Measurement of Immune Infiltration in ER, PR, HER2 IHC Subtypes Reveals Different Populations That May Benefit from Immunotherapy

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Background

Expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), as measured by immunohistochemistry, is routinely used to guide appropriate therapy in breast cancer. HER2 targeted therapy is used to treat HER2 overexpressing patients and endocrine therapy is used to treat ER+ or PR+ patients. However, therapeutic options are limited for patients who are triple negative, relapsed HER2 overexpressed patients, and ER+ or PR+ patients who are refractory to endocrine therapy. For these breast cancer patients, immunotherapy has the potential to improve survival by targeting cancer cells. Although, the role of immune response in breast cancer is not fully understood, studies have observed high number of natural killer (NK) cells, B cells, and cytotoxic T cells suppress tumor growth while high number of macrophages, and regulatory T cells (Treg) promote tumor growth. The purpose of this study is to measure levels of immune cells infiltration (cytotoxic, helper, and regulatory T cells, NK cells, and macrophages) between different ER, PR, and HER2 IHC subtypes.

Methods

Tissue microarrays were constructed from 106 breast cancer patients consisting of 36 triple negatives, 11 HER2 overexpressed (ER^-/PR^-), 7 $ER^+/PR^-/HER2^-$, 24 $ER^+/PR^+/HER2^-$, 8 $ER^+/PR^-/HER2^+$, and 20 $ER^+/PR^+/HER2^+$ assessed by a pathologist based on immunohistochemical stains. MultiOmyx™ hyperplexed immunofluorescence assay was performed on the constructed TMAs to profile IHC4 (HER2, ER, PR, Ki67), and immune (CD3, CD4, CD8, CD45RO, CD56, CD68, FOXP3) markers.

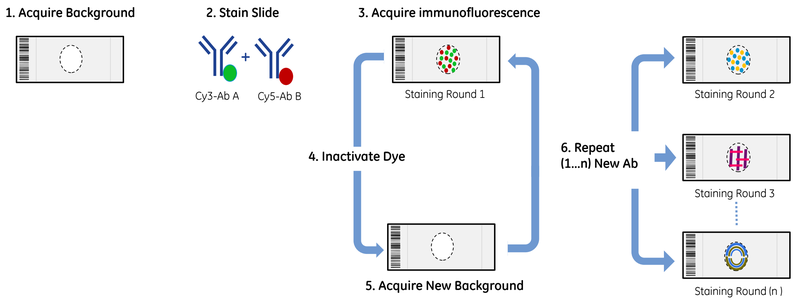

Figure 1: MultiOmyx IF multiplexing scheme from a single tissue section
Conjugated fluorescent antibodies were applied to a slide, followed by whole slide imaging. The dye was chemically inactivated, enabling a second round of staining with another fluorescent antibody. The process was performed multiple times from a **single slide.**

Discussion

Combined IHC4 and immune markers were profiled from a single FFPE slide using a hyperplexed MultiOmyx assay. Total of 106 breast tumor TMAs were assessed for immune cells infiltration in each of the following IHC4 subtypes: $ER^+/PR^+/HER2^-$, $ER^+/PR^+/HER2^+$, $ER^+/PR^-/HER2^-$, $ER^+/PR^-/HER2^{overexpressed}$, $ER^-/PR^-/HER2^{overexpressed}$, and triple negative. Immune cells infiltration is defined as a percentage of positive immune cells relative to the total number of stromal cells. PanCK was used to differentiate between tumor and stromal regions. $ER^-/PR^-/HER2^{overexpressed}$ subtype displayed the highest percentage of not only CD4, CD8 positive cells, but also Ki67 positive cells, followed by the triple negatives, and $ER^+/PR^-/HER2^{overexpressed}$. In the remaining three subtypes, the percentage of CD4, and CD8 positive cells are 1-2% (Fig 2C). Percentage of CD56 positive natural killer cells remained consistent across all subtypes (data not shown). Co-expression analysis (Fig 2D) in $ER^-/PR^-/HER2^{overexpressed}$ illustrates, despite high percentage of CD8+ cytotoxic T lymphocytes, Ki67 positive cells were abundant in the tumor. The data suggests:

- Presence of high intratumoral T cells in $ER^-/PR^-/HER2^{overexpressed}$ does not indicate functionally active T cells
- Possible evasion of host tumor-immune response by immunoinhibitory factors
- Potential benefit from immunotherapy targeting immune checkpoints (anti-PD-L1, anti-CTLA-4)

Comprehensive immunophenotyping requires identification of specific immune cell types, and differentiation of activated immune cells from inhibitory immune cells.

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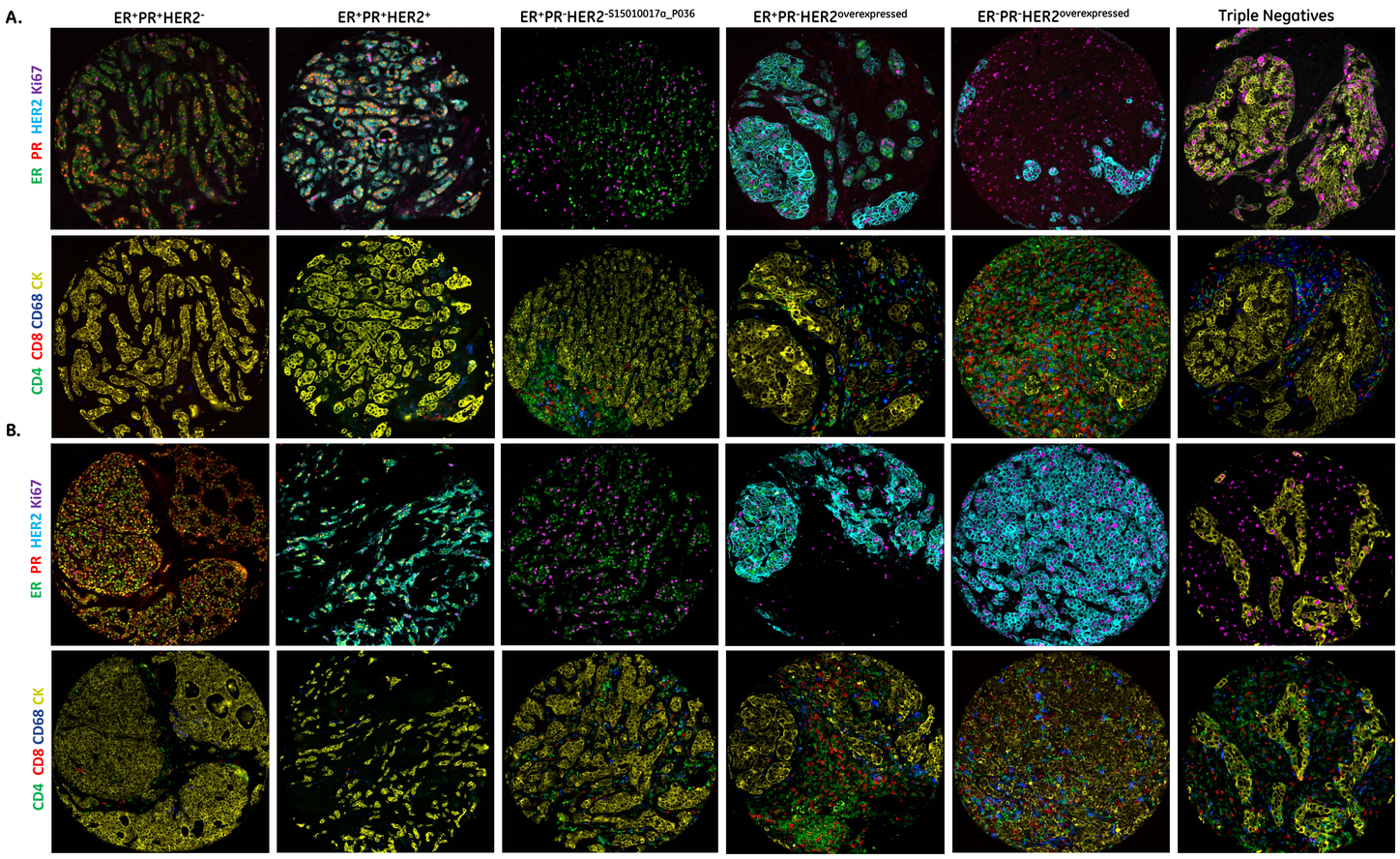
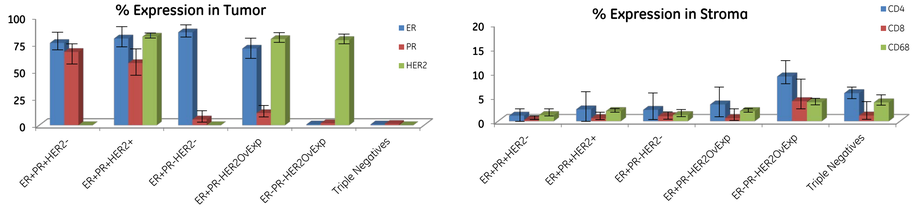
Results: Integrated Analysis of IHC4 and Immune Markers



C.

IHC Subtypes (n=106)	Tumor (% + cells)			Stroma (% + cells)			Total (% / mm²)
	ER	PR	HER2	CD4	CD8	CD68	Ki67
ER+PR+HER2- (24)	75.8	67.5	-	1.1	<1	1.3	2.7
ER+PR+HER2+ (20)	79.9	57.2	81.7	2.4	<1	2.1	2.3
ER+PR-HER2- (07)	85.6	5.4	-	2.3	1.0	1.3	4.6
ER+PR-HER2OverExp(08)	70.7	11.2	79.4	3.5	<1	2.1	4.3
ER-PR-HER2OverExp (11)	-	1.9	78.4	9.2	4.1	3.9	9.3
Triple Negatives (36)	-	1.2	-	5.8	1.2	3.9	5.6



D.

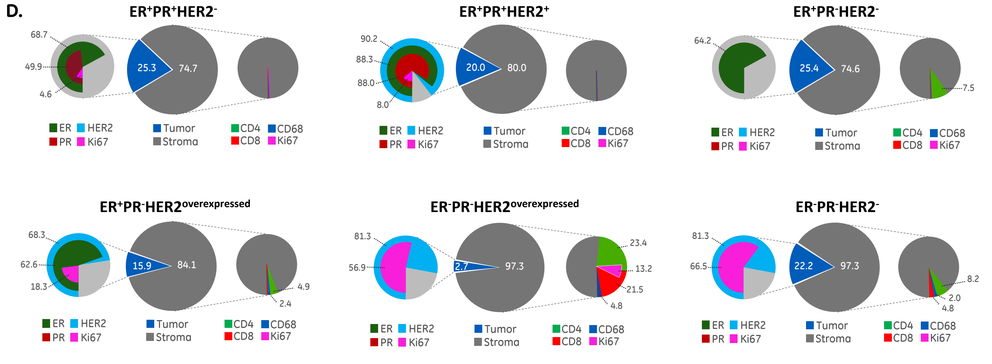


Figure 2:

A,B. Representative multiplexed IF color blended images across six IHC4 subtypes for ER,PR, HER2, Ki67 (top row), CD4, CD8, CD68, and CK (bottom row). 2 sets of representative images are shown.

C. Classification and quantitation of cells positive for IHC4 and immune biomarkers across all 106 samples. ER, PR, HER2 is reported as percentage of positive cells in tumor. CD4, CD8, CD68 is reported as a percentage of positive cells in stroma. Ki67 is reported as a percentage of Ki67 positive cells/mm² (total cells).

D. Co-expression analysis of ER, PR, HER2, and Ki67 in tumor and CD4, CD8, CD68, and Ki67 in stroma. Each marker is calculated as percentage of cells positive in their respective regions. Matching color blended images are shown in figure 2A above.