Prognostic value of PD-L1 expression for surgically treated localized renal cell carcinoma: implications for risk stratification and adjuvant therapies

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Abstract

Background: We aimed to evaluate the prognostic role of programmed-death receptor ligand (PD-L1) in a multinational cohort of patients with localized renal cell carcinoma (RCC).
Methods: Formalin-fixed paraffin-embedded blocks of 1017 patients from the Latin American Renal Cancer Group were analyzed. Tissue microarrays were immunostained for PD-L1 using a commercially available monoclonal antibody. Expression of PD-L1 in ⩾5% tumor cells was considered positive. PD-1 expression in immune cells was also assessed. All cases were reviewed twice based on antibody expression and compared with a positive control. Cox proportional hazard regression models were used to identify predictors of recurrence-free survival (RFS) and overall survival (OS).
Results: A total of 738 cases with complete follow up met criteria. Median age was 57 [interquartile range (IQR): 49–64] years, and median follow up was 34 (IQR: 15–62.9) months. Median tumor size was 5 cm (IQR: 3.0–7.5 cm). Approximately 8.2% and 7.6% of tumors were PD-L1 and programmed cell-death 1 (PD-1) positive, respectively. PD-L1 and PD-1 positivity were significantly associated with higher tumor stage (both $p < 0.001$), and presence of tumor necrosis and lymphovascular multivariable analyses; PD-L1 positivity was found as a predictor of worse RFS [hazard ratio (HR) = 2.08, $p = 0.05$] and OS (HR = 2.61, $p = 0.02$).
Conclusions: PD-L1 positivity was significantly associated with worse outcomes for patients with localized RCC at intermediate follow up. This marker may help stratify patients for stricter surveillance after surgical treatment and provide a basis for checkpoint-inhibitor therapy in the adjuvant setting.

Keywords: programmed-death ligand, renal cell carcinoma, stratification

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Introduction

Renal cell carcinoma (RCC) is a lethal urologic malignancy corresponding to 62,700 new cases and 14,240 deaths per year in the Unites States.[1] For years, agents targeting the vascular epithelial growth factor (VEGF) and the mammalian target of rapamycin have been the standard of care for patients with metastatic disease and clear-cell component.[2,3] Despite the improvement from the pretargeted-therapy era, prognosis continues to be poor, with a 2-year overall survival (OS) of approximately 47% after treatment with tyrosine kinase inhibitors (TKIs).[4] Current new combinations with checkpoint inhibitors are being tested and approved due to improved survival, thus highlighting the need for continued understanding of newer therapeutic agents with novel mechanisms of action.

Increased understanding of tumor cell signaling and interactions with immune-cell receptors have resulted in an increased understanding of immune-checkpoint inhibition.[5] The blockade of cytotoxic T-lymphocyte-associated antigen-4 and programmed cell-death-1 (PD-1) have shown promising results in several malignancies, including advanced RCC.[6–8] These emergent immunomodulatory approaches have been recently incorporated in the treatment armamentarium of RCC and have changed treatment paradigms. Despite these breakthroughs, the role of immune-checkpoint inhibition in the earlier stages of the disease has not yet been elucidated.

Current trials are evaluating the role of checkpoint inhibition as adjuvant therapy for high-risk RCC after nephrectomy, and are expected to alter treatment strategies for localized RCC. Herein, we aimed to assess the prognostic value of PD-1 and ligand (PD-L1) expression in a large, multicenter cohort of patients with localized RCC. We compared clinicopathologic characteristics and survival outcomes based on immunohistochemical (IHC) expression status for patients who underwent extirpative surgery for nonmetastatic clear-cell RCC (ccRCC).

Material and methods

Patient selection and sample collection
The cohort was derived from an international collaborative study group of large academic referral centers in Latin America and the United States.[9] The Institutional Review Boards approved the study, and all patients signed an informed consent form to participate. Based on tissue availability, tissue microarrays (TMAs) were prepared from archival formalin-fixed paraffin-embedded specimens from 1017 cases of nonmetastatic, surgically treated ccRCC.

TMA construction and immunohistochemical analysis
Hematoxylin and eosin slides of each archival specimen were evaluated, and a block representing the overall tumor was chosen for TMA preparation. Three cores of 1 mm diameter per case were selected. Sections were cut 4 μm thick and placed on positively charged slides for immunostaining. Staining was performed using the Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ, USA) using commercially available antibodies against PD-1 on tissue-infiltrating lymphocytes (clone NAT105, diluted 1:100; Abcam, Cambridge, UK) and PD-L1 on tumor cell membranes (clone E1L3N, diluted 1:200; Cell Signaling Technology, Beverly, MA, USA). These assays had been previously validated using Formalin-Fixed Paraffin-Embedded cell-line controls.[10]

All TMAs were reviewed twice, based on antibody expression and compared with a positive control. Scoring was performed by an experienced uropathologist who was blinded to clinical outcomes. PD-L1 expression was evaluated based on the intensity and proportion of tumoral cells showing membranous or cytoplasmic staining, and was scored as follows: 0, negative (no immunoreactivity); 1, weak (5% to less than 25% of cells); 2, moderate (between 25 and 60% of cells); and 3, strong (more than 60% of cells). The numbers of PD-1 cytoplasmic-positive lymphocytes were assessed semiquantitatively.[11]

Statistical analysis
Pathologic stage was assigned according to the 2010 7th American Joint Committee on Cancer (AJCC) staging manual.[12] Associations were assessed with χ^2 tests or Fisher's exact test to compare histopathologic features between clone-positive and -negative tumors. The Kaplan–Meier method was used to evaluate recurrence-free survival (RFS) and OS, with the log-rank test used for comparison. Based on sensitivity analyses, univariable Cox's proportional hazards regression was used to assess pertinent clinicopathologic variables. A backward-stepwise selection procedure was used for the multivariable model. The hazard proportionality was verified by computing the log minus log against time. Statistical analysis was performed using the SPSS 25.0 software package (SPSS, Chicago, IL, USA).

Results

Patient characteristics
The final study cohort consisted of 738 patients after exclusion criteria were applied (Supplementary Figure 1). Median age for the cohort was 57 [interquartile range (IQR): 49–64] years, and the median follow up was 34 (IQR: 15.0–62.9) months. The median tumor size was 5 cm (3.0–7.5 cm). The majority of tumors (68%) were of AJCC pT1 type. Approximately 52% of patients
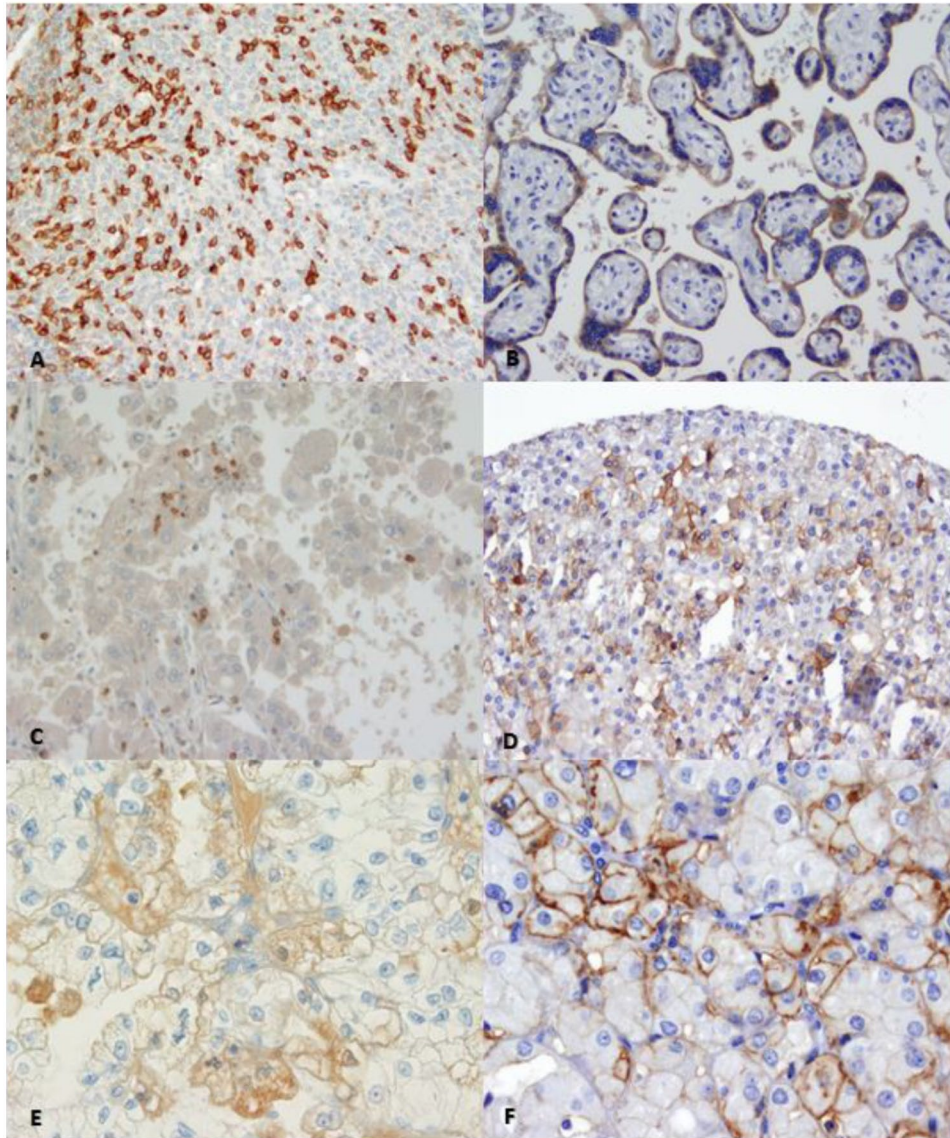
Figure 1. Immunohistochemical staining showing PD-1 (a) and PD-L1 (b) positive controls. PD-1 positive immune cells in 20X (c) and 40X (e) magnification. PD-L1 positive tumor cells in 20X (d) and 40X magnification (f).

were treated with radical nephrectomy and the rest treated with partial nephrectomy. Patient characteristics are summarized in Supplementary Table 1. There were a total of 119 recurrences (16.2%). Approximately 83% of patients were alive at the time of analysis.

Expression profile

On IHC staining, 8.3% and 7.6% of tumors were found to be PD-L1 and PD-1 positive, respectively (Figure 1). Approximately 4.9%, 2.0%, and 1.4% tumors received a PD-L1 score of 1, 2, and 3, respectively; whereas 4.5% and 3.1% received a PD-1 score of 1 and 2, respectively. On univariable analysis, PD-L1-positive tumors had a higher AJCC pT stage ($p < 0.001$), and were associated with tumor necrosis ($p = 0.012$) and lymphovascular invasion ($p = 0.037$). PD-1-positive tumors were also higher stage ($p < 0.001$), and associated with tumor necrosis ($p = 0.019$), lymphovascular invasion ($p < 0.001$), and higher Fuhrman grade ($p < 0.001$; Table 1).

Survival analysis

Kaplan–Meier analyses demonstrated nonsignificant, worse RFS and OS for PD-L1-positive cases (log rank $p = 0.111$ and 0.181, respectively; Figure 2). After adjusting for age, Eastern

Table 1. Association of PD-L1 and PD-1 expression with histopathological parameters.

	PD-L1−	PD-L1+	*p* value	PD-1−	PD-1+	*p* value
Patients	677	61		682	56	
AJCC tumor stage			<**0.001**			<**0.001**
pT1	475 (70.2)	29 (47.5)		471 (69.1)	23 (41.1)	
pT2	152 (22.5)	11 (18)		159 (23.3)	11 (19.6)	
≥pT3	50 (7.3)	21 (34.4)		52 (7.6)	22 (39.3)	
Tumor necrosis			**0.012**			**0.0185**
No	519 (76.7)	38 (62.3)		512 (75)	34 (60.7)	
Yes	158 (23.3)	23 (37.7)		170 (25)	22 (39.3)	
Lymphovascular invasion			**0.037**			<**0.001**
No	604 (89.2)	49 (80.4)		610 (89.4)	41 (73.2)	
Yes	73 (10.8)	12 (19.6)		72 (10.6)	15 (26.8)	
Fuhrman grade			0.237			<**0.001**
≤2	386 (57)	30 (49.2)		392 (57.5)	19 (33.9)	
≥3	291 (43)	31 (50.8)		290 (42.5)	37 (66.1)	

Bold values indicate statistical significance.
AJCC, American Joint Committee on Cancer; PD-1, programmed cell-death 1; PD-L1, programmed cell-death ligand 1.
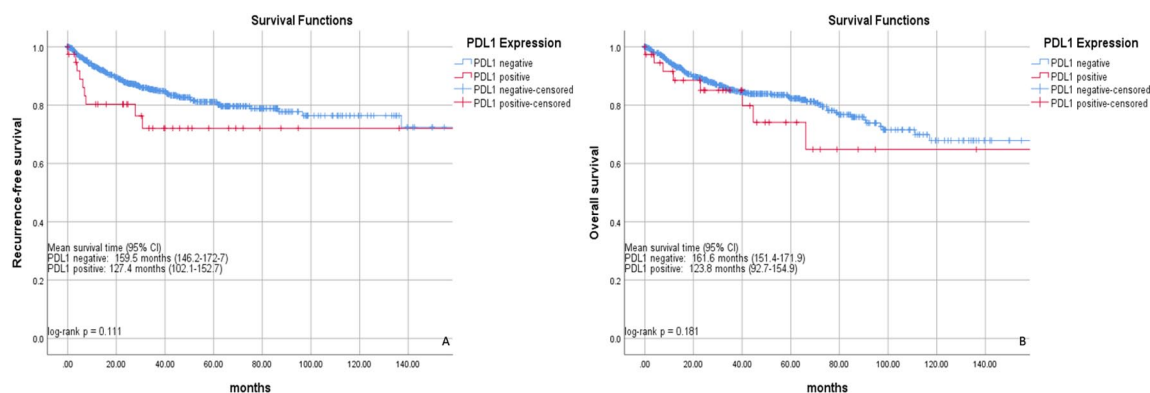

Figure 2. Recurrence-free (a) and overall survival (b) stratified by PD-L1 expression.
CI, confidence interval; PD-L1, programmed cell-death ligand 1.

Cooperative Oncology Group performance status, tumor stage, grade, and presence of lymphovascular invasion or tumor necrosis, PD-L1 positivity was a significant predictor for worse RFS [hazard ratio (HR) = 2.08, 95% confidence interval (CI): 1.01–4.34] and OS (HR = 2.61, 95% CI: 1.15–5.96; Table 2).

Discussion

In this large multi-institutional study, we report the prognostic value of PD-L1 expression for patients with clinically localized ccRCC. We found expression to be associated with high-risk histopathologic features on univariable analyses. After adjustment for pertinent covariates, we found

Table 2. Cox proportional hazard regression analysis for recurrence-free and overall survival.

Survival	Univariable		Multivariable[a]	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value*
Recurrence-free survival				
PD-L1 positivity	1.73 (0.87–3.42)	0.116	2.08 (1.01–4.34)	0.050
PD-1 positivity	2.35 (1.09–5.05)	0.029	–	
Overall survival				
PD-L1 positivity	1.59 (0.80–3.31)	0.185	2.61 (1.15–5.96)	0.022
PD-1 positivity	0.84 (0.27–2.65)	0.769	–	

*Results of backward selection multivariable analysis.
–Dropped from statistical model.
[a]Covariables included age, performance status, tumor stage, grade, and presence of lymphovascular invasion or tumor necrosis.
CI, confidence interval; HR, hazard ratio; PD-1, programmed cell-death 1; PD-L1, programmed cell-death ligand 1.

PD-L1 positivity to be a significant predictor of worse RFS and OS. Our findings highlight PD-L1 positivity as a driver of inferior outcomes notwithstanding other patient and tumor characteristics. These patients merit strict follow-up surveillance and consideration for clinical trial enrollment.

Immunomodulatory agents targeting PD-1/PD-L1 have revolutionized treatment of several malignancies. PD-1 is a member of the B7-CD28 family and serves as a cell-surface inhibitory receptor on T cells.[13,14] Expression of PD-1 and PD-L1 has been associated with poor outcomes in several tumor types.[15–19] Thompson and colleagues were one of the first groups to demonstrate PD-L1 expression as a predictor of cancer progression and mortality in a subset of 268 patients with localized RCC.[20] Abbas and colleagues demonstrated PD-L1 positivity associated with lymph node and distant metastasis, higher AJCC stage, and advanced disease.[21] This same group also performed analyses on nonclear-cell histologies, although expression did not significantly impact tumor aggressiveness or clinical outcome.[22] Studies of metastatic RCC have shown an increased risk of death for those with PD-L1 positivity on IHC staining.[23–25] Our findings corroborate those studies, as PD-L1 positivity was associated with poor histopathologic features and worse outcomes in a multi-institutional cohort of patients of lower-stage ccRCC.

Recent clinical trials have focused on angiogenesis inhibitors such as TKIs and other molecular-targeted agents. So far, randomized phase III trials of adjuvant therapy have led to conflicting results. Sunitinib has shown improved disease-free survival (DFS) by a median of 1.2 years when compared with placebo, while two other studies involving sunitinib, sorafenib, or pazopanib had no impact on DFS while suffering from a high discontinuation rate due to treatment toxicity.[26–28] On the other hand, immunotherapy trials involving nivolumab, or combination of nivolumab and ipilimumab, have shown a survival benefit over VEGF-targeted therapy for patients with previously treated or untreated advanced RCC, respectively.[7,8] This leads us to believe that immunotherapy could have a beneficial role when given at an earlier stage for patients with high-risk RCC after nephrectomy.

Immunotherapeutic agents are now being investigated in the adjuvant and perioperative setting. Ongoing randomized phase III trials with pembrolizumab, atezolizumab, and nivolumab [ClinicalTrials.gov identifiers: NCT03142334, NCT03024996, NCT03055013, respectively] are currently underway and will help elucidate the role of checkpoint inhibition for localized RCC. Our results bear clinical importance, given the potential role for checkpoint inhibition for those with ccRCC PD-L1-positive tumors.

We acknowledge the limitations to this study that cannot be overcome. All cases included only retrospectively collected tissue samples. However, our sample is relatively large and interpreted by an experienced uropathologist blinded to clinical outcomes. Heterogeneity of RCC is well recognized and may not be representative of PD-L1

positivity for all tumor samples. Our study was performed on TMAs, which may not be representative of whole-tissue specimens and could lead to discordance in the expression of PD-L1 between tumor samples and primary tumors. However, we used three cores per patient to more adequately represent the expression of each antigen.[29] Our short follow up and low number of events limit our conclusions. We limited our study to clear cell and no other histological subtypes. Although we correlate PD-L1 expression with cancer outcomes, causality cannot be established. Prospective studies are needed to further characterize the pathophysiological role of PD-L1 and its prognostic and therapeutic implications.

Conclusion

Tumor PD-L1 positivity was a significant predictor of worse outcomes for patients with clinically localized RCC at short follow up. This immunohistochemical marker may help identify patients that merit strict surveillance after nephrectomy and could potentially benefit from checkpoint-inhibitor therapy in the adjuvant setting.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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