Spatial profiling reveals molecular determinants of CD8+ dynamics during chemo-immunotherapy in MSS colon cancer

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Immune checkpoint inhibitors (ICIs) have led to paradigm shifts in the treatment of several tumour types, yet microsatellite stable (MSS) colon cancer remains a major challenge and currently has no approvals for ICI-based therapies. Nevertheless, recent neoadjuvant trials introducing ICIs to early-stage MSS colon tumours have reported encouraging findings. To further improve ICI efficacy on this subset, it is first necessary to mechanistically understand how ICIs function in this otherwise immune-cold disease.

Previous studies profiling pre- and post-treatment ICI colorectal tumors (e.g., Feng et al., Nat Commun 2024) are compromised by significant sampling bias in post-treatment samples: our analysis reveals that responders are primarily composed of normal adjacent stroma while non-responders are predominantly tumor core. To overcome this limitation and characterize the effect of treatment in action, we utilized paired pre- and on-treatment samples from a window-of-opportunity study administering pembrolizumab (anti-PD-1) with XELOX chemotherapy in non-metastatic MSS colon cancer. Pre-treatment samples were composed of 3-4 biopsy pieces, while on-treatment samples were obtained from surgical resection following two cycles of therapy. To achieve a comprehensive spatial overview, large pieces from on-treatment samples (1 cm x 2 cm, encompassing both tumor nest and peripheral stroma) were selected for profiling. Using high-resolution spatial transcriptomics (10x Genomics Xenium), we profiled both pre- and on-treatment samples, generating ~17 million cells across 7 patients with detailed cell-state annotations.

As this is a window-of-opportunity study, clinical response evaluation was not the primary endpoint. Instead, to approximate treatment effect, we focused on CD8+ T-cell infiltration as a key indicator of immune modulation. Consistent with the immune-cold phenotype of MSS disease, only a subset of cases (2/7) showed increased intratumoural CD8+ effector T-cell infiltration during treatment. In contrast, most tumours (5/7) exhibited increased CD8+ pre-effector T cells restricted to peripheral stroma, showing an immune-excluded phenotype.

To link on-treatment CD8+ T-cell localization to local molecular signals, we developed linear regression models that predict CD8+ T-cell spatial distribution from secreted-factor expression. Distinct sets of secreted factors were found to be spatially associated with the two CD8+ T-cell populations: factors associated with infiltrating effector T cells included chemokines like CXCL9, CXCL10, and CXCL11 among others, whereas factors associated with stroma-restricted pre-effector T cells included CXCL12 and a broader collection of suppressive programmes. Our analysis identified specific fibroblast and macrophage states as the source of these secreted factors, suggesting distinct infiltration- and stroma-restricted immune neighborhoods. Furthermore, the infiltration- and exclusion-associated programmes were recapitulated in independent validation datasets, including on-treatment breast cancer samples from a window-of-opportunity trial (n=40; Bassez et al., Nat Med 2021) and treatment-naïve primary colorectal tumours.

Overall, our study provides a comprehensive, high-resolution spatial view into how neoadjuvant chemo-immunotherapy remodels the MSS tumour microenvironment. Notably, key findings, including pre-effector T cells and their associated secreted programs, were predominantly detected at the peripheral stroma, highlighting the critical importance of characterizing this often-overlooked region. By revealing distinct spatial molecular programs that govern T-cell infiltration versus stroma-restriction, our work identifies potential therapeutic strategies to overcome immune exclusion in MSS colon cancer.