

Title: Spatial Analysis of the tumor microenvironment reveals an immunosuppressive phenotype of tertiary lymphoid structures in localized high-risk and progressive prostate cancer

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Background:

Prostate cancer (PC) has historically been considered immunologically “cold” due to low immune infiltration and a predominantly immunosuppressive tumor microenvironment (TME). However, this understanding is largely based on immune profiling studies in metastatic androgen pathway modulation resistant (APMR) disease. The composition and organization of the immune TME in primary PC remains poorly defined, especially in relation to Gleason score and progression status. We hypothesized that the local PC immune TME is more heterogeneous than previously appreciated, with differences in immune composition and spatial organization between High Gleason (HG) and Low Gleason (LG) PC and between progressors and non-progressors.

Methods:

We analyzed 29 radical prostatectomy samples (15 LG, 14 HG, 20 non-progressors, 9 progressors) using a 40-marker multiplex immunofluorescence panel to profile the TME at single-cell resolution. Immune cell phenotypes were defined with respect to the marker panel using pattern-based relative expression of cells. Cell composition was computed within tumor, stroma, and tertiary lymphoid structures (TLSs). Patients were classified as progressors if they had biochemical or radiographic evidence of recurrence.

Results:

We discovered that HG PC exhibited higher total immune infiltration ($p < 0.05$) and specifically TLSs ($p < 0.05$) relative to LG PC. Furthermore, advancing Gleason grade was associated with increased T cell infiltration ($p < 0.05$) and tumor-promoting M2-like tumor-associated macrophage (TAM) density ($p < 0.05$). Strikingly, TLSs were enriched in exhausted (PD1+) CD4 and CD8 T cells, especially near Tregs ($p < 0.001$) and demonstrated a higher M2-like to M1-like macrophage ratio compared to surrounding tumor ($p < 0.001$) and stroma ($p < 0.05$). TLSs in patients that progressed had increased proportions of PD1+CD8T cells and PD1+CD4 T cells ($p < 0.001$, $p < 0.01$).

Conclusions:

Our findings challenge the notion that primary PC is uniformly immunologically inert. We show that HG PC has increased levels of immune infiltration. We describe a paradoxically immunosuppressive TLS phenotype in HG PC, where TLS harbor high levels of exhausted T cells near Tregs and have an unfavorable tumor-promoting M2-like TAM composition. Furthermore, we show that disease progression is associated with exhausted TLS characteristics. This data suggests that HG PC and progressive disease

have a distinct immune microenvironment with unfavorable TLS biology. The spatial organization of immunosuppressive cells in HG PC and in patients that progressed provides a rationale for novel immunotherapeutic targeting of the TLS-rich immunosuppressive landscape of HG PC to improve clinical outcomes in the neoadjuvant setting.

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Conflicts of Interest Disclosure:

The authors have no conflicts of interest relevant to this abstract to disclose.

Immune Composition in Low vs. High Gleason Tumors. **A.** Total immune fraction across tumor and stroma, showing significant differences between HG and LG Gleason samples. **B.** Mean number of cells within TLSs varies between HG and LG Gleason samples. **C.** TLS counts per patient sample. **D.** Treg (CD4FOXP3) proportion is higher in HG stroma and HG tumor TLSs. **E.** Density of M2-like macrophages by Gleason status is higher in HG tumor. **F.** Increasing Gleason grade groups (g1 to g5) correlate with higher counts of helper, cytotoxic, and regulatory T cells. **G.** M2-like macrophage density is significantly increased in g5 relative to g1 and g2. Gleason grade groups are labeled as: g1 = 3+3, g2 = 3+4, g3 = 4+3, g4 = 4+4, and g5 = 4+5, 5+4, 5+5. **H.** Proportion of exhausted (PD1+) CD8T and CD4T cells to non-exhausted (PD1-) T cells in TLSs in high and low Gleason tumors showing increases in T cell exhaustion in high Gleason TLSs. **I.** The ratio of M2-like to M1-like macrophages by compartment is increased in TLSs. **J-K.** Proportion of PD1+ Cytotoxic T cells (K) and PD1+ Helper T cells (L) within TLSs is higher in progressors. Statistics assessed by Wilcoxon Rank Sum Test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).