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

Nandini Pal Basak, Kowshik Jaganathan, Vasanth K, Sindhu Govindan, Ritul Malhotra, Pradeep Kar, Rachita Rao, Satish Sankaran
Farcast Biosciences India Pvt. Ltd., India

Introduction:
There is an unmet need for robust and accurate preclinical models 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 and 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 consenting 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 (100U/ml) or immune checkpoint inhibitor Nivolumab (anti-PD-1, 130µg/ml), Ipilimumab (anti-CTLA4, 90µg/ml) and their combination for 72 hours. Culture supernatant was collected every 24 hours and stored for cytokine analysis. Media was changed every 24 hours.

Flow cytometry analysis: The tumor explants were dissociated post culture with various treatments into single cells and stained 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.

NapoloStaining Analysis: The RNA extracted arm-wise from the explant TMA (Tissue Micro Array) FFPE blocks was quantified using Tape Station and 30-50ng of RNA based on O2V0 concentration was used for running on the NanoString nCounter panel. Data was normalized and analysed using the nSolver™ Data Analysis System for T0 (baseline) and post treatment RNA samples.

TIME Histo-culture platform:

Results:
Farcast™ TIME preserves the tumor immune microenvironment:

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

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

Study Plan for understanding functional fidelity of TILs

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

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

References: J Clin Invest. 2017 May 1;132(9):2836-2840