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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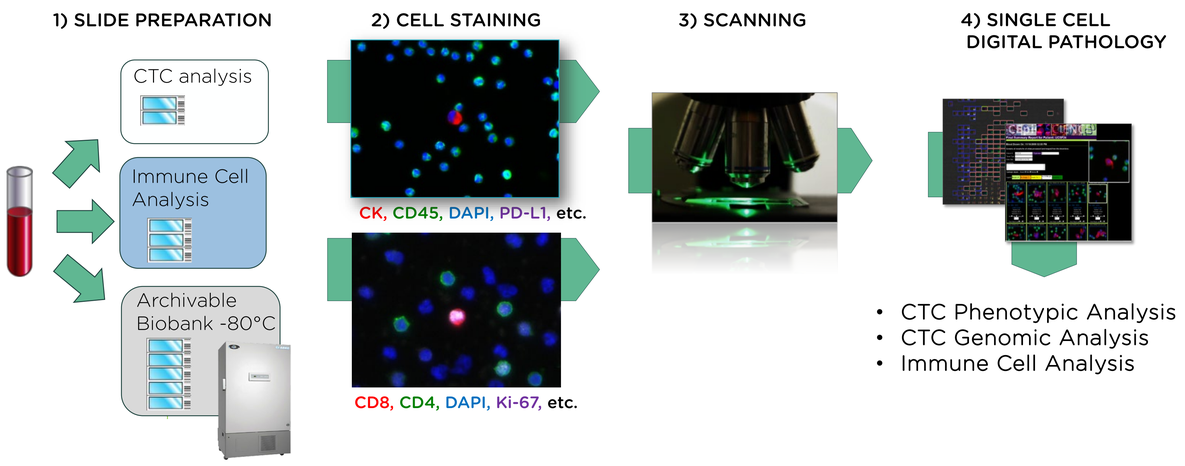
Background

- Expression of PD-L1 on tumor and tumor infiltrating lymphocytes has been associated with improved response to PD-1 and PD-L1 checkpoint inhibitors, however clinical utility is limited.
- Multimodal characterization of both the tumor and host immune system is an unmet medical need for the improved prediction of response to immunotherapy.
- Metastatic lesions are likely to be under-sampled by biopsy given tumor heterogeneity, clonal evolution, and temporal changes in the host immune system under therapeutic pressure.
- Therefore, we sought to expand the existing Epic Sciences' non-invasive liquid biopsy platform to simultaneously examine expression of PD-L1 on circulating tumor cells (CTCs) as well as quantify changes in activated immune cell populations from a single sample in patients undergoing checkpoint inhibitor therapy.

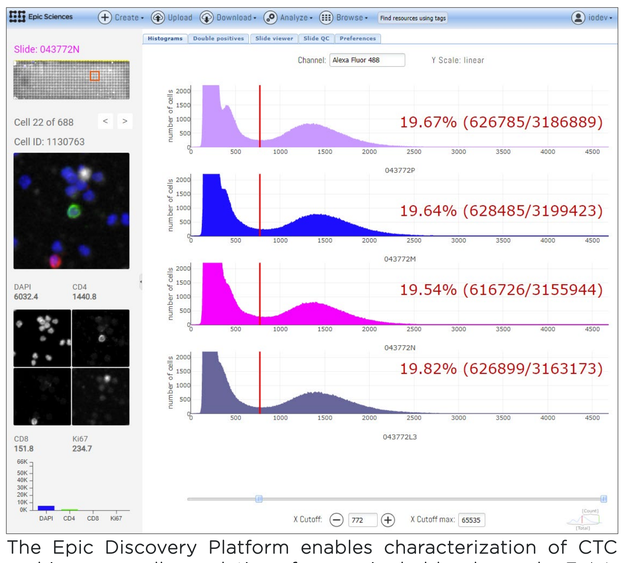
Methods

Blood samples from bladder, kidney, and prostate cancer patients undergoing checkpoint inhibitor therapy were collected at baseline and on-therapy (when available) and sent to Epic Sciences for processing. Control samples were developed using cancer cell lines and healthy donor (HD) blood. Slides were stained with pan-CK/CD45/PD-L1/DAPI or CD4/CD8/Ki-67/DAPI. Approximately 3 million cells per slide were imaged through advanced digital pathology pipelines to detect and quantify changes in immune cell populations and to assess circulating tumor burden.

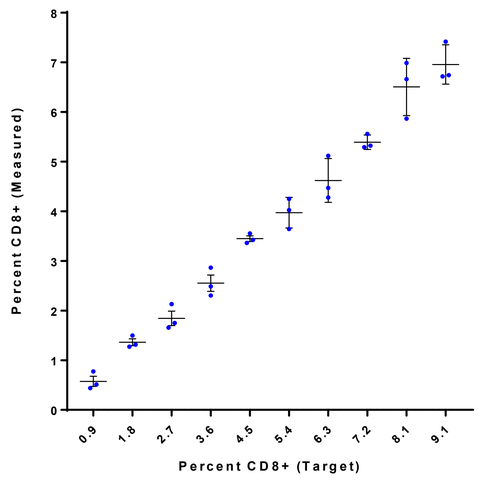


Schematic of Epic's CTC and Immune cell platform. A single blood draw is used to make slides for CTC and immune cell analyses. Slides are stained with CTC and immune cell markers then scanned using a rapid fluorescent scanning method that images each nucleated cell. A multi-parametric digital pathology algorithm is used to detect all cells and assess nuclear and cytoplasmic morphological features, and protein expression. Single cells of interest can be isolated directly from stained slides and processed for genomic sequencing to assess genomic instability and mutational burden.

Tools to Characterize Immune Cell Populations

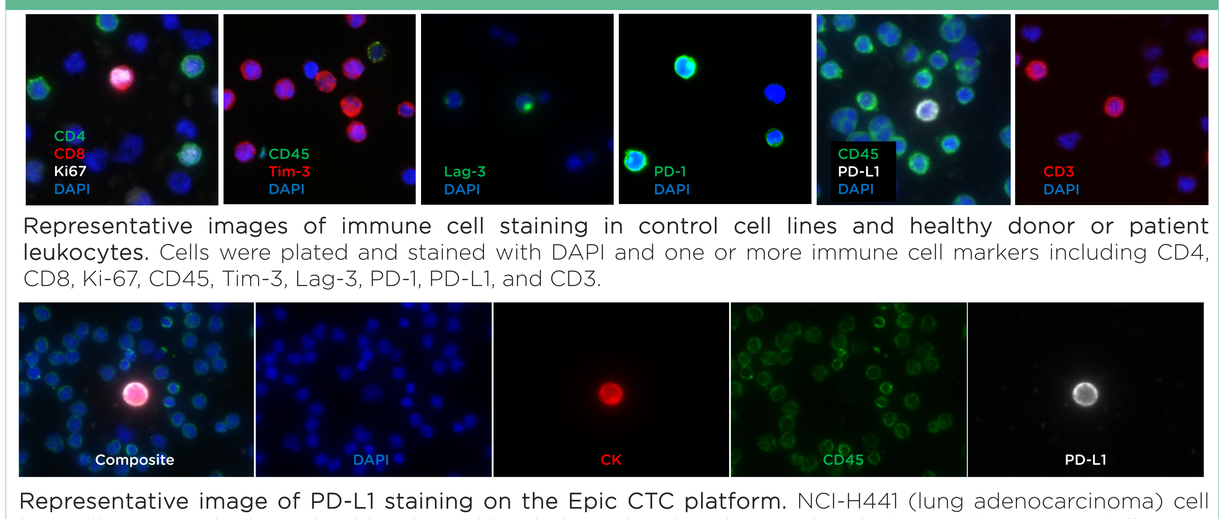


The Epic Discovery Platform enables characterization of CTC and immune cell populations from a single blood sample. Epic's proprietary software performs cloud-based image analysis to characterize 3 million cells per slide. Representative histograms (above) are shown for healthy donor samples that were stained with CD4, CD8, Ki-67, and DAPI. The histograms were used to determine mean fluorescent intensity (MFI) thresholds for each marker and to quantify leukocyte subpopulations.



Detecting immune cell changes: quantitation and linearity of the CD8 assay. $CD8^+$ cells were spiked into healthy donor leukocytes at target ratios of approximately 1 - 9%. After staining and quantification of $CD8^+$ cells, percentages of detected spiked $CD8^+$ cells for three technical replicates (Percent $CD8^+$ Measured) were plotted against target percentages (Percent $CD8^+$ Target). Bars show mean and standard error of the mean.

CTC and Immune Cell Imaging



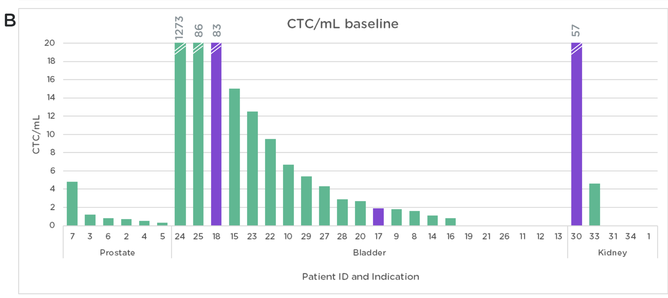
Representative images of immune cell staining in control cell lines and healthy donor or patient leukocytes. Cells were plated and stained with DAPI and one or more immune cell markers including CD4, CD8, Ki-67, CD45, Tim-3, Lag-3, PD-1, PD-L1, and CD3.

Representative image of PD-L1 staining on the Epic CTC platform. NCI-H441 (lung adenocarcinoma) cell line cells were spiked into healthy donor blood, then plated and stained with Epic's PD-L1 assay. Cells were stained with CK, PD-L1, CD45 antibodies and DAPI.

Patient CTC and Immune Cell Characterization

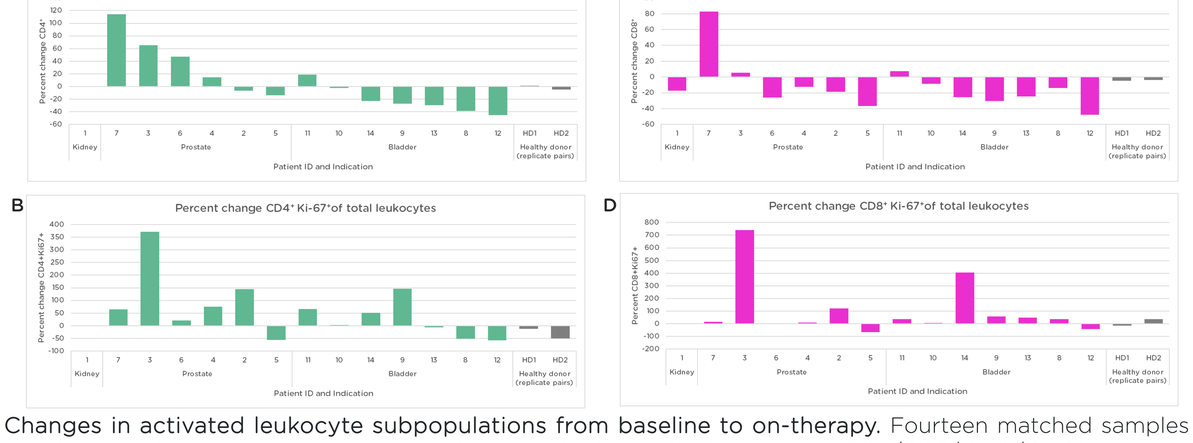
A

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%)



CTC and PD-L1 enumeration and activated CD4 and CD8 quantification of baseline samples. Baseline samples were stained with the PD-L1 CTC assay and activated CD4/CD8 assay (CD4/CD8/Ki-67/DAPI). A) Table shows percentage and range of CTC and PD-L1 positive CTC counts per milliliter (mL) of blood. B) CTC counts per mL per patient are shown, categorized by disease indication. Patients with > 1 PD-L1+ CTC/mL are shown in violet. Percent $CD4^+Ki\text{-}67^+$ cells (C) and $CD8^+Ki\text{-}67^+$ cells (D) out of total leukocytes were quantified for baseline samples. Samples from healthy donors HD1 (n=4) and HD2 (n=8) were run with patient samples.

Immune Cell Changes with Checkpoint Inhibitor Tx



Changes in activated leukocyte subpopulations from baseline to on-therapy. Fourteen matched samples and two healthy donor samples were stained with the activation assay (CD4/CD8/Ki-67/DAPI) and cell counts were determined using the Epic Discovery Platform. Single positive CD4 and CD8 subpopulations were quantified for each time point and percent change was determined (graphs A and C, respectively). Double positive CD4 Ki-67 and CD8 Ki-67 subpopulations were quantified and percent change was determined (graphs B and D, respectively).

Conclusions

- Epic Sciences has developed tools to simultaneously detect, characterize, and quantify CTCs and rare immune cell subpopulations from a single blood draw.
- Examining dynamic biomarker changes with minimally invasive liquid biopsies in longitudinal samples could enable the development of novel diagnostic tools for response prediction and pharmacodynamics studies related to checkpoint inhibition and other immunotherapies.