Simultaneous quantification of activated immune cells and PD-L1 expressing circulating tumor cells (CTCs) in peripheral blood of cancer patients receiving checkpoint inhibitor therapy

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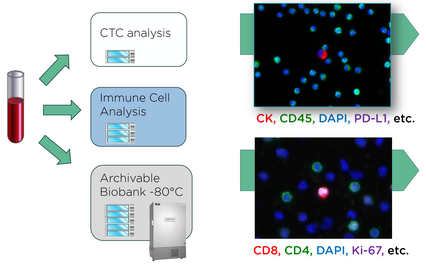
Background

- Expression of PD-L1 on tumor and tumor infiltrating lymphocytes has been associated with improved response to PD-1 and PD-L1 immune checkpoint inhibitors (ICI), however clinical utility is limited and tumor biopsies are not always available.
- Analysis of the peripheral immune cell subpopulations could aid in the prediction of response to immunotherapy, however current technology robust enough for a clinical setting utilizing flow cytometry suffers from low sensitivity and a lack of standardization.
- Epic's Functional Cell Profiling (FCP) platform uses high-throughput microscopy to analyze all nucleated cells within a tube of blood in a robust and reproducible manner.
- Here we sought to characterize activated T-Cell subpopulations using FCP in patients receiving ICI therapy as a proof of concept with the overarching aim of developing better diagnostic tools for immunotherapy response prediction and pharmacodynamics studies.

Methods

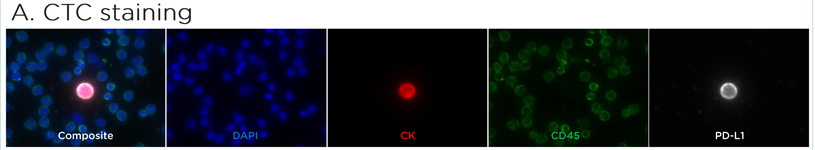
The Epic Sciences Functional Cell Profiling platform



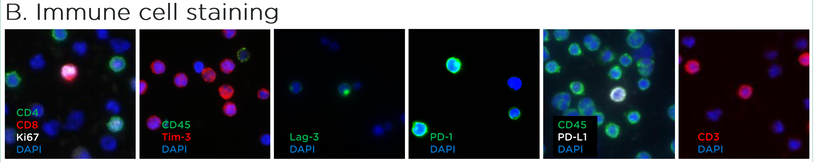
- Imaging and automatic phenotyping of every cell within a tube of blood
- Accurate identification of rare cell populations in a background of 10^6 - 10^7 other cells

CTC and Immune Cell Imaging

A. CTC staining

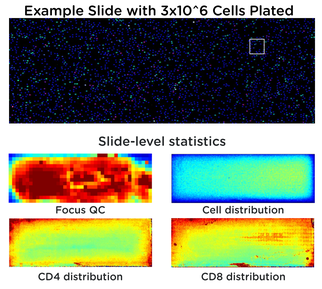


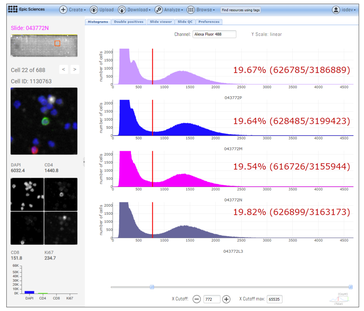
B. Immune cell staining



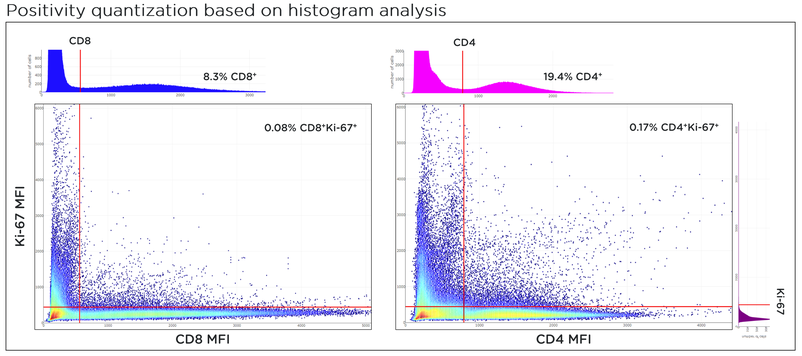
Cloud-based Epic Discovery Platform

- Epic Discovery Platform provides a new way to quantify leukocyte subpopulations by analyzing all cells and supports image storage, analysis, and provenance management.
- Cell Explorer links image-based features, cell location, and any relevant metadata for every cell on the slide.



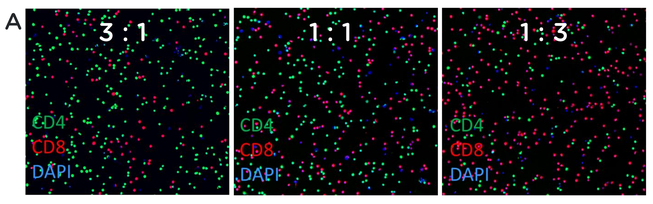


Positivity quantization based on histogram analysis



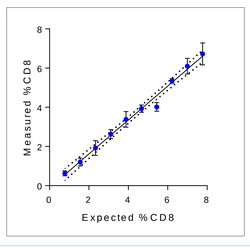
Large and small scale changes can be detected with the T-Cell Activation assay

A



CD4:C8 Ratio	%CD4	%CD8
3:1	70%	23%
1:1	49%	44%
1:3	23%	72%

B



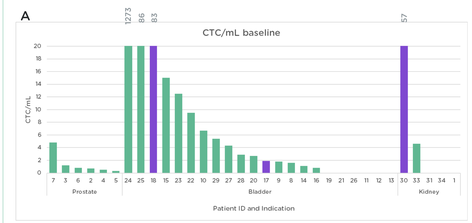
A. Immunomagnetically purified CD4 and CD8 cells plated in ratios of approximately 3:1, 1:1, and 1:3 (left to right). Analyzed ~1 million cells per slide.

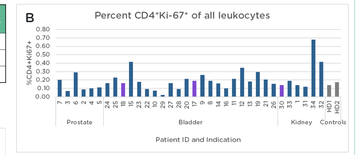
B. Immunomagnetically purified CD8 cells spiked into healthy donor blood at target ratios of approximately 1-8% demonstrate linearity of the assay. Mean and SEM of triplicate values are shown. R^2 = 0.989, slope = 0.87

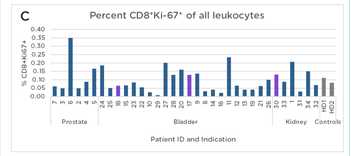
A single patient sample can be used for characterization of both CTCs and immune cell subpopulations

Baseline samples were evaluated for PD-L1 CTC expression and activated T-cell quantification.

Indication	Total patients	CTC/mL (range)	>1 CTC/mL	> 1 PD-L1+ CTC/mL
Prostate	6	0.3-4.8	2 (33%)	0 (0%)
Bladder	22	0-1273	15 (68%)	2 (9%)
Kidney	5	0-57.8	2 (40%)	1 (20%)

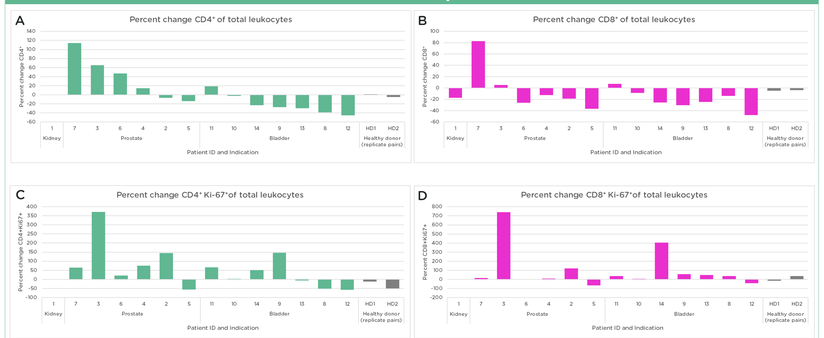






A) CTC counts per mL. Patients with > 1 PD-L1⁺ CTC/mL are shown in violet.
B) Percent CD4⁺Ki-67⁺ cells and (C) CD8⁺Ki-67⁺ cells of total leukocytes

Longitudinal changes were observed upon treatment with checkpoint inhibitor Tx



A and B) Percent change of CD4 and CD8 subpopulations from baseline to on-therapy.
C and D) Percent change of CD4⁺Ki-67⁺ and CD8⁺Ki-67⁺ subpopulations from baseline to on-therapy.

Conclusions

- Epic's Functional Cell Profiling (FCP) platform can simultaneously measure immune related biomarkers in CTCs and leukocytes at single cell resolution from a single blood sample.
- Archived samples can be banked for months to years after procurement enabling retrospective testing with multiple assays.
- The platform has extreme sensitivity and reproducibility over conventional methodologies - Cell sub-populations that exist in a background of 10^5-10^7 other cells are readily identified.
- Changes in immune cell sub-populations in patients receiving ICI therapy were observed across multiple cancer types including bladder, kidney, and prostate.
- Efforts to identify morphological sub-populations within each canonical leukocyte category and comparison of leukocyte and CTC biomarker panels to patient outcomes are ongoing in multiple trials.