

Differential T cell response to anti-PD1 in breast cancer sub-types is driven by activity of intra-tumoral immune cells

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Background

Immune checkpoint inhibition (ICI) holds great promise for triple-negative breast cancer (TNBC), while it shows limited response in hormone receptor positive (ER/PR+) breast cancer (CaBr). Given that the effectiveness of immuno-modulatory drugs depends on immune subpopulations in the tumor microenvironment (TME), we employed the Farcast CaBr TruTumor histoculture platform, that preserves the native TME, to study the role of tumor resident immune cell types in determining T-cell activation levels on ICI treatment, in the two CaBr sub-types.

Methods

Patient tissue samples: Fresh, surgically resected breast cancer (CaBr) tissue sample along with blood sample were collected from consented patients and immediately transported to laboratory for histoculture.

Histoculture workflow: The tumor sample (n=11) was processed to generate thin explants, without enzymatic digestion, to retain near-native tumor microenvironment. Tumor explants were cultured in media with autologous plasma for 72 hrs. Explants were treated with anti-PD1 (Nivolumab:132 µg/ml) or anti-CD3 (0.01 µg/ml) + Interleukin-2 (IL2, 100 IU/ml). Media was replaced along with Nivolumab or antiCD3+IL2 every 24 hours. Response to treatment was evaluated using multi-modal assays (Fig. 1).

Flow cytometry analysis: Post culture, tumor explants were dissociated to generate a single cell suspension, followed by staining with Live/Dead dye and a cocktail of antibodies for immune cell lineage and activation. Data was acquired using BD LSR Fortessa flow cytometer with appropriate compensation controls and analyzed using FlowJo software.

H&E & IHC: H&E staining was performed with 4µm sections obtained from the FFPE block using Leica automated multi-stainer system. CD8 and cleaved Caspase-3 IHCs were performed with 4µm sections using Ventana IHC automated staining system. Scoring was performed by certified pathologists to evaluate tumor content from H&E-stained slides and Cleaved Caspase-3 staining in the tumor compartment.

Cytokine Analysis: The cultured supernatants at T0, T24, T48, T72 were tested for the presence of cytokines (IFN gamma, Peforin, Granzyme B, IL-10, IL-17A, IL-1 Beta, TNF alpha, IL-15, IL-2, MIG (CXCL9), IP-10 (CXCL10), IL-6, VEGF-A, IL-8 (CXCL8), SDF1 alpha, MCP-3 (CCL7), IL-4, IL-5, MIP-1 alpha (CCL3), MCP-4 (CCL13), FGF-2, I-TAC (CXCL11), Eotaxin, G-CSF) using Luminex MAGPIX instrument and data was analysed using MILLIPEX™ Analyst software.

Statistical analysis: All data analysis and graphical representations were done using GraphPad Prism (Version 9). Mann-Whitney t-test was used to generate p-values. p-value significance is represented as *(p<0.05) **(p<0.01) ***(p<0.01). Heat maps were generated on GraphPad and Morpheus (<https://software.broadinstitute.org/morpheus>).

Farcast TruTumor Histoculture work-flow

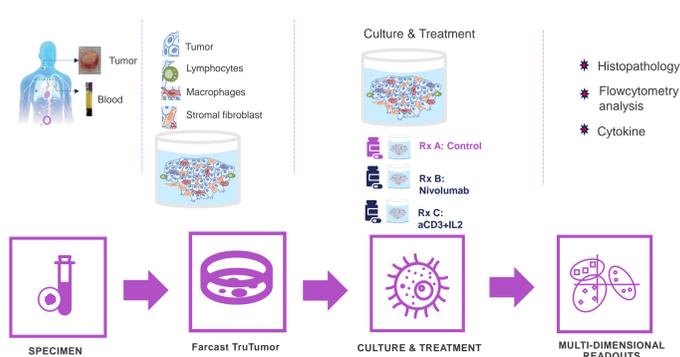


Fig. 1: Schematic representation of Farcast™ TruTumor Histoculture work-flow and downstream assays used for treatment response evaluation.

Patient demography

Parameters	Categories	Values (%)
Age	≤ 47	55
	≥ 47	45
Gender	Female	100
	Male	0
Grade	Grade 1	9
	Grade 2	64
	Grade 3	9
	Unknown	18
Stage	I	18
	II	36
	III	36
	Unknown	9
Primary/Recurrent	Primary	100
Receptor	ER/PR+	55
	TNBC	45
Tumor Site	Left breast	36
	Right breast	63
	Proximal Lymph nodes	1

Table 1: Demography of patient (n=11) sample used for histoculture.

CaBr has low baseline immune content in tumor microenvironment

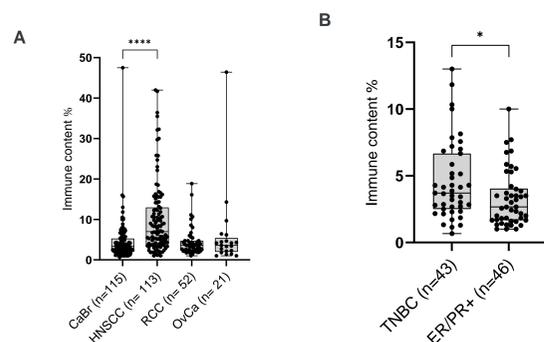


Fig. 2: A. Plot is showing the abundance of immune component in the TME of CaBr, HNSCC, RCC and ovarian cancer (OvCa) samples (H&E). B. Plot is showing the abundance of immune components in the two different CaBr subtypes as evaluated by histopathology analysis.

TNBC has higher proportion of lymphoid population over myeloid population compared to ER/PR+ breast cancer

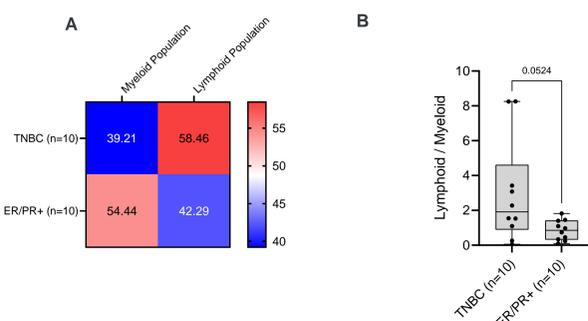


Fig. 3: A. Heatmap is showing the distribution of immune sub-population in TNBC and ER/PR+ TME. B. Plot is showing the ratio of two sub-population of immune cells.

Anti-CD3+IL2 stimulation showed similar response in the two CaBr sub-types

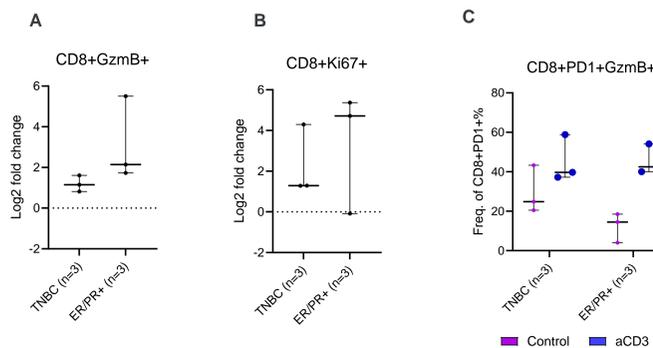


Fig. 4: Graphs showing changes in A. CD8+ Granzyme B+ population (Frequency of CD8+) B. CD8+ Ki67+ population (Frequency of CD8+) and C. Population of exhausted CD8 cells producing Granzyme B in response to aCD3+IL2 stimulation (n=6).

Two distinct sub-populations of exhausted CTLs exhibit dissimilar response to aCD3+IL2 stimulation

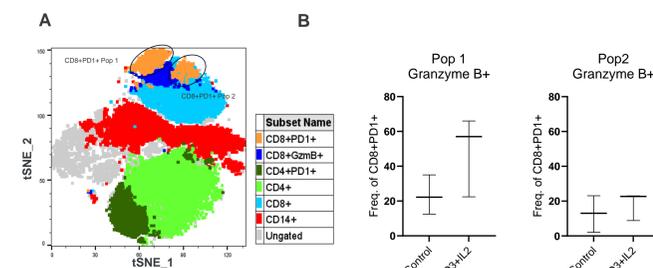


Fig. 5: A. tSNE plot for various immune subsets in the post culture TME identify two populations of exhausted CD8+ T cells. B. Plot showing the increase in Pop 1 but not Pop 2 on stimulation with CD3+IL2.

TNBC exhibited better T cell activation on anti-PD1 treatment compared to HR+ subtype

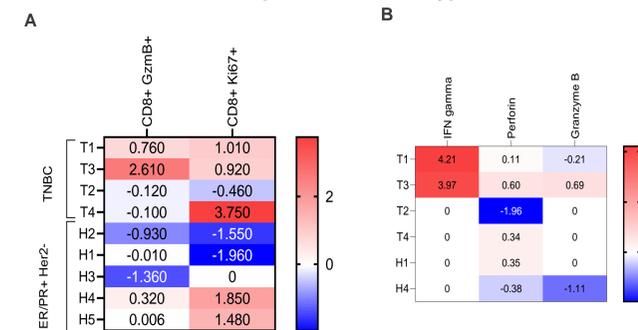


Fig. 6: Heatmap is showing A. log2 fold change for activated and proliferating CTL population upon treatment with Nivolumab and B. cytokine release upon treatment with Nivolumab

Nivolumab non-responders responded to Anti-CD3

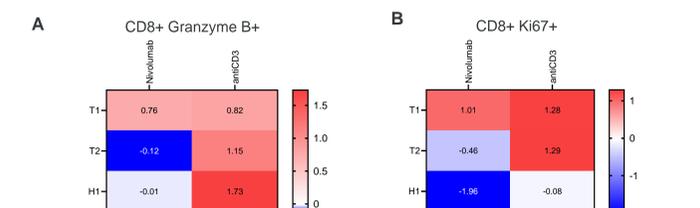


Fig. 7: Heatmap showing log2 fold change with respect to control A. activated and B. proliferating Cytotoxic T cells in response to Nivolumab Treatment and aCD3+IL2 stimulation.

Nivolumab non-responders contained lower proportions of Pop 1 exhausted CD8+PD1+ and higher proportions of monocytes in the control arm

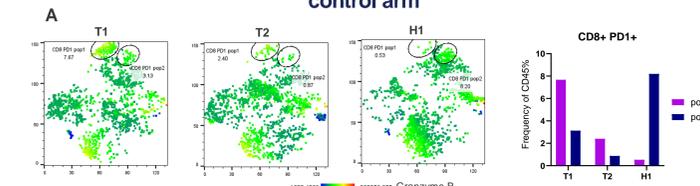


Fig. 8: Graphs showing A. Pop 1 and Pop 2 sub-populations, B. percentage of CD8+PD1+ and C. CD14+ monocyte population in the control arm of TNBC and ER/PR+ samples.

T1 is predisposed to responder phenotype

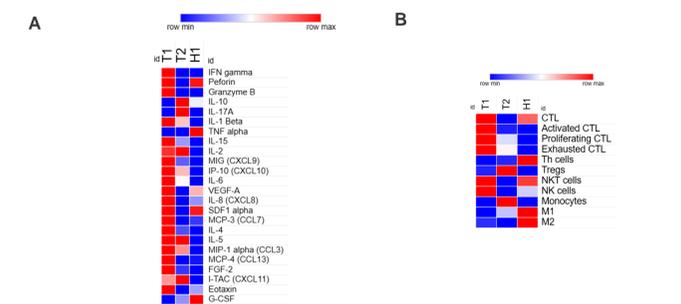


Fig. 9: Heatmap showing A. cytokine released and B. immune cell population in the control arm.

Summary

- The Farcast™ TruTumor platform enables study of immune sub-populations within the tumor microenvironment and their modulation in response to drug treatment.
- Interplay between different TME immune sub-types influencing response to Nivolumab in CaBr is effectively captured in the TruTumor platform.