Quantification of Rare PD-L1 and Other Immunology Biomarker Expressing Leukocytes and CTCs in Peripheral Blood of Cancer Patients
Adam Jendrisak, Nadia Ebrahim, Angel Rodriguez, Rachel Krupa, David Lu, Mahipal Suraneni, Jiyun Byun, Ryon Graf, Yipeng Wang, Mark Landers, Ryan Dittamore
Epics Sciences, Inc., San Diego, California

Background
- Expression of PD-L1 on tumor tissue and immune cell markers (CD3, CD4, CD8, etc.) on tumor infiltrating lymphocytes (TILs) are often associated with improved response to PD-1 and PD-L1 checkpoint inhibitors. However, PD-L1 diagnostic tests suffer from high co-morbidities and from significant false positives and false negatives.
- Expression of PD-1 and Tim-3 on TILs is associated with immune exhaustion.
- Utilizing a non-invasive liquid biopsy, we sought to examine the expression of checkpoint markers (PD-L1, Tim-3) and immune cell markers (CD3, CD8) on circulating tumor cells (CTCs) and leukocyte cell populations for the purpose of developing an improved predictive and pharmacodynamic biomarker for approved PD-1/PD-L1 checkpoint inhibitors and novel immunotherapy drugs in development.

Methods
- Blood samples were drawn from 3 healthy donors, 18 non-cancerous lung disease, 13 lung cancer, and 2 bladder cancer patients and sent to Epic Sciences for processing with Epic Sciences’ immunoassays.
- Panels of immunoassays include staining of checkpoint markers (PD-L1, Tim-3) and immune cell markers (CD3, CD8).

Figure 2: Demonstration of PD-L1 assay specificity: (A) PD-L1-specific antibody and species-matched isotype control were tested in negative Colo205 and high (H820) PD-L1 expressing cell lines. No specific staining was seen in negative control cell lines or with isotype control antibody. (B) Interferon (IFN)-gamma treatment increases PD-L1 expression in Colo205 and A549 cell line cells. PD-L1 expression in IFN-gamma treated SU-DHL-2 cells remain unchanged, likely due to the up-regulation of cytokine signaling suppressor genes in this particular cell line.

PD-L1 Expression in CTC and Leukocytes of Lung Ca

Conclusions
- Epic Sciences’ platform has low limit of detection, ability to archive patient blood samples and ability to quantify biomarker expression on both CTCs and leukocytes simultaneously.
- Detection of leukocyte subtypes such as CD3, CD8, CD14 and CD56 cells, will allow us to further characterize PD-L1 and Tim-3 in T-lymphocytes and other immune cell types.
- Development of a liquid biopsy-based platform that is capable of simultaneously measuring immune biomarkers in CTCs as well as leukocytes will allow real-time assessment of response to immune checkpoint inhibitors.