The Mutational Landscape Defines the Proteome and Spatial Organization of Tumor, Stroma, and Immune cells in Ovarian Cancer

Kruttika Dabke[1], Bassem Ben Cheikh[2], Nicole Gull[1], Amisha Dhawan[2], Katrina Evans[2], Simion Kreimer[1], Pei-Chen Peng[1], Nathan Ing[1], Kate Lawrenson[1], Bobbie Rimel[1], Beth Karlan[3], Simon Knott[1], Simon Gayther[1], Sarah Parker[1], Michelle Jones[1]

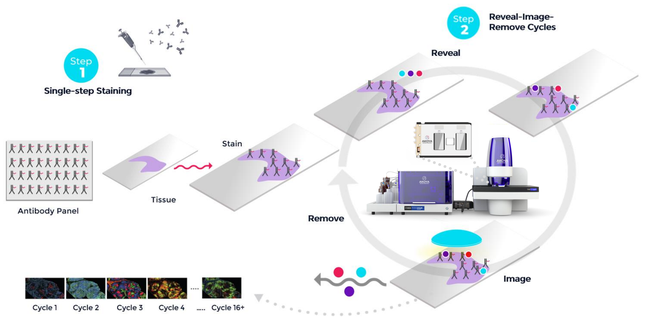
[1]Cedars Sinai Medical Center, Los Angeles, California, USA; [2]Akoya Biosciences, Menlo Park, California, USA;
[3]David Geffen School of Medicine at UCLA, Los Angeles, CA

1. High-grade Serous Ovarian Cancer

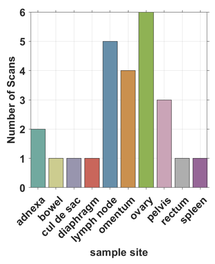
High-grade serous ovarian cancer (HGSOC) is highly aggressive and lethal, with clinical challenges to both diagnosis and treatment. The genomic instability of HGSOC, further complicated by homologous recombination deficiency (HRD), leads to heterogeneity in the HGSOC tumors and patient response to treatment. Proteogenomic studies have provided some insight into HGSOC biology, but our present knowledge regarding the abundance of the tissue-infiltrating immune cell populations and their spatial organization relative to the tumor remains elusive. To gain a deeper understanding, further research employing spatial biology solutions is necessary in order to elucidate this aspect.

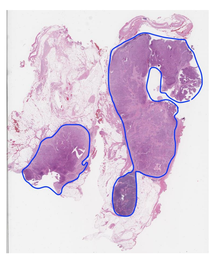
2. Spatial Phenotyping with PhenoCycler-Fusion

The PhenoCycler®-Fusion (PCF) **2.0** technology stands as the fastest whole-slide spatial biology system, allowing for simultaneous detection of over 100 biomarkers through the integration of automated fluidics and iterative imaging with oligo- conjugated antibodies. In this study, we designed a 26-plex antibody panel to explore the evolving alterations in the cellular composition and spatial structuring of the tumor microenvironment (TME) in **primary** and **recurrent** HGSOC tumors, comparing homologous recombination deficiency (**HRD**) against proficiency (**HRP**) statuses.

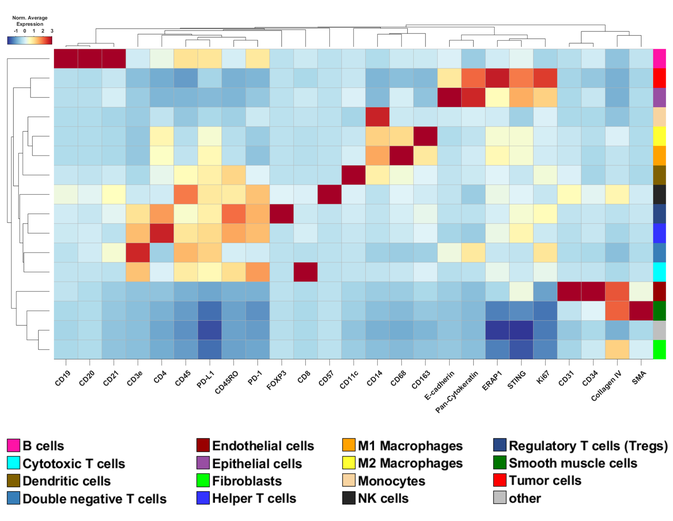


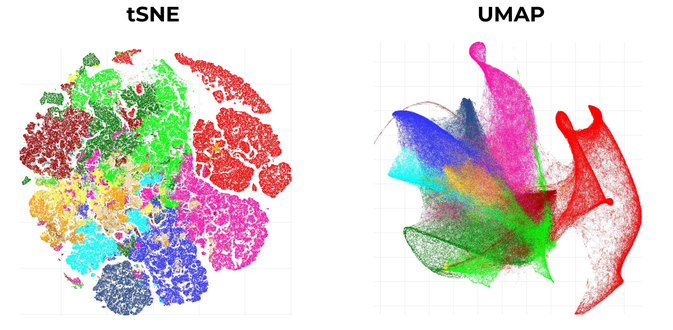
This study was conducted on 23 samples collected from various sites, and Regions of Interest (ROIs) were manually annotated on the H&E images.



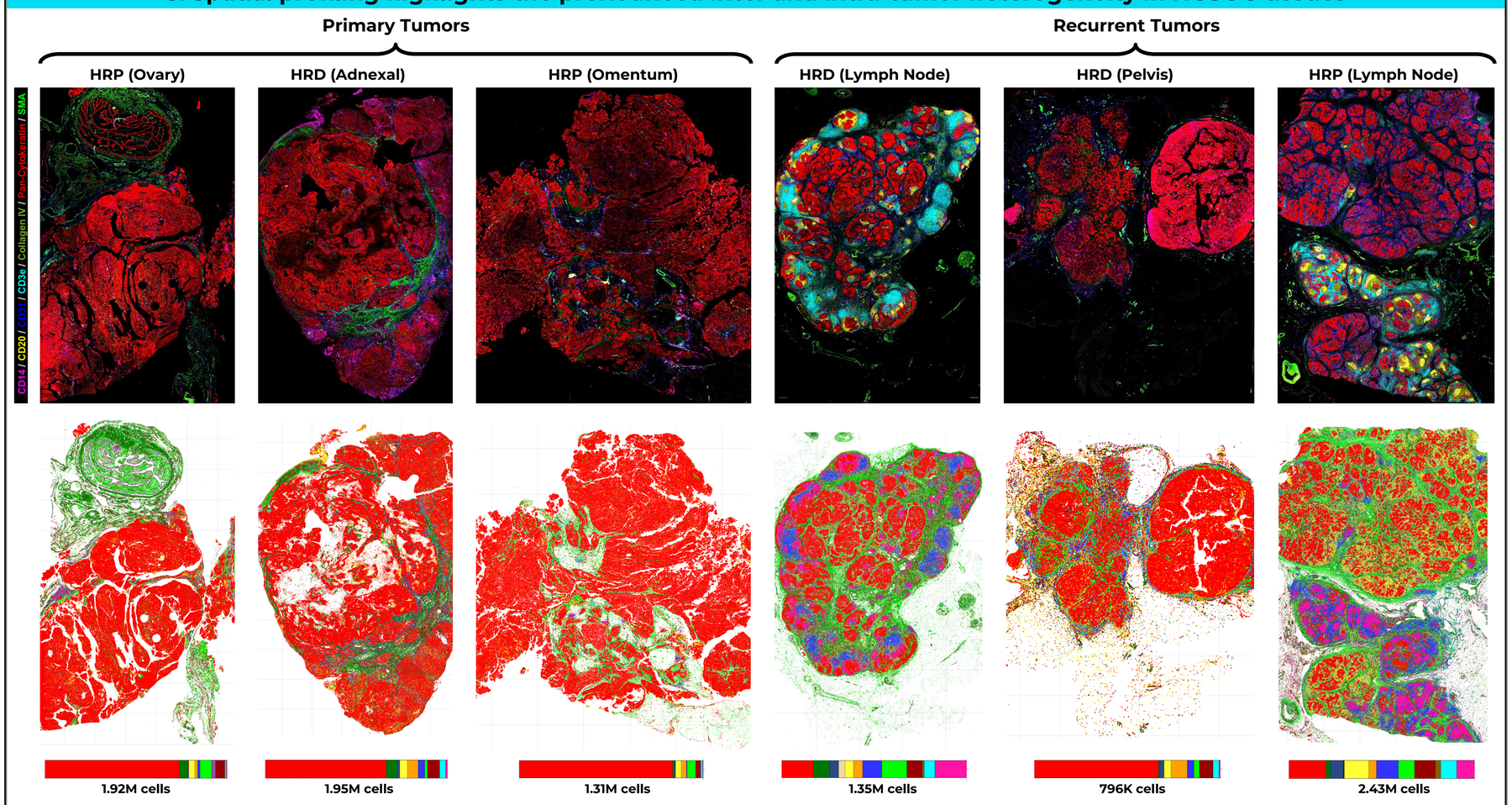


Cell phenotypes are defined by *unsupervised clustering* based on expression of cell lineage and structural markers.





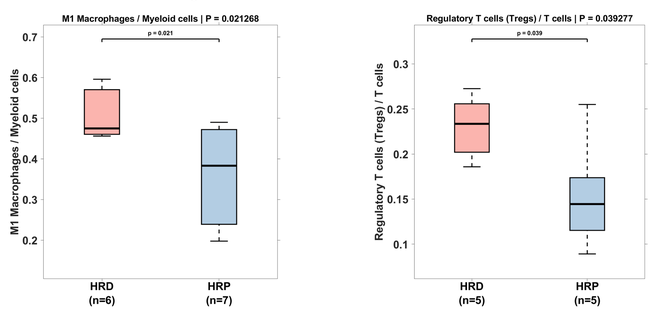
3. Spatial profiling highlights the pronounced inter and intra-tumor heterogeneity in HGSOC tissues



A total of 36.3 million cells were identified from 23 HGSOC tissue samples and classified into 19 cell subtypes. The single-cell spatial maps, accompanied with bar charts, illustrate the differences in the cellular composition and spatial organization of the tumor microenvironment.

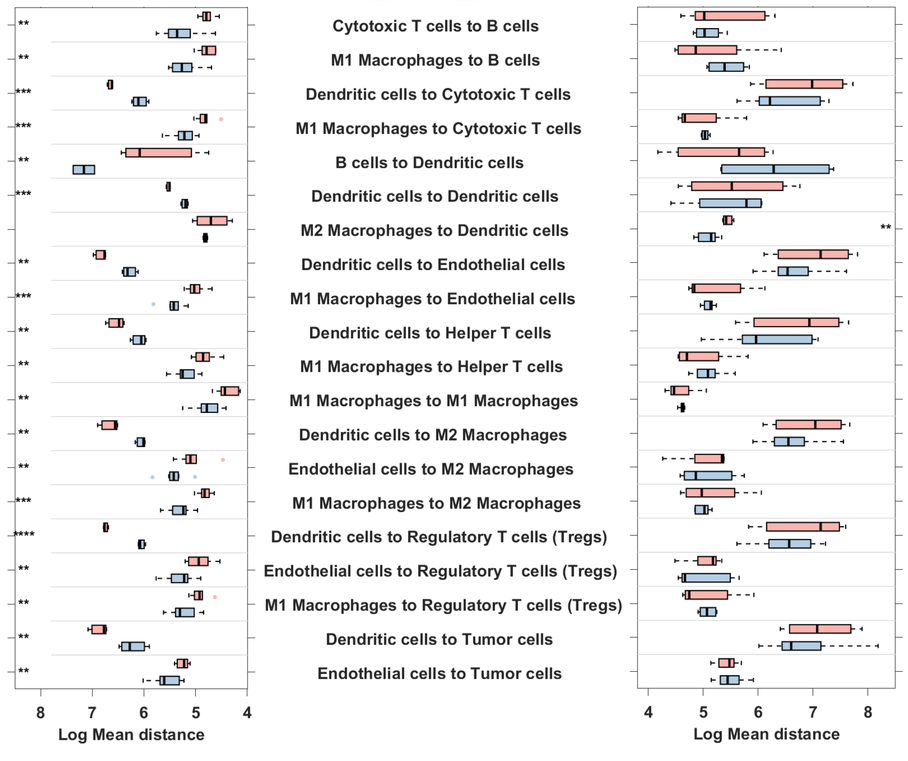
4. Spatial analysis reveals significant differences associated with HRD status

A) Relative Cell Abundance

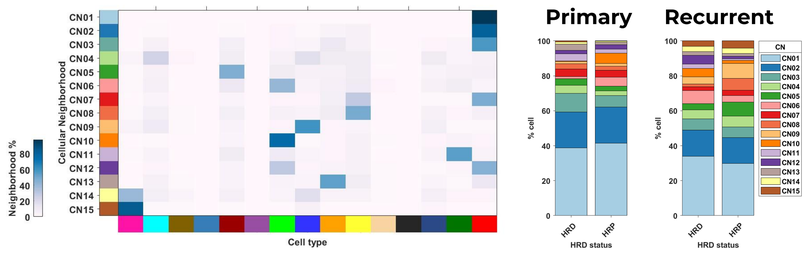


Differential analysis based on relative cell abundance identified significantly higher proportions of M1 Macrophages in the HRD group compared to the HRP group within the primary tumors, and a higher proportions of Tregs within the recurrent tumors.

B) Spatial Proximity Analysis

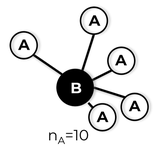


C) Cellular Neighborhood



Cellular neighborhood analysis determines 15 spatial neighborhoods characterized by their unique cell type compositions that vary between the different patient groups. Recurrent tumors have remarkably lower proportions of CN9 (cells interacting with Helper T cells) in the HRD compared to the HRP group, and higher proportions of CN14 and CN15 (cell interacting with B cells) compared to the primary tumors.

Spatial proximity analysis outlining the localization of cell types relative to each other by measuring the average distance of the 10 nearest neighboring cells of type “A” to a focal cell of type “B”. The analysis reveals a larger number of significant differences between the HRD and HRP groups in the primary tumors compared to the recurrent tumors.



5. Conclusion

This study demonstrates the advantages of single-cell spatial phenotyping enabled by the PhenoCycler®-Fusion system for a comprehensive analysis of the cellular composition and spatial structuring of the tumor microenvironment (TME) in primary and recurrent HGSOC tumors. Comparative spatial analyses of homologous recombination deficiency (HRD) and proficiency (HRP) revealed several significant spatial organizational differences. These differences could not be discerned by merely counting cells without knowing their specific locations within the tissue.

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