

PD-L1 Tumor Cell Expression in Upper Tract Urothelial Carcinomas is Associated with Higher Pathological Stage

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Abstract

Background

Upper tract urothelial carcinomas (UTUCs) are a rare and unique subset of urothelial carcinoma (UC). Immune checkpoint inhibitors are showing promise in the treatment of UC of the bladder and UTUC alike. While several large studies have looked at PD-L1 expression in UC in general, most have not investigated UTUC as a separate group. Moreover, comparison between studies of PD-L1 expression is challenging as the different immune checkpoint inhibitors require different PD-L1 scoring systems and cutoffs (i.e. tumor proportion score, combined positive score, and immune cell score).

Methods

This is a retrospective study of 37 cases of resected UTUC. Representative tissue from each case was compiled into tissue microarrays and immunohistochemical stains for PD-L1 (Dako antibody clones 223C and 28 – 8) were performed. PD-L1 staining was evaluated using several established scoring systems: tumor proportion score (TPS), combined positive score (CPS) and immune cell (IC) score. Associations between PD-L1 expression and clinicopathologic features were investigated.

Results

Overall expression of PD-L1 in UTUC was 29.7% when using a TPS cutoff of $\geq 1\%$. 55.6% of cases with higher pathological stage (pT3 or pT4) were positive for PD-L1, compared to only 5.3% of cases with lower pathological stage (pTis, pT1, or pT2; $p = 0.0011$). When using a CPS cutoff of ≥ 10 , there was no significant association between tumor stage and PD-L1 expression. There was no association between PD-L1 positivity and tumor grade, tumor location, gender, or age. There was 100% concordance between 22C3 and 28 – 8 in terms of positivity rate.

Conclusions

Our study shows that 29.7% of UTUCs are positive for PD-L1 TPS expression, comparable to the 20–30% reported in UC literature. Our study also shows that PD-L1 expression in UTUC is more often associated with high pathological stage, which may reflect an immune response evasion mechanism that UC cells acquire later in disease progression. Finally, PD-L1 22C3 and 28 – 8 clones show similar overall patterns of staining in this setting.

Background

Urothelial carcinoma is the 5th most common cancer in the United States and the 9th most common cause of cancer worldwide.¹ Upper tract urothelial carcinomas (UTUCs) are a rare subset of urothelial carcinoma (UC) that occur in the renal pelvis and/or ureter, and represent approximately 5% of total urothelial carcinomas.² UTUC and UC share a similar histologic appearance, both are more common in men (3:1), and both are associated with smoking. However, there are some significant differences as well. UTUC is different at a molecular, anatomic, and

clinical level.^{3,4} Unlike UC, UTUC is highly associated with Lynch syndrome, or hereditary nonpolyposis colorectal cancer (HNPCC), and harbors unique molecular signatures, such as *FGFR3* mutations.⁵⁻⁸ UTUC is the 3rd most common Lynch syndrome associated cancer after colon and endometrial cancer.⁹ The tight anatomic constraints of the upper GU tract make UTUCs more challenging to biopsy, grade, and stage, and they require different treatments.³ Cisplatin-based chemotherapy is the standard of care for muscle invasive bladder UC, but treatment of UTUC usually requires nephroureterectomy, and the resulting loss of renal function may preclude treatment with the standard cisplatin-based chemotherapy because of its known nephrotoxicity.¹⁰ New treatment modalities, such as immune checkpoint inhibitors (ICIs) are a viable, non-chemotherapeutic option. ICIs are becoming more common in the treatment of many types of cancer and PD-L1 expression has been shown in some tumor types to be predictive of response to ICI therapy.

Currently, the United States Food and Drug Administration (FDA) has approved PD-L1 companion diagnostic testing, to qualify patients for treatment with pembrolizumab (Keytruda®, Merck, Kenilworth, NJ, USA), using PD-L1 clone 22C3 (Dako, Agilent, Santa Clara, CA, USA) and atezolizumab (Tecenriq®, Genentech, South, San Francisco, CA, USA), using PD-L1 clone SP-142 (Ventana, Roche, Basel, Switzerland).^{11,12} A third immune checkpoint inhibitor, durvalumab (Imfinzi®, AstraZeneca, Cambridge, UK) has also been approved for use in the setting of urothelial carcinoma, but the associated PD-L1 clone, SP-263 (Ventana, Roche, Basel, Switzerland) currently has no PD-L1 companion diagnostic testing approval.¹³ Finally, nivolumab (Opdivo®, Bristol-Myers Squibb, New York, NY, USA) has similarly been approved for use in this setting, but the associated PD-L1 clone, 28 – 8 (Dako, Agilent, Santa Clara, CA, USA) also currently has no companion diagnostic approval.¹⁴

Patients with locally advanced or metastatic urothelial carcinoma who are not eligible for cisplatin-containing chemotherapy can qualify for monotherapy treatment with pembrolizumab, atezolizumab, or nivolumab. Each of these immune checkpoint inhibitors was FDA approved following a phase 3 clinical trial, and each trial utilized a specific PD-L1 clone, scoring system and expression cutoff value. The PD-L1 testing that accompanies each of these drugs, therefore must use a specific clone, scoring system, and cutoff, and is selected based on which drug is intended for use in the patient. Pembrolizumab requires the use of the 22C3 clone, and requires a combined positive score (CPS) of ≥ 10 .^{11,15} A CPS refers to the sum of positive viable tumor cells (showing membranous staining, irrespective of intensity and completeness) plus the positive, tumor associated lymphocytes and macrophages (membranous or cytoplasmic staining irrespective of intensity) over the total number of tumor cells, multiplied by 100. Qualifying for atezolizumab requires the SP-142 clone, and utilizes an immune cell score (IC) of $\geq 5\%$ of the tumor area, meaning greater than 5% of the tumor area showing positive staining within the immune cells (not the tumor cells).¹⁶ Treatment with nivolumab does not require a companion diagnostic test, however, a complementary diagnostic test is available that utilizes the 28 – 8 clone and a tumor proportion score (TPS) of $\geq 1\%$. TPS is defined as the percentage of tumor cells showing membranous staining (complete or incomplete) at any level of intensity. The variety of PD-L1 antibody clones, scoring systems, and cutoffs used in the clinical trials and in the current literature illustrate the complex nature of PD-L1 testing in general.

Because immune checkpoint inhibitor therapy in UC is quite recent, and given the relative rarity of UTUC, little is known about the prognostic utility of PD-L1 in UTUC, or the status of PD-L1 expression in UTUC in general. UTUC-specific prognostic and predictive models are needed. The aims of this study are to investigate the performance of different FDA-approved PD-L1 clones, and explore any possible association between PD-L1 expression and clinicopathologic features of upper urothelial tract urothelial carcinomas.

Methods

This is a retrospective study of 37 cases of resected UTUC collected at the University of Utah between 2005 and 2014 and performed under appropriate Internal Review Board (IRB) permission (protocol 91019). Two genitourinary pathologists reviewed the H&E slides for each case to confirm diagnosis and associated clinicopathologic features (Table 1).

Table 1
Demographic and Pathologic Features
of 37 cases of Upper Tract Urothelial
Carcinoma

| Feature | n (%) |
|--|---|
| Sex: Female Male | 26 (70.3%) 11 (29.7%) |
| Grade: Low High | 9 (24.3%) 28 (75.7%) |
| Median Age: | 70 years old |
| Pathologic T-Stage: Ta/Tis T1 T2 T3 T4 | 3 (8.1%) 12 (32.4%) 4 (10.8%) 17 (45.9%) 1 (2.7%) |
| Tumor Location: Ureter Pelvis Both | 4 (10.8%) 18 (48.6%) 15 (40.5%) |

Representative tumor tissue from each case was compiled into tissue microarrays and immunohistochemical staining for PD-L1 was performed using 22C3 and 28 – 8 antibody clones (Dako, Agilent, Santa Clara, CA), according to the Dako PD-L1 companion diagnostic package insert and indicated platform. The staining was then evaluated using the three aforementioned scoring systems and the cutoffs were dictated by both the companion diagnostic package insert and/or because of their use in current PD-L1 clinical practice.

Both 22C3 and 28 – 8 stained cases received a TPS, according to the 28 – 8 interpretation manual for urothelial carcinoma.¹⁷ Positive staining was defined as complete circumferential or partial linear cytoplasmic membrane staining at any intensity, and TPS was calculated by dividing the number of tumor cells positive divided by total tumor cell number, multiplied by 100%. Positive PD-L1 expression was defined as a TPS \geq 1%, according to the FDA approved use for 28 – 8 in urothelial carcinomas.

The 22C3 stained cases also received a CPS, according to the 22C3 interpretation manual in urothelial carcinoma.¹⁵ Positive staining was defined as membranous staining of any intensity and of any completeness within the tumor cells, or membranous and/or cytoplasmic staining within the tumor associated lymphocytes and macrophages. Per the interpretation manual, tumor associated lymphocytes and macrophages are

considered those that either infiltrate the tumor cells, abut the tumor cells, or are present within the same 20x field as the tumor cells. CPS was calculated by dividing the number of positive cells (both tumor and immune cells) by the total number of tumor cells, and multiplying by 100. CPS cutoffs of ≥ 10 and ≥ 1 were investigated. These CPS cutoffs were selected because 10 is the FDA approved cutoff for urothelial carcinomas, and 1 is the cutoff utilized for other FDA approved indications (gastric/gastroesophageal junction adenocarcinomas, head and neck squamous cell carcinomas and cervical carcinomas).

An IC score was calculated on the 22C3 stained cases, based on the interpretation manual of SP-142 in urothelial carcinoma. IC staining is scored as the proportion of tumor area covered with any discernable PD-L1 staining of any intensity in immune cells.¹⁶ IC scores of $\geq 5\%$ were considered positive, which is the FDA approved cutoff for SP-142 in urothelial carcinomas.

Two pathologists independently scored all cases and discrepant scores were resolved by consensus review. Finally, Fisher's exact test was used to look for any association between PD-L1 expression (expression vs. non-expression), and clinicopathologic variables, such as gender, grade, stage or age. Stage was stratified into two categorical variables: low stage (including pTis, pT1, or pT2), or high stage (including pT3, pT4). Age was also stratified into 2 groups: < 70 years old, ≥ 70 years old. Potential associations between PD-L1 expression and clinicopathologic features were also investigated utilizing each of the clinically pertinent PD-L1 clones, scoring systems, and positive cutoffs.

Results

Using a TPS threshold of $\geq 1\%$, 22C3 and 28 – 8 clones showed a 100% concordance with regard to overall expression vs. non expression (Fig. 1). Thus, for both 22C3 and 28 – 8, 11 out of the 37 total cases (29.7%) were positive for PD-L1. 10 out of 18 cases (55.6%) with higher pathological stage (pT3 or pT4) were positive for PD-L1 expression, compared to only 1 out of 19 cases (5.3%) of lower pathological stage (pTis, pT1, or pT2), as illustrated in Fig. 2. This difference was statistically significant ($p = 0.0011$).

Using a 22C3 CPS threshold of ≥ 10 , 15 out of 37 cases (40.5%) were positive for PD-L1 expression and 10 out of 18 cases (55.6%) with higher pathological stage were positive, compared to 5 out of 19 (26.3%) of cases with lower pathological stage. This difference was not statistically significant ($p = 0.0991$).

Using a 22C3 CPS threshold of ≥ 1 , 32 out of 37 cases (86.5%) were positive. 17 out of 18 cases (94.4%) with higher pathological stage were positive for PD-L1 expression, compared to 15 out of 19 (78.9%) of cases with lower pathological stage. This difference was not statistically significant ($p = 0.3398$).

Using an IC threshold of $\geq 5\%$ calculated on the 22C3 stained cases, 18 out of 37 cases (48.6%) were positive. 10 out of 18 cases (55.6%) with higher pathological stage were positive for PD-L1 expression, compared to 8 out of 19 (42.1%) of cases with lower pathological stage. This difference was not statistically significant ($p = 0.5171$).

No other significant associations were identified between PD-L1 expression (irrespective of antibody clone, scoring system or cutoff) and gender, histological grade (low vs. high) or age (Table 2).

Table 2
Association of Clinicopathologic Features and PD-L1 Expression

| | % PD-L1 Positive (Using TPS \geq 1) | P value | % PD-L1 Positive (Using CPS \geq 10) | P value | % PD-L1 Positive (Using CPS \geq 1) | P value | % PD-L1 Positive (Using IC \geq 5%) | P value |
|---|---------------------------------------|---------|--|---------|---------------------------------------|---------|---------------------------------------|---------|
| Sex: Female Male | 3/11 (27.3%) 8/26 (30.8%) | 1.000 | 4/11 (36.4%) 11/26 (42.3%) | 1.000 | 11/11 (100%) 21/26 (80.8%) | 0.2947 | 4/11 (36.3%) 14/26 (53.8%) | 0.4756 |
| Grade: Low High | 2/9 (22.2%) 9/28 (32.1%) | 0.6946 | 2/9 (22.2%) 13/28 (44.4%) | 0.2616 | 8/9 (88.9%) 24/28 (85.7%) | 1.000 | 5/9 (55.6%) 13/28 (46.4%) | 0.7140 |
| Pathologic T-Stage: Low (Ta/Tis, T1, or T2) High (T3 or T4) | 1/19 (5.3%) 10/18 (55.6%) | 0.0011* | 5/19 (26.3%) 10/18 (55.6%) | 0.0991 | 15/19 (78.9%) 17/18 (94.4%) | 0.3398 | 8/19 (42.1%) 10/18 (55.6%) | 0.5171 |
| Tumor Location: Renal Pelvis Ureteral/Ureteropelvic | 5/18 (27.8%) 6/19 (31.6%) | 1.000 | 8/18 (44.4%) 7/19 (36.8%) | 0.7431 | 14/18 (77.8%) 18/19 (94.7%) | 0.1797 | 9/18 (50%) 9/19 (47.4%) | 1.000 |
| Age: Below Median Age (< 70 yo) Median Age or Higher (\geq 70 yo) | 5/18 (27.8%) 6/19 (31.6%) | 1.000 | 6/18 (33.3%) 9/19 (47.4%) | 0.5077 | 14/18 (77.8%) 18/19 (94.7%) | 0.1797 | 9/18 (50%) 9/19 (47.4%) | 1.000 |

Discussion:

UTUC is a rare subclass, and as such, treatment guidelines for UTUC are generally extrapolated from more readily available bladder UC data. Our study includes a relatively small number of patients (n = 37) in terms of statistical power, but is highly valuable because it looks specifically at UTUC as a subgroup, rather than extrapolating from UC data. Additional UTUC-specific research, particularly focusing on clinical outcomes after ICI therapy, would be particularly helpful. While our study is retrospective, clinical outcomes data cannot be added because the study was designed and the specimens were obtained prior to FDA approval of immune checkpoint inhibitor treatment for urothelial carcinoma. Moreover, the tissue microarray utilized was de-identified, precluding any subsequent clinical data inquiry.

Because of the rarity of UTUC specimens, our study only included one case of pT4 disease. Despite this possible limitation, the statistical analysis looked at high stage tumors as a group (including pT3 and pT4), and when pT3 tumors are included, there were 18 cases designated as high pathologic stage.

Another potential limitation of this analysis was that the 22C3 was used instead of the FDA approved SP-142 clone which is normally used for IC score calculation, as this was not available. Nevertheless, studies looking at

concordance between PD-L1 clone 22C3 and SP-142 have observed concordance when using respective clinically established scoring systems and cutoffs, and at least one study suggests that 22C3 and SP-142 may show good concordance for IC in urothelial carcinomas.¹⁸

Conclusions:

Our study indicates that there is a significant association between higher pathologic stage UTUC (pT₃ or pT₄) and PD-L1 staining when defined as TPS \geq 1%. There is a similar trend towards increased PD-L1 expression in high stage UTUC when using a CPS cutoff of \geq 10, but the trend fails to achieve statistical significance. An association between high stage UTUC and PD-L1 TPS expression suggests that immune evasion may play a more important role in late stage upper tract urothelial carcinomas. This finding may also have clinical utility, especially in biopsy cases and/or cases where the pathologic stage is not readily appreciable, as increased PD-L1 staining may suggest a higher overall higher pathologic stage. As such, reporting the TPS (using a positive cutoff of \geq 1%), in addition to the FDA-approved CPS may be helpful. In addition, our study shows that the overall rate of PD-L1 positivity in UTUC is 30–50% (depending on the threshold used), which is comparable or slightly higher than the published 20–30% PD-L1 positivity reported in UC of the bladder. Finally, our study indicates that PD-L1 22C3 and 28 – 8 clones show very similar patterns of staining in this setting.

Abbreviations

UTUC: Upper tract urothelial carcinoma; UC: Urothelial carcinoma; TPS: Tumor proportion score; CPS: Combined positive score; IC: Immune cell

Declarations

Ethics approval and consent to participate: Approved under appropriate University of Utah Internal Review Board (IRB) permission (protocol 91019)

Consent for publication: not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article

Competing Interests: The authors declare that they have no competing interests

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Authors Contributions: GD developed the idea for the project and GD and MW were major contributors in writing the manuscript. DA analyzed the histopathologic features of each upper tract urothelial tumor and helped create a tissue microarray. All authors helped score PD-L1 immunohistochemistry, and read and approved the final manuscript.

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Figures

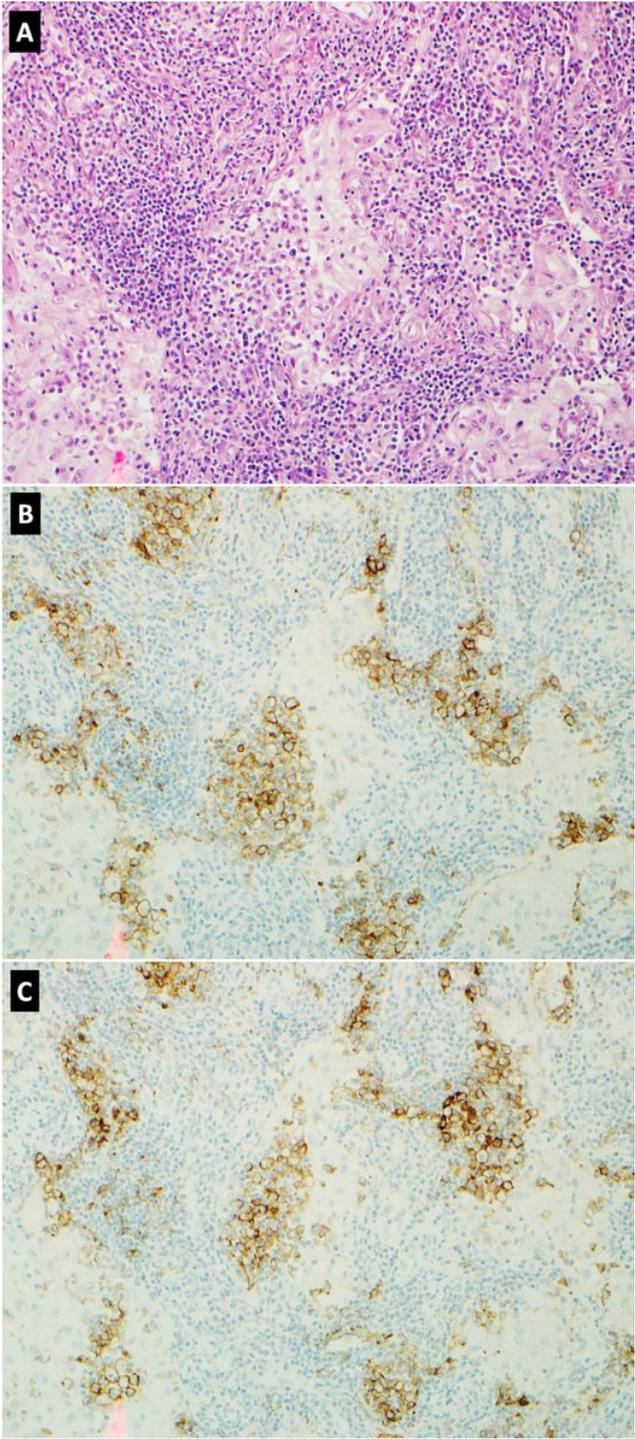


Figure 1

Example of a stage 3, high grade UTUC showing similar staining characteristic with both of the available PD-L1 clones a. H and E section b. PD-L1 staining with 28-8 Clone c. PD-L1 staining with 223C Clone

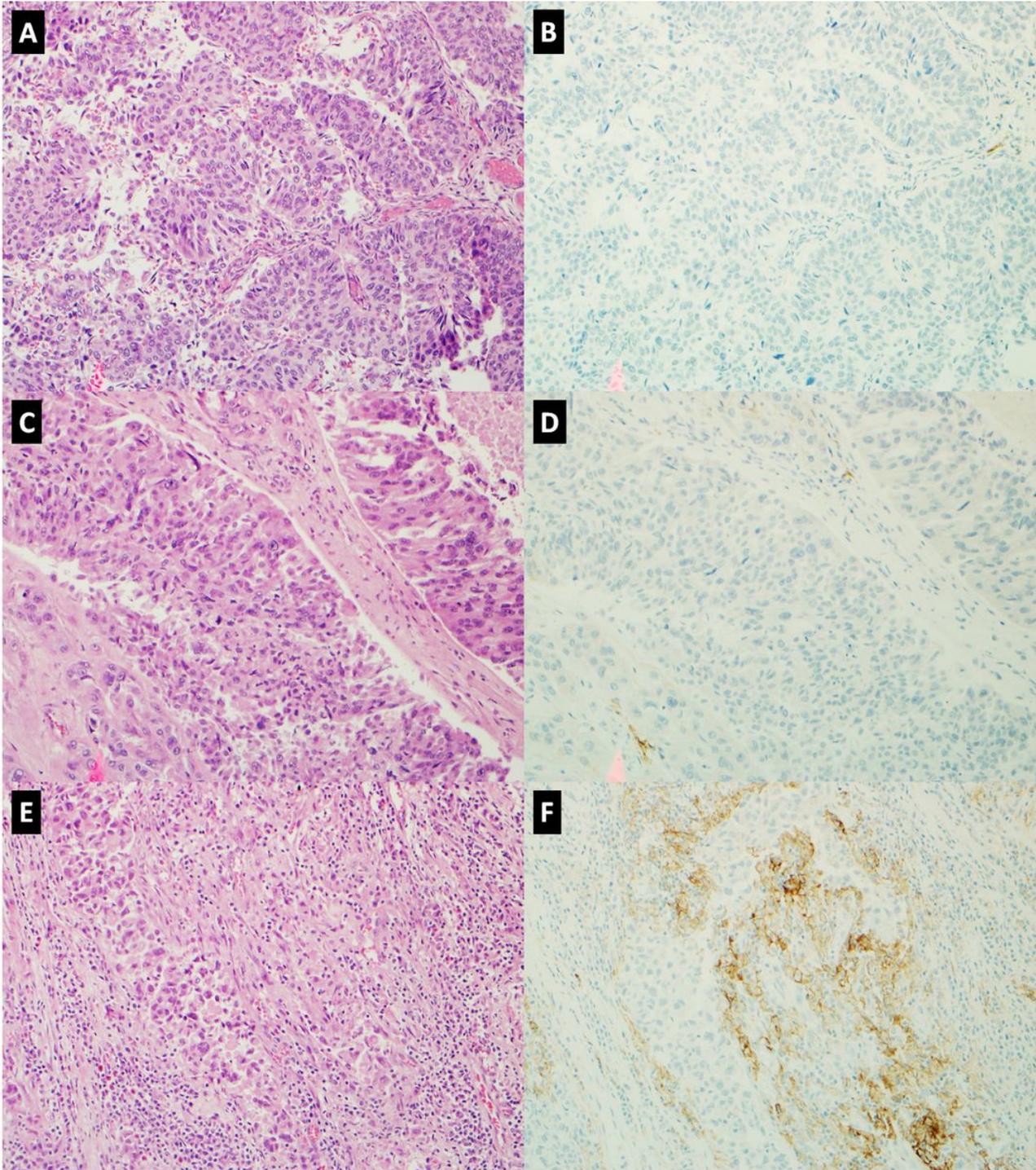


Figure 2

H and E sections and PD-L1 staining in high grade UTUC of different tumor stages a,b. Tis High Grade UTUC c,d. T1 High Grade UTUC e,f. T3 High Grade UTUC.