Analysis of the HR+/HER2- breast cancer tumor microenvironment following immune priming with pelareorep and atezolizumab using imaging mass cytometry - Results from the AWARE-1 trial

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BACKGROUND

- Pelareorep (pela) is an intravenously delivered, non-modified oncolytic reovirus that shows anti-tumor activity through innate and adaptive immune responses as well as direct tumor lysis.
- Previous data from the window of opportunity AWARE-1 study demonstrated synergy between pela in combination with atezolizumab, demonstrating a favorable immunologic response in tumors from early breast cancer (eBC) patients.
- To understand the complex tumor microenvironment (TME) and immune responses in patients before and after treatment, we used imaging mass cytometry (IMC) to perform single cell, highly multiplexed, analysis of their tissue samples.

METHODS

- Newly diagnosed HR+/HER2- eBC patients were enrolled into two cohorts: Cohort 1: pela + letrozole (n=10); and Cohort 2: pela + letrozole + atezolizumab (n=10). Pela was administered on days 1, 2 and 8, 9, and atezolizumab was given on day 3. Tumor biopsies (FFPE samples) collected pre-treatment (D1) and on days 3 (D3, prior to atezolizumab administration) and approximately on day 21 (when tumors were surgically removed) were examined by IMC.
- A marker panel of 37 antibodies was assembled, each of which were conjugated to a unique metal isotope.
- The panel was validated against human tonsil and HR+/HER2- breast cancer control tissue. Image visualization was performed using MCD Viewer from Standard Biotools.
- After validation, the antibody cocktail was used to stain the breast cancer biopsies. MCD Viewer was used again to check the quality of the staining before moving on to the downstream analysis.
- The Steinbock IMC analysis pipeline was used to process and segment the data.
- The data was then exported into RStudio to perform more complex analyses such as: expression normalization, phenotyping (PhenoGraph), UMAP plot generation, pseudocolor image generation, cluster frequency calculation, and significance testing.

RESULTS

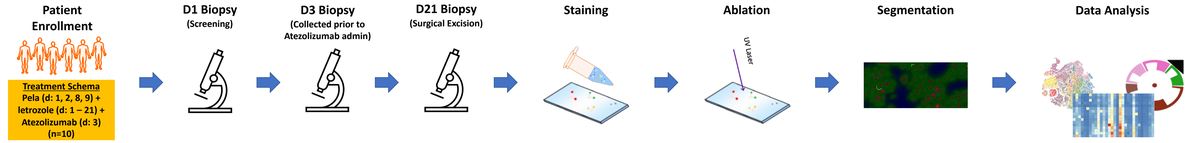

Figure 1

Study treatment and analysis schema. Prior to treatment on day 1, biopsies were collected from each patient. Patients were then treated with a combination of pela, letrozole, and atezolizumab. Biopsies were collected on day 3 prior to treatment. Post-treatment biopsies were then collected on day 21. Each breast cancer biopsy was stained with the antibody cocktail and ablated using the Hyperion™ Imaging System. The raw data was then visualized with MCD Viewer and processed using the Steinbock pipeline. Segmented data was then used for downstream analysis in RStudio.

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

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

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

Checkpoints	
PD1	Checkpoint
PDL1	Checkpoint
IDO	Checkpoint

Functional / Cell State	
GranzymeB	Activation
Ki67	Proliferation
CleavedCasp3	Cell Death

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

Figure 2

Custom IMC panel composed of 37 metal conjugated antibodies.

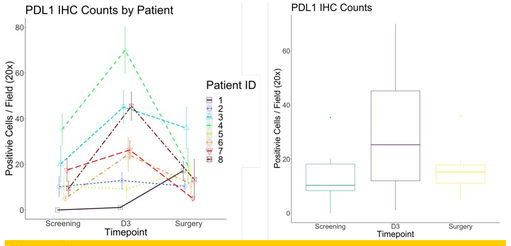

Figure 3

IHC counts of PDL1 positive cells shown by patient and as a summarized boxplot. There was an increase of positive cells between screening and day 3 and a decrease of positive cells between day 3 and day 21.

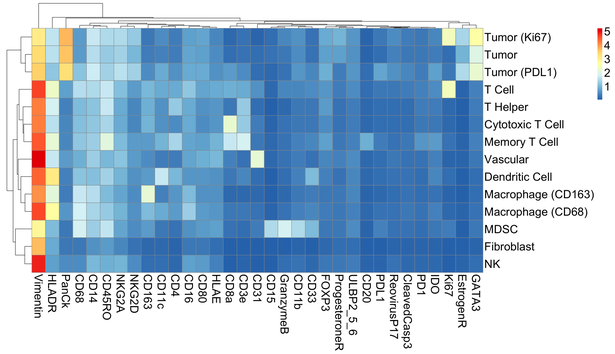

Figure 4

Expression heatmap of phenotypes identified across all samples after performing pixel-classification segmentation in Ilastik and running the PhenoGraph unsupervised clustering algorithm.

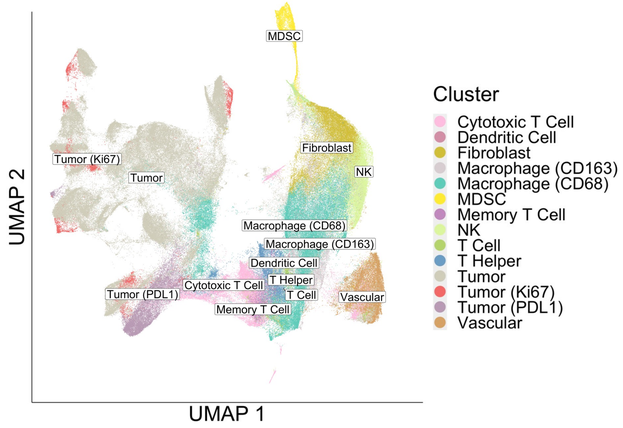

Figure 5

Annotated UMAP of all clusters generated by PhenoGraph.

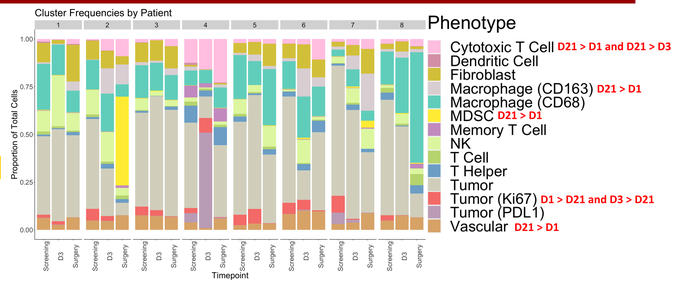

Figure 6

Changes in cluster frequencies across timepoints. There was a significant increase in CD163+ Macrophage, MDSC, and Vascular populations between screening and day 21. There was a significant increase in Cytotoxic T Cells and decrease in proliferating tumor between screening and day 21 as well as between day 3 and day 21.

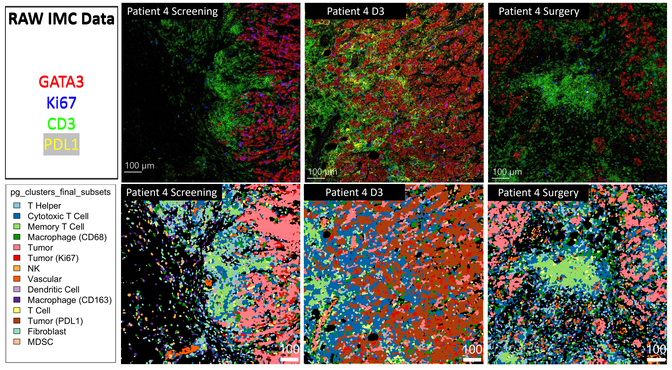

Figure 7

Comparison of raw IMC data (top) and processed/phenotyped data (bottom). Visualization of GATA3, Ki67, CD3, and PDL1 channels on the raw data shows an increase in PDL1 positivity on day 3 consistent with the IHC counts. On the bottom, pseudocoloring of each phenotype identified by PhenoGraph on the segmented cell masks shows the same increase after identification of the PDL1 positive tumor phenotype. Visualization of the raw and phenotyped data shows the changing tumor proportion and architecture throughout the course of treatment. By day 21, tumor cells are much more diffuse and their relative proportion to immune cells has decreased. In the day 3 timepoint, Cytotoxic T Cells can be seen with a higher rate of tumor infiltration relative to screening. The increase of PDL1 expression on day 3 and the following decrease on day 21 as seen by IHC can likely be attributed to the reduction of PDL1 positive tumor cells as a result of treatment.

CONCLUSIONS

- In accordance with the prior AWARE-1 results, IMC demonstrated an enhanced immune state of the tumors after treatment.
- IMC allows us to analyze the potent immune response and cellular interactions in the TME; characterization of these complex interactions provides a better understanding of the key mechanisms of action of such treatments to plan future clinical trials.