Investigation of cellular neighbourhoods and immune checkpoint inhibition in the tumour microenvironment of triple-negative breast cancer.

Maikel L. Colli[-], Wanqiu Zhang[1], Thao Tran[1,2], Rafael Tubelleza[3,4], Alice Ly[1], Sanja Bajovic[1], Marc Claesen[1], Nathan Heath Patterson[1], Arutha Kulasinghe[3,4]

[1] Aspect Analytics, Genk, Belgium
[2] Department of Imaging and Pathology, Laboratory of Translational Cell and Tissue Research, KU Leuven, Leuven, Belgium
[3] Queensland Spatial Biology Centre, Wesley Research Institute, Brisbane, QLD, Australia
[4] Frazer Institute, Faculty of Health, Medicine and Behavioural Sciences, University of Queensland, Brisbane, QLD, Australia

Introduction

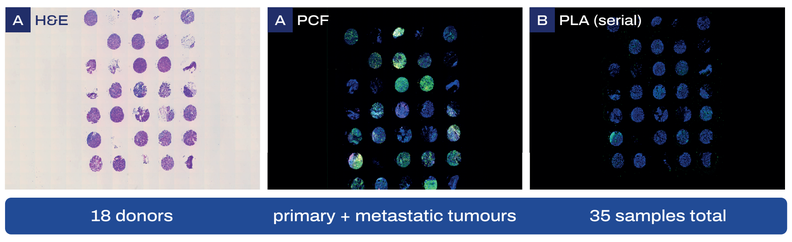
Triple-negative breast cancer (TNBC) represents a major therapeutic challenge due to limited targeted treatment options and an immunosuppressive tumor microenvironment (TME). Immune checkpoint inhibition targeting PD-1 and its ligand PDL1, have proven effective in a subset of TNBC patients. We examined the relationships between PD-1/PDL1 interactions and the TNBC TME to better understand the processes that underpin response and survival.

Methods

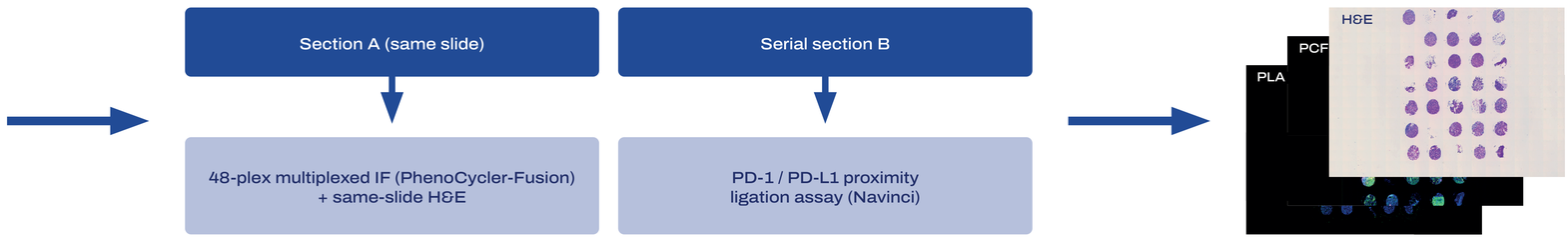
TNBC biopsies (n = 35) were assembled into tissue microarrays consisting of both primary and metastatic tumors. One section underwent multiplexed immunofluorescence using a 48-antibody panel on a PhenoCyclerFusion (Quanterix), followed by same-slide H&E staining. A serial section underwent proximity ligation assay for PD-1/PDL1 (Navinci). Weave (Aspect Analytics) platform used for data analysis and interpretation, pathology annotation, data modality integration, cell typing, and cell neighborhood (CN) analysis.

Results

A | Cohort & tissue microarray



B | Same-slide multi-omic acquisition



C | Processing & cell-type annotation (in Weave)

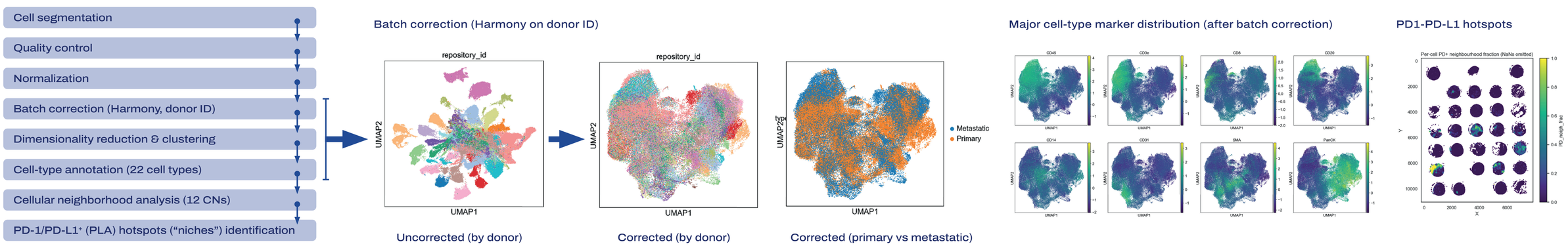
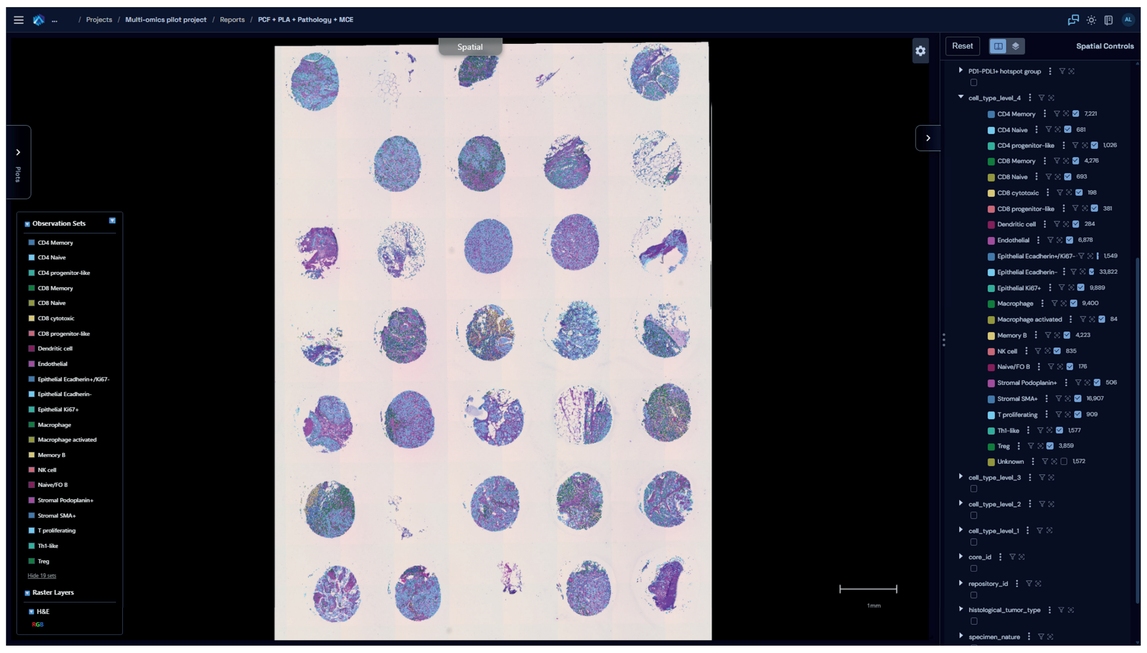


Figure 1: Experimental design and data processing.
A. Biopsies from 18 donors (primary and metastatic tumours; 35 samples in total, as some metastatic samples were unavailable) were assembled into tissue microarrays. **B.** One section underwent 48-plex multiplexed immunofluorescence (PhenoCycler-Fusion) with same-slide H&E, and a serial section underwent PD-1/PD-L1 proximity ligation assay; all three modalities were co-registered in Weave. **C.** After cell segmentation, QC, normalization and Harmony batch correction (donor ID), the distribution of major cell-type markers confirmed the major populations; cells were then annotated and PD-1/PD-L1⁺ hotspots identified.



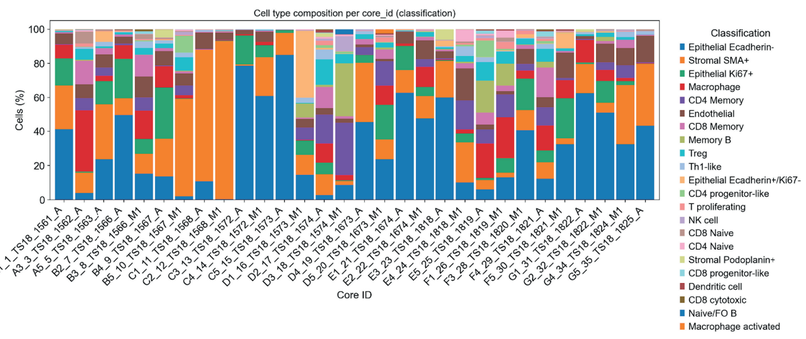


Figure 2: Cell type annotation of TNBC samples.
Cell type annotation was performed using a supervised, marker-based Random Forest (RF) classifier. 22 different cell types were identified across the TNBC samples; the main cell type were Epithelial-Ecadherin-tumor cells, while macrophages were the most frequent immune cell type.

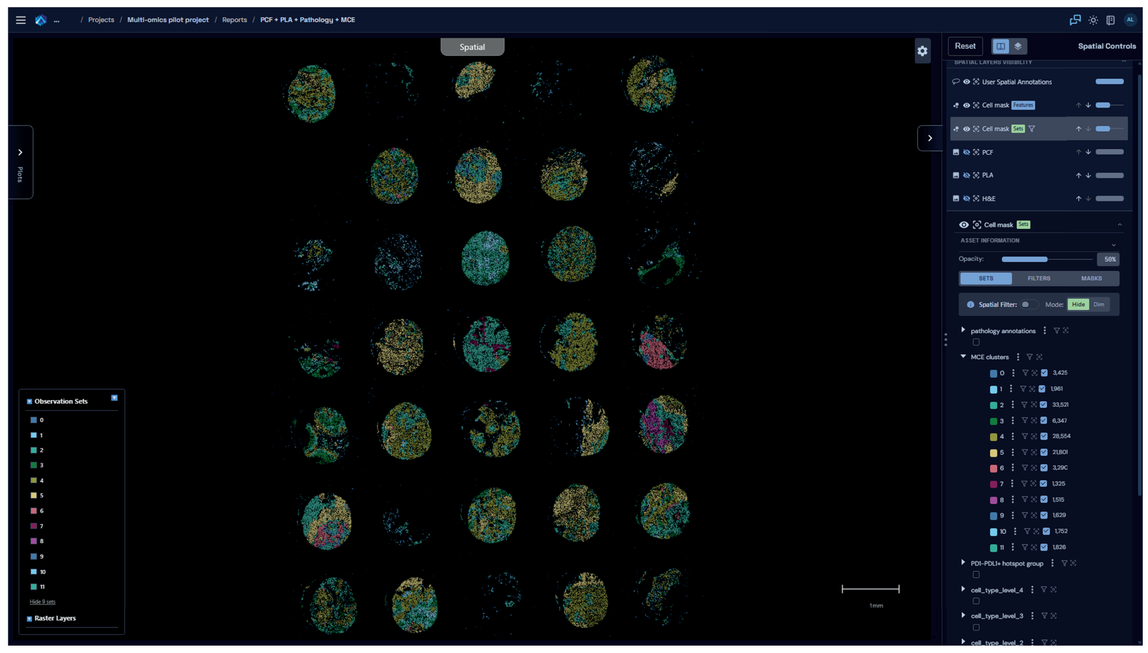
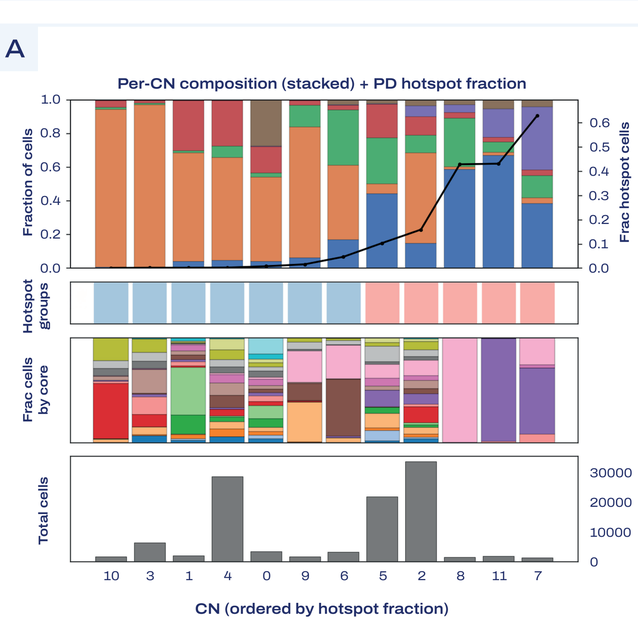
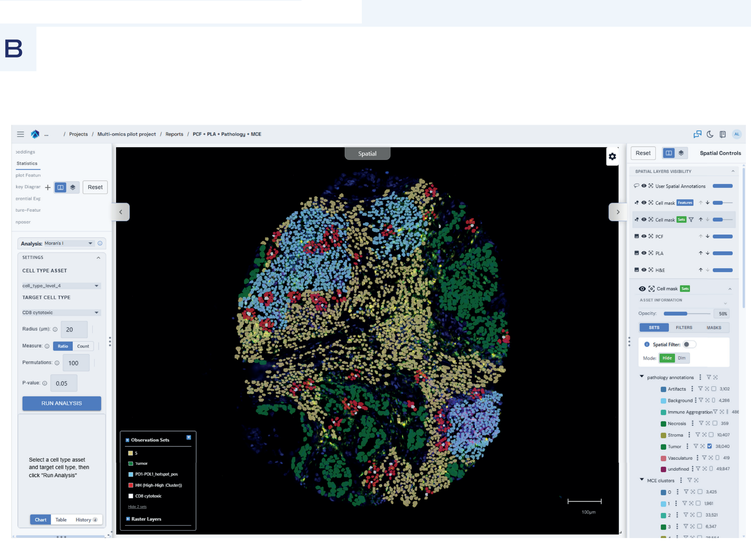
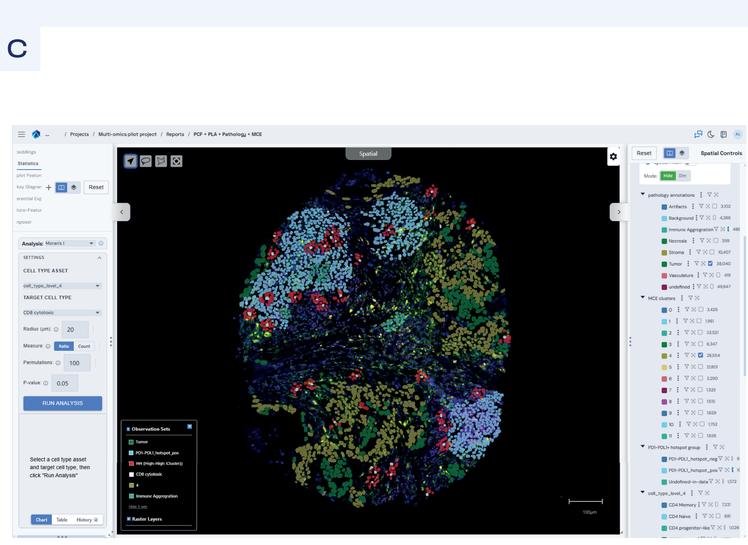


Figure 3: Cellular neighborhood analysis of different TMA cores in Weave
A cell is defined by both its molecular signature and also by its environment. Cells of the same type may present different interactions and functions depending on the composition of nearby cell types. Proprietary CN analysis in Weave takes into consideration cell types and distance to identify spatially-recurring environments. CN analysis detected 12 different spatially recurring neighborhoods across the patient samples.

Figure 4: PD-1/PD-L1 proximity ligation assay (PLA) integration with Cellular Neighborhood (CN) analysis.
PD1-PDL1+ hotspots were defined based on the following criteria: for each cell, the cells lying within a 50 µm radius were taken as its neighbours; a cell was flagged PD-enriched when >30% of those neighbours were PD-1/PD-L1⁺. PD1-PDL1-enriched cells within 50 µm of one another were then linked, and each spatially connected group defines a PD-1/PD-L1+ hotspot. **A:** Per-CN composition vs PD-hotspot fraction — high-hotspot CNs (>10% hotspot cells) are enriched in T and B cells. **B-C**. Representative core presenting: CD8 cytotoxic spatial niches CN4 or CN5, PD-1/PD-L1⁺ hotspots (red), and tumor areas (dark green). CD8⁺ cytotoxic niches were identified using the local indicators of spatial association (LISA). **B:** CN 5 (yellow) is mainly composed of stromal and immune cells, including large areas of PD1-PDL1+ hotspots, retained CD8⁺ cytotoxic niches outside tumour areas. **C:** In comparison, CN 4 (olive green) presents mainly tumour and stroma cells, was depleted of PD-1/PD-L1 and immune cells.







Conclusion & Outlook

- The identified cell types generally express the expected key cell markers.
- The main cell type identified was Epithelial-Ecadherin-tumor cells, reflecting the tumor derived biopsies, while the most frequent immune cell type were macrophages.
- PD1-PDL1+ hotspots show an increase in T cells and a decrease in epithelial cells.
- Cellular neighborhood analysis detected a cluster (#5) enriched in stromal components and PD-PDL1+ hotspots which "retains" a high number of CD8 cytotoxic spatial niches outside of tumor areas, and another one (#4) that represents tumor and stromal components but is depleted of CD8 and other immune cells.

With the support of