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

Rachel Krupa, Robin Richardson, Priscilla Ontiveros, Joseph Schonhoft, Jiyun Byun, David Lu, Aaron Oh, Sean Nisperos, Yipeng Wang, Mark Landers, Ryan Dittamore
Epic Sciences, 9381 Judicial Dr., Suite 200, San Diego, CA 92121 • epicsciences.com

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-CX/CD45/CD-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 to quantify leukocyte subpopulations.

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 B and D, respectively). Double positive CD4 Ki-67 and CD8 Ki-67 subpopulations were quantified and percent change was determined (graphs A and C, 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.