Background: Lung cancers are the leading cause of cancer-related deaths globally and have a 5-year survival of ~20%. Whilst immunotherapies have led to durable and prolonged survival, only a subset of patients remain responsive. Additional biomarkers are needed to better predict which patients will respond or develop resistance against immune checkpoint inhibitor (ICI) therapies. Spatial phenotyping of the tumor microenvironment (TME) is now recognized as a key factor in understanding immunological composition and status that are predictive of ICI therapy benefits.

Methods: Here, we profiled biopsies from a cohort of non-small cell lung cancer (NSCLC) patients treated with single-agent Nivolumab. We developed high-dimensional immune profiling (57 antibody panel) and conducted whole-tissue PhenoCycler®-Fusion spatial phenotyping. Spatial features, including cellular composition and cellular neighborhoods were analyzed and compared against clinicopathological findings and response to immunotherapy.

We then expanded our spatial analysis to a larger NSCLC cohort using customizable PhenoCode™ Signature Panels (PSP) for high-throughput immunoprofiling (CD3/CD8/CD20/CD68/PanCK + CD4 add-in) and immunocontexture (CD8/CD68/PD-L1/FoxP3/PanCK + PD-1 add-in) imaging. The PSP panel content combines the barcode-based antibody chemistry from the PhenoCycler® platform with the signal amplification of Opal chemistry from the PhenoImager® platform. The PSP panel was profiled across 20 biopsies to investigate correlations between spatial phenotypic signatures, clinicopathological findings, and response to ICI therapy.

Results: Our PhenoCycler®-Fusion data revealed more than 10 unique phenotypes within the TMEs of ICI therapy response groups. High-dimensional spatial analyses furthermore revealed intratumoral and intertumoral heterogeneity and cell-specific metabolic signatures. Concurrently, multispectral spatial analysis of PSP panel data uncovered putative spatial signatures within NSCLC cohorts, indicating the enrichment and significance of certain phenotypic frequencies and cellular interactions associated with therapy response.

Conclusions: There is an increasing need for the development of predictive biomarkers of response to ICI therapy. We demonstrate the complementary use of high-dimensional, deep spatial phenotyping analysis with high-throughput multiplex imaging for identifying new spatial signatures with the immune landscape of NSCLC. These methods are amenable to revealing novel cell types and spatial signatures that can be associated with ICI outcomes and therefore serve as clinical biomarkers.

Ethics Approval: This study has Metro South Human Research Ethics Committee (HREC) Approval (LNR/2019/QMS/51117) and University of Queensland ethics ratification.