Utilization of Multiparameter Flow Cytometry for Immune Monitoring of Tumor Infiltrating Lymphocytes Isolated from Fresh Tumor Tissue

Rachel Owen PhD, Angelina Bisconte, Bhagyaraj Ella PhD, Deborah Phippard PhD, Precision for Medicine, Frederick, MD, USA

Abstract# 2180

Introduction

Understanding the role of infiltrating lymphocytes into tumors during clinical trials can provide insights into the complex interplay between the immune system and tumor microenvironment during treatment with novel immuno-oncology therapeutics.

Obtaining viable immune cells from fresh tissue for analysis by flow cytometry can be challenging. Precision for Medicine (PfM) developed optimized shipping conditions and isolation procedures for fresh solid tumor biopsy resection samples or fine needle aspirate (FNA) biopsy samples from a variety of solid tumor indications, including melanoma, colorectal adenocarcinoma, sarcoma, triple negative breast cancer, renal cancer, head and neck cancer, lung cancer and stomach cancer.

Objective

Multi-parameter flow cytometry was established as a method to perform immunophenotypic analysis of fresh tumor tissues within 48 hours of collection. Three flow cytometry panels were used across a variety of tumor indications allowing analysis of 62 tumor samples. Following overnight shipment from collection sites to PfM's laboratory, a real time sample processing procedure using a mechanical dissociation protocol followed by multiparametric flow cytometric analysis was performed to assess the immune populations infiltrating solid tumors.

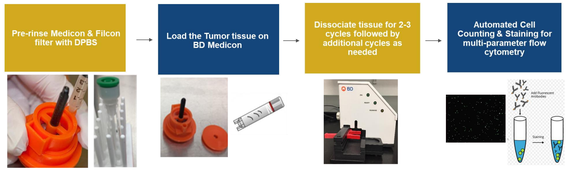
TIL Isolation Method

Tumor tissues were procured by Precision's internal sourcing program. Informed consent was obtained for the collection of tissues for research purposes under site specific collection protocols and Precision for Medicine's Clinical Biospecimen Procurement Protocol to Support Advancements in Medical Diagnoses of Diseases.

Tissues were collected as fine needle biopsies (~1-2mm) or resection of bulk fresh tumor (weight ranging from 91-1400mg, average 330mg) into Miltenyi MACS storage media prior to overnight shipment to Precision's laboratory. Tissues were typically received by 24 - 48 hours post-collection.

Tumor tissue was dissociated by mechanical or manual dissociation. Mechanical dissociation was performed with the BD MediMachine. The Medicon chamber was pre-rinsed with DPBS three times. Fine needle biopsy pieces were loaded with the MACS media from shipment to a maximum volume of 1mL. Two to three dissociation cycles were performed with visual inspection after each cycle. Additional short pulse cycles were performed as needed to obtain full dissociation. Manual dissociation of large tumor pieces was performed with a scalpel in a 6 well plate in MACS buffer.

Following dissociation, the cells were transferred to 15mL Falcon tubes, and the Medicon chamber or plate wells were rinsed. Cells were filtered through a 70uM filter to remove large debris. Cells were resuspended and counted by a Nexcelom Cellometer using Acridine Orange and Propidium Iodide (AO/PI) to determine viability.



Conclusion

This study shows that real time multiparametric flow cytometric analysis of fresh tumor tissues, within 48 hours of collection, can be implemented in clinical trials with careful planning and consideration of clinical operations. Variation in the frequency and phenotype of infiltrating cells present in the tumor tissues was observed. This method is of value for early phase clinical studies evaluating the impact of immune therapies on immune cell infiltration within solid tumors and provides an additional immune monitoring tool.

Multi-parametric Flow Cytometry Method

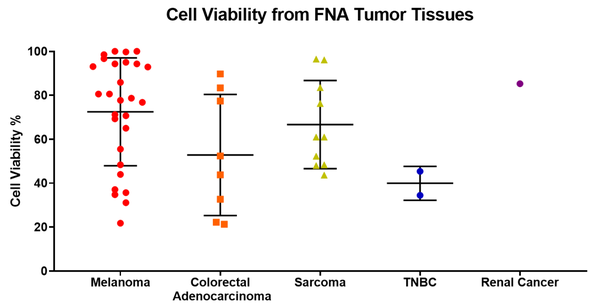
Immunophenotyping panels consisting of markers to identify immune cell subsets were used to stain cells isolated from dissociated tumor tissues.

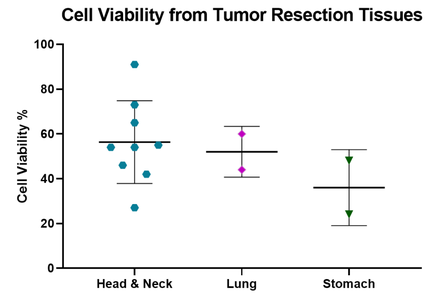
Markers in the three panels included: CD45, CD3, CD4, CD8, CD11c, CD14, CD15, CD16, CD56, CD123, CD86, CD45RA, CCR7, CD25, FoxP3, Ki67, PD-1, ICOS, LAG-3, PD-L1, CD141. Antibodies were purchased from BD Biosciences, eBiosciences or Biolegend.

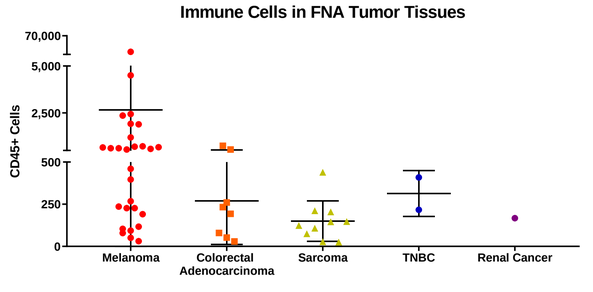
Cells were stained with a Live/Dead viability dye (Life Technologies) for 15 minutes, prior to addition of Fc block for a further 10 minutes incubation. Cells were washed with staining buffer by centrifugation at 500xg for 5 minutes. Cells were resuspended in fixation/permeabilization buffer (eBioscience) for 45 minutes, then washed in permeabilization buffer by centrifugation. The cell pellet was resuspended and stained with an antibody panel cocktail for 30 minutes at room temperature. Cells were washed and resuspended in 200µL of staining buffer prior to acquisition on a LSRFortessa X-20 flow cytometer using FACSDiva software. Data was analyzed with FlowJo.

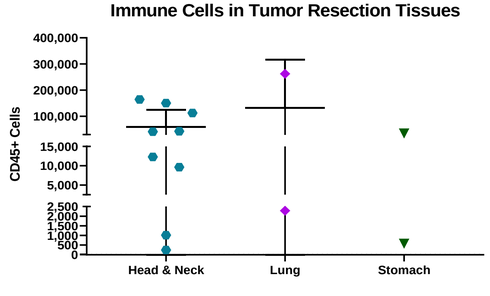
Results

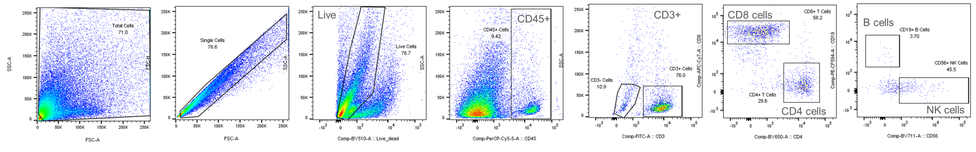
Post-dissociation cell viabilities were determined by automated cell counting with AO/PI with a Nexcelom Cellometer and viabilities ranged between 21 – 100% across the different tissues collected as either fine needle aspirate or tumor biopsy pieces. Flow cytometric analysis showed the isolated cells were tumor infiltrating lymphocytes expressing CD45. A greater number of CD45+ cells were isolated from the larger resection tissue biopsies than the small FNA biopsies, however, our data shows that it is still feasible to isolate TILs from small FNA biopsies. Additional characterization of the infiltrating immune subsets was possible, with an example from a melanoma FNA below showing the presence of T cells, both CD4+ and CD8+, B cells (CD19+) and NK cells (CD56+).







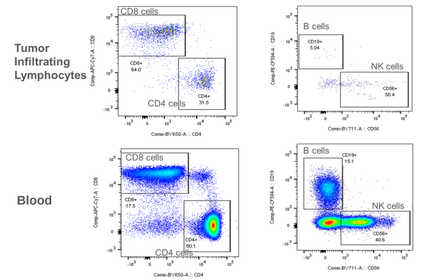


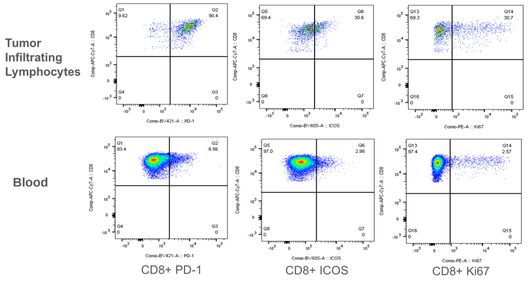


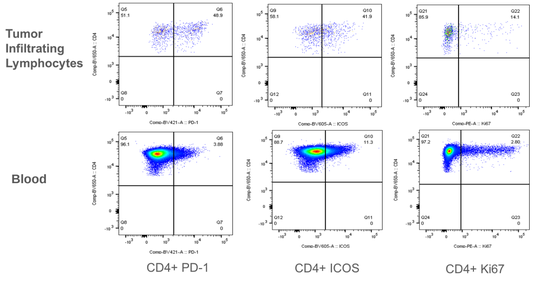
Results

A comparison of immune cell subsets identified infiltrating in tumors and circulating in the periphery could be performed when matched blood was available. An example from melanoma is shown below, where CD4 and CD8 T cells, B cells (CD19+) and NK cells (CD56+) were measured.

The CD4+ and CD8+ T cells were further characterized for the expression of PD-1, ICOS and Ki67, which showed variation in staining patterns between the tumor and periphery.







Presented April 2025

PRECISION for medicine®