

HIGH-PLEX SPATIAL PROTEOMIC PROFILING OF IMMUNOTHERAPY RESPONSE GROUPS IN HEAD AND NECK CANCER IDENTIFIES TISSUE SIGNATURES ASSOCIATED WITH THERAPY RESPONSE

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Background Immunotherapies have improved the treatment landscape for recurrent and metastatic head and neck cancers (HNC). Immune checkpoint inhibitors (ICI) have led to durable benefit in approximately 20% of HNSCC patients. Therefore, predictive biomarkers are needed to identify those likely to respond or develop resistance to ICI therapy.

Methods Our retrospective study profiled pre-treatment formalin-fixed paraffin-embedded (FFPE) tissues from metastatic HNC patients treated with immunotherapy at the Royal Brisbane and Women's Hospital. Here, we used image analytics tool (Oncotopix Discovery[®]) to demarcate gross structures from H&E images (tumour, stroma, tertiary lymphoid structures, muscle, fat and vasculature) and per-region cell counts, which informed our high-plex profiling of the tumour microenvironment using the Nanostring GeoMx Digital Spatial Profiler. Tumour and stromal compartments were measured for 80-proteins simultaneously spanning immune cell typing, immuno-oncology drug targets, immune activation, pan-tumour, cell death, and PI3K/AKT panels. Each GeoMx ROI (H&E and 4-plex IF (DNA, PanCK, CD8 and CD3) was analysed for percent tissue type (tumour, stroma, etc), per-subregion cell counts, simple cellular phenotypes, and spatial metrics between cell types. The protein findings were measured against response to ICI therapy (RECIST criteria) and overall survival parameters to identify tissue signatures associated with patient outcomes. High-plex validation of markers was performed using orthogonal multiplex tools.

Results Our data revealed robust structural segmentation strategies to annotate tissues using H&E, which may sit upstream of high-plex spatial proteomic assays to identify regions of interest for selection and profiling. The study identified VISTA, CD66b, CD44 which were upregulated, and PD-L1, PD-L2, IDO-1 were downregulated in the progressive disease (PD) vs partial response (PR) comparison, respectively. Within the tumour microenvironment compartment, BAD, BIM and Phospho-PRAS40 were upregulated in the PD group, whereas Cleaved Caspase 9, HLA-DR and CD68 was upregulated in the PR group. A multi-marker signature composed of Pan-CK, PD-1, PD-L2 and cleaved caspase 9, co-localised in the tumour compartment was associated with a worse overall survival.

Conclusions There is an increasing need to comprehensively profile tumour tissues from HNC using high-plex automated imaging and tissue profiling methodologies. Our study demonstrates a new combined workflow enabling the development of tissue-based signatures predictive of response/resistance to ICI therapy.

Ethics Approval This study has Human Research Ethics Approval (LNR/2020/QRBW/66744) from the Royal Brisbane and Women's Hospital Human Research Ethics Committee (RBWH HREC) and the University of Queensland

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0117>