118 DELINEATION OF SPATIAL TISSUE SIGNATURES OF IMMUNOTHERAPY RESPONSE GROUPS IN NON-SMALL CELL LUNG CANCER (NSCLC)

[1]Arutha Kulasinghe*, [1]James Monkman, [2]Honesty Kim, [2]Aaron Mayer, [1]Ahmed Mehdi, [3]Marie Cumberbatch, [3]Milan Bhagat, [4]Fabian Schneider, [4]Jeppe Thaagard, [4]James Mansfield, [5]Rahul Ladwa, [6]Scott Mueller, [7]Mark Adams, [5]Kenneth O'byrne. [1]*The University of Queensland, Brisbane, Australia*; [2]*Enable Medicine, California, CA, USA*; [3]*Tristar Technologies, London, DC, UK*; [4]*Visiopharm, Horsholm, Denmark*; [5]*The Princess Alexandra Hospital, Brisbane, Australia*; [6]*University of Melbourne, Melbourne, Australia*; [7]*Queensland University of Technology, Brisbane, Australia*

Background Lung cancers remain the leading cause of cancer related mortality and have a poor 5-year survival. Immunotherapies have led to durable benefit in a cohort of non-small cell lung cancer (NSCLC) patients. Identifying those patient likely to achieve benefit remains a clinical unmet need. Whilst predictive biomarkers such as PD-L1 and tumour mutation burden (TMB) have shown utility, the underlying tumour-immune biology is unlikely represented. The composition and activation status of the cellular milieu contained within the tumour microenvironment (TME) is becomingly increasingly recognised as a driving factor dictating response to immunotherapies.

Methods In this study, we employed multiplex IHC (mIHC), and digital spatial profiling (DSP) to capture the targeted immune proteome and transcriptome of tumour and TME compartments from ICI-treated (n=41) and standard of care (n=47) NSCLC patient cohorts. Oncotopix® Discovery was also used to analyse the highplex imagery. The analysis pipeline consisted of tissue segmentation (tumor, stroma, necrosis, etc), nuclear detection using a deep-learning algorithm for DAPI, a threshold-based cellular phenotyping step, and spatial analyses.

Results Patients sensitive to ICI therapy expressed higher levels of IL2 receptor alpha (CD25, p=0.028) within the tumour compartments, which corresponded with increased *IL2* mRNA (p=0.001) within their stroma. *IL2* mRNA levels within the stroma positively correlated with the expression of pro-apoptotic markers cleaved caspase 9 (p=2e-5) and BAD (p=5.5e-4) and negatively with levels of memory T cells (CD45RO) (p=7e-4). Immuno-inhibitory markers CTLA-4 (p=0.021) and IDO-1 (p=0.023) were supressed in ICI-responsive patients. Tumour CD44 (p=0.02) was depleted in the response group and corresponded inversely with higher stromal expression of one of its ligands, *SPP1*(osteopontin, p=0.008). Cox survival analysis indicated tumour CD44 expression was associated with poorer prognosis (HR=1.61, p=0.01), consistent with its depletion in ICI sensitive patients. The SOC cohort paralleled similar roles for immune checkpoints and pro-apoptotic markers, with LAG3 (HR=3.81, p=0.04) indicating poorer outcome, and BIM (HR=0.16, p=0.014) with improved outcome.

Conclusions Through multi-modal approaches, we have dissected the characteristics of NSCLC treatment groups and provide evidence for the role of several markers including *IL2*, CD25, CD44 and *SPP1* in the efficacy of current generations of ICI therapy. The signatures are being validated in prospective larger cohort studies.

Consent This study has Queensland University of Technology (QUT) Human Research Ethics Committee Approval (UHREC #2000000494).

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0118