Integrative analyses of patterns of CD3 T-cell distribution in metastatic hormone-sensitive and castration-resistant prostate cancer

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Background: The development of bispecific T-cell engagers (BiTES) targeting tumour antigens and CD3 has increased interest in T-cell infiltration in metastatic castration-resistant prostate cancer (mCRPC). To facilitate this, the immune landscape of mCRPC needs to be better understood. This study characterises CD3+ T-cell distribution in mCRPC using advanced spatial imaging and molecular profiling techniques, exploring clinical implications and utility.

Methods: We analysed 847 prostate cancer biopsies, including matched hormone-sensitive prostate cancer (HSPC) and mCRPC samples, using CD3 immunohistochemistry (IHC) and deep learning-based image analysis. Spatial distribution of CD3+ cells was examined using custom algorithms that quantified cell density across the tumour-stroma interface. A subset of samples underwent hyperplex immunofluorescence (IF) to further characterise the immune cell composition. Genomic profiling, including targeted DNA sequencing and RNA sequencing, was performed on selected samples. Survival analysis was conducted using Cox proportional hazards models, adjusting for established prognostic factors in mCRPC.

Results: We observed a significant decrease in CD3+ T-cell density from HSPC to mCRPC (median 260 vs 90.5 cells/mm², $p<0.001$). However, a subset of mCRPC samples showed high CD3+ infiltration. Spatial analysis revealed multiple distinct patterns of CD3+ cell distribution across the tumour-stroma interface (Figure 1). These patterns were independently associated with overall survival in multivariate analysis. High intratumour and stromal CD3+ density (spatial group 6) associated with better prognosis; low CD3+ cell density associated with worse prognosis (HR 1.63, 95% CI: 1.17-2.28, $p=0.00428$). Interestingly, high stromal but low intratumour CD3+ density (spatial group 5) also associated with poor outcomes (HR 1.42, 95% CI: 0.91-2.21, $p=0.12672$; Figure 2). Genomic analysis revealed an association between PTEN loss and reduced CD3+ infiltration. mCRPC with high intratumour CD3+ cells in RNAseq analyses had upregulated inflammation-related gene sets, T cell, B cell and Senescence-Associated Secretory Phenotype (SASP) associated genes in mCRPC. Hyperplex IF analysis confirmed these findings and provided further insights into the immune cell composition of these tumours (Figure 3).

Conclusions: mCRPC can be subclassified by distinct spatial distribution patterns of CD3+ cells around the tumour-stromal interface that correlate with survival independently of established prognostic factors. These findings may have significant implications for immunotherapy strategies, including CD3 targeting BiTES.

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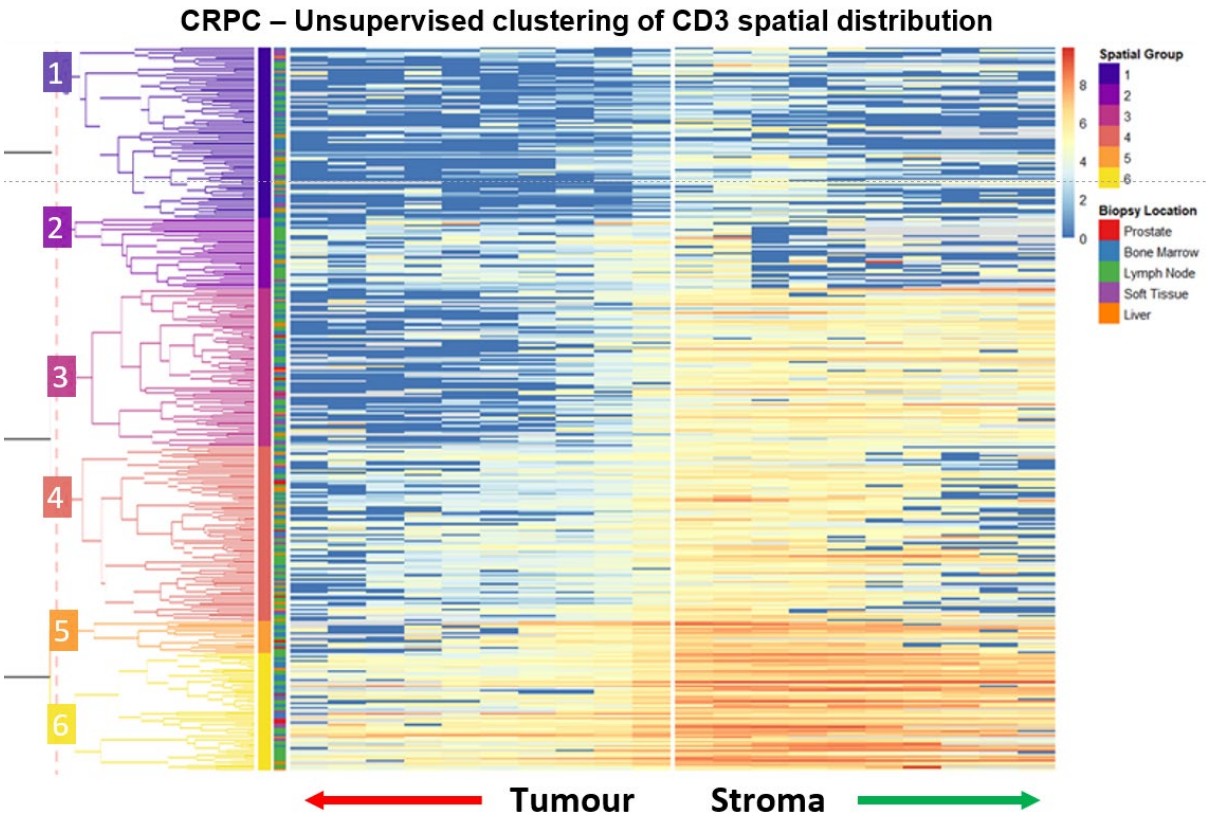

Figure 1: CD3 IHC spatial distribution heatmap

Multivariate Cox model for tumour CD3 density, spatial features and clinical variables

Variable		N	Hazard ratio	p
CRPC to Biopsy Duration		369	1.00 (1.00, 1.00)	<0.001*
Tumour CD3 Density (mm²)		369	1.00 (1.00, 1.00)	0.008*
Spatial Group	6	64	Reference	
	5	31	1.62 (1.01, 2.62)	0.047*
	4	69	1.28 (0.85, 1.91)	0.241
	3	90	1.38 (0.92, 2.06)	0.116
	2	23	1.86 (1.06, 3.25)	0.030*
	1	92	1.58 (1.04, 2.38)	0.031*
log(Neuts)		369	1.30 (1.01, 1.69)	0.044*
log(Lymphs)		369	0.88 (0.71, 1.09)	0.229
log(ALP)		369	1.15 (0.98, 1.35)	0.094
log(PSA)		369	1.15 (1.08, 1.22)	<0.001*
Hemoglobin	Normal	176	Reference	
	Low	193	1.16 (0.91, 1.47)	0.234
LDH	Normal	232	Reference	
	High	137	2.15 (1.66, 2.78)	<0.001*
ECOG	0	48	Reference	
	≥1	321	1.77 (1.23, 2.55)	0.002*
Liver Metastasis	No	291	Reference	
	Yes	78	1.69 (1.26, 2.24)	<0.001*

Figure 2: Multivariate Cox analysis

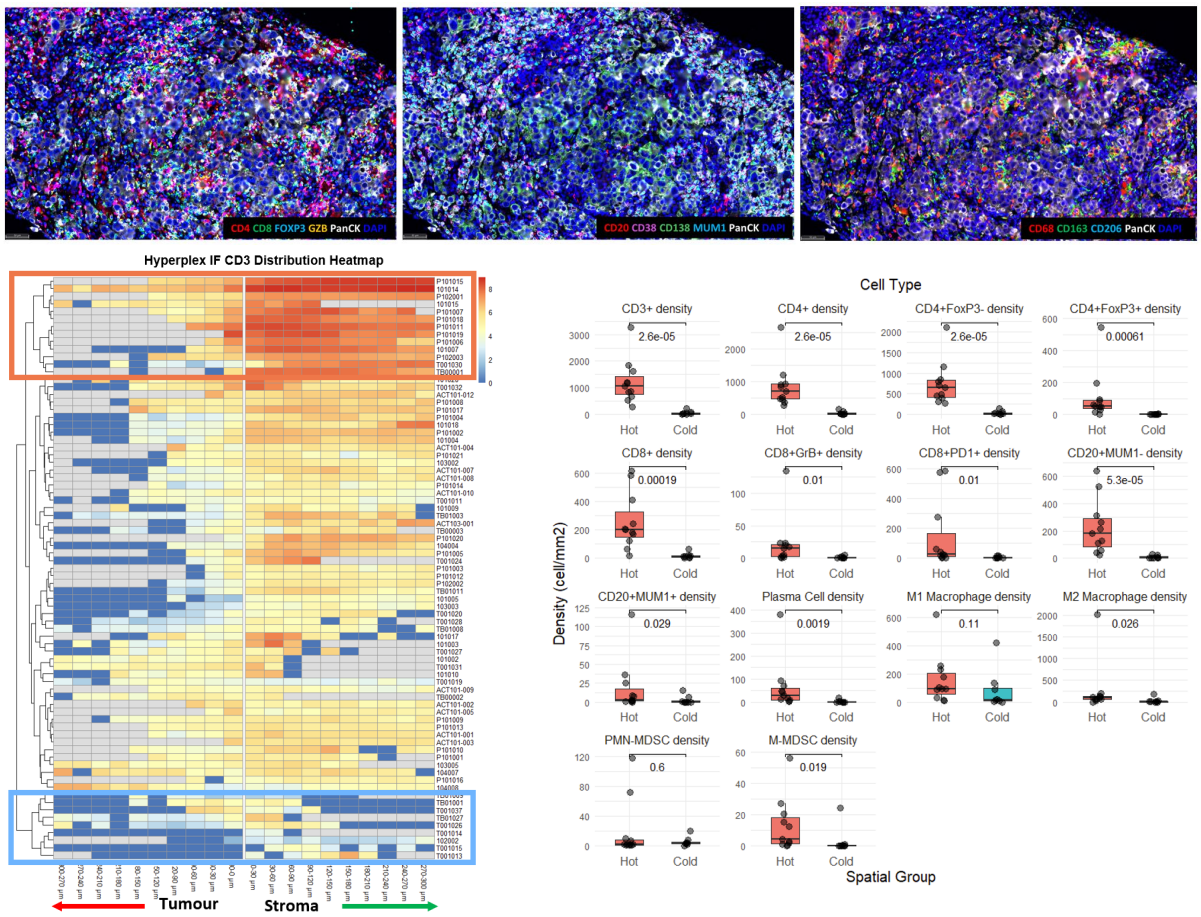

Figure 3: Hyperplex IF-based spatial analyses