Digital spatial profiling in HER2 positive breast cancer: The road to precision medicine

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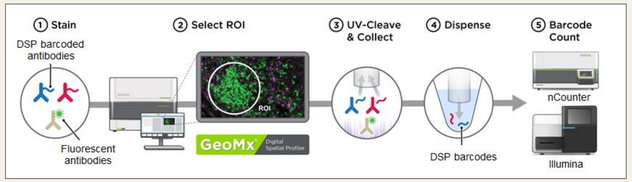
Background

Breast cancer (BC) has been considered immunologically quiescent, this could be explained by characteristics of tumor immune microenvironment (TIME).

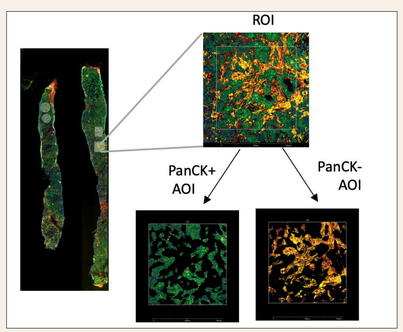
We aimed to characterize the TIME of human epidermal growth factor receptor 2 positive (HER2+) BC using GeoMx® Digital spatial profiling (DSP) in primary and metastatic tumors.

Methods

DSP (described below) was performed on 15 primary and metastatic FFPE samples from 8 patients; including 2 brain metastases.



Visualization markers for Pan-Cytokeratin (PanCK, tumor), CD45 (immune), CD3 (T cells) were used to guide selection of immune cold, warm and hot regions of interest (ROI). Each ROI segmented tumor (PanCK+) or stroma (PanCK-) based on PanCK tumor + or - areas of illumination (AOI).



For each AOI 71 protein targets were tested (Human Immune Cell Profiling Protein Core, Cell Death, Immune Activation Status, Human IO Drug Target, Pan-Tumor, PI3K/AKT Signaling Modules]
Gene expression profiling for PAM50 subtypes and Tumor Inflammation Signature (TIS) was done using the nCounter PanCancer IO 360 assay with a PAM50 spike-in. TIS high or low status was determined by a threshold of 6.5 the mean of the cohort.

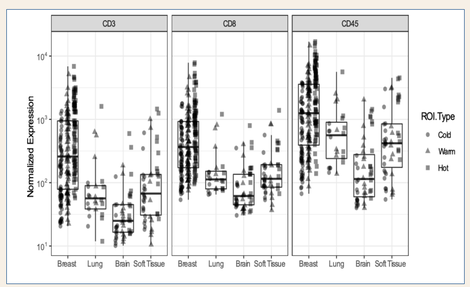
Results

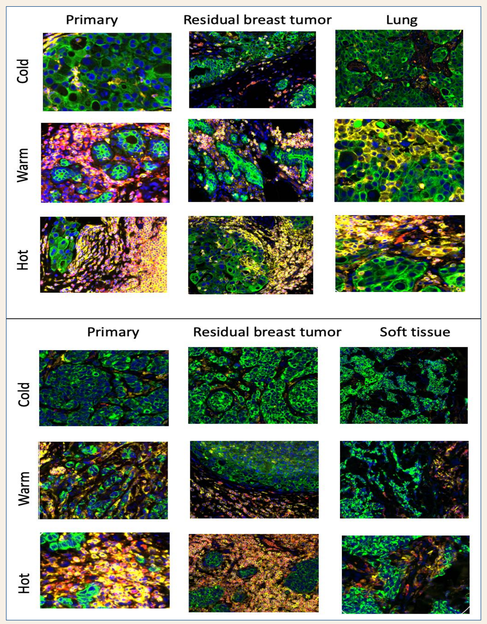
Primary HER2+ samples had the highest amount of tumor infiltrating lymphocytes (CD3, CD8) and overall immune cells (CD45) relative to all metastatic disease in tumor and stroma. These findings were salient in paired samples, particularly in those with three timepoints (primary tumor, residual disease and distant metastatic site). Immune hot, warm and cold (based on CD45 abundance) ROIs from each of the patients revealed varied immune infiltration in each patient, primary tumors had the highest number of immune cells and the metastatic site the lowest.

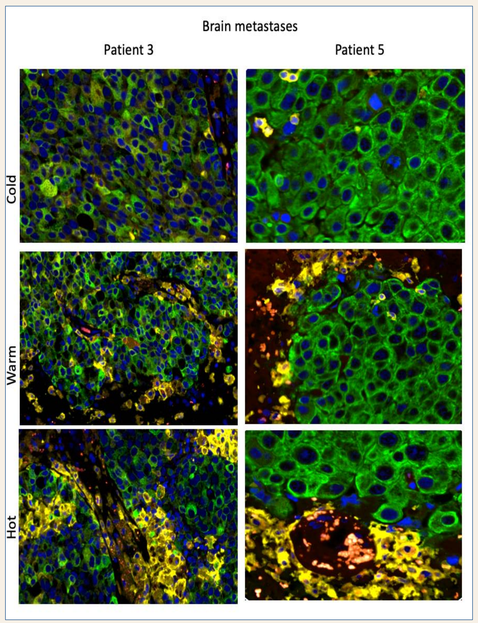
We also calculated the TIS for each sample. Nine of our samples had high TIS (>6.5) and six had low TIS (<6.5). One patient (patient 2) had discordant TIS, meaning that the primary tumor and soft tissue recurrence had low TIS and the residual disease had high TIS.
Protein expression, both tumor and stroma AOIs primary tumors, had higher expression of immune activation and checkpoint markers, including SMA, Bcl-2, CD20, Tim3, CD27, CD8, 41BB, CD56, CD3 and CD4, compared to brain and soft tissue metastases
We also examined the differences in protein and gene expression in tissue type based on breast and brain.

Visualization using DSP shows are less immune and T cells. The largest differences in protein markers was observed in hot ROIs from stromal segments, where the total counts of CD3 and CD8 T cells was not statistically different.

Patient	Race	Tissue	Diagnosis	ER(%)/PR(%)/HER2(+)	PAM50 Subtype	TIS Status
1	Caucasian	Breast	IDC, DCIS	2/0/3	Luminal A	High
		Breast	Tumor emboli	N/A	Luminal A	High
		Lung	Metastatic	0/0/3	HER2 enriched	High
2	Caucasian	Breast	ICD, DCIS	0/0/3	HER2 enriched	Low
		Breast	Residual IDC	0/0/N/A	HER2 enriched	High
		Soft tissue	Local recurrence	0/0/3	HER2 enriched	Low
3	Caucasian	Brain	Metastatic	15/0/3	HER2 enriched	Low
4	Caucasian	Breast	IDC	0/0/3	Basal like	High
		Breast	Local recurrence	N/A	Luminal A	High
5	Caucasian	Brain	Metastatic	0/0/3	Basal like	Low
6	Caucasian	Breast	IDC, DCIS	80/70/2*	Luminal A	High
7	Black/AA	Breast	IDC, DCIS	0/0/3	HER2 enriched	High
		Breast	Local recurrence	N/A	Basal like	High
8	Black/AA	Breast	IDC, DCIS	10/15/1	Luminal A	Low
		Soft tissue	Local recurrence	0/0/3	Basal like	Low







Conclusions

Primary tumors had a higher number of immune cells than metastatic sites, particularly when comparing primary BC and brain metastases. These findings suggest that immunotherapy in early stage BC could be more effective than in the advanced BC. This project will be part of the GeoMx Breast Cancer Consortium.

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