Impact of Programmed Death-ligand 1 Expression on Oncological Outcomes in Patients with Muscle-invasive Bladder Cancer Treated with Radiation-based Therapy

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Abstract

Background: No biomarkers are recommended for patients undergoing radiation-based therapy (RT) for muscle-invasive bladder cancer (MIBC).

Objective: We aim to evaluate the predictive role of programmed death-ligand 1 (PD-L1) expression on the oncological outcomes of patients treated with RT for MIBC.

Design, setting, and participants: A single-center retrospective analysis of tumor specimens collected through transurethral resection (TURBT) from 104 MIBC patients, implemented in a tissue microarray and stained with the SP263 PD-L1 clone (Ventana Medical Systems, Tucson, AZ, USA), was conducted. Two reviewers measured the PD-L1 H-score for tumor and immune cells.

Intervention: RT (maximal TURBT followed by radiation and concurrent chemotherapy when eligible).

Outcome measurements and statistical analysis: Logistic and Cox regression models were used to predict 3-mo complete response (CR) and overall survival (OS) after RT, respectively.

Results and limitations: A total of 88 (85%) patients had cT2 disease and 39 (37.5%) had high immune cell PD-L1 expression. A CR was achieved in 68 (65%) patients. On the multivariable analysis (MVA), a higher clinical stage \( (p = 0.02) \) and a low immune cell PD-L1 H-score \( (p = 0.02) \) were associated with a decreased CR after RT. The median time to death was 43 mo (95% confidence interval 20–66). On Cox MVA, a high immune cell PD-L1 H-score \( (p = 0.0017) \) was associated with better OS, independently of performance status \( (p = 0.0005) \) or tumor stage.
1. Introduction

Muscle-invasive bladder cancer (MIBC) constitutes about 25% of all cases of bladder cancer and carries an overall poor prognosis [1–3]. One of the standards of care for MIBC is radical cystectomy (RC) with cisplatin-based neoadjuvant chemotherapy [1–3]. RC is associated with a 5-yr cancer-specific survival rate of approximately 65% [4,5]. Radiation-based therapy (RT) has emerged in the past decades as an attractive alternative to RC and is being increasingly used as it preserves urinary function and maintains good quality of life. Furthermore, it fulfills a large unmet need where many frail and elderly patients are not candidates for RC. The most commonly adopted therapeutic approach for bladder preservation is trimodal therapy (TMT), consisting of a maximal transurethral resection of bladder tumor (TURBT) followed by RT and concomitant chemotherapy [6,7]. This approach yields 5-yr cancer-specific survival rates ranging from 50% to 82% [6] and is now supported as first-line curative therapy in appropriately selected patients by international guidelines [1,8].

The use of new-generation immunotherapy agents targeting anti–programmed death 1 (PD-1) or its ligand (PD-L1) has impacted the oncological outcomes of patients with metastatic MIBC [9]. PD-L1 is an immune checkpoint in the B7/CD28 pathway that regulates T cell migration, proliferation, and function by binding to its receptors PD-1 (CD80) [10]. While PD-1 is expressed on T-cell surface, PD-L1 is mainly expressed on tumor and immune cells (T and B cells, macrophages, and neutrophils) as part of the tumor microenvironment (TME). A recent report involving bladder cancer patients treated with either TURBT or RC showed that the presence of PD-L1–positive immune cells was an independent prognostic factor for inferior overall survival (OS; \( p = 0.001 \)) and recurrence-free survival [11]. However, little is known for patients undergoing RT and whether the interplay between radiation and the immune TME influences response to therapy. We hypothesize that the impact of TME on therapy outcomes may be different if patients benefit from RT.

Hence, the goal of our study was to evaluate the predictive impact of PD-L1 expression on oncological outcomes following RT in patients with MIBC.

(p = 0.0013). A high tumor cell PD-L1 H-score was not an independent predictor of CR or OS. Limitations of the study include the retrospective design.

Conclusions: MIBC patients with high PD-L1 expression on immune cells appear to have better oncological outcomes following RT. Our results may aid in patient stratification for future clinical trial design.

Patient summary: In this report, we evaluated the role of programmed death-ligand 1 (PD-L1) expressed on tumor and immune cells in the tumor microenvironment for patients treated with a bladder-sparing regimen. We found that PD-L1 overexpression on immune cells is able to predict a better response to radiation-based therapy.

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The H-score was calculated using the following formula [12]:
\[
H = \left( \% \text{ of cells stained at intensity category } 1 \times 1 \right) + \left( \% \text{ of cells stained at intensity category } 2 \times 2 \right) + \left( \% \text{ of cells stained at intensity category } 3 \times 3 \right)
\]

Two H-scores were generated for each tumor-containing core for both tumor and immune cells. Since multiple cores per patient were available, the average H-score for tumor and immune cells was taken into account for statistical analysis.

2.4. Outcomes and statistical analysis

A complete response (CR) was defined by a negative tumor bed biopsy (or the combination of negative urine cytology and normal cystoscopy for patients who did not undergo a control biopsy) within 3 mo after RT, with no signs of locoregional disease or distant metastasis on cross-sectional imaging. A 3-mo cutoff for CR was deemed appropriate to differentiate persistent disease from early relapse [13]. OS was measured from the date of first treatment to the date of death due to any cause. The date of death was systematically retrieved based on data from the patient’s hospital file or from information from Quebec’s Department of Civil Status (the Directeur de l’état civil - Gouvernement du Québec, 2019) prior to statistical analysis.

A descriptive analysis of the cohort was reported. We used median values and interquartile ranges (IQRs) for quantitative variables, and absolute numbers with the respective percentages for qualitative variables.

To identify the predictors of CR to RT, we tested pretreatment variables in a univariable logistic regression model. Variables with \( p < 0.2 \) were included in a stepwise multivariable logistic regression model. The Hosmer-Lemeshow test for goodness of fit was also used to ensure the model’s calibration. To further identify the ideal H-score cutoffs for IHC variable significantly associated with a CR (\( p < 0.05 \)), we used the Youden index based on the receiver operating characteristic curve [14].

Follow-up time was defined by the period between the last day of RT and the last follow-up visit or the date of death. For survival analysis, patients were censored at the last follow-up date and uncensored at the day of death from any cause. Estimates of OS were tested with log-rank and represented by Kaplan-Meier curves. Variables included in Tables 1 and 2 were evaluated with a univariate analysis. A stepwise Cox multivariable regression model including variables with \( p < 0.2 \) in a univariable analysis was generated. Log linearity hypotheses were verified for quantitative variables, and proportional hazard assumptions were verified for all variables. Statistical significance was set at \( p < 0.05 \). Statistical analysis and plots were performed using SAS software (version 9.2; SAS Institute, Cary, NC, USA).

### Table 1 – Patients’ characteristics (N = 104 MIBC patients)

| Characteristic | Yes | No | Total |
|----------------|-----|----|-------|
| **Gender**     |     |    |       |
| Male           | 78  | 26 | 104   |
| Female         | 26  | 78 | 104   |
| **ECOG**       |     |    |       |
| 0              | 47  | 57 | 104   |
| 1              | 33  | 71 | 104   |
| 2              | 19  | 85 | 104   |
| **Tumor stage**|     |    |       |
| T2             | 88  | 16 | 104   |
| T3             | 15  | 89 | 104   |
| **Nodal stage**|     |    |       |
| N0             | 92  | 12 | 104   |
| N1             | 2   | 102| 104   |
| N2             | 9   | 95 | 104   |
| **Concomitant CIS** |   |    |       |
| Absent         | 67  | 37 | 104   |
| Present        | 37  | 67 | 104   |
| **Histology**  |     |    |       |
| NAC            | 82  | 22 | 104   |
| Present        | 40  | 64 | 104   |
| **Median RT dose (Gy)** |   |    |       |
| No             | 26  | 78 | 104   |
| Present        | 78  | 26 | 104   |
| **BCC**        |     |    |       |
| Bacillus Calmette-Guérin | Yes | 20 | 104   |
| No             | 84  | 20 | 104   |
| **BCC**        |     |    |       |
| No             | 13  | 91 | 104   |
| Present        | 91  | 13 | 104   |
| **Median RT fractions** |   |    |       |
| No             | 50  | 54 | 104   |
| Present        | 54  | 50 | 104   |

### 3. Results

**3.1. Patient characteristics and PD-L1 expression**

Our study included 104 patients with a median age at diagnosis of 75 yr (IQR 66–80). Patients’ demographics, and clinical and pathologic data are shown in Table 1. Most patients had good performance status and clinical T2N0 disease. The median percentage of PD-L1–stained cells was 10.5% (1–59%) for tumor cells and 8.5% (4–16%) for immune cells (Fig. 1). The summary of H-scoring is shown in Table 2.

**3.2. Predictors of CR after treatment**

A total of 68 (65%) patients achieved a CR after RT. On multivariable logistic regression, patients harboring clinical non–organ-confined disease (clinical stage T3; odds ratio [OR] = 0.22, 95% confidence interval [CI], 0.06; 0.78, \( p = 0.02 \)) had fewer CRs after RT. The H-score for immune cells (OR = 1.07, 95% CI, 1.01; 1.11, \( p = 0.02 \)) was an independent predictor of a CR after RT. However, a tumor cell H-score was not predictive of a CR after RT (univariable OR = 1.00, 95% CI, 0.99; 1.01, \( p = 0.80 \)).

By using the Youden index, we found that the ideal immune cell H-score cutoff was 15.7. By using this cutoff, we dichotomized our cohort to have low (<15.7) and high (≥15.7) immune cell PD-L1 expression. A total of 39 (37.5%) patients were classified to have high immune cell PD-L1 expression. High PD-L1 expression among immune cells was associated with an increased chance of a CR after RT (OR = 5.08, 95% CI, 1.58; 16.34, \( p = 0.006 \)), independently of clinical tumor stage (\( p = 0.01 \); Table 3). When stratifying patients based on PD-L1 immune cell status, baseline
characteristics were similar between the two groups (including tumor and nodal stage, concomitant carcinoma in situ, hydrenephrosis, type of RT treatment, and performance status) except for lymphovascular invasion (Supplementary Table 1).

### 3.3. Predictors of OS

At the time of this review, 57 patients (54.8%) had died. The median follow-up time for the whole cohort was 58 mo (95% CI, 41; 74). The median time to death from any cause was 43 mo (95% CI, 20; 66).

On multivariable analysis, independent predictors of worse OS were Eastern Cooperative Oncology Group (ECOG) performance status ≥2 (hazard ratio [HR] = 2.78, 95% CI, 1.57; 4.94, p = 0.0005) and clinical stage T3 (HR = 3.19, 95% CI, 1.57; 6.48, p = 0.0013). High PD-L1 expression among immune cells (HR = 0.36, 95% CI, 0.19; 0.68, p = 0.0017) was the sole independent predictor for improved OS (Table 3 and Fig. 2A). A tumor cell H-score was not predictive of OS on univariable Cox regression (p = 0.53) and therefore not implemented into our final model.

Moreover, as an exploratory analysis, we investigated the role of tumor cell PD-L1 expression among patients harboring high PD-L1 expression on immune cells. For this aim, the median tumor cell H-score was used as the cutoff to stratify patients between those with high and low PD-L1 expression on tumor cells. Interestingly, none of the patients having both high immune cell and low tumor cell PD-L1 expression died during follow-up (Fig. 2B).

### 4. Discussion

Our study shows that patients with higher immune cell PD-L1 expression have a superior probability of CR in addition to longer OS following RT for MIBC, independently of other traditional clinicopathological factors, such as tumor stage and performance status. We also found that higher PD-L1 expression on tumor cells did not correlate with either treatment response or OS.

PD-L1 can be expressed on both tumor and immune cells. On tumor cells, PD-L1 inhibits the antitumor responses of PD-L1–expressing CD8+ T cells [15]. Instead, PD-L1 expressed by immune cells plays a role in the escape of tumor cells from immune detection [16]. Moreover, preclinical data suggested that while tumor cell PD-L1 expression is momentary, immune cell expression is prolonged, so

| Table 2 – PD-L1 staining results for immune and tumor cells (per patient analysis) |
|-------------------------|-----------|---------------------|
|                         | N or median | Percent or IQR       |
| **Tumor cells**         |            |                     |
| Highest staining intensity |        |                     |
| 0                       | 17         | 16.6%               |
| 1                       | 20         | 19.2%               |
| 2                       | 28         | 26.9%               |
| 3                       | 33         | 31.7%               |
| Median percentage of PD-L1–positive cells | 10.5% | 1–59               |
| Median H-score | 16.6 | 2.5–65             |
| **Immune cells**        |            |                     |
| Highest staining intensity |        |                     |
| 0                       | 5          | 4.8%                |
| 1                       | 8          | 7.7%                |
| 2                       | 41         | 39.4%               |
| 3                       | 42         | 40.4%               |
| Median percentage of PD-L1–positive cells | 8.5% | 4–16               |
| Median H-score | 10         | 5–23                |

IQR = interquartile range; n = number of patients; PD-L1 = programmed death-ligand 1.

* A PD-L1–positive cell was defined as a cell that had a staining intensity of at least ≥1. The ideal immune cells H-score cutoff was 15.7 according to the Youden index methodology.

![Fig. 1 – PD-L1 expression by tumor and immune cells. (A) Examples of patients with absence of PD-L1 staining in tumor (T) or immune cells (I). (B) Diffuse moderate-to-strong membranous staining and focal weak-to-moderate cytoplasmic staining of neoplastic urothelial cells and immune cells (PD-L1 SP263 clone, 100×). PD-L1 = programmed death-ligand 1.](image-url)
|                | Complete response | Overall survival |
|----------------|-------------------|------------------|
| **ECOG**       |                   |                  |
| 0–1 Ref        |                   |                  |
| 2–3 – – – – – – Ref | 2.782 1.568 – 4.935 0.0005 |
| **Tumor stage**|                   |                  |
| T2 Ref         |                   |                  |
| T3 0.194 0.053 – 0.717 0.0139 3.191 1.572 – 6.478 0.0013 |
| **PD-L1 expression on immune cells** |                   |                  |
| Low Ref        |                   |                  |
| High 5.079 1.579 – 16.337 0.0064 0.355 0.186 – 0.678 0.0017 |

CI = confidence interval; CR = complete response; ECOG = Eastern Cooperative Oncology Group; HR = hazard ratio; OR = odds ratio; OS = overall survival; PD-L1 = programmed death-ligand 1; Ref = reference.

Fig. 2 – Kaplan-Meier survival curves stratified by immune cell PD-L1 staining (A) for the whole cohort and (B) among the patients harboring high immune cell PD-L1 expression. CI = confidence interval; NR = not reached; PD-L1 = programmed death-ligand 1; RT = radiotherapy.
that the majority of PD-L1 in the immunosuppressive TME may be provided by immune cells [16].

Several studies have investigated the prognostic role of PD-L1 expression on both tumor and immune cells for urothelial carcinoma, using different antibodies and cutoffs to define PD-L1 positivity, in various phases of the disease [17–27]. While most of them used tumor cell PD-L1 only, the majority has reported a worse prognosis for patients harboring high tumor cell PD-L1 expression. For studies using immune cell PD-L1 staining also, higher immune cell PD-L1 expression was linked to a better prognosis, potentially as a reflection of an active antitumor immune response [18,27–29]. In our study, although we did not show a negative impact on oncological outcomes for a patient with high tumor cell PD-L1 expression (in the whole cohort), we confirmed that high PD-L1 expression on immune cells was associated with improved outcomes. In patients with high immune cell PD-L1 expression (using immune cell H-score >16, \( p = 0.006 \)), a post-treatment CR was five times more likely to be achieved and a 60% lower risk of death \( (p = 0.04) \) was seen, independently of tumor stage or performance status. To our knowledge, this is the first study reporting and differentiating the impact of tumor versus immune cell PD-L1 expression in a cohort of MIBC patients treated with RT, confirming what was observed for patients undergoing RC [30] or harboring metastatic disease [18].

The only report assessing the role of PD-L1 expression on MIBC patients treated with RT was from Wu et al [31]. In their retrospective study of 72 MIBC patients with a median follow-up of 3.8 yr and treated with chemoradiation, positive PD-L1 expression was significantly linked with lower CR rates, higher locoregional failure rates, reduced disease-free survival, and lower bladder intact survival. However, PD-L1 positivity did not reach statistical significance as a predictor of OS in their multivariable survival analysis. It is important to note that their TMA was different from ours. They build their TMA using one core of bladder cancer and one core of the adjacent nonmalignant epithelium, whereas our TMA contains two cores in the tumor center, two cores of the transition area (tumor edges), and one benign core per patient. In addition, the PD-L1 clone used by Wu et al [31] was not reported, and they failed to mention which cells were stained for PD-L1 (tumor vs immune cells), making it impossible to determine whether immune cell staining was included in their analysis. Lastly, the majority of their PD-L1-positive patients had T3-T4 disease and 40% of them had lymph node involvement. As a result, comparing our outcomes with Wu’s study [31] study is challenging considering that the staining methodology used by them and the population studied are different.

We also analyzed our staining using previously published methods to stratify patients into those with low and high PD-L1 expression. The JAVELIN criterion identified 43% PD-L1-positive patients, while according to the IMVIGOR criterion, 14%, 18%, and 68% of our cohort were ICO, IC1, and IC2, respectively. Both methods do not differentiate between immune and tumor cell PD-L1 staining on the final results, whereas our study showed that tumor cell PD-L1 staining was not predictive of outcomes for MIBC patients undergoing RT. The JAVELIN and IMVIGOR criteria were not independent predictors of CR after RT on multivariable logistic regression (data not shown). Moreover, using the same OS multivariable Cox regression model (including ECOG status and tumor stage), we found that the JAVELIN criteria were associated with improved OS \( (HR = 0.57, 95\% CI, 0.32; 0.99, p = 0.048) \), while the IMVIGOR criteria were not \( (HR = 0.62, 95\% CI, 0.27; 1.44, p = 0.26 \) for IC1 vs IC0; \( HR = 0.56, 95\% CI, 0.29; 1.10, p = 0.09 \) for IC2 vs IC0). These results highlight the need for in-depth analyses when assessing the role of PD-L1 staining in the TME.

Even if no biomarkers are currently recommended in clinical practice for patients undergoing RT, several teams have identified other potential promising IHC targets. Proteins involved in DNA repair such as MRE11 (DNA nuclease) have been reported to significantly predict worse cancerspecific survival following RT for MIBC [32]; this was also reported for patients undergoing chemoradiation therapy [33]. However, a recent study did not find the predictive impact of MRE11 reproducible [34]. Other proteins involved in DNA repair and identified by IHC such as ERCC1 (nucleotide excision repair pathway) and XRCC1 (protein involved in repair of single-strand DNA breaks) were associated with improved disease-specific survival on multivariate analysis in a TMT cohort [35]. Finally, alterations in signal transduction pathways investigated by IHC for EGFR [36], HER2 [37], or VEGF-B [38] have also been reported to have a prognostic role for patients undergoing RT for MIBC.

Our study has several limitations, including its retrospective design and all the inherent issues related to this type of review. Although our TMA had a robust methodology (five cores per patient), we did not analyze whole section slides. Moreover, only one PD-L1 clone was used. However, despite the lack of assay standardization among all clones used, it has been shown that PD-L1 expression can be scored reproducibly [39,40]. Our methodology used the H-score to quantify PD-L1 expression. Potential variability among readers needs to be acknowledged. Our cohort represented a relatively small number of patients (a relatively low number of events) limiting our statistical power. In addition, the type of RT varied among our cohort as some patients received bladder-only RT (40%), whereas others did not receive concurrent chemotherapy (12.5%), limiting the extrapolation of our results. Moreover, 19 patients in our cohort received bacillus Calmette-Guérin (BCG) instillation before MIBC diagnosis, which could have impacted immune cells analysis. Lastly, we did not provide an in-depth analysis of the TME; several key immune cells types such as tumor-infiltrating lymphocytes were not analyzed [41]. Further ongoing research will address this limitation.

5. Conclusions

Our study has shown that PD-L1 expression on immune cells of MIBC specimens may aid in predicting a response to RT in patients undergoing a bladder preservation approach. Our results should prospectively be validated and may assist in segmenting MIBC patients in specific risk stratification for a therapeutic response.
Author contributions: Wassim Kassouf had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Marcq, Mansure, Kassouf.

Acquisition of data: Marcq, Evaristo, Kool, Shinde-Jadhav, Skowronski.

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Appendix A. Supplementary data

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