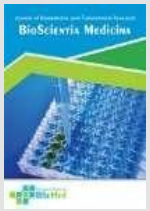
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Beyond Tumor Grade: Investigating the Heterogeneity of PD-L1 Expression in Soft Tissue Sarcomas and the Need for Subtype-Specific Analysis

Hamka Muhammad Nasir Laitupa[1,2*], Eviana Norahmawati[1,2], RA Rose Khasana Dewi[1,2], Rachmad Sarwo Bekti[1], Diah Prabawati Retnani[1,2]

[1]Department of Anatomical Pathology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

[2]Anatomical Pathology Laboratory, Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia

ARTICLE INFO

Keywords:
Immunohistochemistry
Immunotherapy
PD-L1
Soft tissue sarcoma
Tumor grading

***Corresponding author:**
Hamka Muhammad Nasir Laitupa

E-mail address:
hamkalaitupamd@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i8.1350

A B S T R A C T

Background: Soft tissue sarcomas (STS) represent a diverse group of malignant mesenchymal neoplasms with considerable histological variety and differing degrees of malignancy. Programmed Death-Ligand 1 (PD-L1) expression is a crucial immunotherapy target in various cancers, but its role and expression patterns in STS, particularly within the Indonesian population, remain inadequately defined. This study aimed to investigate the differences in PD-L1 expression between low-grade and high-grade STS and to determine the correlation between PD-L1 expression and histological grading in an Indonesian cohort. **Methods:** This analytical observational study utilized a cross-sectional design, incorporating 29 archival paraffin-embedded tissue blocks from STS patients diagnosed at Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia. PD-L1 expression was assessed immunohistochemically using the monoclonal antibody clone 22c3, and scoring was performed using the Combined Positive Score (CPS). Statistical analyses, including the Mann-Whitney U test and Spearman correlation, were employed to evaluate differences and correlations. **Results:** The majority of STS cases (89.7%) exhibited negative PD-L1 expression. The mean PD-L1 CPS was 0.1429 in low-grade STS and 0.233 in high-grade STS. No statistically significant difference in PD-L1 expression was observed between the low-grade and high-grade groups (p=0.620). Furthermore, Spearman correlation analysis revealed no significant association between PD-L1 expression (numeric CPS and categorical positivity) and histological grade (r=0.094, p=0.629 for CPS; r=0.102, p=0.600 for interpretation). **Conclusion:** This study found no significant difference in PD-L1 expression between low-grade and high-grade soft tissue sarcomas, nor a significant correlation with histological grade in the investigated Indonesian patient cohort. These findings suggest that PD-L1 expression, when assessed independently, may not be a reliable prognostic biomarker based solely on tumor grading in STS. Further research with larger sample sizes, encompassing diverse histological subtypes and incorporating additional immune biomarkers, is warranted.

1. Introduction

Soft tissue sarcomas (STS) constitute a heterogeneous collection of rare malignant neoplasms originating from mesenchymal tissue. These tumors are characterized by considerable diversity in their histological appearance and a wide spectrum of clinical aggressiveness and biological behavior. Globally, STS are recognized for their complexity, with over 150 distinct histological subtypes identified, each potentially possessing unique molecular characteristics and clinical trajectories. Generally, STS are considered aggressive malignancies, often associated with a poor prognosis, particularly when diagnosed at an advanced stage. A significant

proportion, approximately 50-60%, of STS cases arise in the extremities, commonly presenting as large, painless, or minimally painful masses. While there is no definitive evidence of significant global shifts in STS incidence or marked geographical variations, data from the United States indicate approximately 7,800 new cases annually, with a sobering mortality rate approaching 50%. In Indonesia, comprehensive epidemiological data regarding STS incidence, whether hospital-based or population-based, remain scarce, highlighting a critical gap in understanding the national burden of this disease. Existing literature suggests that about 75% of STS cases are found in the extremities, with a particular predilection for the thigh, while the abdominal wall and retroperitoneum each account for approximately 10% of cases; a slight male predominance has also been reported in the occurrence of STS.[1,2]

Histologically, STS are broadly categorized into benign and malignant forms, with some subtypes classified as intermediate or borderline malignancies, which are characterized by a high propensity for local recurrence but a relatively low risk of metastasis. Typically, the pattern of differentiation observed in the primary lesion is maintained in recurrent or metastatic lesions, although shifts in differentiation patterns can occur in some instances. The grading of STS is a critical component of pathological assessment, providing crucial prognostic information and guiding therapeutic decisions. Systems such as the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade sarcomas based on parameters including tumor differentiation, mitotic count, and tumor necrosis, categorizing them into low, intermediate, and high grades of malignancy, which generally correlate with their aggressiveness and metastatic potential.[3,4]

In the evolving landscape of oncology, immunotherapy has emerged as a transformative treatment modality for various cancers. Central to many immunotherapeutic strategies is the Programmed Death-1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) pathway. PD-L1, a transmembrane glycoprotein, is expressed on the surface of tumor cells as well as on immune cells such as macrophages and dendritic cells within the tumor microenvironment (TME). The interaction of PD-L1 with its receptor, PD-1, predominantly found on activated T cells, leads to the inhibition of T-cell activation, reduced cytokine production, and a diminished cytotoxic capacity of CD8+ T cells against tumor cells. This mechanism effectively allows cancer cells to evade immune surveillance. Consequently, therapeutic agents known as immune checkpoint inhibitors (ICIs), which block the PD-1/PD-L1 interaction, have demonstrated remarkable success in treating advanced-stage cancers, including melanoma, non-small cell lung cancer, renal cell carcinoma, and bladder cancer, especially in cases with high PD-L1 expression and specific T-cell presence in the TME.[5,6]

While PD-L1 immunohistochemistry (IHC) has become a routine diagnostic and predictive biomarker in several cancer types, its application and interpretation in STS are not as well-established. The use of ICIs targeting the PD-1/PD-L1 axis has shown promise in certain solid tumors, even in unresectable cases. The treatment response is often related to PD-L1 expression and the presence of TILs in the tumor. However, clinical trial data and laboratory findings for ICIs in STS have yielded variable results. Some studies have reported PD-L1, PD-1, and PD-L2 expression in approximately 30% of subcutaneous angiosarcomas and retroperitoneal sarcomas, suggesting a potential prognostic role in these subtypes. Other research has indicated a possible link between PD-L1 expression and histological grade, with higher expression noted in sarcomas with pleomorphic morphology. For instance, one study utilizing the FNCLCC grading system found PD-L1 expression in 18.75% (3/6) of grade I, 56.25% (9/16) of grade II, and 25% (4/6) of grade III STS cases. Although these expression rates were not exceedingly high, they offered a glimmer of hope for a potential correlation, acknowledging that small sample sizes, histological subtype heterogeneity, and technical assay variations could influence such findings. The overall response to immunotherapy in

STS remains inconsistent across studies, likely due to factors including limited sample numbers in many investigations, inherent genetic diversity among sarcomas, and the wide array of distinct tumor subtypes.[7,8]

In Indonesia, research focusing on PD-L1 expression in STS is particularly limited. This paucity of local data contributes to the infrequent routine testing for PD-L1 as a prognostic indicator or as a predictive biomarker to guide immunotherapy decisions in Indonesian STS patients. Given the aggressive nature of many STS and the urgent need for improved therapeutic strategies, understanding the molecular landscape, including immune checkpoint expression, within this specific patient population is of paramount importance.

The novelty of the present study lies in its focused investigation of PD-L1 expression (utilizing the 22c3 antibody clone and Combined Positive Score) specifically in relation to histological grade (low versus high) within a cohort of Indonesian soft tissue sarcoma patients. While international studies have explored PD-L1 in STS, data from Southeast Asian populations, particularly Indonesia, are scarce. This research addresses this regional knowledge gap and provides baseline data that could inform future, larger-scale investigations and potentially influence regional diagnostic and therapeutic considerations. Furthermore, by meticulously analyzing the correlation between PD-L1 expression and a fundamental prognostic factor like histological grade, this study aims to clarify the utility of PD-L1 as a standalone biomarker in this context, especially given the conflicting reports in existing global literature.[9,10] The aim of this study was, therefore, to determine and compare the expression levels of PD-L1 between low-grade and high-grade soft tissue sarcomas and to meticulously evaluate the statistical correlation between PD-L1 expression and the histological grading of these tumors in patients treated at Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia. The findings are anticipated to contribute to a better understanding of the immunobiology of STS in this population and to assess the potential of PD-L1 as a prognostic biomarker stratified by tumor grade.

2. Methods

This research was conducted as an analytical observational study employing a cross-sectional design. The primary objective was to investigate the differences in Programmed Death-Ligand 1 (PD-L1) expression between low-grade and high-grade soft tissue sarcomas (STS) and to ascertain the relationship between PD-L1 expression and histological grading. The study was performed at the Anatomical Pathology Laboratory of Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia. Ethical approval was implicit through the use of archival materials for diagnostic validation purposes, a common practice in retrospective pathology studies aimed at improving diagnostic and prognostic capabilities. The research activities, including sample processing and analysis, were projected to be carried out between August 2024 and February 2025.

A total of 29 archival paraffin-embedded tissue blocks were selected for this study. These samples were derived from patients who had undergone surgical resection or biopsy and were subsequently diagnosed with soft tissue sarcoma through comprehensive histopathological and immunohistochemical examinations at the Department of Anatomical Pathology, Dr. Saiful Anwar Regional General Hospital, Malang. The inclusion criteria mandated a confirmed diagnosis of STS, availability of adequate tissue in the paraffin block for further studies, and complete clinicopathological data, including histological subtype and grade. Samples were categorized into low-grade and high-grade STS based on established histopathological grading criteria, typically following systems like the FNCLCC.

The archival tissue blocks had undergone standardized histopathological processing. Briefly, tissue specimens obtained from surgery or biopsy were fixed in 10% neutral buffered formalin for a minimum duration of 4 to 6 hours to ensure adequate

preservation of tissue morphology and antigenicity. Following fixation, the tissues were processed through an automated tissue processor involving sequential dehydration with graded alcohols, clearing with xylene, and infiltration with molten paraffin wax to create paraffin blocks. For the current study, these paraffin blocks were sectioned using a rotary microtome at a thickness of 4-5 micrometers. The resulting sections were floated on a water bath and mounted onto glass slides. One set of slides from each case underwent routine Hematoxylin and Eosin (H&E) staining for morphological assessment and confirmation of diagnosis and grade. The H&E staining protocol involved deparaffinization in xylene (or using a microwave oven for 2 hours as mentioned for deparaffinization), rehydration through graded alcohols to water, staining with Hematoxylin solution, differentiation in acid alcohol, bluing in tap water or a specific bluing agent, counterstaining with Eosin solution, followed by dehydration, clearing, and mounting with a permanent mounting medium. All H&E stained slides were re-evaluated by experienced pathologists to confirm the original diagnosis, histological subtype, and tumor grade.

Immunohistochemical staining for PD-L1 was performed on newly sectioned slides from the selected paraffin blocks. The procedure was initiated with the deparaffinization of 4-micrometer thick tissue sections by immersing them in xylene, followed by rehydration through a series of decreasing concentrations of alcohol solutions and finally in distilled water. Antigen retrieval, a critical step for unmasking epitopes, was performed by immersing the slides in DIVA Decloaker solution (a heat-induced epitope retrieval solution) and placing them in a decloaking chamber at 90°C for 45 minutes. Following antigen retrieval, endogenous peroxidase activity was blocked by incubating the sections with a peroxide block solution (likely hydrogen peroxide-based) for 25 minutes to prevent non-specific background staining. The slides were then rinsed with Phosphate Buffered Saline (PBS). The primary antibody used was a monoclonal mouse anti-human PD-L1 antibody, clone 22c3 (Catalogue No. 156-B7-100, Dako Agilent Technologies Inc., Santa Clara, CA), which is a widely utilized and validated clone for PD-L1 detection. The primary antibody was applied at a dilution of 1:100 and incubated for 60 minutes at room temperature in a humidified chamber. After primary antibody incubation, a polymer-based detection system was applied. This was followed by the application of 3,3'-Diaminobenzidine (DAB) chromogen, which produces a brown-colored precipitate at the site of antigen-antibody reaction, visualizing the PD-L1 expression. Finally, the sections were counterstained with Hematoxylin to provide nuclear detail, followed by treatment with lithium carbonate for bluing. The slides were then dehydrated through graded alcohols, cleared in xylene, and mounted using a permanent mounting medium and coverslips for microscopic examination. All IHC steps were performed manually. Known positive and negative control tissues were included in each staining run to ensure the validity and reliability of the staining procedure. The immunohistochemically stained slides were evaluated under a light microscope at 100x magnification for overall assessment and at higher magnifications for detailed scoring. PD-L1 expression was considered positive if staining was observed on the membrane of tumor cells and/or on the membrane or in the cytoplasm of immune cells (lymphocytes, macrophages/histiocytes) within the tumor microenvironment. The scoring of PD-L1 expression was performed using the Combined Positive Score (CPS). The CPS was calculated as the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The maximum possible CPS value was 100; if the calculation yielded a result greater than 100, it was reported as 100. For categorical analysis, PD-L1 expression was interpreted based on CPS thresholds: CPS < 1 was considered negative, and CPS ≥ 1 was considered positive. The study also noted a category of CPS ≥ 20, though its specific analytical use beyond general categorization was not detailed for the primary comparisons. The intensity of staining was also noted

(0=negative, 1=weak, 2=moderate, 3=strong), but the main analyses relied on CPS values. All scoring was performed by pathologists.

All collected data, including patient demographics, histological subtype, tumor grade, and PD-L1 CPS values, were entered into a database for statistical analysis. The normality of the PD-L1 expression data (CPS values) was assessed using the Shapiro-Wilk test, as the sample size was less than 50. The data were found to be not normally distributed. Consequently, non-parametric tests were employed for the analysis. The Mann-Whitney U test was used to compare the median PD-L1 expression (CPS values) between the low-grade and high-grade STS groups. The Spearman rank correlation coefficient (r) was used to assess the strength and direction of the association between PD-L1 expression (both as numeric CPS values and as categorical positive/negative interpretation) and histological grading. For categorical analysis of PD-L1 interpretation (positive vs. negative) against grade (low vs. high), the Chi-square test or Fisher's exact test was planned, with odds ratios (OR) and 95% confidence intervals (CI) calculated for 2x2 tables. A p-value of less than 0.05 was considered statistically significant for all tests. Statistical analyses were performed using a standard statistical software package.

3. Results

The study cohort comprised 29 patients diagnosed with soft tissue sarcoma. A summary of their demographic and clinicopathological features is presented in Table 1. The patient cohort demonstrated a wide age range, with a mean age in the fifth decade and a predominance of male patients. Tumor grades were almost equally distributed between low and high categories. GIST and MPNST were the most commonly identified histological subtypes. Crucially, PD-L1 expression was predominantly negative across the entire cohort, with only a small fraction of tumors showing positivity by CPS criteria. The overall mean CPS was very low, reflecting this general lack of strong PD-L1 immunoexpression.

Table 1. Characteristics of the study sample (N=29).

Characteristic	Value
Age (Years)	
Mean (±SD, if available)	43.31
Median	47
Range	0 – 76
Most frequent age group (51-60 yrs)	24.1% (7 patients)
Gender	
Male	69.0% (20 patients)
Female	31.0% (9 patients)
Tumor grade	
Low grade	48.3% (14 patients)
High grade	51.7% (15 patients)
Tumor location (Most frequent)	
Femur sinistra	13.8% (4 patients)
Histological subtype (Most frequent)	
Gastrointestinal stromal tumor (GIST)	17.2% (5 patients)
Malignant peripheral nerve sheath tumor (MPNST)	17.2% (5 patients)
PD-L1 expression (Overall)	
Negative (CPS < 1)	89.7% (26 patients)
Positive (CPS ≥ 1)	10.3% (3 patients)
Mean CPS (Overall)	0.189 ange: 0 – 2.0)

PD-L1 expression in soft tissue sarcomas by Grade PD-L1 positivity was identified in a small subset of both low-grade and high-grade STS. In the low-grade STS group (n=14), one case (7.1%) showed positive PD-L1 immunoexpression. This was a Malignant Peripheral Nerve Sheath Tumor (MPNST) with a CPS of 11.3. The mean PD-L1 CPS for this group was 0.1429 ± 0.535 (Figure 1). In the high-grade STS group (n=15), two cases (13.3%) demonstrated positive PD-L1 expression. These were an epithelioid sarcoma and an extraskeletal Ewing sarcoma, with CPS values of 2.0 and 7.5, respectively. The mean PD-L1 CPS for this group was 0.233 ± 0.623 (Figure 2).

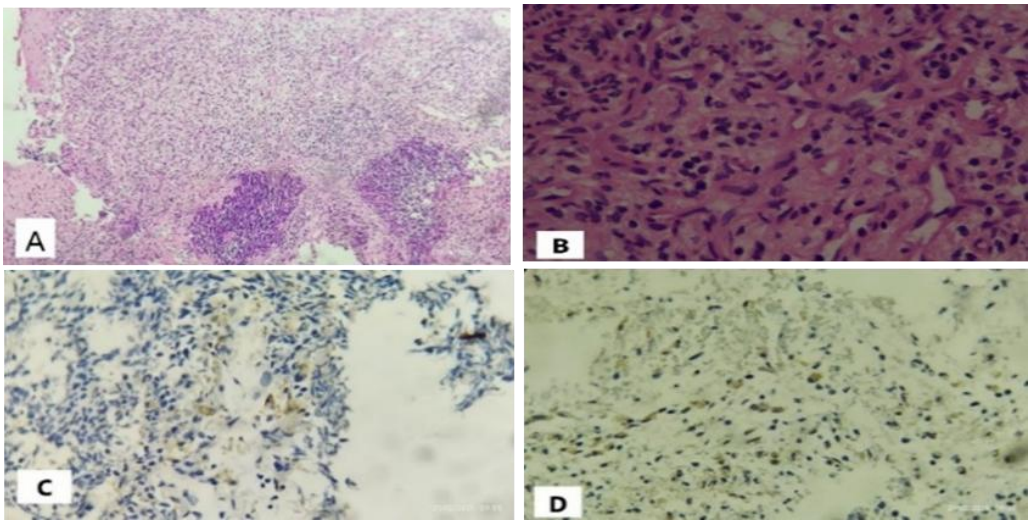


Figure 1. The immunohistochemical expression of PD-L1 in a low-grade malignant peripheral nerve sheath tumor research sample. Panels A (100x magnification) and B (400x magnification) illustrate Hematoxylin and Eosin (H&E) staining. Panels C and D demonstrate PD-L1 expression on tumor cell membranes and on the membranes and in the cytoplasm of inflammatory cells.

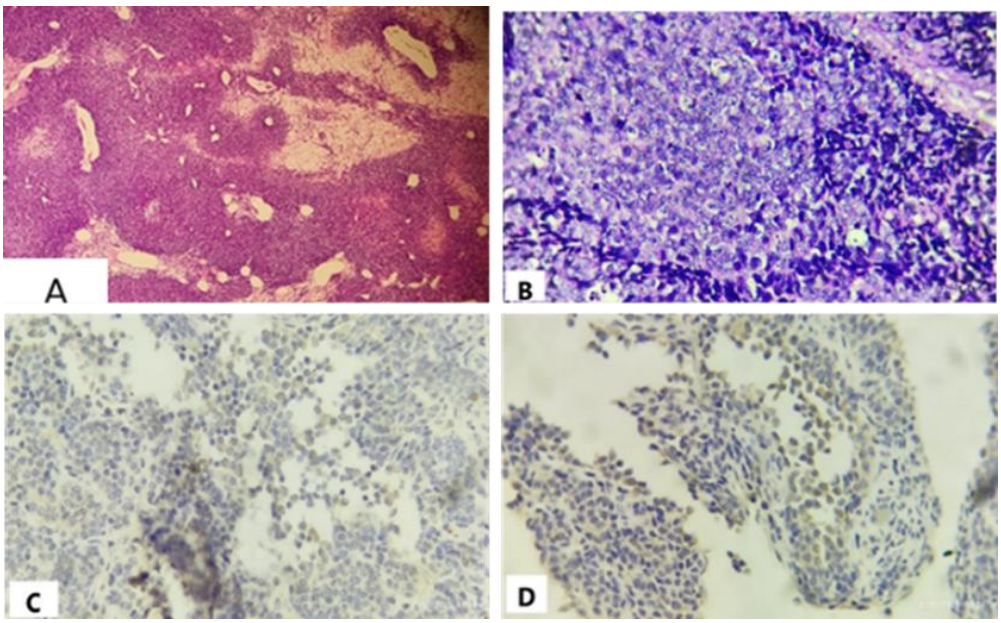


Figure 2. PD-L1 immunoexpression in the Atypical Extraskeletal Ewing Sarcoma, High Grade. A (100x magnification) and B (400x magnification) show Hematoxylin and Eosin (HE) staining. C and D illustrate PD-L1 expression on tumor cell membranes and on the membranes and in the cytoplasm of inflammatory cells.

The relationship between PD-L1 expression and histological grading was assessed using Spearman correlation, with the results summarized in Table 2. The data in Table 2 clearly demonstrate a lack of significant statistical association between PD-L1 expression and tumor grade. When PD-L1 was considered both a continuous variable (CPS score) and a categorical variable (positive/negative), the correlation coefficients (r) were very close to zero, indicating an extremely weak positive relationship. The p-values were substantially greater than 0.05, confirming that these weak correlations were not statistically significant. This suggests that the level of PD-L1 expression, or the likelihood of a tumor being PD-L1 positive, did not significantly vary with the histological grade of the STS in this cohort. Further analysis using a Chi-square test for the categorical interpretation of PD-L1 (Positive vs. Negative) against tumor grade (Low vs. High) is presented in Table 3.

Table 2. Correlation between PD-L1 expression and histological grading of soft tissue sarcomas.

Variables correlated	**Correlation coefficient (r)**	**p-value**
PD-L1 expression (Numeric CPS) vs. Grade	0.094	0.629
PD-L1 interpretation (Categorical: Positive/Negative) vs. Grade	0.102	0.600

Table 3 shows the distribution of PD-L1 positive and negative cases within the low-grade and high-grade STS groups. While a slightly higher percentage of high-grade tumors were PD-L1 positive (13.3%) compared to low-grade tumors (7.1%), this difference was not statistically significant, as evidenced by the Chi-square p-value of 0.584. The odds ratio of 2.00 suggests that high-grade tumors had twice the odds of being PD-L1 positive compared to low-grade tumors; however, the extremely wide 95% confidence interval (0.16 to 24.87) includes 1.0, indicating that this finding is not statistically significant and the estimate is imprecise due to the small number of positive cases.

Table 3. Crosstabulation of PD-L1 interpretation by histological grade.

PD-L1 interpretation	**Low grade (n=14)**	**High grade (n=15)**	**Total**	**Odds ratio (95% CI)**	**p-value (Chi-square)**
	n (%)	n (%)	n (%)		
Negative (CPS < 1)	13 (92.9%)	13 (86.7%)	26 (89.7%)	Reference	0.584
Positive (CPS ≥ 1)	1 (7.1%)	2 (13.3%)	3 (10.3%)	2.00 (0.16 – 24.87)	

The Mann-Whitney U test was performed to directly compare PD-L1 expression levels (mean CPS) between the two grade groups, with findings presented in Table 4. As shown in Table 4, the mean PD-L1 CPS was numerically slightly higher in the high-grade STS group (0.233) compared to the low-grade STS group (0.1429). However, this difference did not reach statistical significance, with the Mann-Whitney U test yielding a p-value of 0.620. This key result indicates that, within this study cohort, there was no significant distinction in the quantitative levels of PD-L1 expression when comparing low-grade versus high-

grade soft tissue sarcomas. The results consistently demonstrated that PD-L1 expression was infrequent in this STS cohort. There was no statistically significant difference in PD-L1 expression levels between low-grade and high-grade tumors, and no significant correlation was found between PD-L1 expression (either quantitative CPS or categorical positivity) and the histological grade of the tumors.

Table 4. Comparison of PD-L1 expression (CPS) between low-grade and high-grade soft tissue sarcomas.

Group	N	Mean PD-L1 CPS (± Std. Dev.)	p-value (Mann-Whitney U)
Low Grade	14	0.1429 (± 0.535)	0.620
High Grade	15	0.233 (± 0.623)	

4. Discussion

The intricate relationship between the immune system and cancer has paved the way for immunotherapy, revolutionizing treatment paradigms for numerous malignancies. Central to this revolution is the PD-1/PD-L1 immune checkpoint pathway, where PD-L1 expression on tumor cells or immune cells within the tumor microenvironment can lead to T-cell exhaustion and immune evasion. Consequently, the assessment of PD-L1 expression has become a critical biomarker in guiding therapeutic decisions for various cancers. However, in the diverse and complex world of soft tissue sarcomas (STS), the role and regulatory landscape of PD-L1 remain less clearly defined. This study embarked on an investigation into PD-L1 expression within an Indonesian cohort of STS patients, specifically dissecting its association with histological grade—a fundamental prognostic indicator. The findings, characterized by a low overall prevalence of PD-L1 positivity and a notable absence of significant correlation or difference in expression between low-grade and high-grade STS, contribute valuable insights into the immunobiology of these tumors, particularly in an underrepresented patient population. A primary observation from this research was the predominantly negative PD-L1 status across the STS samples, with nearly 90% of cases exhibiting a Combined Positive Score (CPS) of less than 1. This low rate of PD-L1 positivity is not entirely unexpected in the context of STS, as numerous studies have reported variable but often modest expression levels across different sarcoma subtypes. The inherent heterogeneity of STS, comprising over 150 distinct histological entities, is a major confounding factor. Each subtype possesses unique genetic and epigenetic characteristics that can influence the tumor microenvironment and, consequently, the expression of immune checkpoint molecules like PD-L1.[11,12]

The pathophysiology of PD-L1 expression is complex and can be driven by several mechanisms. Innate immune resistance involves constitutive PD-L1 expression by tumor cells, often driven by oncogenic signaling pathways (such as PI3K/AKT, MAPK, or STAT3 activation) that can upregulate PD-L1 transcription. Alternatively, adaptive immune resistance occurs when PD-L1 expression is induced in response to an active anti-tumor immune response, primarily through cytokines like interferon-gamma (IFN-γ) secreted by activated T cells and NK cells. The low overall PD-L1 expression observed in this STS cohort might suggest that either the oncogenic pathways driving constitutive expression are not universally active across these sarcomas, or that many of these tumors do not elicit a robust IFN-γ-mediated adaptive immune response—they may be immunologically "cold" or ignorant. Tumors with low mutational burden, which is characteristic of many sarcoma subtypes (excluding a few like undifferentiated pleomorphic sarcoma or dedifferentiated liposarcoma), often have fewer neoantigens, leading to weaker T-cell priming and infiltration, and consequently, less IFN-γ production to induce PD-L1. The choice of antibody clone (22c3) and scoring system (CPS) is critical in PD-L1

assessment. CPS evaluates PD-L1 staining in both tumor cells and immune cells relative to the number of tumor cells, potentially capturing a broader aspect of the immune interaction than scoring systems focused solely on tumor cell staining (like TPS). However, even with this comprehensive scoring, the expression remained low. This finding underscores that if PD-L1 positivity is a prerequisite for benefit from anti-PD-1/PD-L1 therapies, then only a small fraction of the general STS population, as represented by this cohort, might be considered prima facie candidates based on this biomarker alone.[13,14]

Histological grade in STS reflects tumor differentiation, mitotic activity, and necrosis, collectively serving as a surrogate for tumor aggressiveness and metastatic potential. It is often hypothesized that high-grade tumors, with their increased cellular atypia, proliferation, and genomic instability, might harbor a more inflamed or reactive tumor microenvironment, potentially leading to higher PD-L1 expression either through increased neoantigen load and subsequent IFN-γ signaling or through oncogenic pathway activation. This study, however, did not find a statistically significant difference in PD-L1 expression (mean CPS) between low-grade (0.1429) and high-grade (0.233) STS (p=0.620). Similarly, the correlation analyses showed no significant association between PD-L1 levels (numeric or categorical) and grade. This lack of a clear grade-dependent PD-L1 expression pattern challenges the simplistic view that higher grade automatically equates to a more immunomodulatory phenotype via PD-L1. Several pathophysiological considerations could explain this. Firstly, the mechanisms driving PD-L1 expression may be independent of those determining grade. While grading reflects cellular morphology and proliferation, PD-L1 expression is more directly tied to specific signaling pathways (intrinsic) or immune cell infiltration and cytokine milieu (extrinsic). These factors might not scale linearly with grade across the diverse spectrum of STS. Secondly, high-grade tumors, despite their potential for increased neoantigenicity, might also employ other potent immune evasion mechanisms beyond PD-L1, or they might exhibit a dysfunctional immune infiltrate where IFN-γ production is impaired. Conversely, some low-grade STS subtypes might have specific molecular alterations or microenvironmental features that, despite their lower overall aggressiveness, induce PD-L1.[15,16]

The other study also reported no significant link between PD-L1 expression and FNCLCC grade, supporting the current findings. They noted PD-L1 expression across all grades, suggesting that grade is not a primary determinant. The findings of the current study also align with other studies, who stated that PD-L1 expression in sarcomas does not always correlate with clinicopathological factors such as grade. This implies that relying on histological grade to predict PD-L1 status, and by extension, potential suitability for PD-1/PD-L1 inhibitors, would be unreliable in STS. However, it is important to contextualize these findings with conflicting reports. Some studies did observe higher PD-L1 expression in high-grade sarcomas. Other studies specifically highlighted subtypes like undifferentiated pleomorphic sarcoma (UPS) and alveolar soft part sarcoma (ASPS), known for their aggressive nature, as having higher PD-L1 expression linked to an active immune microenvironment. Other studies even correlated higher PD-L1 in high-grade tumors with increased TILs and metastatic risk. Other studies also leaned towards higher PD-L1 in high-grade sarcomas being associated with poorer prognosis. These discrepancies across studies likely reflect the profound heterogeneity within STS. Even within "high-grade" or "low-grade" categories, the specific mix of histological subtypes can dramatically influence overall findings. For instance, if a study's high-grade cohort is enriched in subtypes known for high PD-L1 (like ASPS or some UPS), it might show a positive correlation with grade, whereas a cohort with different subtype distributions might not. The current study, with a sample size of 29, encompassed a variety of subtypes, including GIST and MPNST as the most common; the specific immunogenic profiles of the

particular subtypes within its low-grade and high-grade arms would be influential.[17,18]

Despite the overall negative findings concerning grade, the study did identify PD-L1 positivity in a few specific cases, hinting at subtype-specific expression patterns. One low-grade Malignant Peripheral Nerve Sheath Tumor (MPNST) showed PD-L1 positivity (CPS 11.3). MPNSTs are aggressive sarcomas often arising from peripheral nerves or in patients with Neurofibromatosis Type 1 (NF1). NF1 is caused by mutations in the *NF1* gene, a tumor suppressor that negatively regulates RAS signaling. Aberrant RAS pathway activation is common in MPNSTs and could theoretically contribute to PD-L1 upregulation through downstream signaling cascades like PI3K-AKT or MEK-ERK. Furthermore, MPNSTs can sometimes have a notable immune infiltrate. The finding aligns with other studies that reported PD-L1 expression in about a third of MPNSTs, suggesting a subset might indeed be immunogenic. One high-grade epithelioid sarcoma was PD-L1 positive (CPS 2.0). Epithelioid sarcoma is a rare, aggressive STS subtype often characterized by loss of INI1 (SMARCB1) expression, a core subunit of the SWI/SNF chromatin remodeling complex. Loss of INI1 can lead to epigenetic dysregulation and potentially influence the expression of various genes, including immune-related ones. Some studies have reported remarkably high rates of PD-L1 expression in epithelioid sarcoma, making it a particularly interesting candidate for immunotherapy. The relatively low CPS in this single case warrants further investigation in larger series of this subtype. One high-grade extraskeletal Ewing sarcoma was also PD-L1 positive (CPS 7.5). Ewing sarcomas are defined by specific chromosomal translocations, most commonly involving the *EWSR1* gene and an ETS family transcription factor gene (like *FLI1*). The resulting fusion oncoproteins drive tumorigenesis. While generally not considered highly immunogenic, other studies have shown PD-L1 expression in a subset (around 13%) of Ewing sarcomas. The mechanisms for PD-L1 expression in Ewing sarcoma are not fully clear but could involve the oncogenic fusion protein's downstream effects or microenvironmental interactions. These individual positive cases, though few, underscore the critical message that PD-L1 expression in STS is likely more dependent on the specific histological subtype and its underlying molecular drivers than on broad categorizations like histological grade alone. Each subtype has a unique biology, and lumping them together can obscure important signals.[19,20]

The tumor microenvironment (TME) in STS is a complex ecosystem of cancer cells, stromal cells (fibroblasts, endothelial cells), and immune cells (lymphocytes, macrophages, myeloid-derived suppressor cells). The composition and activation state of this TME significantly dictate tumor progression and response to therapy, including immunotherapy. PD-L1 expression is a dynamic feature of this TME. In many STS, the immune infiltrate can be sparse or skewed towards immunosuppressive cell types. The "immunological ignorance" of some sarcomas—whereby they fail to elicit a strong T-cell response due to low neoantigen load or defective antigen presentation—would result in low IFN-γ levels and consequently low PD-L1 expression. This seems to be the case for the majority of tumors in the current study. Even when TILs are present, their functionality can be impaired. Regulatory T cells (Tregs), M2-polarized macrophages, and MDSCs can create an immunosuppressive milieu that dampens cytotoxic T-cell activity and may also influence PD-L1 expression patterns on tumor and immune cells. For example, some studies have shown an association between PD-L1 expression and FOXP3+ Treg infiltration in STS, correlating with poor prognosis. This suggests that PD-L1 expression, when it occurs, might be part of a broader immunosuppressive network. The role of specific oncogenic pathways in STS subtypes could also directly modulate PD-L1. As mentioned, pathways like PI3K/AKT and MAPK, which are dysregulated in many cancers including some sarcomas, have been shown to upregulate PD-L1. The loss of tumor suppressor genes like PTEN (activating PI3K/AKT) or NF1

(activating RAS/MAPK) in certain sarcoma subtypes could thus directly contribute to PD-L1 expression independent of immune stimuli. However, if these pathways are not the dominant drivers of PD-L1 in the majority of STS within this cohort, it would contribute to the observed low expression.

The generally low expression in low-grade STS in this study (mean CPS 0.1429) is consistent with the WHO's description of low-grade tumors typically having a less active immune microenvironment. Low-grade tumors often have fewer genetic alterations, lower proliferation rates, and consequently, may present fewer neoantigens to the immune system, leading to a quiescent TME with minimal IFN-γ and thus low PD-L1. Other study found that PD-L1 expression in STS correlated with low TILs, a feature often seen in low-grade tumors. The slightly higher, yet still not significantly different, PD-L1 expression in high-grade STS (mean CPS 0.233) is interesting. High-grade tumors, by definition, have higher mitotic rates and often more genomic instability, which could lead to increased neoantigen production and a more inflamed TME. However, if this inflammation is not effectively translated into IFN-γ production by functional effector T cells, or if other immune escape mechanisms are dominant, then PD-L1 upregulation might not be a prominent feature. Moreover, the heterogeneity within high-grade STS is vast; some subtypes might be highly immunogenic while others are adept at creating an "immune-excluded" phenotype where T cells are present in the stroma but cannot penetrate the tumor bed. Other study noted that PD-L1 expression in high-grade STS was subtype-dependent, prominent in myxofibrosarcoma and UPS but not all high-grade types, emphasizing that grade itself isn't the sole driver.

The findings from this study, particularly the lack of association between PD-L1 expression and grade, and the overall low positivity, have significant clinical implications for STS management in the Indonesian context and contribute to the global understanding. If PD-L1 expression is to be used as a predictive biomarker for anti-PD-1/PD-L1 therapies in STS, its utility appears limited when applied broadly without considering histological subtype. The results suggest that patient selection based on histological grade alone for PD-L1 testing or immunotherapy consideration is not supported. The observation that 10.3% of patients did show PD-L1 positivity indicates that there is a subset of STS patients who might be candidates for checkpoint inhibitors. Identifying these patients accurately is key. The current study hints that focusing on specific subtypes—like epithelioid sarcoma, MPNST, or Ewing sarcoma as suggested by the positive cases —might be more fruitful than broad-based screening. For these subtypes, even if overall positivity is low in a mixed cohort, the expression within that specific histology might be more consistent or clinically meaningful. The broader challenge in STS immunotherapy is that PD-L1 is likely not the sole determinant of response. The TME's complexity, including the presence and functionality of TILs, tumor mutational burden (TMB), MSI status (though rare in most STS), and the expression of other immune checkpoint molecules (TIM-3, LAG-3) or co-stimulatory molecules, all play a role. Therefore, a multi-biomarker approach may be necessary for more accurate patient stratification in STS.

The data from Indonesia are particularly valuable given the limited research from this region. It provides a local benchmark and highlights the importance of conducting studies in diverse populations, as genetic backgrounds and environmental factors could potentially influence tumor biology and immune responses. While the current study focused primarily on the relationship between PD-L1 and grade, the broader discussion in the source material about immunotherapy responses being variable in STS is pertinent. The success of ICIs in other cancers has often been linked to higher PD-L1 expression or high TMB. Many common STS subtypes have relatively low TMB. However, specific subtypes like UPS can have higher TMB, and others like alveolar soft part sarcoma (ASPS), while often PD-L1 positive, have unique biological features (TFE3 fusions) that make them responsive to ICIs despite typically low TMB. This

again points to the critical importance of subtype-specific understanding. The findings of this study temper enthusiasm for using PD-L1 as a simple, universal biomarker tied to grade in STS. Instead, they advocate for a more sophisticated approach where PD-L1 is considered one piece of a larger puzzle, with histological subtype being a primary lens through which its expression should be interpreted and further investigated. The path forward likely involves deep dives into the immunogenomics of individual sarcoma subtypes to identify those most likely to benefit from current immunotherapies or to develop novel immune-based strategies for those that are immunologically "cold." The data showing a lack of correlation with grade reinforces the idea that other biological drivers are more central to PD-L1 expression in this diverse group of cancers.

5. Conclusion

This study investigated the expression of PD-L1 in 29 cases of soft tissue sarcoma, comparing low-grade and high-grade tumors within an Indonesian patient cohort. The overall expression of PD-L1 was low, with 89.7% of cases demonstrating negative PD-L1 expression (CPS < 1). There was no statistically significant difference in the mean PD-L1 expression (Combined Positive Score) between low-grade STS (mean CPS 0.1429) and high-grade STS (mean CPS 0.233) (p=0.620). No significant correlation was found between PD-L1 expression levels (either numeric CPS or categorical interpretation) and the histological grade of soft tissue sarcomas (r=0.094, p=0.629 for numeric; r=0.102, p=0.600 for categorical). PD-L1 expression alone, as assessed in this study, does not appear to be a robust prognostic biomarker based on histological grading in this cohort of soft tissue sarcoma patients. The heterogeneity of STS and the complexity of the tumor immune microenvironment suggest that a more individualized approach, likely incorporating histological subtype and a broader panel of immune biomarkers, is necessary to identify patients who might benefit from PD-1/PD-L1 targeted immunotherapies.

6. References

1. Ranade M, Shah A, Yadav S, Menon S, Gupta S. Extra-nodal follicular dendritic cell sarcoma with unusual morphological features and PDL1 expression. Indian J Med Paediatr Oncol. 2022.
2. Bertucci F, Finetti P, Perrot D, Leroux A, Collin F, Le Cesne A, et al. PDL1 expression is a poor-prognosis factor in soft-tissue sarcomas. Oncoimmunology. 2017; 6(3): e1278100.
3. Toulmonde M, Adam J, Bessede A, Ranchère-Vince D, Velasco V, Brouste V, et al. Integrative assessment of expression and prognostic value of PDL1, IDO, and kynurenine in 371 primary soft tissue sarcomas with genomic complexity. J Clin Oncol. 2016; 34(Suppl_15): 11008.
4. Case Medical Research. MEK inhibitor and a PDL1 inhibitor patients with locally advanced and/or metastatic soft tissue sarcoma. Case Med Res. 2020.
5. Kim C, Kim EK, Jung H, Chon HJ, Han JW, Shin K-H, et al. Prognostic implications of PD-L1 expression in patients with soft tissue sarcoma. BMC Cancer. 2016; 16(1): 434.
6. Pollack S, He Q, Yearley J, Emerson RO, Vignali M, Zhang Y, et al. Correlation of T-cell infiltration and clonality with PD-L1 expression in soft tissue sarcomas. J Clin Oncol. 2017; 35(Suppl_7): 23–23.
7. Que Y, Xiao W, Guan Y-X, Liang Y, Yan S-M, Chen H-Y, et al. PD-L1 expression is associated with FOXP3+ regulatory T-cell infiltration of soft tissue sarcoma and poor patient prognosis. J Cancer. 2017; 8(11): 2018–25.
8. Torabi A, Amaya CN, Wians FH Jr, Bryan BA. PD-1 and PD-L1 expression in bone and soft tissue sarcomas. Pathology. 2017; 49(5): 506–13.
9. Patel KR, Martinez A, Stahl JM, Logan SJ, Perricone AJ, Ferris MJ, et al. Increase in PD-

L1 expression after pre-operative radiotherapy for soft tissue sarcoma. Oncoimmunology. 2018; 7(7): e1442168.

10. Kelany MR, Barth T, Salem D, Mosaad M. PD-L1 expression in soft tissue sarcomas and its prognostic implication. J Clin Oncol. 2019; 37(Suppl_15): e14261.
11. Asanuma K, Nakamura T, Hayashi A, Okamoto T, Iino T, Asanuma Y, et al. Soluble programmed death-ligand 1 rather than PD-L1 on tumor cells effectively predicts metastasis and prognosis in soft tissue sarcomas. Sci Rep. 2020; 10(1): 9077.
12. Orth MF, Buecklein VL, Kampmann E, Subklewe M, Noessner E, Cidre-Aranaz F, et al. A comparative view on the expression patterns of PD-L1 and PD-1 in soft tissue sarcomas. Cancer Immunol Immunother. 2020; 69(7): 1353–62.
13. Zhou M, Bui N, Lohman M, van de Rjin M, Hwang G, Ganjoo K. Long-term remission with ipilimumab/nivolumab in two patients with different soft tissue sarcoma subtypes and no PD-L1 expression. Case Rep Oncol. 2021; 14(1): 459–65.
14. Kim SK, Kim JH, Kim SH, Lee YH, Han JW, Baek W, et al. PD-L1 tumour expression is predictive of pazopanib response in soft tissue sarcoma. BMC Cancer. 2021; 21(1): 336.
15. Kelany M, Barth TF, Salem D, Shakweer MM. Prevalence and prognostic implications of PD-L1 expression in soft tissue sarcomas. Pathol Oncol Res. 2021; 27: 1609804.
16. Hashimoto K, Nishimura S, Ito T, Kakinoki R, Akagi M. Immunohistochemical expression and clinicopathological assessment of PD-1, PD-L1, NY-ESO-1, and MAGE-A4 expression in highly aggressive soft tissue sarcomas. Eur J Histochem. 2022; 66(2).
17. Liu J, Gao T, Tan Z, Li S, Xu J, Bai C, et al. Phase II study of TQB2450, a novel PD-L1 antibody, in combination with anlotinib in patients with locally advanced or metastatic soft tissue sarcoma. Clin Cancer Res. 2022; 28(16): 3473–9.
18. Clarke C, Istl AC, Bedi M, Singh R, Charlson J, Neilson J, et al. The impact of hypofractionated pre-operative radiation therapy on PD-L1 expression in soft tissue sarcomas. Int J Radiat Oncol Biol Phys. 2024; 120(2): S88.
19. Kurt İnci B, Acar E, Gürler F, İlhan A, Yıldız F, Ardıç F, et al. Prognostic role of OX40, LAG-3, TIM-3 and PD-L1 expression in bone and soft tissue sarcomas. J Clin Med. 2024; 13(12): 3620.
20. Yoshida K, Asanuma K, Matsuyama Y, Okamoto T, Hagi T, Nakamura T, et al. Release of exosomal PD-L1 in bone and soft tissue sarcomas and its relationship to radiotherapy. Cancers (Basel). 2024; 16(13): 2489.