

RESEARCH ARTICLE

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Prognostic Value of *PD-L1* and Its Correlation with *GATA3*, *CK5/6*, and *CK14* Expression in Muscle-Invasive Bladder Cancer

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Abstract

Background: Muscle-invasive bladder cancer (MIBC) is an aggressive urothelial carcinoma with poor outcomes. This study investigated programmed death ligand 1 (*PD-L1*) expression and its association with key immunohistochemical markers (*GATA3*, *CK5/6*, *CK14*) and overall survival (OS). **Methods:** We analyzed formalin-fixed, paraffin-embedded tissue from 61 patients with MIBC who underwent radical cystectomy and chemoradiation. Immunohistochemistry was performed for *PD-1*, *PD-L1* (SP263), *GATA3*, *CK5/6*, and *CK14*. *PD-L1* expression was classified as high or low based on VENTANA guidelines. **Results:** *PD-L1* was detected in 34% of tumor tissues and 79% of peritumoral immune cells. High *PD-L1* expression (18% of cases) was significantly associated with advanced tumor stage, lymphovascular invasion, metastasis, and poor outcomes. Patients with high *PD-L1* had markedly reduced 2-year OS (18.2% vs. 57.2%; HR: 3.16, 95% CI: 1.59–7.24). A negative correlation was observed between *PD-L1* and *GATA3*, while *PD-L1* overexpression positively correlated with basal-like markers *CK5/6* and *CK14*. **Conclusion:** High *PD-L1* expression predicts aggressive clinicopathological features and poor survival in MIBC. Routine *PD-L1* assessment, alongside luminal and basal markers, offers a critical strategy for refining risk stratification and guiding personalized therapeutic decisions in patients undergoing radical cystectomy.

Keywords: urinary bladder cancer, *PD-L1*, *GATA3*, prognostic markers

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Introduction

Urinary bladder cancer (BCa) represents a significant public health challenge worldwide, with an estimated global number of 573,000 new cases and 213,000 deaths in 2020 [1]. The etiology of BCa is multifactorial, with risk factors including tobacco smoking, exposure to certain industrial chemicals, chronic bladder inflammation, and genetic predispositions. In general, BCa is characterized by the malignant growth of tissue within the urinary bladder. Histologically, urothelial (transitional cell) carcinoma constitutes the majority of bladder cancer cases, although other types, such as squamous cell carcinoma and adenocarcinoma, are also observed [2]. BCa are subcategorized according to their invasiveness into non-muscle-invasive bladder cancer (NMIBC)

and muscle-invasive bladder cancer (MIBC). MIBC, accounting for 25% of all BCa, is a more aggressive form that has a poorer prognosis due to its higher chance of recurrence and metastasis [3]. When management of NMIBC is usually limited to transurethral resection of the pathology; MIBC usually requires more aggressive treatments such as radical cystectomy and systemic chemotherapy [4]. As patients with MIBC are mostly in advanced age, a significant proportion of them cannot tolerate chemotherapy. Recent advances in immunotherapy and targeted therapies have shown promise in improving outcomes for patients with advanced disease.

Urinary bladder cancer (BCa) represents a significant public health challenge worldwide, with an estimated global number of 573,000 new cases and 213,000 deaths in 2020 [1]. The etiology of BCa is multifactorial, with

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risk factors including tobacco smoking, exposure to certain industrial chemicals, chronic bladder inflammation, and genetic predispositions. In general, BCa is characterized by the malignant growth of tissue within the urinary bladder. Histologically, urothelial (transitional cell) carcinoma constitutes the majority of bladder cancer cases, although other types, such as squamous cell carcinoma and adenocarcinoma, are also observed [2]. BCa are subcategorized according to their invasiveness into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). MIBC, accounting for 25% of all BCa, is a more aggressive form that has a poorer prognosis due to its higher chance of recurrence and metastasis [3]. When management of NMIBC is usually limited to transurethral resection of the pathology, MIBC usually requires more aggressive treatments such as radical cystectomy and systemic chemotherapy [4]. As patients with MIBC are mostly of advanced age, a significant proportion of them cannot tolerate chemotherapy. Recent advances in immunotherapy and targeted therapies have shown promise in improving outcomes for patients with advanced disease.

Immunotherapy, particularly immune checkpoint inhibitors (ICIs), has emerged as a transformative approach to managing MIBC, offering new therapeutic options for patients with advanced disease [5]. These agents target inhibitory pathways in T cells, such as the programmed cell death-1 (*PD-1*)/programmed cell death-ligand 1 (*PD-L1*) axis and cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*), to enhance the immune system's ability to recognize and destroy cancer cells. Clinical trials have demonstrated the efficacy of ICIs, including pembrolizumab, atezolizumab, and nivolumab, in MIBC, showing significant improvements in overall survival and response rates compared with traditional chemotherapy in certain patient cohorts [6]. The use of ICIs is now approved in various settings, including as second-line therapy for platinum-refractory advanced urothelial carcinoma [6]. Despite their promise, responses to ICIs are variable, and research continues to identify predictive biomarkers and develop combination strategies to overcome resistance. *PD-L1* expression in bladder cancer tissues has been associated with tumor aggressiveness, stage, and prognosis, suggesting its potential as a disease marker [7]. Recent studies have explored *PD-L1* expression as a predictive biomarker for ICI efficacy, which blocks the *PD-1*/*PD-L1* interaction to enhance anti-tumor immune responses. The rationale is that tumors expressing high levels of *PD-L1* may be more susceptible to ICIs, such as pembrolizumab and atezolizumab, making *PD-L1* expression a relevant factor in selecting patients for immunotherapy [8]. However, the correlation between *PD-L1* expression and response to ICIs in bladder cancer is complex and not absolute, with responses observed in both *PD-L1*-positive and *PD-L1*-negative tumors. Moreover, the prognostic value of *PD-L1* in MIBC patients who received standard chemotherapy was controversial [9–11]. This inconsistency highlights the need for further research to fully understand the utility of *PD-L1* as a biomarker for patient selection and prognosis in bladder cancer.

Recent genomic studies have led to the classification of MIBC into distinct molecular subtypes, primarily basal and luminal, which display distinct clinical behaviors and sensitivities to chemotherapy [12]. Crucially, these subtypes are also thought to possess unique immune microenvironments. For instance, basal-type tumors are often associated with greater immune infiltration and *PD-L1* expression than luminal types, potentially influencing their responsiveness to checkpoint blockade. However, the interplay between these established molecular markers (such as *GATA3*, *CK20*, *CK5/6*, and *CK14*) and *PD-L1* expression remains underexplored in clinical settings.

Our recent research established a correlation between subtype markers (*GATA3*, *CK20*, *CK5/6*, and *CK14*) and outcomes in MIBC, showing that *GATA3* and *CK5/6* were positively associated with survival, whereas a double-negative status for both markers was associated with a poorer prognosis [12]. Building on these findings, this study aims to investigate the prognostic value of *PD-L1* expression in a distinct cohort of MIBC patients who underwent cystectomy at our institute, specifically in relation to molecular subtype markers [9–11]. We hypothesize that *PD-L1* expression varies significantly across molecular subtypes and that combining *PD-L1* status with subtype classification will provide superior prognostic stratification compared with either marker set alone, ultimately offering better therapeutic guidance for this patient group.

Materials and Methods

Patients

The study included 61 adult patients with MIBC who underwent radical cystectomy at Songklanagarind Hospital from January 2017 to July 2021. Preoperative evaluation and postoperative adjuvant treatment were performed according to the standards of the Thai Urological Association [13]. Cases in this cohort did not overlap with our prior study [12]. A pathologic review was performed by a pathologist specializing in genitourinary tract pathology (KK), who was blinded to the outcome data. Clinical data, including age at diagnosis, sex, and clinical course, were retrieved from the hospital's electronic medical record (HIS system). Pathologic staging followed the TNM classification and the eighth edition of the American Joint Committee on Cancer Staging Manual. NMIBC cases were excluded from this study. Cases with operative mortality were excluded from the survival analysis. Survival data were derived from clinical follow-up and death registry data from the Thai citizen registration system. The study protocol was approved by the Human Research Ethics Committee of the Faculty of Medicine, Prince of Songkla University (REC61-233-10-1). All methods were carried out in accordance with the World Medical Association Declaration of Helsinki. Informed consent was obtained from all patients or legally authorized representatives.

Immunohistochemistry

Archived pathological specimens from all included

cases were retrieved from formalin-fixed, paraffin-embedded tissue blocks. Blocks were selected if the sampling contained more than 70% viable tumor area. Specimens were cut into 5- μ m sections for immunostaining. Immunohistochemistry for *PD-L1* (Abcam anti-PD1 antibody [NAT 105], 1:100 dilution), *PD-L1* (VENTANA SP263, 1:100 dilution; Roche, USA), *GATA3* (UMAB218, 1:100 dilution; OriGene, MD, USA), *CK5/6* (D5/16, 1:50 dilution; Dako, Glostrup, Denmark), and *CK14* (OIT4A7, 1:100 dilution; OriGene) was performed by a pathology technician. Counterstaining was performed using the EnvisionTM+ system (DAKO) according to the manufacturer's protocol. Quantification of positive staining for *GATA3*, *CK5/6*, *CK14*, and *PD-L1* involved measuring the area of immunoreactivity (estimated percent of positive staining per tumor area) and staining intensity (0: no staining; 1: weak positivity; 2: moderate positivity; 3: strong positivity). The percentage of positive staining was stratified into proportion scores: 0 for 0% (no positive cells), 1 for <1%, 2 for 1%-10%, 3 for 11%-33%, 4 for 34%-66%, and 5 for >67%. The Allred score was obtained by summing the intensity and proportion scores, with a range of 0-8. Interpretation of *PD-L1* staining followed the guidelines of the VENTANA *PD-L1* Assay for urothelial carcinoma [14]. Briefly, *PD-L1* status is determined by the percentage of tumor cells with any membrane staining above background or the percentage of tumor-associated immune cells with staining (IC+) at any intensity above background. The percent of tumor area occupied by tumor-associated immune cells (ICP) was used to determine IC+, representing the percent area of ICP showing *PD-L1*-positive immune cell staining. *PD-L1* status was considered high when more than 25% of tumor cells showed membrane staining, or when positive staining in tumor-associated immune cells (IC+) exceeded 25%, provided the percentage of tumor-associated immune cells in the tumor area (ICP) was over 1%, or IC+ was 100% when ICP was low (1%).

When none of these criteria for high expression were met, *PD-L1* status was classified as low (negative).

Statistical analysis

Categorical data, i.e., the proportion of positive staining, were reported as percentages and compared between groups using the chi-square test and Fisher's exact test, as appropriate. Continuous data were reported as medians and compared using the Mann-Whitney U-test. The correlation coefficient (R) was used to quantify the correlation between the expression levels of each marker. Overall survival (OS) probabilities after radical cystectomy were estimated using the Kaplan-Meier method. A log-rank test was used to compare survival probabilities between marker status groups. The hazard ratio was estimated using Cox regression. Two-sided p-values < 0.05 were considered statistically significant. Statistical analyses and visualization were performed using Python 3.9 and Stata 14.0 (Stata Corp., TX).

Results

A total of 61 cases, including 55 males and 6 females, underwent surgery during the study period. The first enrolled case was operated on in January 2017, and the last case in this series was operated on in July 2021. Most cases (88.5%) had high-grade histology, and 54.1% had microscopic lymphatic-vascular invasion. Nodal metastasis was reported in 17 cases (27.9%), and distant metastasis in 9 (14.8%) (Table 1).

PD1 immunoreactivity was negative in all samples. *PD-L1* stained positive in tumor cells in 21 cases (34.4%) and in surrounding immune cells in 48 cases (78.7%). The average *PD-L1* staining area was 9.8% in the tumor area and 9.5% in the surrounding immune cell area. The immunoreactivity pattern of *PD-L1* in urothelial tumors was typically membranous, with discrete positivity in surrounding tumor-associated immune cells (Figure 1). In

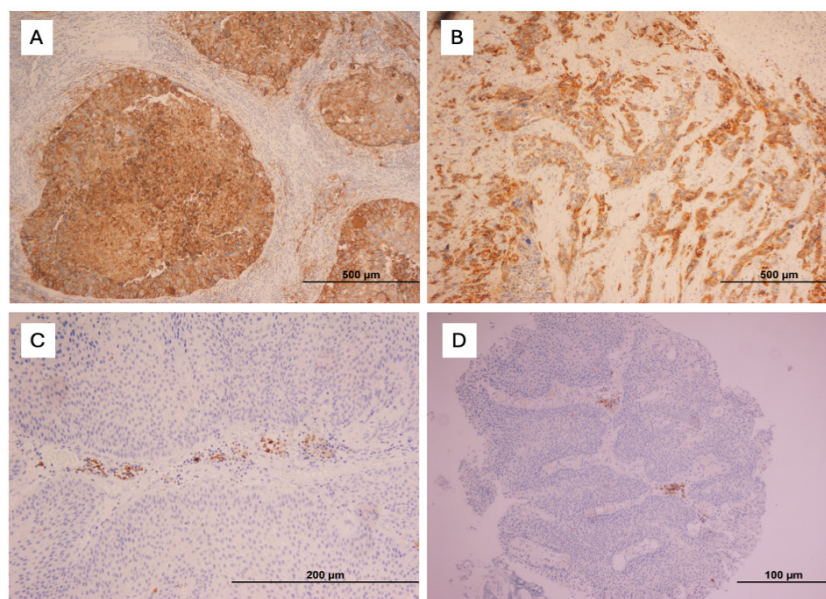


Figure 1. Immunoreactivity Patterns of *PD-L1* in Muscle-Invasive Bladder Cancer A) A hundred percent tumor cell positivity B) A fifty-percent positive tumor cell positivity C-D) No positivity in tumor cells when the tumor-associated inflammatory cells show positive staining. Scales are in the figure.

Table 1. Comparing Clinical and Pathological Features between Cases with High and Low Expression of *PDL1*

Parameter	N (%)	PDL1 Low	PDL1 High	p-value
All	61 (100.00%)	50 (81.97%)	11 (18.03%)	-
Sex				
Male	55 (90.16%)	45 (81.82%)	10 (18.18%)	0.927
Female	6 (9.84)	5 (83.3%)	1 (16.67%)	
Age group				
< 65 years	27 (44.26%)	20 (74.07%)	7 (25.93%)	0.153
> 65 years	34 (55.74%)	30 (88.24%)	4 (11.76%)	
Grade				
Low	7 (11.48%)	7 (100.0%)	0 (0.0%)	0.187
High	54 (88.52%)	43 (79.63%)	11 (20.37%)	
T-stage				
T1-2	28 (45.90%)	23 (82.14%)	5 (17.86%)	0.974
T3-4	33 (54.10%)	27 (81.82%)	6 (18.18%)	
N-stage				
N0	44 (72.13%)	39 (88.64%)	5 (11.36%)	0.029
N1-2	17 (27.87%)	11 (64.71%)	6 (35.29%)	
M-stage				
M0	52 (85.25%)	45 (86.54%)	7 (13.46%)	0.026
M1	9 (14.75%)	5 (55.56%)	4 (44.44%)	
LVI				
No	28 (45.90%)	26 (92.86%)	2 (7.14%)	0.042
Yes	33 (54.10%)	24 (72.73%)	9 (27.27%)	
<i>GATA-3</i> intensity				
Low/Moderate	13 (21.31%)	4 (30.77%)	9 (69.23%)	0.000
High	48 (78.69%)	46 (95.83%)	2 (4.17%)	
<i>GATA-3</i> area				
< 10%	6 (9.84)	3 (50.00%)	3 (50.00%)	0.032
> 10%	55 (90.16%)	47 (85.45%)	8 (14.55%)	
<i>CK56</i> intensity				
Low/Moderate	17 (27.87%)	15 (88.24%)	2 (11.76%)	0.429
High	44 (72.13%)	35 (79.55%)	9 (20.45%)	
<i>CK56</i> area				
< 10%	26 (42.62%)	24 (92.31%)	2 (7.69%)	0.07
> 10%	35 (57.38%)	29 (74.29%)	9 (25.71%)	
<i>CK14</i> intensity				
Low/Moderate	44 (72.13%)	38 (86.36%)	6 (13.64%)	0.151
High	17 (27.87%)	12 (70.59%)	5 (29.41%)	
<i>CK14</i> area				
< 10%	35 (57.38%)	31 (88.57%)	4 (11.43%)	0.120
> 10%	26 (42.62%)	19 (73.08%)	7 (26.92%)	

LVI, Lymphatic-vascular infiltration

correlation matrix analysis among the percent expression areas of all markers, the strongest positive correlation was between *CK14* and *CK5/6* (R 0.60), while the strongest negative correlation was between *GATA3* and *CK14* (R -0.19). When paired with the others, *PD-L1* percent expression on tumor cells had the highest correlation with *CK5/6* percent expression and *PD-L1* (R 0.43), and *PD-L1* percent expression on tumor-associated immune

cells had the highest correlation with *CK14* (R -0.008). Fisher's exact test demonstrated that high *PD-L1* status was significantly associated with non-high staining intensity but with a higher positive staining area in *GATA3* (Figure 2).

When Allred scores for *GATA3*, *CK5/6*, and *CK14* were calculated and compared between the *PD-L1* High and *PD-L1* Low groups, the scores for the 3 markers

Table 2. Comparison of Allred Scores of GATA3, CK5/6, and CK14 between the 2 Statuses of PD-L1 Expression

	All (n= 61)	PD-L1 Low (n=50)	PD-L1 High (n=11)	p-value
GATA-3	8 (8,8)	8(8,8)	7(4,7)	0.000
CK56	6(4,8)	6(3,7)	8(6,8)	0.013
CK14	0(0,5)	0(0,5)	5(3,7)	0.020

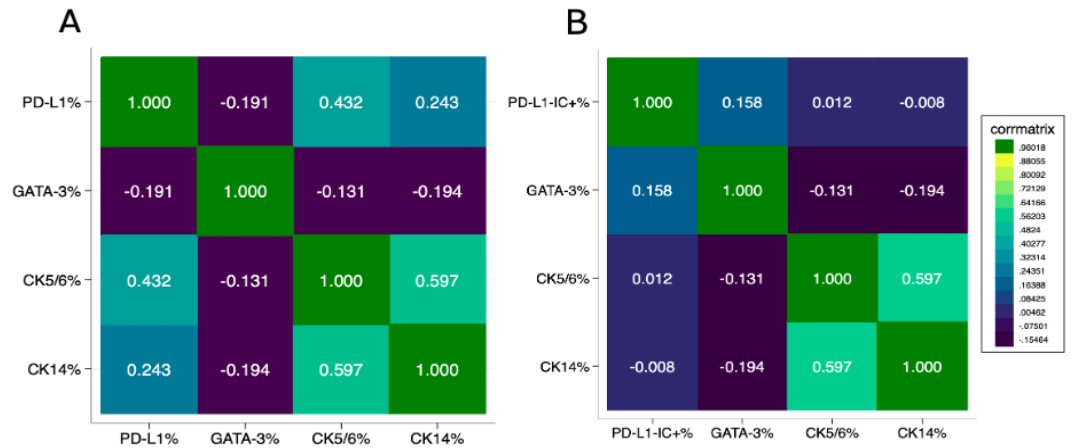


Figure 2. Heat Maps of Correlation Coefficients among A) percentage of tumor cells with positive PD-L1 staining (PD-L1%) and percentage of the other markers; B) percentage of tumor-associated inflammatory cells with positive PD-L1 staining (PD-L1-IC+%) and percentage of the other markers.

significantly differed by PD-L1 status (Table 2 and Figure 3). The mean follow-up period was 65.3 months (31.8-86.7 months). Kaplan-Meier survival analysis

showed that 1-year and 5-year overall survival (OS) were 58.5% and 32.0%, respectively (Figure 4). On univariate analysis, higher T, N, and M stages were

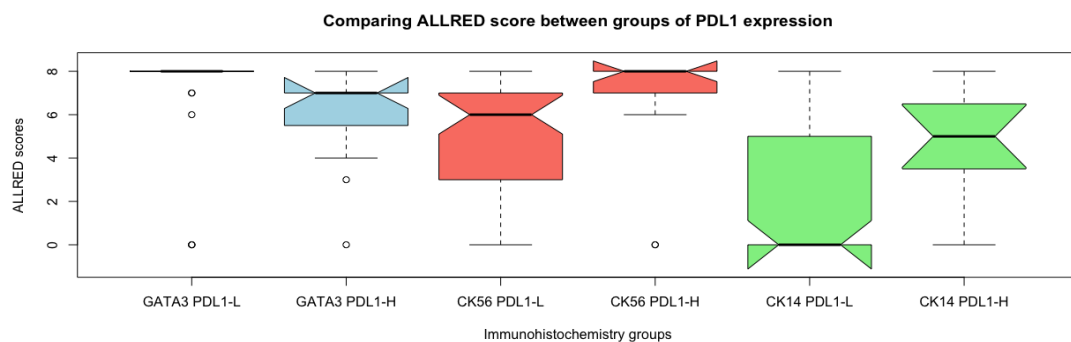


Figure 3. Boxplots with interquartile notches comparing Allred scores of GATA-3, CK5/6, and CK14 between the 2 statuses of PD-L1 expression

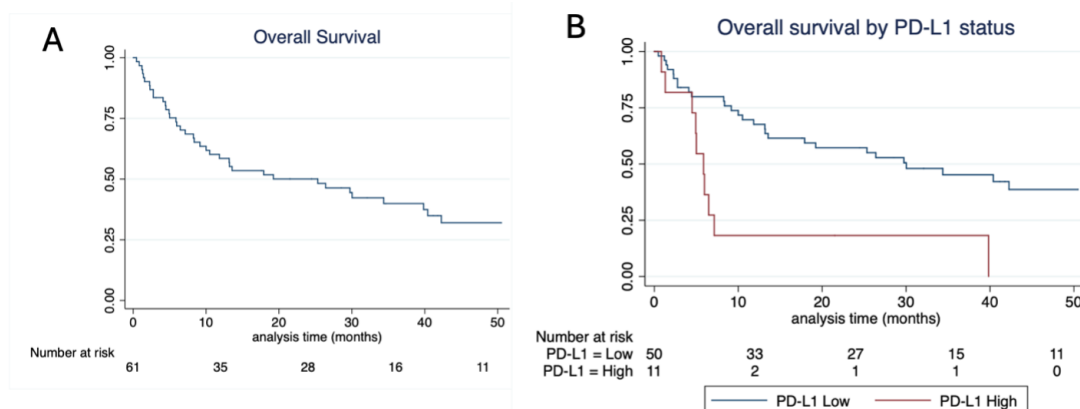


Figure 4. Kaplan Meier Curves Showing Overall Survival and Survival Comparisons between PD-L1 Low Expression and PD-L1 High Expression

Table 3. Comparing Survival Functions on Clinical and Immunohistochemistry Profiles

Parameter	N (%)	2-year survival*	Cox's Hazard Ratio	p-value**
All	61 (100.00%)	50.06% (36.87%-61.88%)	-	-
Sex				
Male	55 (90.16%)	53.75% (39.63%-65.94%)	ref	0.059
Female	6 (9.84)	16.67% (0.77%-51.68%)	2.43 (0.94-6.29)	
Age group				
< 65 years	27 (44.26%)	62.96% (42.12%-78.01%)	ref	0.225
> 65 years	34 (55.74%)	39.65% (23.26%-55.62%)	1.48 (0.77-2.84)	
Grade				
Low	7 (11.48%)	68.57% (21.28%-91.21%)	ref	0.071
High	54 (88.52%)	47.35% (33.52%-59.96%)	3.44 (0.82-14.36)	
T-stage				
T1-2	28 (45.90%)	67.67% (47.03%-81.69%)	ref	0.000
T3-4	33 (54.10%)	34.67% (19.00%-50.88%)	3.45 (1.67-7.11)	
N-stage				
N0	44 (72.13%)	62.81% (46.61%-75.33%)	ref	0.004
N1-2	17 (27.87%)	17.65% (4.35%-38.30%)	3.83 (1.96-7.53)	
M-stage				
M0	52 (85.25%)	56.94% (42.27%-69.18%)	ref	0.002
M1	9 (14.75%)	11.11% (0.61%-38.77%)	3.18 (1.49-6.82)	
LVI				
No	28 (45.90%)	63.91% (43.27%-78.72%)	ref	0.014
Yes	33 (54.10%)	37.94% (21.62%-54.17%)	2.21 (1.15-4.24)	
<i>PDL1</i>				
Low	50 (81.97%)	57.22% (42.24%-69.66%)	ref	0.001
High	11 (18.03%)	18.18% (2.85%-44.17%)	3.16 (1.59-7.24)	
<i>GATA-3</i> intensity				
Low/Moderate	13 (21.31%)	23.08% (5.58%-47.46%)	ref	0.001
VHigh	48 (78.69%)	57.50% (42.17%-70.15%)	0.32 (0.16-0.66)	
<i>GATA-3</i> area				
< 10%	6 (9.84)	33.33% (4.61%-67.56%)	ref	0.073
> 10%	55 (90.16%)	51.91% (37.89%-64.22%)	0.45 (0.19-1.10)	
<i>CK56</i> intensity				
Low/Moderate	17 (27.87%)	41.18% (18.58%-62.64%)	ref	0.766
High	44 (72.13%)	53.53% (37.64%-67.05%)	0.90 (0.45-1.78)	
<i>CK56</i> area				
< 10%	26 (42.62%)	57.69% (36.76%-73.91%)	ref	0.127
> 10%	35 (57.38%)	44.02% (27.07%-59.73%)	1.65 (0.86-3.17)	
<i>CK14</i> intensity				
Low/Moderate	44 (72.13%)	56.76% (39.43%-70.84%)	ref	0.033
High	17 (27.87%)	39.58% (20.21%-58.44%)	1.98 (1.05-3.73)	
<i>CK14</i> area				
< 10%	35 (57.38%)	52.08% (36.46%-65.59%)	ref	0.051
> 10%	26 (42.62%)	44.34% (0.48%-28.49%)	1.90 (0.99-3.68)	

LVI, Lymphatic-vascular infiltration; *estimated time-specified survival function with 95% confidence interval; **p-value of Log-rank test

significantly associated with poorer survival. Regarding histopathological profiles, lymphatic-vascular infiltration, high *PD-L1* expression, low *GATA3* intensity, and high *CK14* intensity were associated with poorer outcomes (Table 3 and Figure 4). Multivariable Cox proportional

hazards analysis showed that only metastatic status was independently associated with survival (adjusted OR 2.80, 95% CI 1.15-6.77).

Discussion

Our study explored the role of *PD-1* and *PD-L1* as disease markers in MIBC, an aggressive malignancy in the elderly. The study found that only *PD-L1* was expressed in tumor tissue in 34% of cases and in surrounding immune cells in 79% of cases. *PD-L1* was expressed at a high level in 18% of cases, and this high expression was correlated with higher tumor stages and poorer survival outcomes. Although the prevalence of high *PD-L1* expression in our study was lower than that reported in other international studies, primarily from Western populations, which reported a range of 48%-54% [15–17], it was comparable to the study from Korea using the same antibody and reading criteria (SP263), which reported an incidence of 23.5% [10]. This variation suggests potential ethnic differences in immune profiles or discrepancies in antibody sensitivity and scoring algorithms used across global studies. In addition, the area of positivity in tumor tissue was 9.8% in our study, comparable to the aforementioned Korean study. Our study also highlighted a correlation between high *PD-L1* expression and the expression of known immunohistochemistry markers in MIBC, *GATA3*, *CK5/6*, and *CK14* [12]. Moreover, high *PD-L1* was associated with lymphovascular invasion, an aggressive histopathological feature.

GATA3, a crucial transcription factor in T-cell development and immune regulation, has garnered significant attention in MIBC [18]. Previous studies, including one from our group, have shown that reduced *GATA3* expression is associated with poor prognosis, more aggressive tumor behavior, and increased risk of recurrence in MIBC patients [12, 19]. In this study, we observed an inverse correlation between *GATA3* and *PD-L1* expression. When *PD-L1* was low, the *GATA3* Allred score was invariably high. Conversely, in the high *PD-L1* group, *GATA3* expression was lower and more variable. This inverse relationship aligns with the molecular classification of bladder cancer, in which basal-type tumors (characterized by low *GATA3* and high *CK5/6* or *CK14*) exhibit distinct immune landscapes compared to luminal types. Mechanistically, basal-type tumors are known to be more immune-infiltrated (“hot tumors”) and often constitutively express *PD-L1*, potentially driven by activation of the *EGFR* pathway or *STAT3* signaling, both of which are upregulated in basal subtypes. This suggests that loss of luminal identity (*GATA3* loss) may create a permissive environment for immune evasion via the *PD-1/PD-L1* axis.

A recent pan-cancer expression analysis of The Cancer Genome Atlas (TCGA) cohort reported that high *GATA3* expression was preferentially observed in the luminal subtype and was mutually exclusive with other immune checkpoint regulators, including *PD-1*, *PD-L1*, and *CTLA-4* [20]. Both *GATA3* and *PD-L1* were significantly associated with survival outcomes in MIBC, though they were not independent of each other. These findings suggest a potential functional interaction between the two proteins. In certain cancers, loss of *GATA3* has been shown to promote immune evasion by directly upregulating *PD-L1*, a key immune checkpoint

molecule. This suggests that *GATA3* may act as a tumor suppressor, in part, by negatively regulating *PD-L1* expression and preventing cancer cells from escaping immune surveillance. Restoring *GATA3* function may therefore represent a promising therapeutic strategy to enhance anti-tumor immunity in cancers with low *GATA3* expression. A study in triple-negative breast cancer cell lines suggested that downregulation of *GATA3* was associated with aberrant expression of *GATA* binding protein 3 antisense RNA 1 (*GATA3-AS1*), a long non-coding RNA, and that this molecule might also function in the stabilization of *PD-L1* [21].

With the standardization of interpretation criteria, *PD-L1* immunoreactivity has emerged as a potential prognostic marker in various cancers, including urothelial carcinoma [8, 22]. In addition to tissue expression, a study demonstrated that urine *PD-L1* correlated well with tissue expression and was associated with poorer clinical outcomes in bladder cancers [22, 23]. While its role as a predictive biomarker for response to immune checkpoint inhibitors is established, its prognostic value remains controversial. Our results were consistent with previous studies showing that high *PD-L1* expression in tumor cells or the tumor microenvironment may be associated with poor prognosis in urothelial carcinoma, indicating aggressive tumor behavior and potential for immune evasion [24]. In addition to evidence that high *PD-L1* expression was found at a significantly higher frequency in metastatic MIBC and in those with poorer survival, the inverse correlation between *PD-L1* and *GATA3*, a known prognostic marker in urothelial cancer, further supported the prognostic role of *PD-L1* in MIBC patients who underwent radical cystectomy and did not receive immunotherapy. However, other studies have reported no significant correlation between *PD-L1* expression and clinical outcomes [9, 16, 25]. The complex interplay between *PD-L1* expression, tumor-infiltrating lymphocytes, and other immune markers in the tumor microenvironment likely influences the prognostic impact of *PD-L1*.

Several limitations of our study must be acknowledged. First, this retrospective study was conducted at a single institution, which may introduce selection bias. Second, the sample size was relatively small, limiting the statistical power to detect weaker associations. Third, only one pathologist read the slides. Finally, the heterogeneity of *PD-L1* expression within the tumor tissue itself can pose challenges for accurate scoring, although we used standardized SP263 criteria to mitigate this issue. Future multicenter prospective studies with larger cohorts are needed to validate these findings.

In conclusion, *PD-L1* expression correlates inversely with *GATA3* and directly with basal markers (*CK5/6*, *CK14*), identifying a more aggressive subtype with poorer prognosis. Moreover, the study showed the prognostic impact of *PD-L1* in MIBC patients who underwent radical cystectomy and received standard chemotherapy. Therapeutically, these findings suggest that patients with the “double-hit” phenotype of low *GATA3* and high *PD-L1* may benefit most from immune checkpoint blockade, and that routine assessment of both markers could refine

patient selection for immunotherapy in the adjuvant or metastatic setting.

Author Contribution Statement

Conceptualization: SS, JS; Methodology: JS, SS, KK; Formal analysis and investigation: SS, KK, TB, PC, NK, NP, JS; Writing-original draft preparation: SS, NK, PC; Writing-review and editing: SS; Resources: TB, KK, NP, PC; Supervision: SS, KK. All authors agreed with this submission.

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Data availability

Data is available on request from the authors.

Conflict of interest

None of the authors has any potential conflicts to disclose.

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