Multiplex analysis of the tumor immune microenvironment during treatment with atezolizumab/pelareorep/letrozole reveals novel immune-tumor interactions

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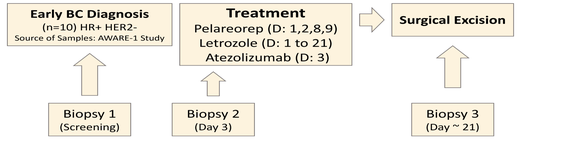
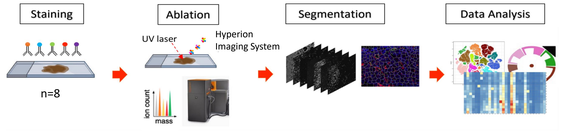
Introduction

- Breast cancer is the most common tumor and the leading cause of cancer death in women worldwide.
- Oncolytic viruses can selectively target malignant tissues without affecting normal cells.
- Pelareorep (pela) is a non-modified intravenously administered oncolytic reovirus exhibiting effective tumor suppression through both innate and adaptive immune responses, as well as direct tumor lysis.
- Our previous study demonstrated the synergistic potential of combining pela with atezolizumab, showcasing promising immunological reactions within tumors of early breast cancer patients.
- Tumor biopsies were taken from patients enrolled in Cohort 2 of the AWARE-1 study. Patients received pela, atezolizumab , and letrozole. Biopsies were taken before treatment, on day 3 (D3) before atezolizumab, followed by surgical excision on day 21 (D21).
- We employed imaging mass cytometry (IMC) to conduct high-dimensional, single-cell analysis of tissue samples, to gain deeper insights into the complex tumor immune microenvironment (TiME) pre- and post-treatment.

Materials and Methods



Imaging Mass Cytometry



Breast Cancer TiME Antibody Panel

NK Cells	
NKG2A	NK Cells
NKG2D	NK Cells
ULBP2	NK Cells
Granzyme B	NK / T Cells
CD16	NK / Macrophage

T Cells	
CD45RO	Memory T Cells
CD3	T Cells
CD4	Helper T Cells
CD8a	Cytotoxic T Cells
FOXP3	Regulatory T Cells

Dendritic Cells	
CD11c	DC

B Cells	
CD20	B Cells

Nuclei	
Histone H3	Chromatin

Macrophages	
CD14	Monocytes / Macrophages
CD68	Monocytes / Macrophages
CD80	Activation Marker
CD163	Macrophages (M2)
HLA-DR	Macrophages (M1)

Immune Regulator	
HLA-ABC	Immune Regulator
IDO	Immune Regulator
PD-L1	Checkpoint
PD-1	Checkpoint
HLA-E	NKG2A Ligand

Myeloid	
CD15	Gran / MDSC
CD33	Myeloid
CD11b	MDSC

Breast Cancer	
ER	Hormone Receptor
PR	Hormone Receptor
GATA3	Luminal TF
Pan-CK	Cytokeratin

Mesenchymal Markers	
Vimentin	epithelial-mesenchymal transition (EMT)

Cell Death	
Caspase 3	Apoptosis

Reovirus	
Reovirus p17	Reovirus p17

Cell Growth and Division	
Ki-67	Proliferation

Endothelial	
CD31	Vascular

Summary of Key Findings

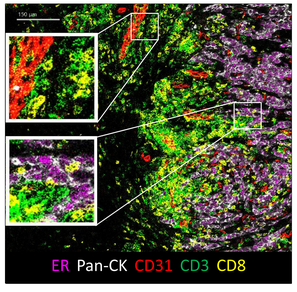


Figure 1.
Interaction of immune cells with breast cancer cells in Patient #4, Screening.

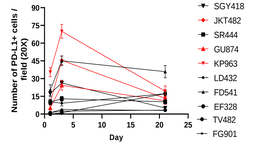


Figure 2.
- Plots of PD-L1 expression were obtained with the 28-8 anti-PD-L1 clone quantifying expression in both tumor tissue and immune cells by IHC.
- PD-L1 expression was induced on D3 following Pela but decreased on D21 following atezolizumab.

Summary of Key Findings

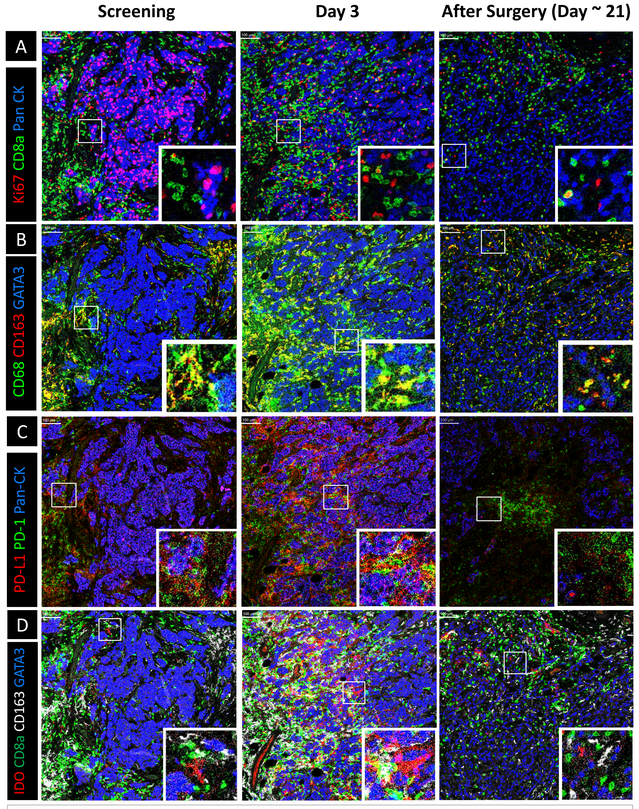


Figure 3. TiME of proliferating breast cancer cells on Screening, D3 and After Surgical Excision (D21). The presented results include a selection of key antibodies from the panel.
(A) Increase in proliferating (Ki67+) cytotoxic T cells adjacent to tumor cells post-treatment (D3 and D21).
(B) CD68+ macrophages infiltrated tumor on D3. Increased CD68+/CD163+ macrophages were noted on D21.
(C) On D3, there was an increase in PD-1 and PD-L1 expression. By D21, PD-L1 expression decreased in tumor cells, while PD-1 expression remained.
(D) Treatment induced IDO expression in tumor cells.

- We noticed an increase in proliferating (Ki67+) cytotoxic T cells adjacent to apoptotic (caspase 3+) tumor cells post-treatment (both D3 and D21) samples indicating immunogenic cell death.
- We observed a decrease in Ki67+ and ER+ tumor cells after treatment indicating decreased tumor cell proliferation and hormone expression.
- In 3 out of 8 patients, treatment was associated with a shift in monocyte/macrophages from an M1 (CD68+/CD163-) to M2 (CD68+/CD163+) phenotype associated with significant tumor infiltration and a significant increase in apoptotic M2 macrophages on D21.
- Consistent with the known immune priming effects of pela, both PD-1 and PD-L1 increased on D3. Subsequently, on D21, post atezolizumab, tumor PD-L1 decreased in tumor cells while PD-1 expression persisted. The decrease in PD-L1 expression may be explained by the clear reduction of tumor cells.
- Treatment induced IDO expression on both tumor and monocyte/macrophage cells.
- Unlike its effect on PD-L1, atezolizumab did not appear to attenuate IDO expression on D21.

Conclusion

- Our study utilizing IMC revealed the immune-tumor interactions during and post treatment with atezolizumab/pelareorep/letrozole.
- Characterization of these complex interactions allows for a deeper understanding of the key mechanisms of action of these treatments and planning of future combination studies.

References

1. Shen, L., et al., *Exploration of prognosis and immunometabolism landscapes in ER+ breast cancer based on a novel lipid metabolism-related signature*. Front Immunol, 2023. **14**: p. 1199465.
2. Mostafa, A.A., et al., *Oncolytic Reovirus and Immune Checkpoint Inhibition as a Novel Immunotherapeutic Strategy for Breast Cancer.* Cancers (Basel), 2018. **10**(6).
3. Keren, L., et al., *A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging.* Cell, 2018. **174**(6): p. 1373-1387.e19.