Demonstrating the role of spatial contexture in determining response to immune check point inhibitors using a tumor histo-culture platform

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Introduction:

Baseline signatures have so far failed to accurately predict response to immune checkpoint inhibitors. A model that mimics the clinical response of tumor immune microenvironment (TiME) upon treatment in culture, would dramatically improve the chances of identifying true responders. Tumor histocultures capture the complex interactions between various tumor microenvironment components effectively. They retain the immune cell repertoire along with spatial context. The Farcast™ TiME histoculture platform provides a living microenvironment with near native fidelity to elucidate treatment response patterns that closely mimic patient response.

Methodology:

Patient tissue samples: Fresh surgically resected Head and Neck Squamous Cell Carcinoma (HNSCC) tissue samples were collected from consented patients . A matched blood sample from the patient was also collected.

Histo-Culture workflow: The tumor sample was processed to generate thin explants without enzymatic digestion to retain the tumor microenvironment. The tumor explants were cultured with media and autologous plasma. The explants were treated with immune checkpoint inhibitor Nivolumab (anti-PD1, 132 µg/ml) and Nivolumab + Ipilimumab (anti-CTLA4, 90.8 µg/ml) for 72 hrs. Fresh media was replaced every 24 hrs and the collected supernatant was used for cytokine analysis.

Flow cytometry analysis: The tumor explants were dissociated into single cells. And stained with with Live/Dead dye, surface antibodies and intracellular antibodies. Data was acquired in BD LSR Fortessa using appropriate compensation controls and analysed using FlowJo software. CD45, CD3, CD4, CD8, Granzyme-B, Ki67, PD1 antibodies were used to understand T cell activation post treatment.

Cytokine Analysis: The supernatants collected at T0, T24, T48, T72 were used for analysis using Luminex Magpix instrument using a 5 plex (IFNg, IL10, TNFa, Perforin and GranzymeB) Procarta Plex kit and data was further analysed using Milliplex analyst software.

H&E & IHC: H&E and Cleaved Caspase 3 IHC was performed with 4µm sections obtained from the FFPE block using Leica automated multi-stainer system and Ventana IHC automated staining system respectively. Scoring was performed by certified pathologists. From H&E-stained slides, tumor content, Immune content and Infiltrating immune cells (% immune cell proximal to tumor nest) were evaluated. Cleaved caspase 3 staining was evaluated in the tumor compartment.

mIHC: mIHC was performed using T-reg FixVUE (CD8, CD4, FoxP3, panCK and nucleus counterstain) from Ultivue on 4µm FFPE sections from post treatment samples. Data was analysed using QuPath analysis software.

NanoString GeoMx DSP: This was performed using a 72-protein marker assay panel using panCK,CD8 and DNA as morphological markers. Using panCK segmentation masking was performed and the data was analyzed by GeoMx DSP software.

Farcast™ TiME Histo-culture platform:

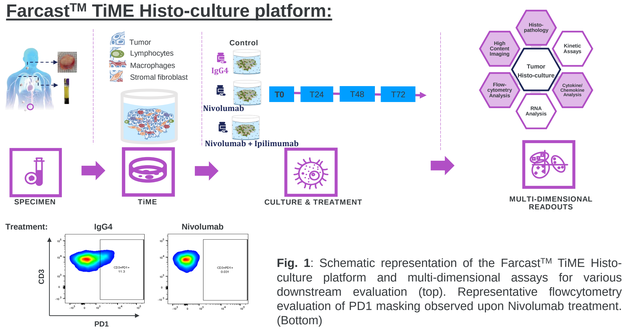

Fig. 1: Schematic representation of the Farcast™ TiME Histo-culture platform and multi-dimensional assays for various downstream evaluation (top). Representative flowcytometry evaluation of PD1 masking observed upon Nivolumab treatment. (Bottom)

Patient Demography:

Patient Demographics	Gender		Age		Grade		
	Male	Female	35–60	>60	1	2	3
No. of Patients	19	26	32	13	31	13	1

Results:

Interferon gamma secretion does not always lead to tumor cytotoxicity

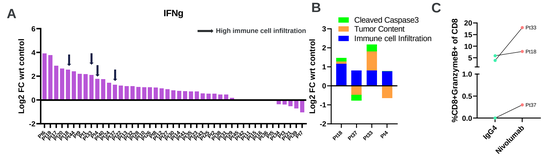

Fig.1: [A] Stratification of patient cohort based on IFNg secretion. Marked with arrows are the samples with highest immune infiltration. [B] Samples with highest immune infiltration and its correlation with tumor cytotoxicity [C] CD8+GranzymeB+ prevalence in sub-set of high immune infiltration samples.

Characterization of a treatment resistant sample using spatial analysis

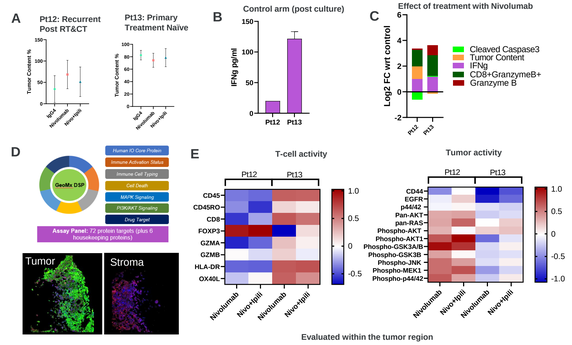

Fig.2 : [A] Tumor content evaluated using H&E-stained images post treatment. [B] Low T cell activity assessed by Interferon gamma secretion in Pt12, compared to Pt13. [C] Effect of treatment with Nivolumab evaluated with multi-modal assays: H&E (Tumor content); IHC (cleaved Caspase), Cytokine secretion (Interferon gamma, GranzymeB), Flow cytometry (CD8+GranzymeB+) [D] GeoMx DSP panel details and the segmentation strategy using panCK masking [E] Heatmap of Log2 protein expression fold change upon treatment within the Tumor segment in the two samples. Cytotoxic T cell (CTL)/Treg markers are shown as T cell activity and tumor proliferation markers are shown as Tumor markers.

Elucidating temporal response to treatment by combining bulk with spatial distribution of CTL

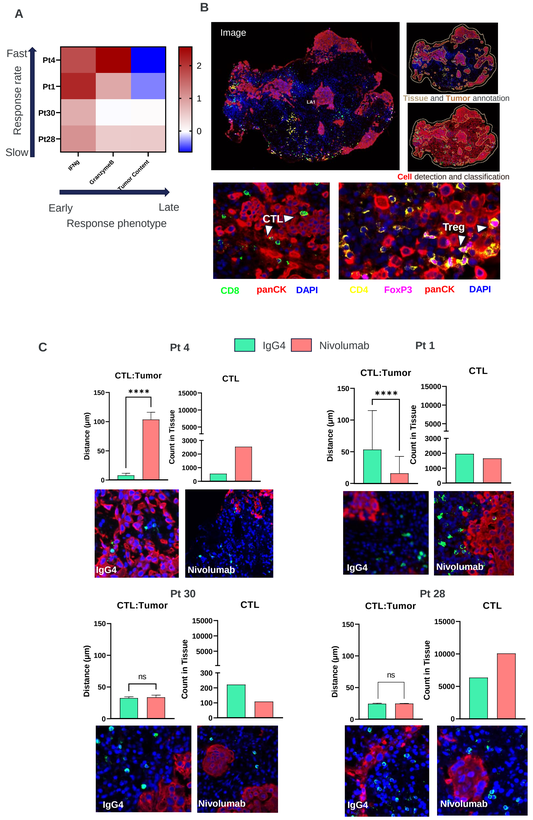

Fig.3 : [A] Multiple bulk assay readouts used to stratify response rates in four samples [B] QuPath based annotation and segmentation strategy used for multiplex IHC spatial data analysis. [C] Total CD8+ cell count and their spatial distribution pattern is shown graphically for each sample. Representative mIHC images from the control (IgG4) and treated (Nivolumab) arms are shown.

Ipilimumab used in combination with Nivolumab effects the distance between CTL and Treg

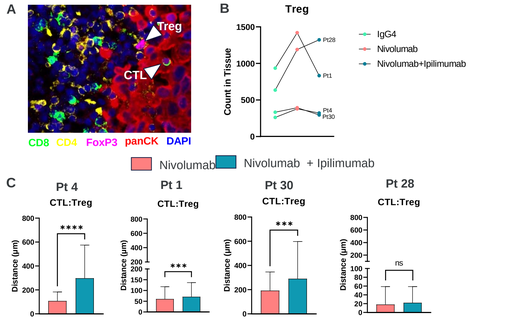

Fig.4 : [A] Representative image of a tumor explant stained with CTL, Treg and tumor markers is shown. [B] QuPath based quantification of Treg numbers is shown graphically across various treatments. [C] Distance between CTL and Treg is graphically represented post treatment.

Spatial response to treatment with Immune checkpoint inhibitor

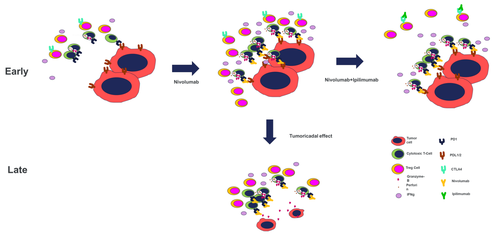

Fig.5: Schematic representation of the spatial dynamics observed in different tumor microenvironments upon Nivolumab and Nivolumab+Ipilimumab combination

Summary

Farcast TiME thus provides a unique platform that can combine spatial dynamics of immune cells overlaid with bulk data, providing important insights into understanding underlying mechanisms that counter T-cell response to immune check point inhibitors.

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