Uncovering spatial relationships of the tumor microenvironment in the Carolina Breast Cancer Study

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UNC LINEBERGER COMPREHENSIVE CANCER CENTER

Study Objectives

- Measure immune marker expression in immune hot spots and tumor regions from whole slides and TMAs
- Analyze differences in immune marker expression by subtype
- Correlate immune marker expression with epidemiologic and clinical features

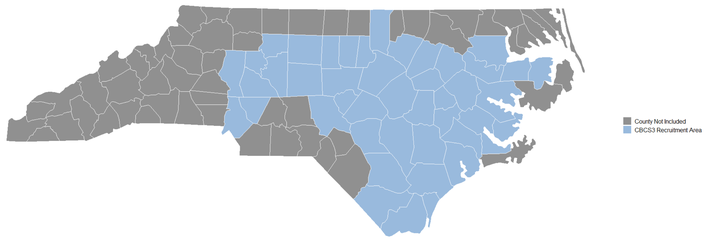
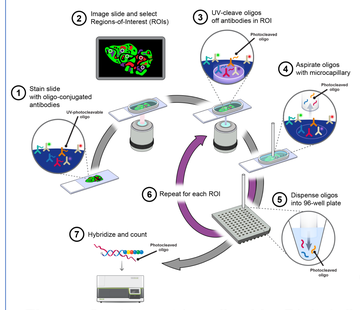


Figure 1.) The CBCS has been conducted in three phases: Phase 1 (1993-1996), Phase 2 (1996-2001), and Phase 3 (2008-2013). In all phases of CBCS, we employed a population-based sampling schema that deliberately oversampled black and young women (<50) with breast cancer to address risk factors specific to those generally underrepresented groups.

Sample Selection

	Whole Slides	TMAs
Race		
Black	4(67%)	37(49%)
Non-Black	2(33%)	38(51%)
PAM50 Subtype		
HER2	0(0%)	1(2%)
LumA	3(50%)	25(33%)
LumB	0(0%)	10(13%)
Basal-like	3(50%)	15(20%)
Missing	0(0%)	24(32%)
IHC Subtype		
ER-/HER2+	0(0%)	5(7%)
LumA	3(50%)	31(41%)
LumB	0(0%)	21(28%)
Basal-like	3(50%)	14(19%)
Missing	0(0%)	4(5%)

Table 1.) For our analysis, we used whole tumor slides from 6 patients, in addition to 4 tissue microarrays (TMAs) that incorporated a total of 76 patients, 6 of which were the same as our whole slide analysis. Patient demographic information is listed above.



4-1BB	CD20	CD45	EpCAM	Histone H3	Mouse IgG2a	Rabbit IgG
aSMA	CD25	CD45RO	ER alpha	HLA-DR	NY-ESO-1	S100b
B7-H3	CD27	CD56	FAPa	ICOS	OX40L	S6 Ribosomal Protein
Bcl-2	CD3	CD66b	Fibronectin	IDO-1	Pan-Cytokeratin	STING
beta-2-microglobulin	CD34	CD68	FoxP3	Ki-67	PD-1	TGF beta 1
CD11c	CD4	CD8	GAPDH	LAG3	PD-L1	TIM-3
CD127	CD40	CD80	GITR	ARG1	PD-L2	VISTA
CD14	CD40L	CD86	GZMB	MART-1	PR	
CD163	CD44	CTLA4	HER2/ErbB2	Mouse IgG1	PTEN	

https://www.nanostring.com/products/geomx-digital-spatial-profiler/geomx-dsp

Figure 2.) Nanostring GeoMx Digital Spatial Profiling is a tissue morphology guided protein expression assay. Tissues are stained with 2-3 cell surface markers for cell types of interest (CD45, CD68, Pan-CK), along with approximately 40 oligo-tagged antibodies for other markers of interest. Regions of interest (ROI) are picked based on cellularity, and UV light cleaves oligo tags from antibodies. Oligos are then read and counted to determine protein expression.

Digital Spatial Profiling

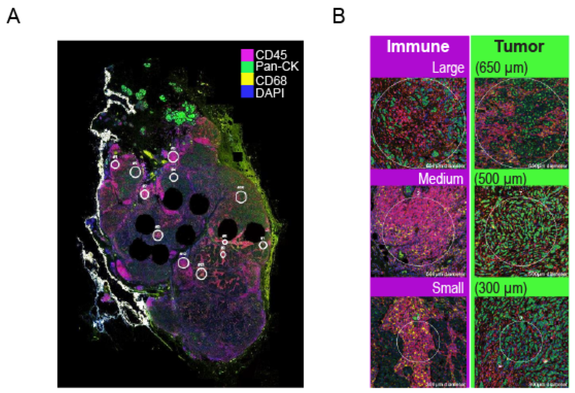


Figure 3.) A) Highly immune infiltrated whole tumor slides from 6 patients were stained with CD45 (red), CD68 (yellow), and Pan-CK, in addition to antibodies listed above. **B)** 12 ROIs were selected based on cellularity, 4 large (650 µm), 4 medium (500 µm), and 4 small (300 µm).

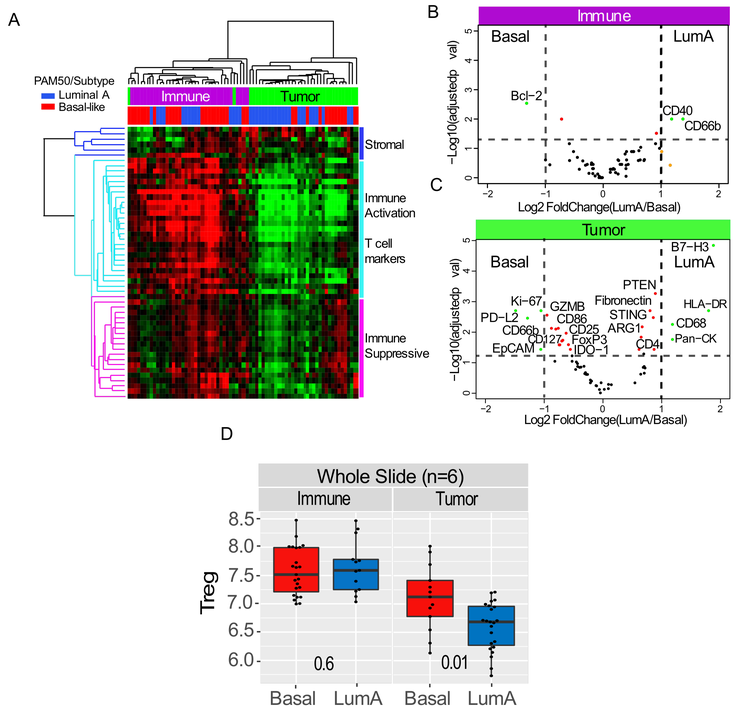
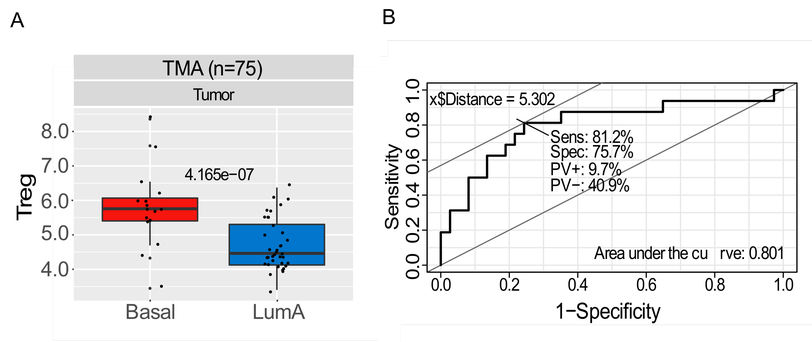


Figure 4.) A) Heatmap of protein expression for pilot dataset with PAM50 subtype and cellularity labeled. **B&C)** The dataset was split into two, with one encompassing the immune high ROIs and one with the tumor high ROIs. Volcano plots of each dataset are shown, with proteins expressed 2-fold or greater and $p < 0.05$ labeled green. **D)** Immune marker profiling was performed based on marker expression. Overall, in immune high ROIs, all immune cell markers were high. In tumor high ROIs, there were significant differences in some cell populations, such as Tregs being higher in Basal tumors.

Immune Cell Markers Differ by Subtype



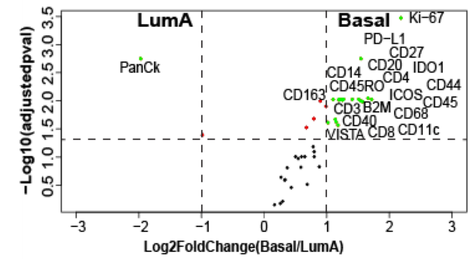


Figure 5.) In order to expand our analysis to more samples, specifically samples enriched for tumor cell content, we analyzed 4 TMAs encompassing 76 patients, including the 6 we already examined by whole slide. For each TMA core, we chose 1-3 ROIs based on Pan-CK staining, 384 ROIs in total. After filtering hot spots and outliers, there were 346 ROIs and 75 patients. **A)** Immune marker profiling shows higher Treg marker expression in Basal like tumors **B)** ROC analysis shows 80% sensitivity in Basal vs Luminal A classification based on Treg marker expression. **C)** Volcano plot of Basal vs Luminal A. There were significant differences in immune marker expression between Luminal A and Basal-like tumors.

Key Findings

- Probing differences immune marker expression by subtype is more prolific in tumor rich regions
- ICC shows strong correlation between probing immune markers by whole slides or TMAs
- Treg marker expression is higher in Basal-like versus Luminal A cancers

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