Evaluation of programmed cell death ligand 1 expression in a contemporary cohort of penile squamous cell carcinoma and its correlation with clinicopathologic and survival parameters

A study of 134 patients

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ABSTRACT

Objectives: Penile squamous cell carcinomas (PCs) are rare malignancies with a dismal prognosis in a metastatic setting; therefore, novel immunotherapeutic modalities are an unmet need. One such modality is the immune checkpoint molecule programmed cell death ligand 1 (PD-L1). We sought to analyze PD-L1 expression and its correlation with various clinicopathologic parameters in a contemporary cohort of 134 patients with PC.

KEY POINTS

- Programmed cell death ligand 1 (PD-L1)–positive tumors may define a subset of penile squamous cell carcinoma (PC) associated with adverse clinicopathologic features and worse survival outcomes.
- PD-L1 expression is associated with high-grade and metastatic tumors, and PD-L1 positivity, including higher expression, portends lower overall and cancer-specific survival.
- Immune checkpoint inhibitors targeting the programmed cell death 1 protein/PD-L1 pathway may be a therapeutic option in PC.

KEY WORDS

penile; squamous cell carcinoma; PD-L1; overall survival; cancer-specific survival

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Methods: A cohort of 134 patients with PC was studied for PD-L1 immunohistochemistry. The PD-L1 expression was evaluated using a combined proportion score with a cutoff of 1 or higher to define positivity. The results were correlated with various clinicopathologic parameters.

Results: Overall, 77 (57%) patients had positive PD-L1 expression. Significantly high PD-L1 expression was observed in high-grade tumors ($P = .006$). We found that 37% of human papillomavirus (HPV)–associated subtypes and 73% of other histotype tumors expressed PD-L1, while 63% of HPV-associated tumors and 27% of other histotype tumors did not (odds ratio, 1.35; $P = .002$ when compared for HPV-associated groups vs all others). Similarly, PD-L1–positive tumors had a 3.61-times higher chance of being node positive than PD-L1–negative tumors ($P = .0009$). In addition, PD-L1 high–positive tumors had a 5-times higher chance of being $p16^{ink4a}$ negative than PD-L1 low–positive tumors ($P = .004$). The PD-L1–positive tumors had a lower overall survival and cancer-specific survival than PD-L1–negative tumors.

Conclusions: Overall, PD-L1 expression is associated with high-grade and metastatic tumors. Lower PD-L1 expression is observed more frequently in HPV-associated (warty or basaloid) subtypes than in other, predominantly HPV-independent types. As a result, PD-L1 positivity, including higher expression, portends lower overall and cancer-specific survival. These data provide a rational for further investigating PD-L1–based immunotherapeutics in PC.

INTRODUCTION

Advanced penile squamous cell carcinomas (PCs) have a dismal prognosis. According to GLOBOCAN 2020 data, 36,068 new cases of PC were diagnosed, and there were 13,211 deaths.[1] India has one of the highest incidences of PC worldwide, with 3.32 cases per 100,000 men in some regions.[2] Advanced disease is associated with significant morbidity and mortality as well as poor disease outcome. The etiology of PC is multifactorial, and the incidence varies according to circumcision practice, phimosis, having multiple sexual partners, human papillomavirus (HPV) infection status, personal hygiene, exposure to tobacco products, treatment with ultraviolet B (UVB) radiation (for patients with psoriasis), penile rash for longer than 1 month, immunosuppression, and radiation therapy (RT), among other factors.[3-5] Traditionally, the presence of inguinal lymph node involvement is considered the single most important unfavorable prognostic factor in PC, irrespective of T stage.[6] Histopathologic variables, including morphologic subtype, grade, T stage, lymphovascular invasion (LVI), and perineural invasion (PNI), are predictors of nodal metastases.[6] Effective therapeutic options for advanced PC are an unmet need, and the scope of immunotherapy in PC is an area of intense research.[7,8]

Human papillomavirus infection has been associated with the development of PC in approximately 30% to 80% of the cases,[9,10] and $p16^{ink4a}$ overexpression has widely been used as a surrogate marker for HPV infection in various squamous malignancies, including PCs.[9-12] Programmed cell death 1 protein (PD-1) is a co-inhibitory receptor present on CD8-positive cytotoxic T cells; it interacts with its ligand, programmed cell death ligand 1 (PD-L1), on tumor cell membranes, resulting in suppression of T-cell activation and proliferation and dampening of the host antitumor immune response.[7-9,13] The expression of PD-L1 is induced in response to inflammation and high cytokine levels. Tumor cells thus express PD-L1 as an adaptive immune response mechanism to attenuate the host antitumor immune response. As a result, inhibiting the PD-1/PD-L1 immune checkpoint pathway should augment tumor cell killing by cytotoxic T cells. Several studies have shown that PD-L1 expression is associated with adverse clinicopathologic features and worse outcomes in other urologic malignancies, including renal cell carcinoma and bladder cancer.[9,14,15] In addition, PD-L1 may be a biomarker to predict oncologic outcome and treatment response.[9,16,17]

In PC, isolated studies have demonstrated an association between PD-L1 expression and higher tumor stage, higher nodal stage, and reduced cancer-specific survival (CSS).[7,9] Although cisplatin- and paclitaxel-based chemotherapies remain the mainstay of treatment in advanced disease, newer therapeutic modalities are necessary in this neoplasm.[8] There is an associated need to explore immune checkpoint inhibitors as possible treatment options in PC. We sought to analyze PD-L1 expression and its correlation with various clinicopathologic parameters and survival outcomes in a contemporary cohort of 134 patients with PC.

METHODS

Patient and Cancer Characteristics

This study was approved by the institutional review boards of the authors' respective institutions. It included 134 men diagnosed with PC between January 2009 and August 2020 who had received treatment and for whom paraffin tissue blocks were available for further analysis. Demographic, clinical, histopathologic, treatment-related, and survival data were collected from the medical records. These data included patient age at time of surgery, type of surgical procedure, histopathologic subtype, tumor grade, LVI status, PNI status, depth of invasion, margin status, disease stage, adjuvant therapy, and survival. The histopathology slides were reviewed by 4 study pathologists (S.K.M., A.L., S.J., and A.V.P.), and the microscopic variables were tabulated. Overall survival (OS) was calculated from the date of surgery to the date of death or last follow-up, with no restriction on the cause of death. We estimated CSS from the date of surgery until death from PC. Patients who were treated for PC, were clinically node negative, and did not undergo inguinal lymph node dissection were considered pathologically node negative if they remained relapse free during the course of their follow-up.

Immunohistochemical Analysis

Whole sections of the tumors from all 134 cases of primary PC were stained with $p16^{ink4a}$, p53, and PD-L1 immunostains (immunohistochemistry [IHC]). PD-L1 IHC was carried out using rabbit monoclonal antibody (mAb) (E1L3N; cell signaling; prediluted) on an autostainer. Positive control of human tonsil

tissue was included in each run. Positive PD-L1 staining was defined as complete or partial, circumferential, or linear plasma membrane staining of any intensity that could be differentiated from the background and diffuse cytoplasmic staining. The percentage of tumor cells with membranous staining was assessed by the pathologists, who were blinded to the clinicopathologic parameters. The PD-L1 expression was evaluated using the combined positive score (CPS), with a cutoff of 1 or higher to define positivity. The CPS includes the number of positive tumor cells, lymphocytes, and macrophages for assessing PD-L1 expression and is defined by the number of PD-L1 staining cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells multiplied by 100.[18] Based on the Keynote-048 trial for head and neck squamous cell carcinomas, a 2-tier system was devised wherein tumors with a CPS between 1 and 19 were considered PD-L1 low positive and those tumors with a CPS of 20 or higher were considered PD-L1 high positive.[19] In tumors with a heterogeneous staining pattern, the entire PD-L1–stained tumor section was divided into grids from 1 end to the other, covering the entire tumor area; scoring was performed in each grid, then an average was calculated. This technique covered the entire tumor area, taking into account both negatively and positively stained tumor cells and associated immune cells. The results were correlated with various clinicopathologic parameters.

p53 and $p16^{ink4a}$ staining was performed using $p16^{ink4a}$ (Ventana Medical Systems; clone E6H4 mouse; prediluted) and p53 (Ventana Medical Systems; clone BP53-11 mouse; prediluted) on whole tumor sections. Positive controls (cervical carcinoma for $p16^{ink4a}$ and tonsil for p53) and negative controls (tonsil for $p16^{ink4a}$ and colon for p53) were included in each staining run. Overexpression of $p16^{ink4a}$ was defined as a continuous strong nuclear or nuclear and cytoplasmic staining of more than 50% of the tumor cells. For the assessment of p53, staining intensity (weak, moderate, and strong) and percentage of tumor cells staining positive were estimated. p53 positivity has previously been defined as either the complete absence of p53 staining in the tumor cells (null immunophenotype); 75% or more nuclear staining in the tumor cells, regardless of the staining intensity (overexpression); or aberrant cytoplasmic staining of the tumor cells. These patterns were used as the surrogate for *TP53* alteration. p53 negativity (surrogate for *TP53* wild type) was defined by an admixture of negatively stained and positively stained tumor (<75%, regardless of the intensity) cells, imparting a mosaic pattern of staining. For a subset of these cases, p53 and p16 status has been assessed previously, with the assessment described in detail.[20]

Statistical Analyses

All continuous variables (age, OS, and CSS) were expressed in median (interquartile ranges [IQRs]), a measure of variability, based on dividing a data set into quartiles. Categorical variables (surgical procedure, tumor grade and subtype, T stage, LVI, depth of invasion, PNI, margin status, adjuvant therapy, IHC results, and nodal involvement) were expressed as frequencies. Survival analyses were performed using the Kaplan-Meier method, where *P* values were calculated using the log-rank test. Associations between IHC status, nodal involvement, OS, and CSS were assessed.

RESULTS

Demographics and Clinical Characteristics

A total of 134 patients with PC were studied. The median (IQR) age was 65 years: 64 (41-80) years for PD-L1–positive tumors and 65 (49-80) years for PD-L1–negative tumors. Sixty-two (46%), 48 (36%), and 24 patients (18%) were treated with partial penectomy, total penectomy, and less invasive wide local excision, respectively. The majority (87 [64.9%]) had clinically N0 disease at the time of diagnosis, while 47 (35.1%) patients had clinically node-positive disease.

Histopathologic Characteristics

The histopathologic subtypes were HPV-associated subtypes, including warty or basaloid (total = 59; warty = 17; basaloid = 28; warty-basaloid = 14), and others (predominantly HPV-independent subtypes, n = 75). The other histologies included adenosquamous (n = 3), papillary (n = 5), sarcomatoid (n = 3), pseudoglandular (n = 10), usual type (pseudohyperplastic; n = 45), and mixed (n = 9). Low-grade histology was observed in 85 tumors, and 49 were of high-grade histology. Three tumors had sarcomatoid dedifferentiation; PNI was observed in 82 tumors, and 52 tumors did not show PNI. Seventy tumors had 5 mm or less depth of invasion, and the remaining 64 tumors had 5 mm or more depth of invasion. Forty-seven tumors exhibited LVI, while 87 were negative. When margin status was assessed, 6 tumors had positive margins, which included 2 with proximal urethral margin, 1 with proximal shaft and corpora cavernosa, and 3 with proximal periurethral connective tissue. The T stages were 41, 49, 39, and 5 in pT1, pT2, pT3, and pT4 categories, respectively. The pathologic N stages were 87, 20, 11, and 16 in pN0, pN1, pN2, and pN3 categories, respectively.

Follow-Up and Survival Characteristics

Fifty-two patients (39%) received adjuvant therapies, including RT in 11, chemotherapy in 19, and combined chemoradiation in 22 patients. Median follow-up for OS in the entire cohort was 44 months (range, 2-120 months). Seventy-two patients (54%) died during the follow-up period; of these, 50 (69%) patients died from PC and 22 (31%) died from other causes. The median follow-up for CSS was 36 months (range, 2-103 months).

Immunohistochemical Characteristics

PD-L1 Expression

Seventy-seven (57%) tumors expressed PD-L1, while 57 (43%) were negative. The percentage of tumor cell membrane staining ranged from 1% to 90%. Of the PD-L1–positive tumors, 48 (62%) showed low PD-L1 positivity and 29 (38%) showed PD-L1 high positivity. Twenty-two of 59 (37%) HPV-associated warty, basaloid, or warty-basaloid tumors and 55 of 75 (73%) other-histotype tumors expressed PD-L1, whereas 37 of 59 (63%) warty, basaloid, or warty-basaloid tumors and 20 of 75 (27%) other-histotype tumors did not show PD-L1 expression. Among the PD-L1–positive tumors, 59% (43/73) expressed p53 and 50% (32/64) expressed $p16^{ink4a}$, while 41% (30/73) of p53-positive tumors and 50% (32/64) of $p16^{ink4a}$-positive tumors did not express PD-L1 **TABLES 1** and **2** and **FIGURE 1**.

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TABLE 1 Clinicopathologic Characteristics of PD-L1–Positive and PD-L1–Negative Tumors

Characteristic	PD-L1–positive status (CPS ≥1) (n = 77)	PD-L1–negative status (CPS <1) (n = 57)	*P* value	OR
Age at time of surgery, median (range), y	64 (41-80)	65 (49-80)	.314	1.41
Tumor grade/differentiation, No.			.013	5.02
Low (I and II)	42	43		
High (III)	35	14		
Histopathologic type, No.			.002	1.35
Other	55	20		
Warty or basaloid	22	37		
Lymph node involvement, No.			.0009	3.67
Positive	36	11		
Negative	41	46		
Adjuvant therapy, No.			.45	1.32
Yes	32	20		
No	45	37		
p53 IHC, No.			.71	1.14
Positive	43	30		
Negative	34	27		
p16 IHC, No.				
p16 positive	32	32	.095	0.58
p16 negative	45	25		
p16 positive/p53 positive	15	18	.453	
p16 negative/p53 negative	17	14		

CPS, combined positive score; IHC, immunohistochemistry; OR, odds ratio; PD-L1, programmed cell death ligand 1.

TABLE 2 Clinicopathologic Characteristics of PD-L1 Low–Positive and PD-L1 High–Positive Tumors

Characteristic	PD-L1 low–positive status (CPS 1-19) (n = 48)	PD-L1 high–positive status (CPS ≥20) (n = 29)	*P* value	OR
Age at time of surgery, median (range), y	64 (41-80)	63 (49-74)	.271	1.81
Tumor grade/differentiation, No.			.006	1.72
Low (I and II)	32	10		
High (III)	16	19		
Histopathologic type, No.			.087	0.38
Other	31	24		
Warty or basaloid	17	5		
Lymph node involvement, No.			.25	1.72
Positive	20	16		
Negative	28	13		
Adjuvant therapy, No.			.65	1.24
Yes	19	13		
No	29	16		
p53 IHC, No.			.18	1.9
Positive	24	19		
Negative	24	10		
p16 IHC, No.				
p16 positive	26	6	.004	0.22
p16 negative	22	23		
p16 positive/p53 positive	10	5	.202	1.69
p16 negative/p53 negative	8	10		

CPS, combined positive score; IHC, immunohistochemistry; OR, odds ratio; PD-L1, programmed cell death ligand 1.

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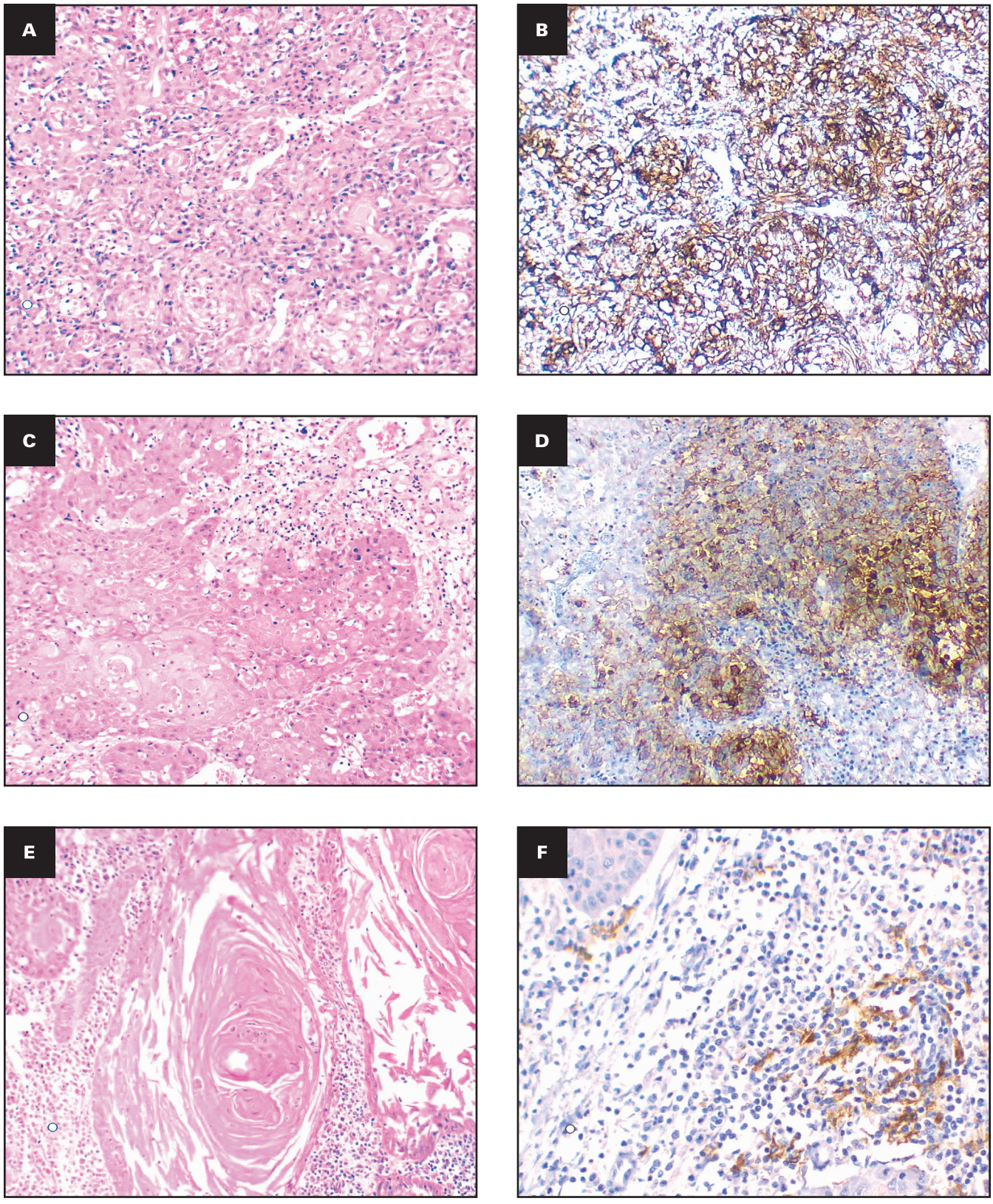

FIGURE 1 Evaluation and reporting of programmed cell death ligand 1 (PD-L1) (×20). **A**, Poorly differentiated and high-grade invasive penile squamous cell carcinoma (H&E). **B**, High PD-L1 expression, combined positive score (CPS) ≥20. **C**, Poorly differentiated, high-grade invasive penile squamous cell carcinoma (H&E). **D**, High PD-L1 expression, CPS ≥20. **E**, Well-differentiated invasive penile squamous cell carcinoma (H&E). **F**, Low PD-L1 expression, CPS <20.

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p53 Expression

Seventy-three tumors had a mutated-type staining pattern for p53; of these, 43 tumors expressed PD-L1 and 30 were PD-L1 negative. Among the 61 p53-negative tumors, 34 had PD-L1 staining and 27 did not show PD-L1 staining.

$p16^{ink4a}$ Expression

Sixty-four tumors expressed $p16^{ink4a}$, of which 32 were PD-L1 positive and 32 were PD-L1 negative. Various histologic subtypes and their $p16^{ink4a}$ positivity were as follows: HPV-associated warty or basaloid (total = 49/59 [83%]; warty = 14/17 [82%]; basaloid = 21/28 [75%]; warty-basaloid = 14/14 [100%]) and other histologies (total = 12/75 [16%]; adenosquamous = 0/3 [0%]; papillary = 1/5 [20%]; sarcomatoid = 0/3 [0%]; pseudoglandular = 0/10 [0%]; usual type = 3/45 [7%]; mixed = 8/9 [89%]). Of the $p16^{ink4a}$-negative tumors, 45 were PD-L1 positive and 25 were PD-L1 negative. Only 6 of 32 (19%) $p16^{ink4a}$-positive tumors had high PD-L1 CPS score, but 23 of 45 (51%) $p16^{ink4a}$-negative tumors showed a high PD-L1 score ($P = .004$).

Statistical Comparisons

On correlating the grade of tumors with PD-L1 expression, high-grade tumors (35/49 [71%]) were 5 times more likely than low-grade tumors to be PD-L1 positive (42/85 [48%]; odds ratio [OR], 5.02; $P = .013$). Because the interpretations are based on 2×2 χ^2 analysis, the corollary that PD-L1–positive tumors were significantly more likely to be higher grade than PD-L1–negative tumors is also appropriate. Similarly, on correlating nodal status with PD-L1 expression, node-positive tumors (36/47 [76.5%]) were 3.67 times more likely to be PD-L1 positive than node-negative tumors (41/87 [47%]; OR, 3.67; $P = .0009$). The corollary that PD-L1–positive tumors are more likely to be node positive is also appropriate. There was an inverse relation between $p16^{ink4a}$ expression and PD-L1 high positivity (CPS ≥20). $p16^{ink4a}$-positive patients (6/32 [19%]) were significantly (78%) less likely to be PD-L1 high positive than $p16^{ink4a}$-negative patients (23/45 [51%]; OR, 0.22; $P = .004$).

Twenty-two of 59 (37%) warty, basaloid, or warty-basaloid tumors and 55 of 75 (73%) other-histotype tumors expressed PD-L1, whereas 37 of 59 (63%) warty, basaloid, or warty-basaloid tumors and 20 of 75 (27%) other-histotype tumors did not show PD-L1 expression (OR, 1.35; $P = .002$ for comparing HPV-associated groups vs all others). Not surprisingly, the proportion of warty, basaloid, or warty-basaloid tumor subtypes showed significant $p16^{ink4a}$ positivity (83% vs 16%; $P = .0001$) compared with other subtypes; however, 89% of the mixed tumors expressed $p16^{ink4a}$. p53 was positive in 73 (54%) and negative in 61 (46%) tumors. There was no specific association of any histologic subtype with p53 expression.

No statistically significant association was observed between PD-L1 expression and the patient age, all histologic subtypes individually, depth of invasion, LVI, PNI, p53 IHC, and adjuvant therapy, either in a univariate or multivariate model. Also, no statistically significant difference was observed in PD-L1 expression, including the type of expression (low vs high) between the dual-positive (p16-positive/p53-positive) and dual-negative (p16-negative/p53-negative) tumors. Overall, PD-L1–positive PC was associated with adverse clinicopathologic characteristics and vice versa **TABLES 1** and **2**.

PD-L1 Expression and Survival

Patients with PD-L1–positive tumors had significantly lower OS than those with PD-L1–negative tumors (median OS for patients with PD-L1–positive PC: 27 months [range, 2-111 months]; median OS for patients with PD-L1–negative PC: 65 months [range, 10-120 months]; hazard ratio [HR], 1.85 [95% CI, 1.3-2.6]; $P = .00037$]) **FIGURE 2**. Even within the PD-L1–positive cohort, the OS was worse in the PD-L1 high cohort (CPS ≥20) than in the PD-L1 low cohort (CPS <20) (median OS among those with PD-L1 high–positive disease, 20 months [range, 2-87 months]; median OS among those with PD-L1 low–positive disease, 34.5 months [range, 6-111 months]; HR, 2.27 [95% CI, 1.3-3.97]; $P = .00054$) **FIGURE 3**. The CSS was also lower among PD-L1–positive tumors than among PD-L1–negative tumors (median CSS for those with PD-L1–positive tumors, 18 months [range, 2-103 months]; median CSS for those with PD-L1–negative tumors, 70 months [range, 19-102 months]; HR, 2.43 [95% CI, 1.39-4.21]; $P = .001$) **FIGURE 2**. Similar to the trend in OS, within the PD-L1–positive population, the CSS was much lower in the PD-L1 high cohort than in the PD-L1 low cohort (median CSS among those with PD-L1 high–positive tumors, 11.5 months [range, 2-43 months]; median CSS among those with PD-L1 low–positive tumors, 34 months [range, 7-103 months]; HR, 3.32 [95% CI, 1.46-7.55]; $P = .0005$) **FIGURE 3**. Thus, PD-L1 positivity was a prognostic marker in PC, with higher expression portending worse survival outcomes. In summary, on multivariate analyses, significantly high PD-L1 expression was observed in high-grade tumors ($P = .006$). PD-L1–positive tumors had a 3.61 times higher chance of being node positive than PD-L1–negative tumors ($P = .0009$). PD-L1 high–positive tumors had a 5 times higher chance of being $p16^{ink4a}$ negative than PD-L1 low–positive tumors ($P = .004$). Patients with PD-L1–positive tumors had a lower OS and CSS than patients with PD-L1–negative tumors.

$p16^{ink4a}$ and p53 Expression and Survival

On univariate analyses, HPV-associated subtypes (warty, warty-basaloid, and basaloid tumors) (HR, 0.32 [95% CI, 0.22-0.48]; $P < .0001$), $p16^{ink4a}$ positivity (HR, 0.26 [95% CI, 0.18-0.38]; $P < .0001$), $p16^{ink4a}$ and p53 co-expression (HR, 0.29 [95% CI, 0.17-0.51]; $P < .0001$), and nodal status (HR, 1.78 [95% CI, 1.22-2.60]; $P = .003$) were significant predictors of OS. Similarly, on univariate analyses, HPV-associated pathologic subtypes (warty, warty-basaloid, and basaloid tumors) (HR, 0.33 [95% CI, 0.15-0.72]; $P = .005$), $p16^{ink4a}$ positivity (HR, 0.31 [95% CI, 0.17-0.59]; $P < .0001$), $p16^{ink4a}$ and p53 co-expression (HR, 0.33 [95% CI, 0.13-0.83]; $P = .018$), and nodal status (HR, 3.28 [95% CI, 1.74-6.17]; $P < .0001$) were significant predictors of CSS. However, p53 expression (for OS: HR, 1.12 [95% CI, 0.78-1.62]; $P = .52$; for CSS: HR, 1.07 [95% CI, 0.60-1.90]; $P = .81$) and adjuvant therapy (for OS: HR, 1.23 [95% CI, 0.85-1.76]; $P = .27$; for CSS: HR, 1.39 [95% CI, 0.79-2.47]; $P = .25$) were not statistically significant for either OS or CSS. On multivariate

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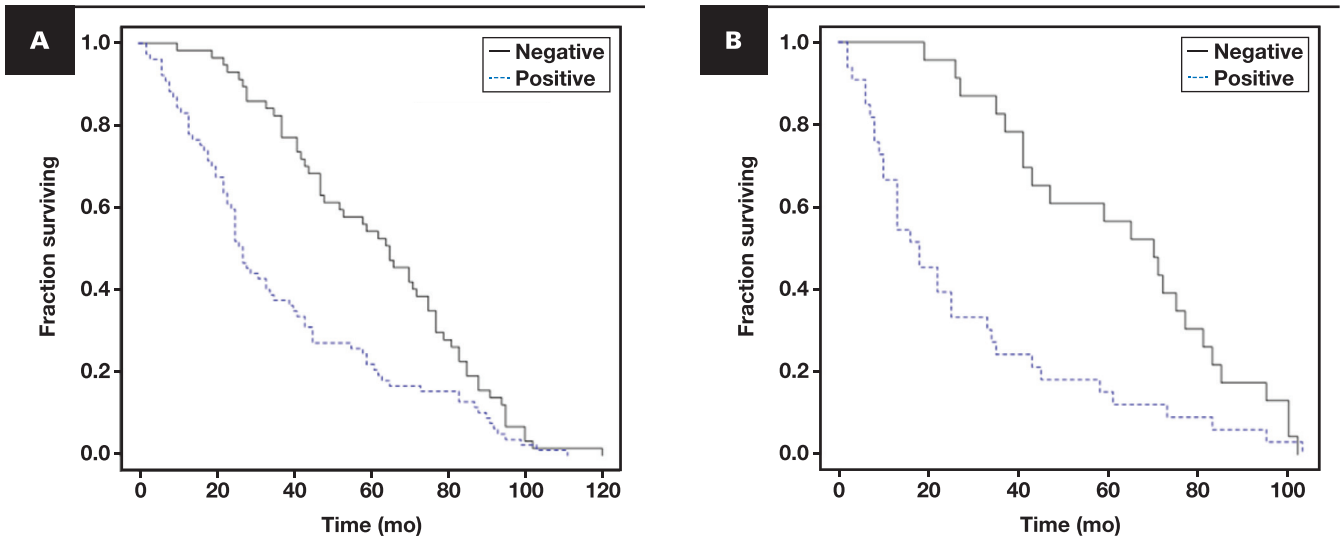

FIGURE 2 OS (**A**) and CSS (**B**) outcomes for patients with respect to PD-L1–positive vs PD-L1–negative status. **A**, PD-L1–positive status median OS, 27 months (range, 2-111 months); PD-L1–negative status median OS, 65 months (range, 10-120 months); log-rank *P* = .00037; HR, 1.848 (95% CI, 1.311-2.606). **B**, PD-L1–positive status median CSS, 18 months (range, 2-103 months); PD-L1–negative status median CSS, 70 months (range, 19-102 months); log-rank *P* = .001; HR, 2.428 (95% CI, 1.399-4.213). CSS, cancer-specific survival; HR, hazard ratio; OS, overall survival; PD-L1, programmed cell death ligand 1.

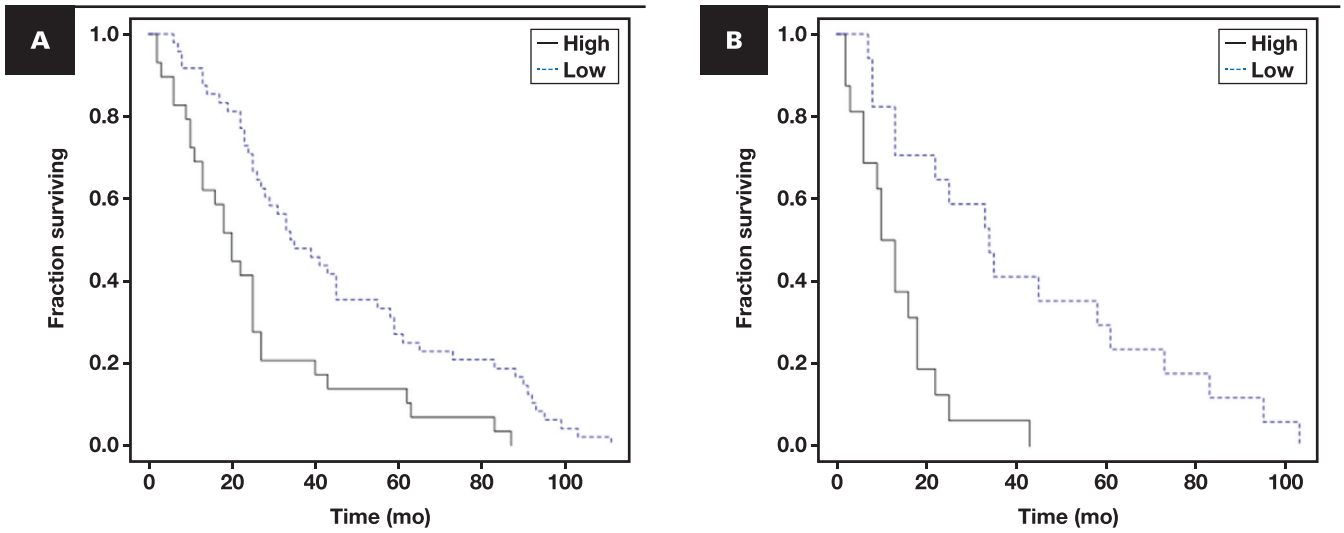

FIGURE 3 OS (**A**) and CSS (**B**) outcomes for patients with respect to PD-L1 high–positive vs PD-L1 low–positive status. **A**, PD-L1 high–positive status median OS, 20 months (range, 2-87 months); PD-L1 low–positive status median OS, 34.5 months (range, 6-111 months); log-rank *P* = .00054; HR, 2.27 (95% CI, 1.3-3.97). **B**, PD-L1 high–positive status median CSS, 11.5 months (range, 2-43 months); PD-L1 low–negative status median CSS, 34 months (range, 7-103 months); log-rank *P* = .0005; HR, 3.32 (95% CI, 1.46-7.55). CSS, cancer-specific survival; HR, hazard ratio; OS, overall survival; PD-L1, programmed cell death ligand 1.

analyses, HPV-associated pathologic subtypes (warty, warty-basaloid, and basaloid) (HR, 0.38 [95% CI, 0.23-0.61]; *P* < .0001) and $p16^{ink4a}$expression (HR, 0.32 [95% CI, 0.20-0.50]; *P* < .0001) were associated with improved OS, while the presence of nodal metastasis (HR, 2.80 [95% CI, 1.87-4.20]; *P* < .0001) was a significant predictor of reduced OS. Similarly, on multivariate analyses, HPV-associated pathologic subtypes (HR, 0.23 [95% CI, 0.09-0.54]; *P* = .001) and $p16^{ink4a}$ expression (HR, 0.15 [95% CI, 0.07-0.33]; *P* < .0001) were associated with improved OS, while the presence of nodal metastasis (HR, 8.78 [95% CI, 3.92-19.74]; *P* < .0001) was a significant predictor of reduced CSS. In Kaplan-Meier analysis, the unadjusted estimated OS was significantly longer in $p16^{ink4a}$-positive tumors (median 75 months vs 25 months; HR, 0.26 [95% CI, 0.18-0.38]; *P* < .0001), as was the CSS (median 70 months vs 19 months; HR, 0.315 [95% CI, 0.17-0.59]; *P* < .0001). P53 expression, however, did not appear to have a significant impact on OS (median 52 months vs 41 months; HR, 1.12 [95% CI, 0.78-1.62]; *P* = .52) or CSS (median 43 months vs 25 months; HR, 1.07 [95% CI,

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TABLE 3 Compilation of Published Data on PD-L1 in Penile Squamous Cell Carcinoma

Study	Cohort size, No.	Clone used	Scoring system used	PD-L1 positive, No. (%)	PD-L1 negative, No. (%)	PD-L1 high, No. (%)	PD-L1 low, No. (%)	p16 positive, PD-L1 positive, %	p16 positive, PD-L1 negative, %	Node positive, PD-L1 positive, %	Node positive, PD-L1 negative, %	OS for PD-L1–positive disease, mo	OS for PD-L1–negative disease, mo	CSS for PD-L1–positive disease, mo	CSS for PD-L1–negative, mo
Present study, 2022	134	E1L3N (cell signaling)	CPS	77 (57)	57 (43)	29 (38)	48 (62)	50	50	77	23	27	65	18	70
De Bacco et al, 2020	35	ZR3 (Zeta)	CPS	18 (51)	17 (49)	NA	NA	37	63	69	31	5-y OS high in PD-L1 (59%)		NA	NA
Davidsson et al, 2019	222	Unknown	CPS	70 (32)	152 (68)	NA	NA	NA	NA	NA	NA	Worse OS		Worse clinical outcome	
Cocks et al, 2017	53	E1L3N (cell signaling	CPS	21 (40)	32 (60)	NA	NA	NA	NA	38	62	NA	NA	NA	NA
Udager et al, 2016	37	5H1 (Merck)	CPS (cutoff of 5)	23 (62)	14 (38)	NA	NA	50	50	91	9	NA	NA	7	0
Deng et al, 2016	116	E1L3N (cell signaling	CPS	54 (47)	62 (53)	NA	NA	NA	NA	NA	NA	Correlates with poor outcome		NA	NA

CPS, combined positive score; CSS, cancer-specific survival; NA, data not available; OS, overall survival; PD-L1, programmed cell death ligand 1.

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0.60-1.90]; $P = .81$). Patients with dual positivity ($p16^{ink4a}$ positive/p53 positive) had a significantly higher OS (median 77 months vs 27 months; HR, 0.29; 95% CI, 0.17-0.51; $P < .001$) and CSS (median 83 months vs 27 months; HR, 0.33 [95% CI, 0.13-0.83]; $P = .012$). In Kaplan-Meier analysis for the lymph node–positive cases, the median OS for patients with dual-positive disease was significantly higher than for those with dual-negative disease (77 months vs 13 months; $P < .0001$). The median CSS for patients with dual-positive disease, however, was not significantly different from those with dual-negative disease (34 months vs 13 months; $P < .064$), although there was a trend toward improved CSS in the dual-positive subset.

DISCUSSION

Newer therapeutic modalities are an unmet need in advanced PC. Recently, however, the US Food and Drug Administration approved immunotherapy with anti–PD-1/PD-L1 mAbs in advanced genitourinary malignancies such as renal cell carcinoma and urothelial carcinoma.[9,16,21,22]

Immunotherapy has been studied in only a few trials enrolling patients with PC (NCT02837042 and NCT02834013), but encouraging data have been published.[23] In a single case report, a patient with chemotherapy/RT-refractory advanced PC with nodal metastases attained partial response using nivolumab.[9,24] Previous studies had indicated that higher expression of PD-L1 was associated with adverse pathologic features and worse clinical outcomes in PC and other urologic malignancies **TABLE 3**.[9,14-16] Importantly, it had been reported that the likelihood of response to anti–PD-1 therapy was correlated with tumor PD-L1 expression.[16,17] Thus, to predict the possible utility of immune checkpoint blockade in PC, it is essential to understand the expression pattern of PD-L1 in this disease. In this context, ours is the second-largest study so far and the largest study from India to assess the expression of PD-L1 in a clinically well-annotated cohort of patients with PC. In the present cohort, PD-L1 positivity was observed in 57% of these patients, which is in concordance with prior studies that reported PD-L1 positivity rate ranging from 32% to 62%.[7,9,25,26] Of the PD-L1–positive tumors in our study, 62% showed low PD-L1 positivity and 38% showed high PD-L1 expression. Results from immunotherapy trials in head and neck squamous cell carcinomas suggest that patients whose tumors show higher PD-L1 expression may have better response to monotherapy with PD-1 blockade.[19] Extrapolating these data, our findings may suggest that the subset of PC tumors with high PD-L1 expression may be susceptible to enhanced immune-mediated killing with anti–PD-1 or anti–PD-L1 mAbs.

Davidson et al[25] assessed the expression of PD-L1 and its association with clinical outcomes in 222 patients with PC. They demonstrated that one-third of patients had PD-L1–positive tumors, and these patients had a lower CSS. Most studies on PD-L1 expression in PC have used the CPS rather than the tumor proportion score because the former has a higher likelihood of picking PD-L1 positive cases (the CPS considers PD-L1 expression both on tumor cells and on immune cells). The CPS has also been used in pivotal trials of immunotherapy in head and neck squamous cell carcinomas, thus providing the rationale for PD-L1 expression by CPS in PC.[19]

Udager et al[7] demonstrated PD-L1 expression in 26 of 37 (72%) patients with PC. PD-L1 expression was more frequent in primary tumors with usual-type histology (unfavorable histology) and nodal metastasis; it was associated with a worse CSS.[7] The authors also observed that PD-L1 expression was not found among the warty and verrucous types (favorable subtypes) of PC. Although our study did not show a statistically significant correlation between PD-L1 expression and all individual histologic subtypes of the tumors, we had similar results to those of Udager et al, who showed lower expression in HPV-associated (warty or basaloid) types than other, predominantly HPV-independent types.

In 53 patients with PC, Cocks et al[27] showed PD-L1 positivity in 44% of stage II tumors and 38% of node-positive tumors. PD-L1 expression did not correlate with patient age, tumor location, histologic subtype, tumor grade or stage, or depth of invasion. These results are similar to our study in that PD-L1 expression in PC is associated with adverse clinicopathologic parameters. Deng et al[28] concluded that 53% of the tumors were PD-L1 positive and that higher PD-L1 expression was associated with a shorter CSS. Thus, high PD-L1 expression in PC was associated with a poor prognosis, and these findings indicate that the PD-1/PD-L1 axis may be a potential therapeutic target for such patients.

De Bacco et al[9] assessed PD-L1 and $p16^{ink4a}$ expression in a cohort of 35 patients with PC. A total of 18 (51%) patients had PD-L1–positive tumors, and PD-L1 positivity was associated with larger tumors and $p16^{ink4a}$ positivity. PD-L1 positivity was more frequent in high-grade tumors (78%). It was also associated with nodal involvement. These findings were in concordance with our study, except for $p16^{ink4a}$ co-expression. The results of $p16^{ink4a}$ expression were not in concordance with our study, which instead demonstrated a statistically significant inverse relationship between PD-L1 and $p16^{ink4a}$ expression. In our study, in the overall population, there was no significant correlation between PD-L1 positivity and $p16^{ink4a}$expression, but $p16^{ink4a}$-positive tumors were significantly less likely to be PD-L1 high positive (CPS ≥20) compared with $p16^{ink4a}$-negative tumors ($P = .004$). This discrepancy in results may be explained by the sample size and other, unknown factors.

Ottenhof et al[26] studied the prognostic value and association between multiple tumor microenvironmental factors and clinical outcomes such as nodal metastasis and survival in PC. They found that 48% of tumors were PD-L1 positive, and this positivity was significantly correlated with tumor grade, nodal metastases, and poor survival. Of the patients with PD-L1–positive tumors, 82% were negative for HPV. This finding is in concordance with our study, in which approximately 57% of patients with PC had PD-L1–positive tumors and PD-L1 high–positive tumors were significantly less likely to be $p16^{ink4a}$ positive, which is a surrogate for relation to HPV-related carcinogenesis. Taken together, these findings suggest that future PD-L1 studies, whether clinical or histopathologic, should analyze and compare PD-L1 status across groups of cases defined by relation to HPV (ie, compare the HPV-associated group of subtypes [warty or basaloid] with the HPV-independent group of subtypes) rather than attempting to identify significant differences among a large number of subtypes defined by histology alone.

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Although p53 and p16^{ink4a} status and their correlation were not the major focus of this manuscript and we recently published this work on another study using a smaller subset of the same cohort,[20] the overall observations are as follows: p16^{ink4a} status is an independent predictor of survival in PC. There is a strong association between p16 positivity and histology, with the HPV-associated warty, basaloid, and warty-basaloid subtypes being positive. p53 is a predictor of nodal metastasis irrespective of p16^{ink4a} status. Dual-positive tumors (p16^{ink4a} and p53 positive) have a significantly better outcome than dual-negative tumors (both markers negative); however, no statistically significant difference was observed in PD-L1 expression, including the type of expression (low vs high) between the dual-positive and dual-negative tumors **TABLES 1** and **2**. Overall, we suggest that p16 and p53 prognostic biomarkers should be performed at baseline in all patients with PC.

The major strengths of our study include its large cohort size and geographical context—that is, this is the first study from India exploring PD-L1 expression in PC. We further stratified the PD-L1–positive cohort into low- and high-positive subsets and found significant differences between them. Our study has certain limitations, however. First, although it is a multicenter study with a large cohort, ours was a retrospective analysis, which has inevitable selection bias. Second, the follow-up period was relatively short for a subset of patients.

In conclusion, PD-L1–positive tumors may define a subset of clinically aggressive PC that is associated with worse outcome. It provides a rationale for the subsequent investigation of PD-1/PD-L1 inhibitors in the treatment of PC.

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