Imaging mass cytometry of pelareorep treated early breast cancer samples reveals developing tertiary lymphoid structures

Keck Medicine of USC — Abstract #4560

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BACKGROUND

- Pelareorep (pela) is an intravenously delivered oncolytic virus that stimulates anti-tumor activity through both direct tumor lysis and activation of innate and adaptive immune responses.
- The AWARE-1 study is a window-of-opportunity trial investigating the effects of pelareorep-based combination therapy in early-stage breast cancer.

- The tumor immune microenvironment (TiME) plays a critical role in shaping anti-tumor immunity, yet its dynamic remodeling during therapy remains incompletely understood.
- Imaging mass cytometry (IMC) enables high-dimensional, spatially resolved profiling of immune cell populations within tumor tissue.
- Tertiary lymphoid structures (TLS) are organized immune cell aggregates associated with improved anti-tumor responses, but their development and spatial organization during treatment are not well characterized.
- This study aims to characterize temporal changes in immune cell phenotypes, spatial organization, and cell–cell interactions within the TiME, with a focus on TLS formation following pelareorep-based therapy.

STUDY DESIGN AND METHODS

- Newly diagnosed HR+/HER2− early breast cancer patients were enrolled into two treatment cohorts:
- Cohort 1: pela + letrozole (n = 10)
- Cohort 2: pela + letrozole + atezolizumab (n = 10)
- Pela was administered on days 1, 2, 8, and 9; letrozole was given daily (days 1–21); atezolizumab was administered on day 3.
- Tumor biopsies (FFPE) were collected at baseline (D1), on day 3 prior to atezolizumab administration (D3), and at surgery (~D21).
- Tissue samples were stained using a 37-marker imaging mass cytometry (IMC) antibody panel, with each antibody conjugated to a unique metal isotope.
- The antibody panel was validated using human tonsil and HR+/HER2− breast cancer control tissues.
- Image acquisition and initial visualization were performed using the Hyperion Imaging System and MCD Viewer (Standard Biotools), with post-staining quality control assessment.
- IMC data were processed and segmented using the Steinbock analysis pipeline.
- Downstream analyses were performed in RStudio according to the Steinbock IMC analysis pipeline.
- Samples were re-analyzed using Hyperion XTi whole-slide imaging and MCD SmartViewer (Standard Biotools) to assess the presence and spatial distribution of TLS across non-ablated tissue regions.

RESULTS

Lymphoid Cells	
CD20	B Cell
NKG2A	NK Cells
ULBP 2-5-6	NK Ligand
CD16	NK / Myeloid Cells
NKG2D	NK / T Cells
CD3e	T Cells
CD4	T Helper Cells
CD45RO	Memory T Cell
CD80	T Cell Activation
CD8a	Cytotoxic T Cells
FOXP3	Regulatory T Cell

Tumor Related	
ProgesteroneR	Tumor
EstrogenR	Tumor
GATA3	Tumor
Vimentin	Structural Protein
PanCk	Epithelia
ReovirusP17	Oncolytic Virus

Functional / Cell State	
GranzymeB	Activation
Ki67	Proliferation
CleavedCasp3	Cell Death

Myeloid Cells	
CD14	Monocytes
CD33	Macrophages
CD68	Macrophages
CD163	Macrophages
CD11c	Dendritic Cells
CD11b	MDSC
CD15	MDSC
CD163	Myeloid / NK

Structure / Environmental	
CD31	Vascular/Megakaryocytes
PanCk	Epithelia
Vimentin	Structural Protein

Checkpoints	
PD1	Checkpoint
PDL1	Checkpoint
IDO	Checkpoint

Other	
HLA-ABC	MHC I
HLA-E	MHC I
HLA-DR	MHC II
HistoneH3	Nuclear Stain
191Ir	Nuclear Stain
193Ir	Nuclear Stain

Figure 1

Custom IMC panel composed of 37 metal-conjugated antibodies.

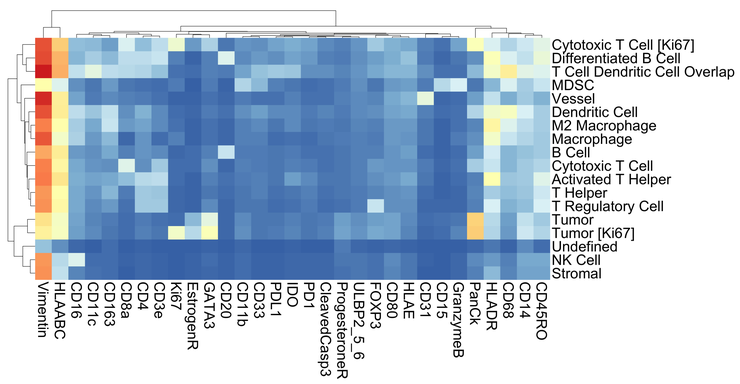


Figure 2

Expression heatmap of phenotypes identified across all samples following object segmentation and unsupervised clustering.

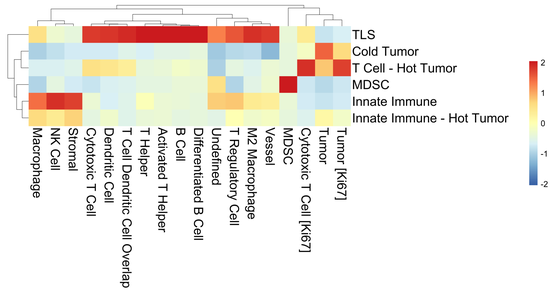


Figure 3

Cellular neighborhood heatmap determined using an algorithm similar to PhenoGraph, identifying the most common cellular organizations.

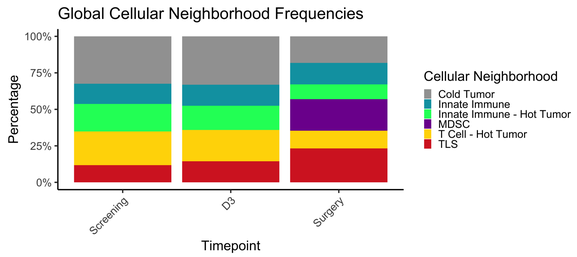


Figure 4

Cellular neighborhood frequencies show an increase in TLS-associated cellular neighborhoods.

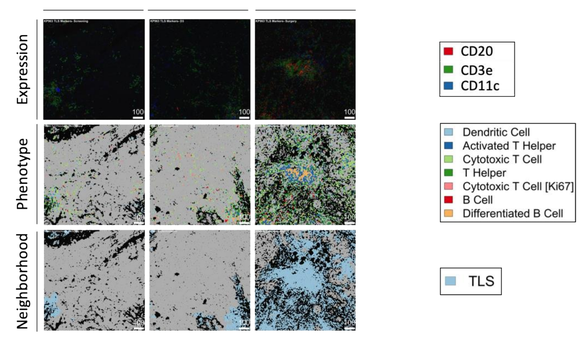
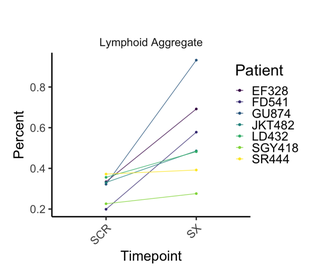
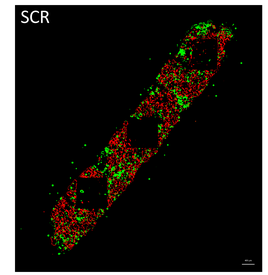


Figure 5

Triplex representative images from a selected patient showing TLS-related cells in three sets of visualizations for three ROIs. Top row: raw marker-level expression of CD20, CD3, and CD11c highlighting B cells, T cells, and dendritic cells. Middle row: corresponding cell masks highlighting phenotyped cells of interest that form the TLS: dendritic cell, activated T helper, cytotoxic T cell, T helper, cytotoxic T cell [Ki67], B cell, and differentiated B cell. Bottom row: corresponding cell masks highlighting TLS neighborhood formed by the interactions of the above cells.





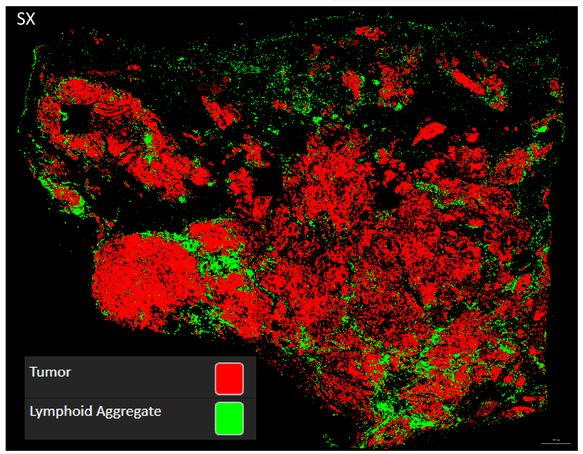


Figure 6

Line plot shows an increase in lymphoid aggregate populations across whole-slide ablated tissue.
Representative whole-slide images from a selected patient (GU874) illustrate the increased presence of lymphoid aggregates within the tumor microenvironment.

CONCLUSIONS

- Unsupervised clustering and cellular neighborhood analysis revealed dynamic remodeling of the tumor immune microenvironment (TiME) following pelareorep-based therapy.
- A distinct TLS-associated cellular neighborhood, composed of cytotoxic T cells, dendritic cells, T helper cells, B cells, differentiated B cells, and M2 macrophages, was identified and shown to increase over time.
- Whole-slide re-analysis using Hyperion XTi further validated the presence and distribution of lymphoid aggregates across non-ablated tissue, indicating that TLS formation is not limited to sampled regions.
- Together, these findings provide the first spatially resolved evidence of TLS development and coordinated cell–cell interaction dynamics in early-stage breast cancer patients treated with pelareorep-based therapy.