

Whole-slide CyCIF and archive-scale H&E digitization characterize immune-organized primary prostate cancer

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Background

While historically considered an immune “cold” tumor, a subset of treatment-naïve prostate cancer instead harbors a lymphocyte-rich immune response that may be targetable with new immunotherapy strategies. As has been well-demonstrated in other solid tumors, the spatial organization of lymphocytes, in particular tertiary lymphoid structures (TLS), may primarily carry biological and prognostic information in prostate cancer. Here we investigate the features of immune-organized primary prostate cancer and their population prevalence using multiplex immunofluorescence and H&E analysis.

Methods

We performed whole-slide cyclic immunofluorescence (CyCIF) on 29 radical prostatectomy specimens stratified by grade (high Gleason grade [HGG], n=14; low [LGG], n=15). Lymphocyte organization was parameterized using a new immune-organization package we developed for single-cell spatial data: *tlsR*. CyCIF identified TCF1⁺ Tpex cells, CD21⁺CD23⁺ follicular dendritic cells, and other cell types diagnostic of bona fide TLSes. To extend immune cluster (IC) analysis to H&E images, we digitized over 20,000 genitourinary H&E slides (2001–2025) from the Gelb Center, Dana-Farber Cancer Institute. Images were scanned to 40x resolution and with extensive pathologist-in-the-loop quality control, label extraction, and medical record number linkage. We identified over 200 additional prostate-cancer cases with H&E features of TLSes for further investigation.

Results

By CyCIF, HGG tumors were significantly more lymphocyte-infiltrated than LGG, with lymphocytes organized into discrete B cell-rich and T cell-rich ICs. Many HGG clusters showed B/T zonation and CD21⁺CD23⁺ FDC networks and were classified as bona fide TLSes, although the majority of ICs did not meet these criteria. True TLS-containing tumors were enriched for precursor exhausted T cells (Tpex) and proliferating B cells, with adjacent tumor compartments containing granzyme B⁺ cytotoxic T cells. To digitize over 20,000 glass H&E slides at-scale, we partnered with HistoWiz to scan approximately 900 slides/day with >90% passing initial quality control. Images were stored as pyramidal OME-TIFF at 0.248 µm/pixel, and OCR resolved most printed labels (~20% of handwritten labels required manual correction) before identifiers were linked to clinical data. Using this dataset, screened over 5000 slides (~4500 low-grade, 500 high-grade; multiple slides per case) and identified an additional 200 patients with TLS-like immune clusters. We are pursuing additional CyCIF, exome sequencing and RNA sequencing on a subset of these cases to identify clinical and genomic features of TLS-rich localized prostate cancer.

Conclusions

Immune cluster-rich prostate cancer marks a subset of localized prostate cancer. High-plex imaging identifies that primarily high-grade cases contain bona-fide TLSes, whereas low-grade prostate cancer is generally more immune-depleted and with less immune organization. Digitization of >20,000 H&E slides at the DFCI Gelb Center will allow for population-scale immune cluster analysis using computational and manual pathology methods.

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Conflicts of Interest Disclosure Statement

A.P. (Parvatikar), A.C., E.P., and R.T. are employees of HistoWiz Inc. P.K.S. is a cofounder and member of the Board of Directors of Glencoe Software and a member of the Scientific Advisory Board for RareCyte and Montai Health; he holds equity in Glencoe and RareCyte. J.W. is a consultant for Echo Immunotherapeutics. P.K.S. is a consultant for Merck. E.V.A is consulting for Enara Bio, Manifold Bio, Monte Rosa, Novartis Institute for Biomedical Research, Serinus Bio, and TracerBio, hold equity in Tango Therapeutics, Genome Medical, Genomic Life, Enara Bio, Manifold Bio, Microsoft, Monte Rosa, Riva Therapeutics, Serinus Bio, Syapse, and TracerDx; editorial board in Science Advances; received funding from Novartis, BMS, Sanofi, NextPoint. A.P. is consulting for Exelixis, Tolmar Therapeutics, Janssen, Novartis and BostonGene and received research support from BMS, Clovis Oncology, Progenics, Janssen, Laekna, Astrazeneca, Xencor, Zenith, and Exelixis. The remaining authors declare no competing interests.

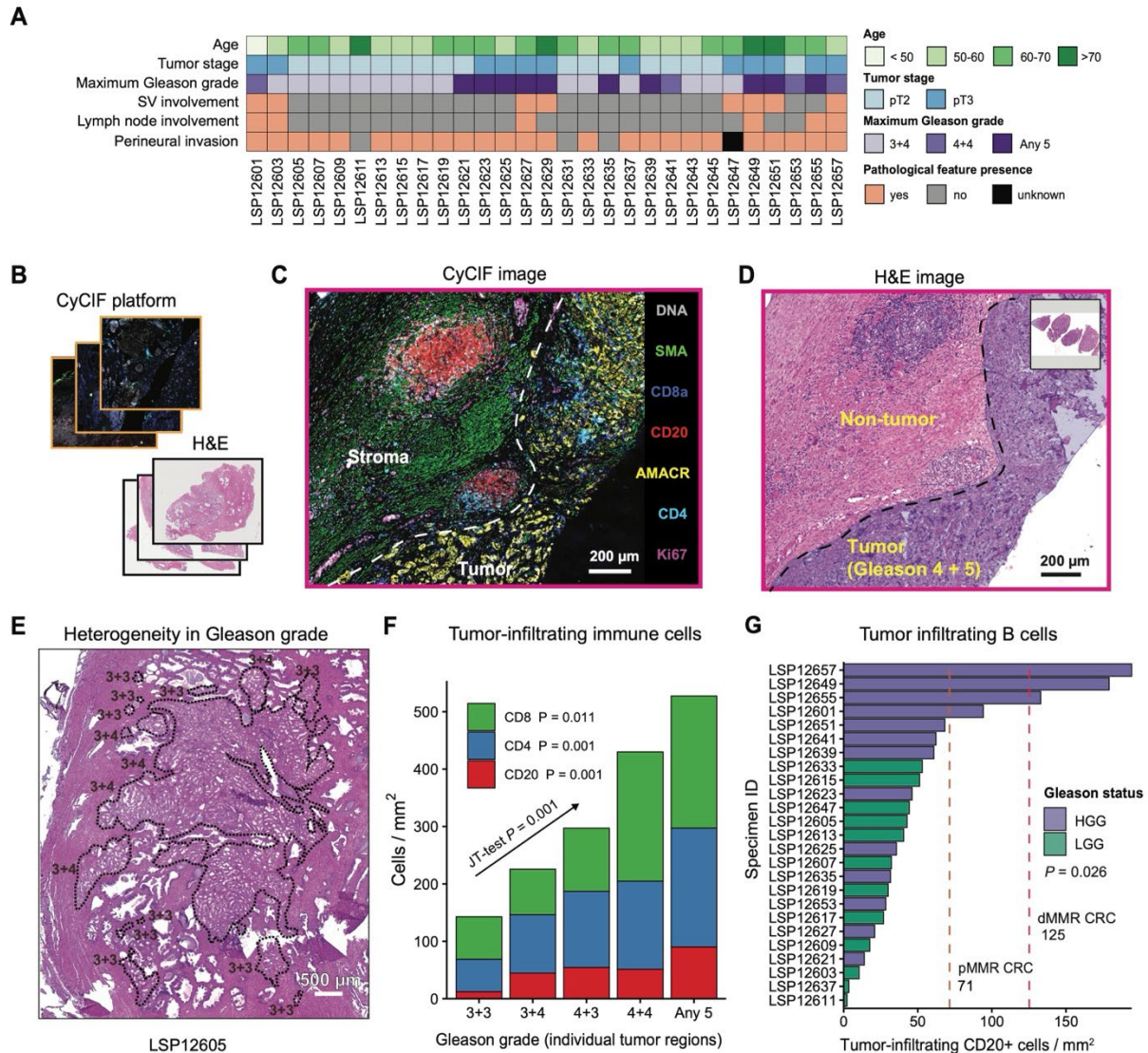


Figure 1: Whole slide CyCIF imaging reveals elevated B and T cell infiltration in high Gleason grade (HGG) PCa. **A**, Cohort overview with key clinical annotations. **B**, Experimental workflow: whole-slide cyclic multiplex immunofluorescence (CyCIF) and adjacent H&E imaging was performed on whole-slide radical-prostatectomy specimens. **C**, CyCIF image with selected markers shown; white dashed line separates the tumor and stroma compartment. **D**, Adjacent H&E image of the same specimen as in panel (C). **E**, Exemplar H&E image with individual tumor domains annotated with Gleason grades. **F**, Infiltration of B cells, CD4 T cells, and CD8 T cells per mm² across Gleason grade groups G1–G5 (“3 + 3”, “3 + 4”, “4 + 3”, “4 + 4”, and “any 5”, respectively) within individually annotated tumor compartments (as in panel E) for all samples. The Jonckheere–Terpstra trend test (*P* value displayed) confirms an increasing lymphocytic immune-cell proportion with grade. Linear fit *P* values for each individual cell type are also shown (*F*-test). **G**, Density of intratumoral CD20⁺ B cells (cells per mm²) by Gleason grade. The one-sided Mann-Whitney U test *P* value compares high- vs. low-grade disease; dashed lines indicate median reference densities in colorectal cancer as indicated. Point-wise one-sided Wilcoxon test *P* values for adjacent grade comparisons are indicated.