Immune profiling of immunotherapy and adjuvant chemotherapy pretreatment of NSCLC tissues by CODEX

James Monkman[1], Afshin Moradi[1], Connor O'Leary[2], Zhenqin Wu[3], Steven Hamel[3], Dan Winkowski[5], Fabian Schneider[5], Chin Wee Tan[4], Ning Liu[4], Melissa Davis[4], James Mansfield[5], Aaron Mayer[3], Ken O'Byrne[2], Arutha Kulasinghe[1]

1) Frazer Institute, University of Queensland, Brisbane, Australia; 2) Princess Alexandra Hospital, Woolloongabba, Australia; 3) Enable Medicine, Menlo Park, CA, USA; 4) The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia 5) Visiopharm, Horsholm, Denmark

Lung Cancer

Non-small cell lung cancer (NSCLC), including adenocarcinoma and squamous cell carcinoma subtypes, are the leading cause of cancer related mortality globally. The 5-year survival of patients ~20%. Whilst targeted therapies are increasing, including multiple generations of tyrosine kinase inhibitors and immune checkpoint inhibitors, there is a growing need to identify predictive biomarkers for these therapies.

Here we profiled an adjuvant chemotherapy (n=61) as well as a second line immunotherapy (n=42) NSCLC cohort by spatial transcriptomics and proteomics) to investigate the association between immune composition and patient outcome. We applied a panel cytotoxic and hyperactivated T cell states, as well as B cells, Tregs and myeloid lineage innate immune cell types.

Our study profiled tumour-immune composition across patients and investigate the spatial neighbourhoods and clusters that these cells software for tissue segmentation (into classes for tumor, stroma, artifacts, blood vessels, etc.), cellular segmentation, and each marker, and then performed spatial analyses (distances, interactions, neighborhoods) using SpatialMap phenotypes.

Methods (Tissue microarray study)

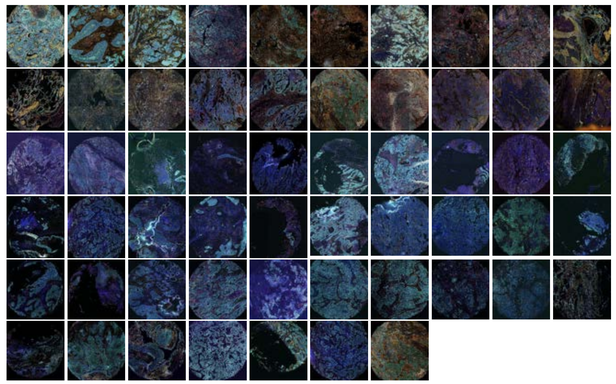


Figure 1. Individual core images of the Tissue microarray profiled on the CODEX (Phenocycler) technology from Akoya Biosciences for 34 markers. Phenoplex™ software for tissue segmentation (into classes for tumor, stroma, artifacts, blood vessels, etc.), cellular segmentation, and cellular phenotyping based on thresholds for each marker, and then performed spatial analyses (distances, interactions, neighborhoods) using SpatialMap to identify cellular motifs associated with clinical phenotypes.

Visualize

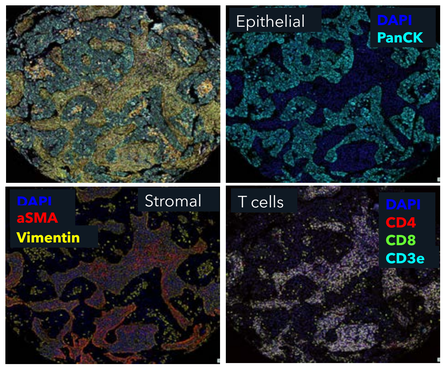


Figure 2. Panels of markers of multiple colors (7+) can be saved for easy visualization of different groups of biologically relevant markers. In this case, three different panels are shown, one for tumor architecture, one for stromal architecture, and one as a basic T cell panel.

Classify Tissue | Segment Cells | Phenotype

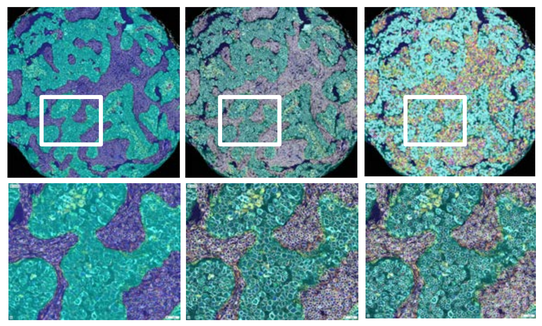


Figure 3. Analysis steps of the Codex images are shown for the same core at two different magnification levels. The first step is a paint-to-train deep-learning-based tissue segmentation into epithelial (cyan) and non-epithelial regions (blue). Next, is a cell segmentation using a pre-trained A.I. algorithm using the DAPI channel. Nuclei are expanded into cell cytoplasmic regions using a watershed method. Last comes the phenotyping of the cells based on the Guided Workflow (Fig. 4).

Phenoplex Guided Workflow

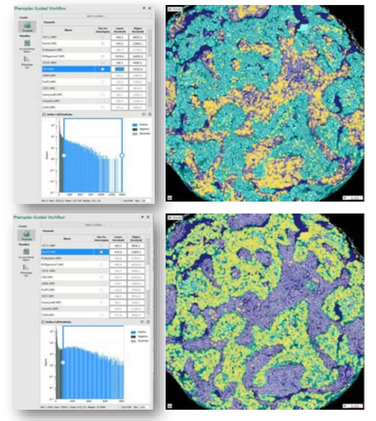
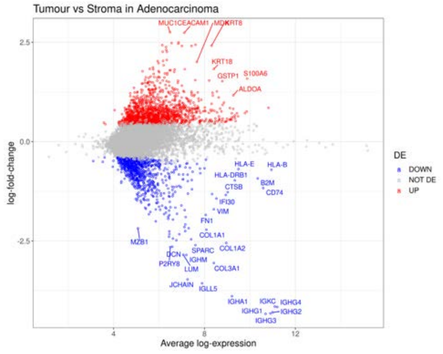


Figure 4. A guided workflow for cellular phenotyping. Users set thresholds for each biomarker based on an assessment of the optimal positivities.

Spatial transcriptomics



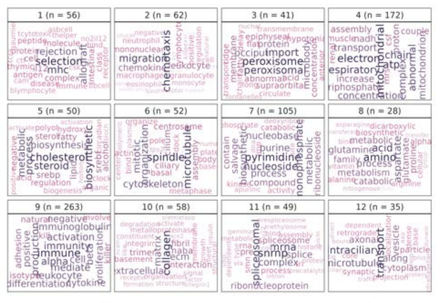


Figure 5. Differential Expression (DE) analysis of tumour vs stroma within the adenocarcinoma subset by compartmentalised spatial transcriptomics. The standR package was used for data gene/sample QC, normalization (relative log expression, RLE analysis and TMM normalization), followed by linear modelling using edgeR and Limma-voom for DE analysis. The vissE plot shows the gene-set enrichment analysis (GSEA) of the differentially expressed genes (GSEA: Fry & vissE).

Data Import

Characterize Cellular Niches

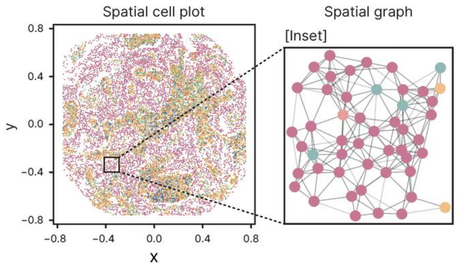


Figure 6. Spatial plot of one tumor core, colored by phenotype. Phenotypes were programmatically imported from Visiopharm outputs (left panel). Inset: Graph of spatial neighbor relationships (k-nearest neighbor algorithm, k = 10). Gray lines indicate a neighbor relationship between connected cells.

Cell Neighborhood Analysis

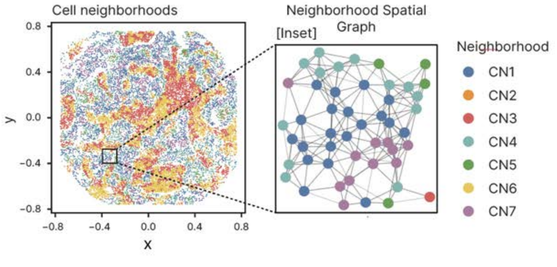


Figure 7. Spatial plot of a tumor core, colored by cell neighborhood identity. Cell neighborhoods were derived by counting neighbor phenotypes for each cell and clustering the resulting cells by phenotype count matrix (right panel; k-means, k = 7). Inset: Graph of spatial neighbor relationships, with grey lines indicating a neighbor relationship between connected cells.

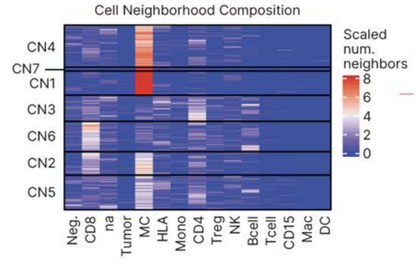


Figure 8. Heatmap of the cell neighborhood counts matrix, depicting the cell compositions of each neighborhood. Counts data were subsampled to 2000 data points.

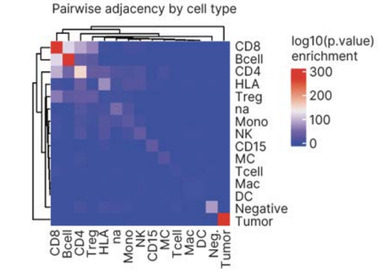


Figure 9. Heatmap of cell interaction enrichments. Each element heatmap shows -log10 p-values for interactions (hypergeometric test). Results represent one tumor core. Further analysis to compare interactions across multiple samples is shown below.

Cellular Heterogeneity

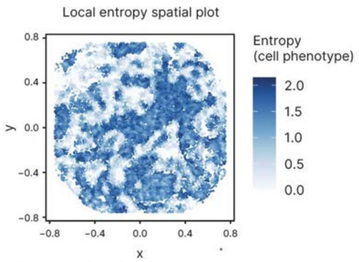


Figure 10. Spatial plot of local entropy. Entropy was computed on phenotype classes of connected cells (knn, k = 10) and is a metric of cellular heterogeneity in the neighborhood of the index cell.

Rapid Insight Generation

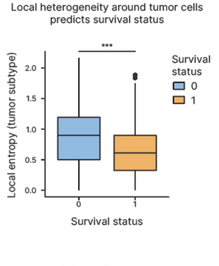


Figure 11. Comparison of local entropy values of single-positive PanCK+ tumor cells (n = 45186), grouped by survival status (0 = deceased, n = 28; 1 = survivor, n = 12; two sided t-test p < 1e-16).

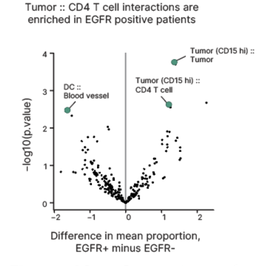


Figure 12. Comparison of cell interaction proportions between patients with and without an EGFR mutation (p-value = two sided t-test).

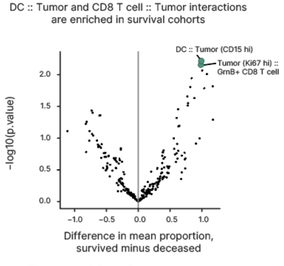


Figure 13. Comparison of cell interaction proportions between deceased and surviving patients (p-value = two sided t-test).

Spatial Analysis Ecosystem

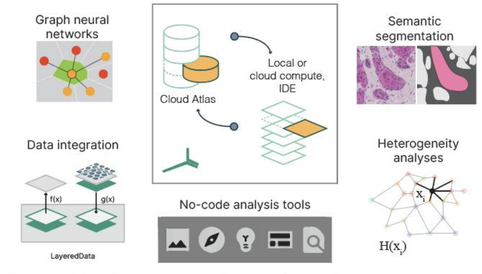


Figure 14. Schematic of Enable Cloud Platform ecosystem, including neural network-based microenvironment analysis, semantic segmentation, multimodal integration, heterogeneity analysis, and a no-code analysis toolkit for visualization, plotting, and exploration of data. Data are readily accessed and shared with collaborators or the broader community.

Conclusion

- Cutting-edge spatial transcriptomic and proteomic technology were used to profile tissue microarrays from NSCLC patients.
- Differential expression analysis of transcriptome data based on the standR pipeline was used to identify DEGs between adeno- and squamous cell carcinoma and the TME
- For proteomic imaging data, we developed an analysis pipeline in Phenoplex that enabled tissue-segmentation, cell segmentation and used a guided workflow to identify cell phenotypes.
- Characterization of cellular features *in situ* on the Enable Cloud Platform identified unique cell neighbourhoods and interactions linked to clinical endpoints (overall survival) and EGFR mutation status.
- A potential role for circum-tumor cellular heterogeneity was uncovered and linked to overall survival.

Cell Interaction Analysis