

## CHARACTERIZATION OF THE HORMONE RECEPTOR-POSITIVE BREAST TUMOR MICROENVIRONMENT USING SINGLE CELL TRANSCRIPTOMICS AND MULTI-PLEX IMMUNOFLUORESCENCE

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**Background** Hormone receptor-positive (HR+) breast tumors are the most frequently diagnosed type of breast cancer (~70%) and are characterized by the expression of the estrogen receptor and/or the progesterone receptor. HR+ breast tumors derive limited benefit from immune checkpoint therapy (ICT), which can be at least partially attributed in part, to low levels of T cell infiltration and low expression of T cell immune checkpoint molecules. In other solid tumors, targeting components of the tumor microenvironment outside of T cells have shown the ability to enhance ICT responses, however, such targets have been relatively unexplored in HR+ breast cancer. We hypothesize that there are unidentified targetable non-lymphocyte immune populations within the HR+ breast tumor microenvironment, which can be targeted to increase response to ICT.

**Methods** We performed paired bulk RNA sequencing and cyclic immunofluorescence (CyCIF) from human HR+ tumors (n=30) as well as single-cell RNA sequencing (scRNA-seq) on a subset (N=17) of the tumors, to characterize the spatial composition of the immune infiltrate and identify gene expression profiles that correlate with T cell infiltration. scRNA-seq samples were derived from fresh frozen tumor biopsies whereas both Bulk RNAseq and CyCIF samples were derived from FFPE tissue sections. scRNA-seq analysis was performed using the Seurat R Package and CyCIF sections were preprocessed using the MCMICRO pipeline developed by the Harvard Laboratory of Systems Pharmacology and analyzed in R. TIL level was determined through pathological scoring of stromal TILs and divided into the two groups based on the population mean.

**Results** We have identified tumor-associated macrophages (TAMs) as being the immune cell population most abundant in HR+ tumors by both scRNA-seq & CyCIF. We identified SPP1+ CD36+ expressing macrophages to be significantly enriched in T cell-low compared to T cell-high HR+ tumors. Inversely, T cell high HR+ tumors displayed a significant enrichment of MUCL1+APOD+ macrophages expressing high levels of MHC class II molecules.

**Conclusions** Collectively, these findings put forth two populations of macrophages within HR+ tumors as novel immunotherapeutic targets for further investigation to enhance T cell responses and ICT.

**Acknowledgements** We thank the Dana-Farber Center for Cancer Genomics for processing and sequencing the tumors for scRNASeq and the Brigham and Women's Center for Advanced Molecular Diagnostics for deriving RNA from FFPE

tissues. This work was supported by the Susan G. Komen Foundation (CCR18547597), NCI Cancer Systems Biology Center of Excellence Grant (U54-CA225088), Terri Brodeur Breast Cancer Foundation, The Harvard Ludwig Center, NIH DF/HCC SPORE in Breast Cancer (P50 CA168504), NIH NCI R01/R37 CA269499, and The Concern Foundation.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.1485>