BACKGROUND: Cancer immunotherapy with monoclonal antibodies targeting the PD-1/PD-L1 axis induces prominent and lasting clinical responses in patients with diverse tumor types. In bladder cancer (BC), the PD-1 blocking antibody pembrolizumab and the anti PD-L1 antibody atezolizumab have shown unprecedented clinical benefit in patients with metastatic disease. Expression of PD-L1 and elevated tumor infiltrating lymphocytes (TILs) has been consistently associated with response to PD-1 axis blockers in advanced solid tumors. However, the expression of PD-L1 and TILs in early stage BC remains poorly understood. Here, we measured the levels of PD-L1 protein and TILs in a retrospective collection of clinically localized tumors and determined their association with major clinical and pathological variables.

METHODS: Formalin-fixed, paraffin-embedded samples from 91 clinically localized BCs were obtained from a cohort of patients treated between 2012 and 2014 in our institution and represented in tissue microarray (TMA) format. Each TMA included 4 representative cores from each tumor, 3 positive and 2 negative control samples. PD-L1 immunohistochemistry was performed using the E1L3N XP antibody (Cell Signaling Technology). TILs were evaluated using Hematoxilin-Eosin stained preparations. Semi-quantitative scores for PD-L1 protein levels in tumor cells and TILs in the tumor and stromal compartment were assessed by a pathologist using 5% increments and represented as means for each case. Association with clinical and pathological variables was determined using descriptive statistics.

RESULTS: Tumor stage distribution was as follows: Ta=49 (54%); Tis=3 (3%); T1=23 (25%) and T2=16 (18%). Most cases were high grade tumors (65 vs. 35%). Tumor PD-L1 signal was detected in all cases with a median score of 9 positive cells and a mean of 19%. Stromal TILs were also identified in all cases and showed a median of 5 and a mean of 10%. Intratumoral TILs were significantly lower than those in the stromal compartment ($p<0.001$). There was no significant correlation between PD-L1 levels and TILs in the studied samples. Likewise, no significant association was found between PD-L1 levels and histological grade, tumor stage, age, sex, presence of lymphovascular invasion and previous history of BCG treatment.

CONCLUSIONS: PD-L1 and TILs are expressed in early-stage BCs, but the levels are lower than those reported in advanced disease. The lack of association between PD-L1 and TILs suggests Interferon gamma-independent PD-L1 upregulation in this setting. The absence of significant relationship between the markers and major clinical and pathological variables suggests homogenous adaptive immune pressure/immunoediting in bladder-restricted disease. Further studies will be required to validate our findings in an independent population and determine the therapeutic potential of PD-1 axis blockade in clinically localized BC.