Chromogenic triplex characterization of breast and gastrointestinal cancers for biomarker discovery and spatial image analysis

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Abstract #65
SITC 2025

Background:

HER2 and PD-L1 expression serve as key biomarkers in breast and gastrointestinal (GI) cancer research. The tumor immune microenvironment, particularly the presence of tumor-infiltrating lymphocytes (TILs), also plays a critical role in these cancers, with mounting evidence of the importance of the signaling between the tumor and its environment. In this study, we evaluated two multiplex chromogenic immunohistochemistry (IHC) assays, HER2/PD-L1/CD3 and HER2/PD-L1/CD8, to assess the relationships and spatial expression with the tumor microenvironment

Methods:

Formalin-fixed, paraffin-embedded (FFPE) tissue sections from HER2-expressing and/or PD-L1-positive breast and GI cancers were stained using CD3/HER2/PD-L1 and CD8/HER2/PD-L1 chromogenic multiplex IHC assays on the BOND RX RUO automated stainer from Leica Biosystems. The assays employed the Chromoplex III Triple Detection RUO system from Leica Biosystems. Whole-slide imaging was performed on the Aperio GT 450 DX scanner, and HALO® digital image analysis from Indica Labs was used to quantify biomarker expression with the Multiplex IHC module leveraging HALO AI nuclear and membrane segmentation. Relationships between identified tumor cells and immune infiltrates were further interrogated with the Spatial Analysis module.

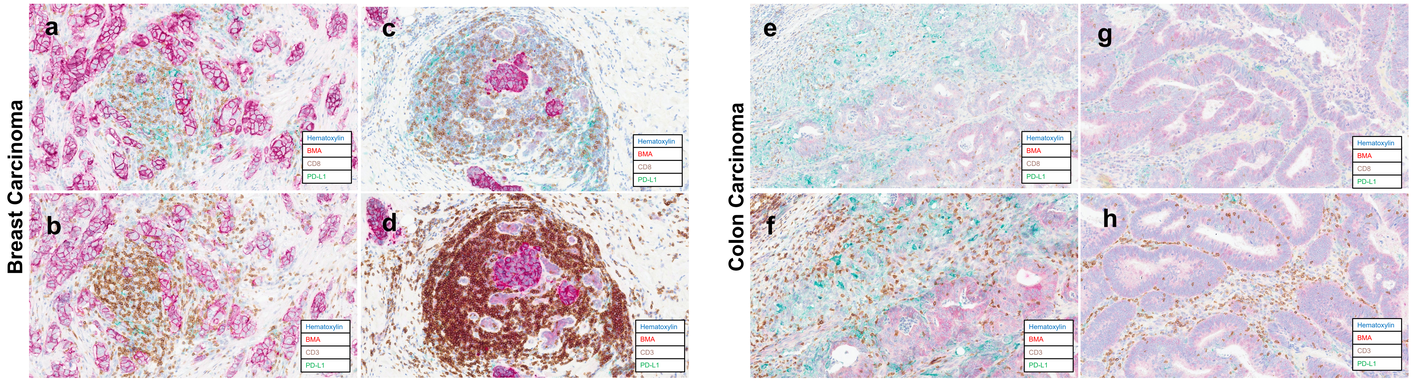

Figure 1: Multiplex IHC staining of Breast and Colon Carcinoma: CD3-DAB, **HER2(BMA)**-Red, **PDL1-**Green shown in b, d, f, h **and CD8-**DAB, **HER2(BMA)-**Red, **PDL1-**Green shown in (a, c, e, g)**. Staining exhibits the spatial interaction of TILs with the carcinoma.** Sample images are shown demonstrating staining of two 3Plex panels on 4 representative cases, 2 colon carcinoma (c, d and g, h) and 2 breast carcinoma (a, b and e, f). Images shown at 20x slides scanned on Leica GT450 Dx.

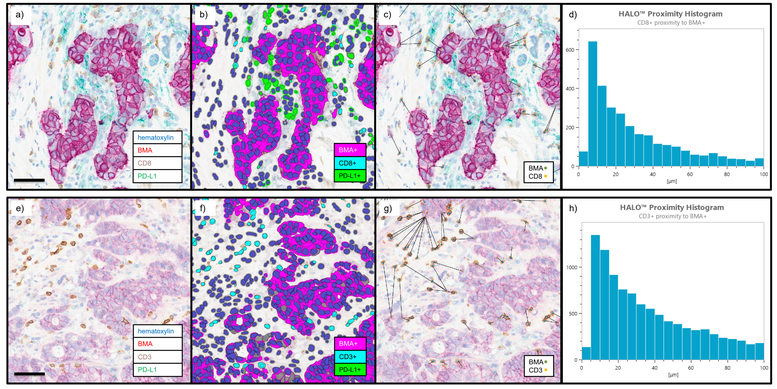

Figure 2: Image analysis performed using HALO digital image analysis software. Breast carcinoma tissue stained with HER2 (BMA), CD8 and PD-L1 (CAL10) visualized with ChromoPlex III Triple Detection RUO (a). Tissue was analyzed with HALO AI to segment sells based off nuclear and membrane morphology (b). Biomarker positivity was measured with HALO's Multiplex IHC analysis module. Cellular compartment positivity is indicated with the colored overlay. HALO proximity analysis measured the distance of each CD8 positive cell to the nearest HER2 positive tumor cell (c). CD8+ cells are listed according to their distance away from HER2+ cells and show a peak at 10 µm (d). Scale bar is 50 µm.
Stomach carcinoma tissue stained with HER2 (BMA), CD3 and PD-L1 (CAL10) visualized with ChromoPlex III Triple Detection RUO (e). Tissue was analyzed with HALO AI to segment sells based off nuclear and membrane morphology (f). Biomarker positivity was measured with HALO's Multiplex IHC analysis module. Cellular compartment positivity is indicated with the colored overlay. HALO proximity analysis measured the distance of each CD3 positive cell to the nearest HER2 positive tumor cell (g). CD3+ cells are listed according to their distance away from HER2+ cells and show a peak at 10 µm (h). Scale bar is 50 µm.

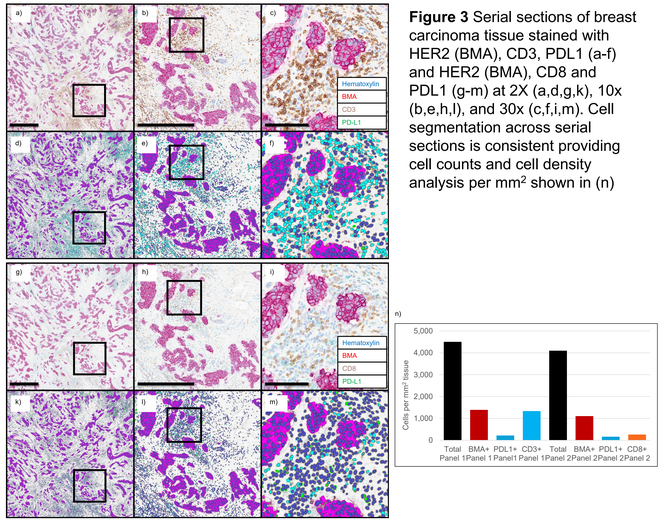

Figure 3 Serial sections of breast carcinoma tissue stained with HER2 (BMA), CD3, PDL1 (a-f) and HER2 (BMA), CD8 and PDL1 (g-m) at 2X (a,d,g,k), 10x (b,e,h,l), and 30x (c,f,i,m). Cell segmentation across serial sections is consistent providing cell counts and cell density analysis per mm^2 shown in (n)

Results

- In HER2-positive breast cancer, expression of PD-L1 and TIL markers (CD3, CD8) varied, with a subset of cases showing high CD8+ T-cell infiltration, potentially indicative of a more immunogenic phenotype.
- HALO AI analysis provides insights into proximity of CD3, CD8 and PDL1 positive infiltrating cells to HER2 positive carcinoma cells within breast and stomach carcinoma cases providing valuable spatial analysis using chromogenic multiplexing.
- Visualization of 3 markers simultaneously using ChromoPlex III Triple Detection RUO allows automated spatial analysis of tumor samples.
- In HER2 positive breast cancer and stomach cancer cases higher density of CD3+ cells and CD8+ cells shown to be in closer proximity to HER2 positive cells with the highest number of CD3 or CD8+ cells seen within 10uM of a HER2+ cell.

Conclusions:

ChromoPlex III Triple Detection RUO can be used as a tool to identify 3 simultaneous markers in FFPE tissue using automated staining (BOND RX Stainer). As an example - multiplex chromogenic IHC staining of HER2, PD-L1, and CD3/CD8 provides valuable insights into the interplay between the tumor and the immune cell microenvironment in breast and GI cancers. The use of whole-slide digital pathology (Aperio GT 450 DX Scanner) and HALO AI Image Analysis, enables high-throughput assessment of multiplex chromogenic stained slides. HALO AI Image Analysis is a powerful tool for automated quantification of marker expression and proximity analysis.