 28 (90.3%) were B7-H3 positive. Twelve of the 13 PD-L1 positive patients were positive for B7-H3. PD-L1 and B7-H3 were widely co-expressed in biphasic and sarcomatoid type tumor cells. These findings might provide a rationale for the use of combination therapy for mesothelioma by targeting PD-L1 and B7-H3, as well as the development of anti-B7-H3 or anti-PD-L1 single agents.

KEYWORDS: B7-H1, B7-H3, mesothelioma, PD-L1
INTRODUCTION

Malignant pleural mesothelioma (MPM) is a rare aggressive malignancy commonly associated with occupational asbestos exposure, with increasing incidence worldwide.\(^1\) Both of diffuse and localized mesothelioma are classified as epithelioid, biphasic, or sarcomatoid subtypes according to the WHO classification.\(^2\) The histological classification reflects the prognosis, with the sarcomatoid type having the worst prognosis followed by the biphasic type and the epithelioid type.\(^3\)-\(^7\) The median overall survival (mOS) of untreated cases was 11 months for the epithelioid type, 10 months for the biphasic type, and 5 months for the sarcomatoid type.\(^8\) After treatment, the mOS was 16.9 months for the epithelioid type, 13.1 months for the biphasic type, and 5.5 months for the sarcomatoid type.\(^4\) A phase III comparative study of combination therapy with cisplatin and pemetrexed for previously untreated unresectable MPM\(^8\) reported a median survival time of only 12.1 months; therefore, novel efficacious therapeutic drugs are required. Recently, the pathogenesis of MPM has been intensively studied and new therapeutic agents for mesothelioma including molecular-targeting therapy and immunotherapy are being developed.\(^9\)-\(^17\)

Among ten B7 family proteins thought to be involved in immunoregulation in human malignancies, including MPM, programmed death ligand 1 (PD-L1: also known as B7 homolog 1 [B7-H1]), and its receptor, human programmed death-1 (PD-1), have received much attention resulting in the development of many immune checkpoint inhibitors. PD-1, a
membrane protein expressed on T cells, binds to PD-L1, a ligand expressed by tumor cells, thereby facilitating the inhibition of T cell activation. Expression of PD-L1 was reported to correlate with a poor prognosis in patients with renal cell carcinoma,18-21 pancreatic carcinoma,22-24 uroepithelial carcinoma,25, 26 and Wilms' tumors.27

Anti-PD-1 antibody/anti-PD-L1 antibody drugs were developed to enhance T-cell activation by inhibiting this pathway, and have been shown to be clinically effective and safe in various solid tumors.28-30 PD-L1 is expressed in MPM, with frequencies between 40% to 70%.31, 32 The expression of PD-L1 correlates with a poor outcome of MPM.31-35 In clinical trials of nivolumab or pembrolizumab in patients with MPM, the progression free survival (PFS) was approximately 6 months and the OS was approximately 18 months.11, 16 Therefore, the prognosis of MPM has not improved sufficiently, and new therapeutic agents or combination therapies are expected to be developed.

B7 homolog 3 (B7-H3), also known as CD276, belongs to the B7 family of immune checkpoint molecules, and is a type I transmembrane protein encoded on chromosome 9 in mice and on chromosome 15 in humans. It has up to 30% amino acid identity with other B7 family members.36, 37 In addition, several studies reported that the overexpression of B7-H3 correlated with poor outcomes in patients with lung cancer,38, 39 esophageal cancer,40 head and neck cancer,41 prostatic cancer,42, 43 colorectal cancer,44, 45 gallbladder cancer,46 endometrial cancer,47 renal cell carcinoma,48-50 breast cancer,51, 52 and malignant melanoma.53, 54 Thus,
B7-H3 expression may be a valuable biomarker for predicting outcomes in patients with various cancers. In tumor tissues, B7-H3 is expressed in tumor cells as well as stromal cells including vascular endothelial cells and fibroblasts.\textsuperscript{55,56} However, B7-H3 is also present at low levels in normal human tissues including the liver, colon, and prostate.\textsuperscript{55-57} Therefore, although B7-H3 is a promising target for tumor immunotherapy, further intensive evaluation and clinical trials are required.

The aims of this study were to investigate PD-L1 and B7-H3 expression in mesothelioma and their potential correlations with histological subtype, to develop drugs targeting PD-L1 and B7-H3 that might be used for combination therapy.

**MATERIALS AND METHODS**

**Tissue samples**

Tissue samples from 31 patients diagnosed with mesothelioma were obtained by surgical resection, biopsy, or autopsy between 1991 and 2019. Two cases of non-tumorous mesothelial tissue were obtained by autopsy of patients with other malignancies. All procedures were performed in accordance with the Ethics Committee at Juntendo University School of Medicine (approval number: M19-083) and with the Declaration of Helsinki. The tumors were classified into 22 epithelioid, 3 biphasic, and 6 sarcomatoid types. PD-L1 and B7-H3 were stained using the serial tissue sections. Two pathologists (KK, HS) reviewed all existing
pathology slides. Staging was based on the seventh edition of UICC-TNM’s staging system.58

**Immunohistochemistry for PD-L1**

Immunohistochemistry (IHC) was performed on formalin-fixed, paraffin-embedded tissues. After deparaffinization, tissue sections were placed in Target Retrieval Solution pH 9 (S2367, DAKO Japan, Tokyo, Japan) for antigen activation, heated in an autoclave at 121°C for 10 minutes, and then allowed to cool to about 40°C. After blocking treatment for endogenous peroxidase activity was performed with 0.3% H2O in methanol, sections were blocked with 5% normal goat serum. Then tissues were incubated overnight at 4°C with rabbit anti-human PD-L1/CD274 monoclonal antibody (clone SP142, 1:100 dilution, Spring Bioscience, CA, USA) as the primary antibody. Then, sections were incubated at room temperature for 60 minutes with EnVision-HRP Labelled Polymer Anti-Rabbit (DAKO, K4003) as the secondary antibody. Next, the slides were incubated in 3,3-diaminobenzidine (DAB) for 8 minutes.

**IHC for B7-H3**

For antigen retrieval, tissue sections were placed in Citrate Buffer Antigen Retriever pH 6.0 (C9999, Sigma-Aldrich Japan, Tokyo, Japan), heated in a decloaking chamber (Bio Care Medical, CA, USA) at 95°C for 20 minutes, cooled to 70°C, removed from the chamber and allowed to cool to about 40°C. After the blocking of endogenous peroxidase activity by 3%
H$_2$O$_2$, sections were blocked with 5% normal rabbit serum. Then, they were incubated for 60 minutes with 0.5 µg/ml of goat anti-human B7-H3 polyclonal antibody (AF1027, R&D systems, MN, USA) as the primary antibody, followed by incubation with Histofine Simple Stain MAX-PO (goat) (414161, Nichirei Bioscience, Tokyo, Japan) as the secondary antibody for 60 minutes. Finally, the slides were incubated in 3,3-diaminobenzidine (DAB) for 5-7 minutes.

**Evaluation of IHC for PD-L1 and B7-H3**

Evaluation of IHC was performed by two pathologists to estimate the percentage of cells exhibiting circumferential membranous staining for both PD-L1 and B7-H3. Regarding PD-L1 expression, we interpreted the results to be positive if at least 1% of the tumor cells were stained, regardless of the intensity, based on the criteria used in a study of non-small cell lung cancer. As for B7-H3, the staining intensity and area were evaluated according to the criteria, which refers to HER2 testing in breast cancer because there is no standard for B7-H3 in clinics. Circumferential membrane staining that is complete, intense and in ≥10% of tumor cells was scored as 3+. Weak-to-moderate complete membrane staining observed in ≥10% of tumor cells was scored as 2+. Incomplete membrane staining that is faint/barely perceptible and in ≥10% of tumor cells was scored as 1+. A score of 1+ or more indicated positive staining.
Flow cytometry analysis

Because few studies have investigated the expression of B7-H3 in mesothelioma compared with PD-L1, we confirmed the expression of B7-H3 *in vitro* by flow cytometry using a mesothelioma cell line. The cell-surface expression of B7-H3 was measured by BD LSR Fortessa flow cytometry and analyzed by FlowJo version 10. It was performed using NCI-H226 (epithelioid), ACC-MESO4 (epithelioid), NCI-H2452 (epithelioid) and MSTO-211H (biphasic) as mesothelioma cell lines, MeT-5A as a non-tumorous mesothelial cell line, Raji (Burkitt lymphoma) and Jurkat (T cell lymphoma) as negative controls, and PC-3 (prostate cancer cell line) as a positive control. Cells were incubated with 0.5 µg/ml anti B7-H3 antibody (R&D Systems) or 0.5 µg/ml purified goat IgG (I5256, Sigma-Aldrich Japan) at 37°C for 30 minutes. After washing with 1% BSA/PBS, the cells were stained with 1.0 µg/ml Alexa488 labeled donkey anti-goat IgG (Molecular Probes, OR, USA) for 30 minutes, washed with 1% BSA/PBS, stained with DAPI (4',6-diamidino-2-phenylindole, dihydrochloride), and the fluorescent intensities were measured by flow cytometry.

Statistical analysis

Statistical analyses were performed using Statcel3 software (OMS Publishing Inc., Saitama, Japan). The χ² test was used to analyze correlations between epithelioid and non-epithelioid types and PD-L1 or B7-H3 expression. IHC-stained areas of PD-L1 and B7-H3 in epithelioid
or non-epithelioid types were compared using the Student’s \( t \) test. Differences were statistically significant at \( p < 0.05 \).

**RESULTS**

The clinical characteristics of patients enrolled in this study are summarized in Table 1. The mean age at diagnosis was 66.1 years, and the male-to-female ratio was 8:2. Smoking history or asbestos exposure were 51.6% and 48.4%, respectively.

Expression of B7-H3 in mesothelioma cell lines was assessed by flow cytometric analysis. NCI-H226, ACC-MESO4, NCI-H2452, MSTO-211H, MeT-5A and PC-3 (positive control) expressed B7-H3. There was no expression of B7-H3 in Raji or Jurkat used as negative controls (Figure 1).

Figure 2 showed that both of PD-L1 and B7-H3 were mainly expressed in the cell membranes of epithelioid, biphasic and sarcomatoid mesothelioma. A part of cells expressing them in the membrane also showed the staining signals in the cytoplasm, and there was no cells expressing signals only in the cytoplasm. The membranous expression was considered to be a positive finding, regardless of their expression status in the cytoplasm, for both of PD-L1 and B7-H3, because they are supposed to function as membranous proteins. Figure 2 also showed the tendency that PD-L1 positive cells were more frequently observed in the area of lymphocyte infiltration than B7-H3 positive cells, in epithelioid and sarcomatoid
mesothelioma.

Thirteen of all 31 cases (41.9%) were positive for PD-L1, with the percentage of positive area between 5% to 90% (Table 2). In histological subtypes, the positive signals were detected in 6 (27.3%) of 22 epithelioid (positive area between 10 and 50%), and 7 (77.8%) of 9 non-epithelioid (biphasic and sarcomatoid) cases (positive area between 5% and 90%) (Table 2). The positive rate of PD-L1 was significantly lower in epithelioid (27.3%) than non-epithelioid cases (77.8%; \( p = 0.00969 \)) (Table 3a). As for B7-H3, on the contrary, both of epithelioid and non-epithelioid cases showed high positivity around 90 % (Table 3b). As for the intensity of B7-H3 signal, there was no histological differences. More than half [59.1% (13/22) of epithelioid and 55.5% (5/9) of sarcomatoid types] showed a strong or moderate signal (IHC 3+ or 2+) (Table 3c). Twelve of 13 PD-L1 positive cases were also B7-H3 positive.

In the three biphasic cases, PD-L1 positive area in epithelioid and sarcomatoid parts were 10 % and 30 % in the first, 10% and 0 % in the second, and 0% and 0% in the third case, respectively. Similarly, B7-H3 positive area in epithelioid and sarcomatoid parts were 100% (2+) and 100% (2+) in the first, 80% (2+) and 50% (2+) in the second, and 60% (2+) and 0 % in the third case, respectively. Non-tumorous mesothelial tissue was negative for the membranous expression of both PD-L1 and B7-H3 (Figure 3a, b). Table 3d showed 7 of 9 non-epithelioid cases were male and two were female. All two women were negative for PD-L1 and had no apparent involvement of extrinsic factors, such as smoking or asbestos. On the
other hand, all seven men were positive for PD-L1, five of seven had a history of exposure to asbestos, and two had an unknown history of exposure to asbestos (Table 3d). In 22 cases of epithelioid type, there was no relationships among these factors.

Our study demonstrated that PD-L1 and B7-H3 were widely expressed in mesothelioma, with heterogeneous expression among tumor cells. The expression level of B7-H3 was significantly higher than that of PD-L1 in the epithelioid type (Figure 4a, \( p < 0.00001 \)). However, in non-epithelioid type, there was no significant difference in the expression levels of PD-L1 and B7-H3 (Figure 4b, \( p = 0.46815 \)).

**DISCUSSION**

Studies of the expression of PD-L1 and B7-H3 in mesothelioma are limited. As for non-small cell lung cancer, Yonesaka et al. reported that the dual blockade of B7-H3 and PD-L1 enhanced antitumor potency compared with single blockade in pre-clinical studies.\(^6\) However, no studies have investigated potential correlations between PD-L1 and B7-H3 expression in mesothelioma. In this study, 13 of 31 (41.9%) patients with mesothelioma were PD-L1 positive. The positive rate of PD-L1 was significantly higher in the non-epithelioid type (77.8%) compared with the epithelioid type (27.3%; \( p = 0.00969 \), Table 3a). Our findings support the results of previous studies\(^{35,62-64} \) where the expression of PD-L1 in the non-epithelioid type was higher than in the epithelioid type.
Table 3d showed the tendency that PD-L1 expression was associated with history of asbestos exposure, in non-epithelioid mesothelioma, although there was no statistical significance because of small number of cases. This kind of tendency was not observed in the epithelioid type (data not shown). These data suggested that asbestos exposure history and gender might be associated with high expression of PD-L1 in non-epithelioid mesothelioma, but not in epithelioid one. Further studies are required to examine this relationships.

Calabro et al.\textsuperscript{65} reported B7-H3 expression in mesothelioma using a tissue microarray. They showed that, in 13 epithelioid cases, high or moderate B7-H3 expression was observed in 7 or 6 cases, respectively, whereas B7-H3 expression was not detected in 9 of 10 non-epithelioid cases, and the remaining 1 case showed moderate expression.\textsuperscript{65} Their data about the B7-H3 expression in the epithelioid type is similar to ours, but that in non-epithelioid type is not. Our study showed that 8/9 (88.9\%) of non-epithelioid cases expressed B7-H3. The tissue microarray used by Calabro et al.\textsuperscript{65} consists of 1.5 mm-sized core tissues [in duplicate] per sample. Samples used in our study were obtained by surgical resection, biopsy, or autopsy, and contained larger tissues than those used in the tissue microarray. This point may be the reason of increased sensitivity in our study to detect signals in non-epithelioid mesothelioma that frequently show morphological heterogeneity. In non-tumorous mesothelial tissues, there was no B7-H3 expression (Figure 3). Flow cytometric analysis showed B7-H3 positive staining for MeT-5A, a non-tumorous mesothelial cell line. Met-5A was established by
immortalization of non-malignant mesothelial cells and contains the proliferative activity that may induce the expression of B7-H3. B7-H3 was widely expressed in mesothelioma cells and not expressed in non-tumorous mesothelial cells. As several studies reported that B7-H3 has been implicated in cancer cell proliferation, cell adhesion, migration, invasion, angiogenesis, metastasis, and anti-cancer drug resistance, B7-H3 might be involved in the intrinsic regulator of cancer growth and progression of mesothelioma.

Although the receptor for B7-H3 remains unknown, many studies reported B7-H3 is a co-inhibitory molecule that negatively regulates T helper (Th)1- and Th2-mediated responses and regulatory T cell accumulation, and that its inhibition triggers anti-tumor immune responses. Our results showed that more lymphocytic infiltrates were observed around PD-L1 expressing cells, suggesting their interaction with immune cells in mesothelioma. On the other hand, less lymphocytic infiltrates were observed around B7-H3 expressing cells. This suggested that the functional roles of B7-H3 in mesothelioma may probably be independent of its interaction with immune cells.

Differences in the prognosis of mesothelioma assessed by histology have been reported. Non-epithelioid types have a poor prognosis compared with epithelioid types, and therefore it is necessary to consider the treatment response to the drug according to the histological type. However, few studies in mesothelioma have reported efficacy by histology. A phase III trial of cisplatin plus pemetrexed did not address histologic type effects. Although a phase II
trial of carboplatin in combination with pemetrexed reported a response in the epithelioid and biphasic types and no response in the sarcomatoid type,\textsuperscript{73} no histological subtype was reported in other trials.\textsuperscript{74} An association between PD-L1 expression and survival was reported in mesothelioma.\textsuperscript{31-35} Recently developed immune checkpoint inhibitors such as nivolumab and pembrolizumab are being investigated in clinical trials. In a recently reported nivolumab trial of 34 previously treated patients with MPM, the overall response rate (ORR) was 29%, the mPFS was 6.1 months, and the mOS was 17.3 months regardless of histology. The ORR in each histological type was 26% (7/27), 25% (1/4), and 67% (2/3) in epithelioid, sarcomatoid, and biphasic cases, respectively. The ORR differed by PD-L1 expression (40% for $\geq 1\%$ vs 8% for $< 1\%$, respectively).\textsuperscript{16} However, a phase II trial of pembrolizumab in 93 patients with MPM reported an ORR of 18%, with a mPFS of 3.1 months, and an mOS of 7.2 months, regardless of histology. Non-epithelioid types expressed significantly higher levels of PD-L1 than epithelioid types ($p = 0.001$). Patients with non-epithelioid type had a longer mPFS than those with epithelioid type (5.6 vs 2.8 months [hazard ratio [HR] = 0.49, 95% confidence interval [CI]: 0.28–0.88, $p = 0.02$]). High PD-L1 expressing ($\geq 50\%$) patients had a longer mPFS than those with negative or intermediate PD-L1 expression (6.2 vs 2.7 months [HR = 0.36, 95% CI: 0.14–0.93, $p = 0.04$]).\textsuperscript{64}

One study reported that B7-H3-deficient mice or mice treated with anti-B7-H3 monoclonal antibody showed reduced growth of tumors.\textsuperscript{75} Currently, several drugs targeting B7-H3 are
being tested in clinical trials. Enoblituzumab (MGA271), an Fc-optimized humanized anti-B7-H3 monoclonal antibody, has shown tolerability and preliminary efficacy (patients experienced disease stabilization [>12 weeks] and tumor shrinkage [2%-69%] across several tumor types) in clinical trials (NCT01391143), and has been studied in combination with other immune checkpoint inhibitors, including anti-PD-1 antibodies (NCT02475213, NCT02381314). In addition, clinical trials of three investigational drugs (MGD009, MGC018, DS-7300a) have begun. MGD009 is a bispecific DART (dual-affinity re-targeting antibody) molecule designed to target B7-H3 expressed on tumor cells and CD3 expressed on normal T cells (NCT02628535, NCT03406949). MGC018 is an antibody-drug conjugate (ADC) comprising a humanized B7-H3 monoclonal antibody conjugated to a novel DNA alkylating payload via a cleavable peptide linker (NCT03729596). DS-7300a is an investigational B7-H3 targeting ADC comprised of a humanized anti-B7-H3 monoclonal antibody, which is attached to a novel topoisomerase I inhibitor payload by a tetra-peptide-based linker (NCT04145622).

This study had some limitations. First, although some drugs are being developed to target B7-H3, none of them has been approved by regulatory authorities. In addition, currently there are no clinical trials investigating each drug for mesothelioma, and the efficacy in each histologic type is unknown. Furthermore, the criteria for IHC-positivity of PD-L1 and B7-H3 have not been defined in mesothelioma. However, this is the first study to investigate the protein expression status of PD-L1 and B7-H3 in human tumor tissue sections of
mesothelioma. We found that B7-H3 was widely expressed in mesothelioma. In particular, PD-L1 and B7-H3 were widely co-expressed in tumor cells of the non-epithelioid type, where unmet medical needs are high. In contrast, there was no PD-L1 and B7-H3 expression in normal mesothelial cells, and therefore, B7-H3 might be considered a potential target for drug development. We are currently planning to investigate the antitumor effects of drugs targeting B7-H3 in rodents-models\textsuperscript{77} of mesothelioma. Our findings provide a rationale for testing combinations of drugs targeting PD-L1 and B7-H3, and for the development of single-agent drugs targeting PD-L1 or B7-H3.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Takashi Yao, Dr. Takeshi Matsunaga, and Dr. Yoshiya Horimoto for their suggestions related to our research. This study was supported in part by a Grant-in-Aid (221S0001) for Scientific Research on Innovative Areas from the Japan Society for the Promotion of Science, Grants-in-Aid (S1311011 and S1511008L) from the Foundation of Strategic Research Projects in Private Universities of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid for Special Research in Subsidies for ordinary expenses of private schools from The Promotion and Mutual Aid Corporation for Private Schools of Japan, and grants from Shizuoka Medical Research Center for Disaster of Juntendo University Shizuoka Hospital, and the Institute for Environmental
and Gender-Specific Medicine of Juntendo University Urayasu Hospital.

**DISCLOSURE STATEMENT**

E. Matsumura, a member of Juntendo University School of Medicine, is also an employee of Daiichi Sankyo Co., Ltd.; however, this study was not funded by Daiichi Sankyo Co., Ltd.. The other authors have declared that no competing interests exist. Data monitoring was performed by independent monitors of clinical research and the trial center at Juntendo University School of Medicine to assure the study results. This study was approved by the Ethics Committee at Juntendo University School of Medicine (approval number: M19-083).

**AUTHOR CONTRIBUTIONS**

EM, KK, MA, HS and OH conceived and designed the experiments. EM, MA, NO, HS and MTH performed the experiments. EM and MA analyzed the data. EM, KK, MA NO and HS contributed reagents/materials. OH provided intellectual advice. EM, KK, MA and HS wrote the manuscript. All authors read and approved the final manuscript.
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**Tables**

Table 1: Clinical characteristics of 31 patients with mesothelioma

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
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<tr>
<td>Mean (SD), years</td>
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<tr>
<td>Median</td>
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<td>Range</td>
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<th>Sex, number (%)</th>
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<td>Female</td>
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<tr>
<td>Male</td>
<td>24 (77.4)</td>
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<th>History of asbestos exposure</th>
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<td>Exposure</td>
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<td></td>
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<td>Unknown</td>
<td>11 (35.5%)</td>
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**Histological subtype**

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<th>Subtype</th>
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<td>Epithelioid</td>
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<tr>
<td>Biphasic</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Sarcomatoid</td>
<td>6 (19.3)</td>
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**Stage, number (%)**

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<th>Number</th>
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<tr>
<td>I</td>
<td>6 (19.4)</td>
</tr>
<tr>
<td>II</td>
<td>3 (9.7)</td>
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<tr>
<td>III</td>
<td>13 (41.9)</td>
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<tr>
<td>IV</td>
<td>9 (29.0)</td>
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**Previous treatment**

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<th>Treatment</th>
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<tr>
<td>None</td>
<td>21 (67.7%)</td>
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<tr>
<td>Chemotherapy</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>2 (6.5%)</td>
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SD, standard deviation
Table 2. Immunohistochemistry of PD-L1 in 31 cases of mesothelioma: percentage of area with positive staining

<table>
<thead>
<tr>
<th>Cell stain, %</th>
<th>31 cases (All)</th>
<th>22 cases (Epithelioid type)</th>
<th>9 cases (Non-Epithelioid type)</th>
</tr>
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<tbody>
<tr>
<td>&lt;1</td>
<td>18 (58.1%)</td>
<td>16 (72.7%)</td>
<td>2 (22.2%)</td>
</tr>
<tr>
<td>5</td>
<td>1 (3.2%)</td>
<td>0</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>10</td>
<td>3 (9.7%)</td>
<td>3 (13.6%)</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>1 (3.2%)</td>
<td>1 (4.5%)</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>3 (9.7%)</td>
<td>0</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>50</td>
<td>2 (6.5%)</td>
<td>2 (9.1%)</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>1 (3.2%)</td>
<td>0</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>90</td>
<td>2 (6.5%)</td>
<td>0</td>
<td>2 (22.2%)</td>
</tr>
</tbody>
</table>
Table 3. Expression of PD-L1 and B7-H3 in mesothelioma

(a) Positive rate of PD-L1 expression in 31 cases of mesothelioma

<table>
<thead>
<tr>
<th>Status</th>
<th>All cases (n=31)</th>
<th>Epithelioid type cases (n=22)</th>
<th>Non-epithelioid type cases (n=9)</th>
<th>Epithelioid vs non-epithelioid p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (&lt;1%)</td>
<td>18 (58.1%)</td>
<td>16 (72.7%)</td>
<td>2 (22.2%)</td>
<td>0.00969</td>
</tr>
<tr>
<td>Positive (≥1%)</td>
<td>13 (41.9%)</td>
<td>6 (27.3%)</td>
<td>7 (77.8%)</td>
<td></td>
</tr>
</tbody>
</table>

(b) Positive rate of B7-H3 expression in 31 cases of mesothelioma

<table>
<thead>
<tr>
<th>Status</th>
<th>All cases (n=31)</th>
<th>Epithelioid type cases (n=22)</th>
<th>Non-epithelioid type cases (n=9)</th>
<th>Epithelioid vs non-epithelioid p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (0)</td>
<td>3 (9.7%)</td>
<td>2 (9.1%)</td>
<td>1 (11.1%)</td>
<td>0.86289</td>
</tr>
<tr>
<td>Positive (≥1+)</td>
<td>28 (90.3%)</td>
<td>20 (90.9%)</td>
<td>8 (88.9%)</td>
<td></td>
</tr>
</tbody>
</table>

(c) Staining intensity of B7-H3 in 31 cases of mesothelioma

<table>
<thead>
<tr>
<th>Intensity of cell</th>
<th>All cases (n=31)</th>
<th>Epithelioid type</th>
<th>Non-epithelioid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining</td>
<td>Cases (n=22)</td>
<td>Type Cases (n=9)</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (9.7%)</td>
<td>2 (9.1%)</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>1+</td>
<td>10 (32.3%)</td>
<td>7 (31.8%)</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>2+</td>
<td>12 (38.7%)</td>
<td>7 (31.8%)</td>
<td>5 (55.5%)</td>
</tr>
<tr>
<td>3+</td>
<td>6 (19.4%)</td>
<td>6 (27.3%)</td>
<td>0</td>
</tr>
</tbody>
</table>

(d) Association between PD-L1 status, history of asbestos exposure, and sex in 9 cases of non-epithelioid mesothelioma

<table>
<thead>
<tr>
<th>PD-L1 Status</th>
<th>History of Asbestos Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
</tr>
<tr>
<td>Positive</td>
<td>5 male</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>
**Figure Legends**

Figure 1. Flow cytometry analysis for the expression of B7-H3 on mesothelioma cell lines (NCI-H226, ACC-MESO4, NCI-H2452, MSTO-211H) and non-tumorous mesothelial cells (Met-5A). PC-3, derived from prostatic carcinoma, are used as a positive control. Raji and Jurkat are used as negative controls.

Figure 2. Representative figures of PD-L1- and B7-H3-positive cases of mesothelioma. Both of PD-L1 and B7-H3 were expressed in the cell membrane. (40×)

Figure 3. PD-L1 and B7-H3 in non-tumorous mesothelial tissues. PD-L1(a) and B7-H3(b) were not expressed in normal peritoneal mesothelium.

Figure 4. Comparison of PD-L1 and B7-H3 IHC-stained areas in (a) epithelioid and (b) non-epithelioid type.
Figure 1.

![Graphs showing cell counts for different cell lines](image)

**Legend:**
- B7-H3 (R&D, AF1027, 0.5μg/mL, 100μL/test)
- Nega. Cont.: Purified Goat IgG (0.5μg/mL, 100μL/test)

**Note:** Cells: 5 x 10^6 cells/test

---

Figure 2.

<table>
<thead>
<tr>
<th>epithelioid</th>
<th>biphasic</th>
<th>sarcomatoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>epithelioid area</td>
<td>sarcomatoid area</td>
<td>sarcomatoid</td>
</tr>
</tbody>
</table>

**Images:**
- PD-L1
- B7-H3
Figure 3.

(a)  
(b)  

Figure 4.

(a)  
(b)  

IHC stained area (%)  

PD-L1  B7-H3  

$\text{p}<0.00001$  

IHC stained area (%)  

PD-L1  B7-H3  

$\text{p}=0.46815$