Understanding the functional fidelity of tumor infiltrated leukocytes (TILs) in a human tumor histo-culture platform

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Introduction:

There is an unmet need for robust and accurate preclinical model to minimize translational failure especially in immuno-oncology (IO) research. The poor correlation between preclinical data with clinical trials remains a major concern. A near native and biologically relevant model is required for better correlation with an in vivo drug efficacy. Farcast™ Tumor immune Micro-Environment (TiME) is a human histo-culture platform which preserves tumor and stroma along with the immune compartment, post culture. In this study we investigate the functional fidelity of TILs by subjecting tumor explants to immune modulators and check point inhibitors.

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 either with immune stimulants like LPS (1µg/ml), anti-CD3 (10 ng/ml) plus IL2 (100units/ml) or immune checkpoint inhibitor Nivolumab (anti-PD1, 132µg/ml), Ipilimumab (anti-CTLA4, 90.8µg/ml) and their combination for 72 hours. Culture supernatant was collected every 24 hour and stored for cytokine analysis. Media was replaced every 24 hours.

Flow cytometry analysis: The tumor explants were dissociated post culture with various treatments into single cells and stained with with Live/Dead dye, and cocktail of immune cell lineage and activation marker antibodies. Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and analysed using FlowJo software.

Cytokine Analysis: The cultured supernatants at T0, T24, T48, T72 were tested for the presence of various cytokines using Luminex Magpix instrument and data was analysed using MILLIPLEX™ Analyst software.

NanoString Analysis: The RNA extracted arm-wise from the explant TMA (Tissue Micro Array) FFPE block was quantified using Tape Station and 30-50ng of RNA based on DV200 concentration was used for running on the NanoString IO360 panel. Data was normalized and analyzed using the nSolver™ Data Analysis software for T0 (baseline) and post treatment RNA samples.

IHC and Multiplex IHC: IHC was performed with 5µm sections obtained from the FFPE block using Ventana IHC automated staining system. Scoring was performed by certified pathologists. For Multiplex IHC, using Opal 520 and Opal 620 detection system, manual staining procedure according the manufacturer's protocol was performed, followed by DAPI staining. The slides were imaged using Zeiss Axio Observer and analysed using ZEISS ZEN Analysis tool.

Farcast™ TiME Histo-culture platform:

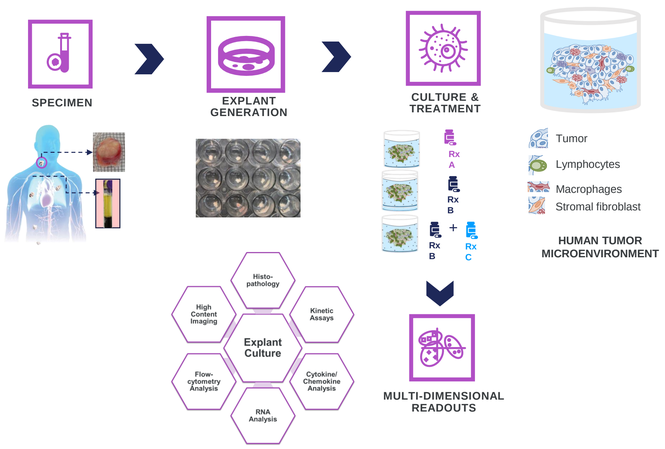


Fig. 1: Farcast™ TiME Histo-culture platform schematic representation of the various steps involved to perform the assay along with the various downstream evaluation

Results:

Farcast™ TiME preserves the tumor immune microenvironment:

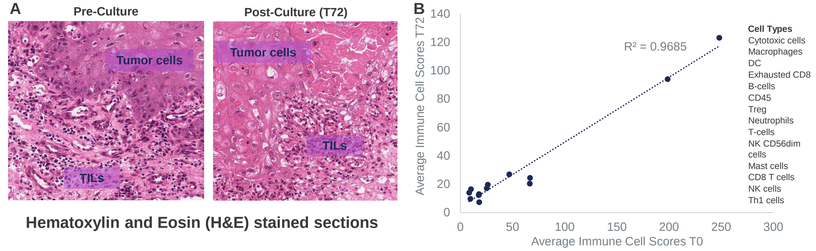


Fig. 2: A. Representative H&E stained HNSCC explants demonstrating the preservation of morphology and the various elements of TiME. B. Correlation of immune cell scores obtained from NanoString evaluation of the RNA expression before and after culture.

Retention of live immune cell population post 72hrs of tumor explant culture

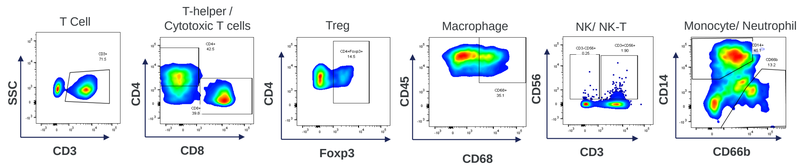
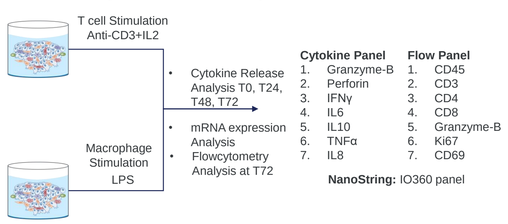


Fig. 3: Flowcytometry analysis of various live immune components after culture for 72hrs.

Study Plan for understanding functional fidelity of TILs



Anti-CD3+IL2 and LPS treated tumor explants shows specific modulation of the targeted immune compartment

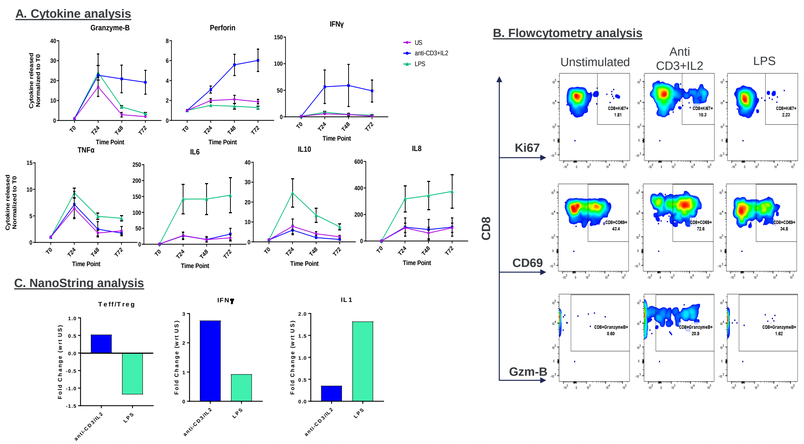


Fig. 4: A. T cell specific cytokine response was observed specifically by anti-CD3+IL2 stimulation while macrophage specific cytokine response was observed only by LPS stimulation. The flowcytometry (B) and NanoString (C) analysis also demonstrated specific response pattern.

Response to Nivolumab treatment

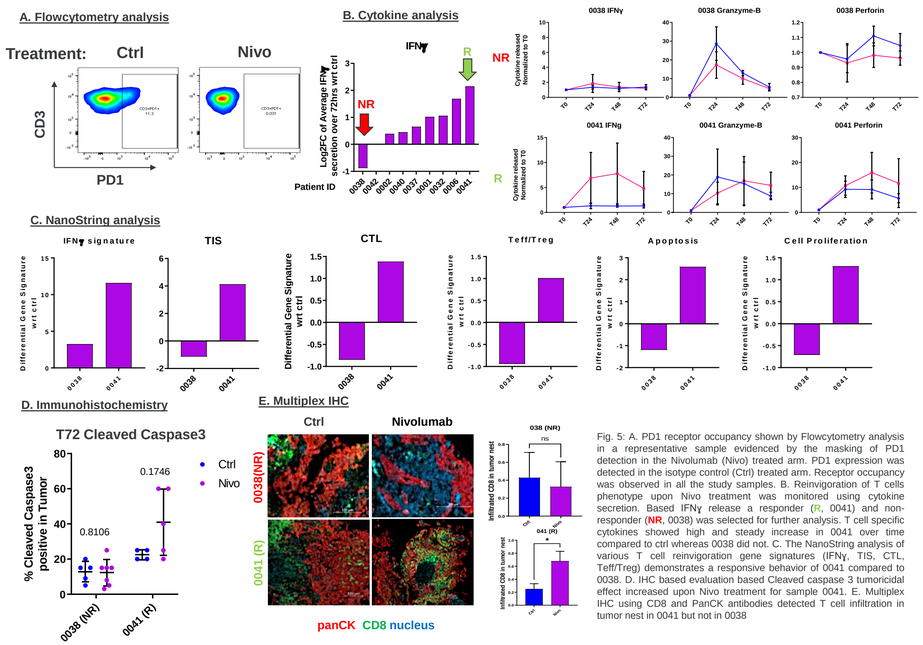


Fig. 5: A. PD1 receptor occupancy shown by Flowcytometry analysis in a representative sample evidenced by the masking of PD1 detection in the Nivolumab (Nivo) treated arm. PD1 expression was also observed in all the study samples. B. Reinvigoration of T cells phenotype upon Nivo treatment was monitored using cytokine secretion. Based IFNγ release a responder (R, 0041) and non-responder (NR, 0038) was selected for further analysis. T cell specific cytokines showed high and steady increase in 0041 over time compared to ctrl whereas 0038 did not. C. The NanoString analysis of various T cell reinvigoration gene signatures (IFNγ, TIS, CTL, Teff/Treg) demonstrates a responsive behavior of 0041 compared to 0038. D. IHC based evaluation based Cleaved caspase 3 tumoricidal effect increased upon Nivo treatment for sample 0041. E. Multiplex IHC using CD8 and PanCK antibodies detected T cell infiltration in tumor nest in 0041 but not in 0038

Baseline gene signature of Responder and Non-responder

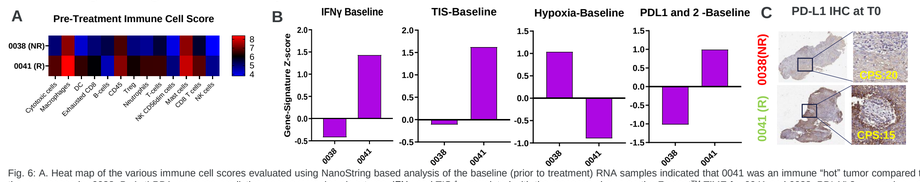


Fig. 6: A. Heat map of the various immune cell scores evaluated using NanoString based analysis of the baseline (prior to treatment) RNA samples indicated that 0041 was an immune "hot" tumor compared to the non-responder 0038. B. Anti-PD1 response predicting gene expression signatures, IFNγ and TIS [1], correlated with the response shown on the Farcast™ TiME for 0041 and 0038. PDL1/L2 expression was comparatively higher in 0041 whereas 0038 had a higher expression of hypoxia related gene signature. C. The PD-L1 IHC at base line showed comparable CPS score for 0041 and 0038 though staining intensity was higher in 0041.

Improved response to Nivolumab (anti-PD1)-Ipilimumab (anti-CTLA4) combination compared to Nivolumab monotherapy: a case study

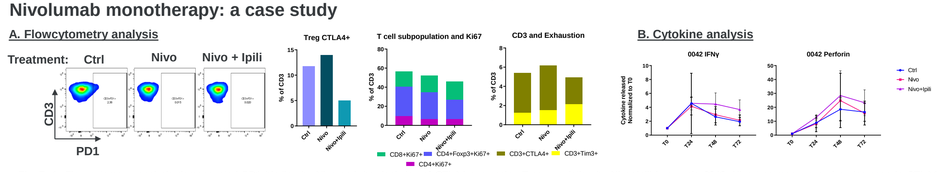


Fig. 7: A. Flow cytometry analysis demonstrated PD1 binding by Nivo and the depletion of CTLA4 expressing Treg population by Nivo+Ipili in sample 0042. Nivo treatment caused an increase in CTLA4 expressing Treg population. Depletion of Tregs in combination with increase of proliferating CD8 was observed in the Nivo+Ipili combination arm. The Nivo+ Ipili also elicited a sustained INFγ and Perforin release (B). The synergistic activity of the combination treatment was clearly elucidated in the Farcast™ TiME platform.

Conclusion:

1. The Farcast™ TiME histo-culture platform preserves both the tumor morphology and the various immune components and retains their functionality in culture.
2. This platform provides a unique near native assay system to explore novel immune oncology-based therapies and compare it with the existing standard of care immuno-oncology agents.
3. The platform showed potential to predict response to immune check point inhibitors.

Reference: 1. J Clin Invest . 2017 Aug 1;127(8):2930-2940

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