Defining PD-1/PD-L1 receptor-ligand interactions in the head and neck cancer tumor microenvironment identifies unique 'immune cell rivers' of cellular interactions

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Introduction

Head and neck cancer encompasses a group of malignancies that arise in the oral cavity, pharynx, larynx, sinuses, and salivary glands, often linked to tobacco use, alcohol consumption, and human papillomavirus (HPV) infection. Immune checkpoint inhibitors have shown benefit in both HPV positive and negative head and neck cancers, yet biomarkers predictive of benefit are currently needed. While PD-L1 is a commonly used biomarker, it does not reflect a dynamically changing tumour microenvironment. To address this challenge, we have here applied an in situ proximity ligation detection assay (isPLA) of PD-1/PD-L1 interactions (Navinci Technology) to visualise these receptor-ligand activity on spatially mapped head and neck cancer tissues.

METHODS

This study has Human Research Ethics Approval (HREC) from the Royal Brisbane & Women's Hospital and Princess Alexandra Hospital. The study was approved by the University of Queensland. Formalin-Fixed Paraffin Embedded (FFPE) sections from 18 Head and Neck squamous cancer carcinoma (HNSCC) tissue samples were subjected to staining using a 60 plex Phenocycler Fusion (Akoya Biosciences) panel followed by PD-1/PD-L1 isPLA on serial or same sections. Tissues were quantified for isPLA signal, cell types and cellular neighborhoods.

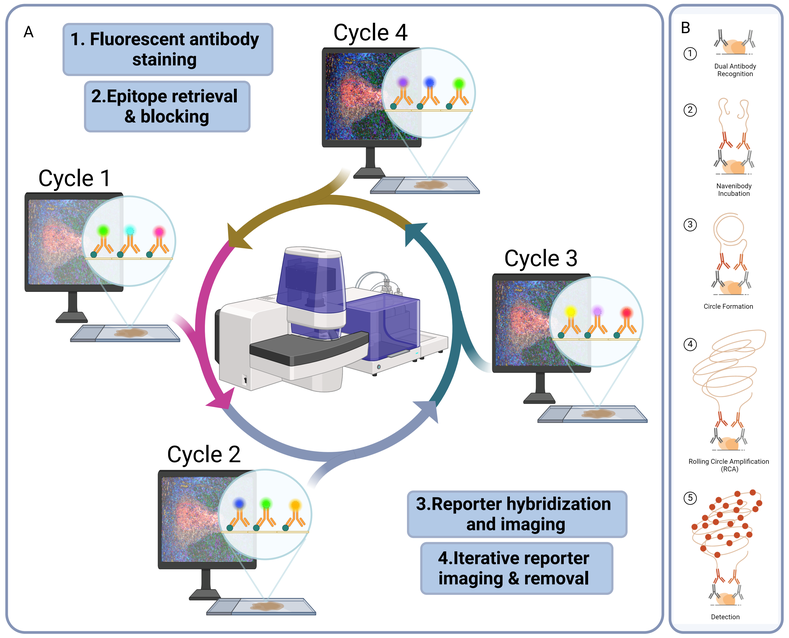


Figure 1. A) Spatial phenotyping of the tumor section using Phenocycler Fusion system. **B)** Navinci isPLA workflow

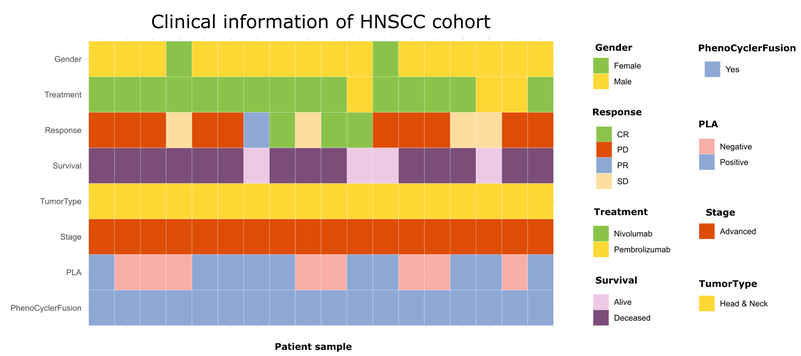


Figure 2. The clinicopathological information for the HNSCC cohort of 18 patients including gender, response, treatment, stage, and survival.

Results

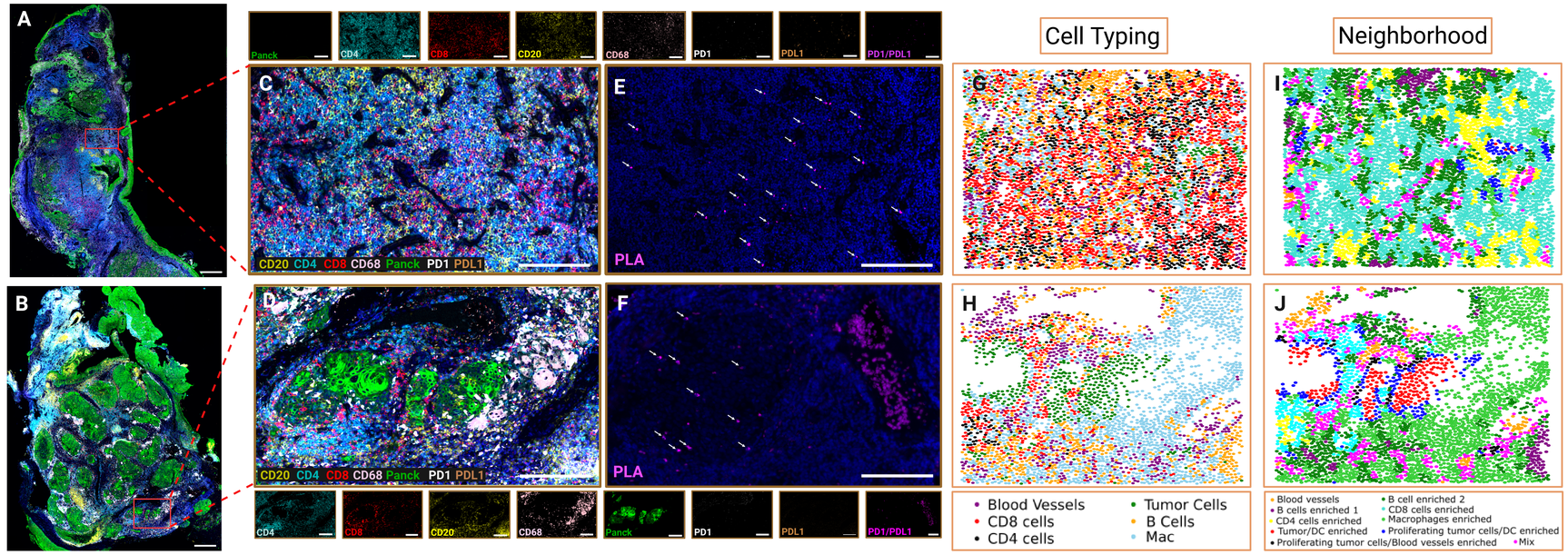


Figure 3. Spatial analysis utilizing Multiplex and isPLA technique. **A, B)** Comprehensive multiplex images of two distinct head and neck cancer tissues, captured through PhenoCycler-Fusion. **C, D)** Multiplex images that highlight selected markers, along with their corresponding channels. **E, F)** isPLA images, pinpointing the regions positive for PD-1/PD-L1 interactions, corresponding to the same areas as depicted in panels C and D, with the PLA channel activated. **G, H)** Analysis of Cellular Neighborhoods and Cell Typing, employing Cluster-Based Annotation Techniques. This section elucidates the cluster annotation process by which individual cells are categorized into distinct cell types identified within the sample. Each color denotes a unique cell type, facilitating visual differentiation and enumeration. Panels G and H display the predominant cell types within the tissue, represented by varying colors. **I, J)** Cellular neighborhoods in relation to the clustering algorithm. scale bar = 100 um.

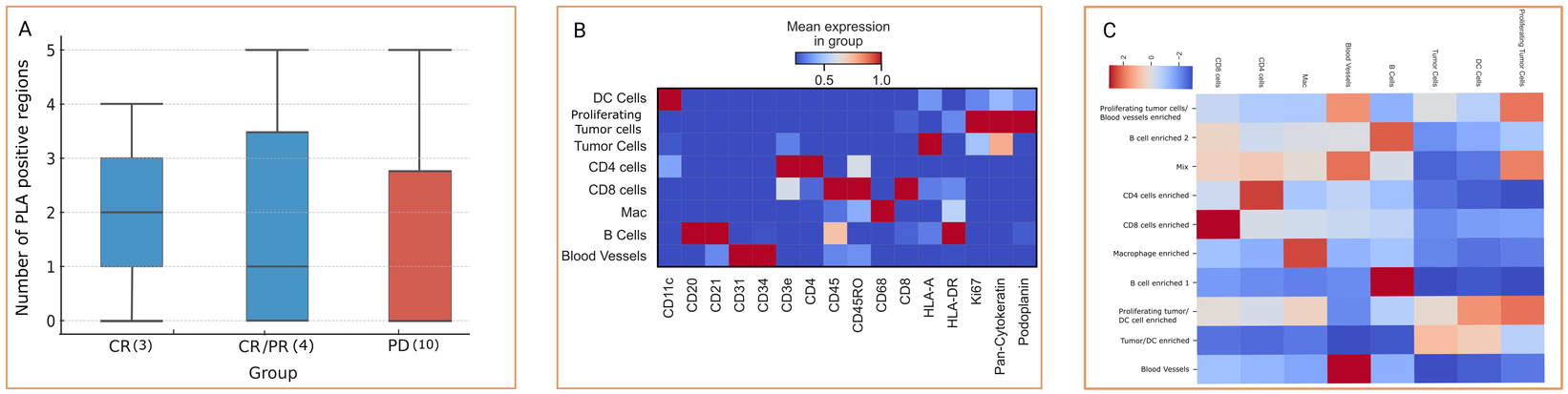


Figure 4. A) Comparative Analysis of Response Categories in Patients with Positive isPLA Results. The figure presents three separate analyses comparing the distribution of 'Number of Positive Regions' between different patient response groups. Positive regions defined as a area with at least 10 isPLA signals for PD-1/PD-L1. Advanced Characterization of Cellular Phenotypes and Spatial neighborhood within Tissues. **B)** Cell type clustering heatmap constructed based on the expression profiles of a selected panel of cell markers. The heatmap provides a visual representation of the clustering analysis, illustrating the intricate relationships and similarities between different cell types based on their marker expression. **C)** A neighborhood clustering heatmap derived from the spatial distribution patterns of various cell types. By analyzing the proximity and arrangement of cell types within the tissue matrix, this heatmap offers insights into the cellular microenvironment, revealing the complex interactions and spatial organization that characterize the cancerous tissue.

Conclusion

This study has uncovered that PD-1/PD-L1 interactions predominantly occurs at the interface between the tumor and the tumor microenvironment (TME), with the majority of cell types including CD20 and CD8 cells. Detailed cell typing and cellular neighborhood analyses have elucidated the contributions of diverse immune cell populations to PD-1/PD-L1 ligation events. The employment of cutting-edge spatial phenotyping techniques in conjunction with the proximity ligation assay has been pivotal in underscoring these observations. This combination of technologies has provided a precise mapping of ligation sites and a comprehensive characterization of their cellular composition, thus enhancing our understanding of the immunological landscape within the tumor milieu.

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