Characterization of PD-L1 Expression in Circulating Tumor Cells (CTCs) of NSCLC

David Lu, Rachel Krupa, Melissa Harvey, Jessica Louw, Adam Jendrisak, Mark Landers, Dena Marrinucci, Ryan Dittamore

Epic Sciences, Inc., La Jolla, California

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Background

Programmed death-ligand (PD-L)-1 is an immunosuppressor via interaction with its receptor, PD-1, expressed on activated T- and B-cells. PD-L1 upregulation in cancer cells enables evasion of immune surveillance by the inhibition of immune cell activation. PD-L1 expression in non-small cell lung cancer (NSCLC) is associated with decreased overall survival and increased tumor invasiveness.

Novel PD-1 and PD-L1 targeting immunotherapies are demonstrating efficacy in multiple tumor types. Recent data have observed increased progression free survival (PFS) with therapy in patients who harbor higher expression of the PD-L1 protein in tumor tissue sections. However, NSCLC patients may have insufficient tumor sample or have high co-morbidities preventing access to tissue IHC to determine PD-L1 expression and potential benefit to novel PD-1/PD-L1 immunotherapies. **Here, we present the development of a PD-L1 protein assay for the examination and molecular characterization of CTCs and CTC subpopulations in newly diagnosed NSCLC patients.**

Methods

25 blood specimens from newly diagnosed NSCLC patients were collected prior to therapy and shipped to Epic Sciences. All nucleated cells were plated onto glass slides and subjected to immunofluorescent (IF) staining and CTC identification by fluorescent scanners and algorithmic analysis. CTCs, defined as traditional (CK+, CD45- with intact and morphologically distinct DAPI+ nuclei), apoptotic (CK+, CD45-, non-intact nuclei) and CK- (CK-, CD45-, intact and distinct nuclei) were identified. Samples were characterized with PD-L1 IF to assess protein expression.

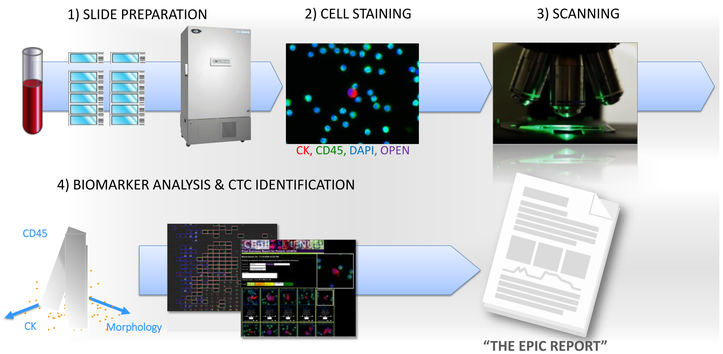


Figure 1: Schematic of Epic's CTC collection and detection process: 1) Nucleated cells from a blood sample are plated onto slides and stored in the -80C biorepository; **2)** Slides are stained with CK, CD45, DAPI and PD-L1; **3)** Slides are scanned, and multi-parametric digital pathology algorithms are run; **4)** Software and human reader confirmation of CTCs and quantitation of biomarker expression are obtained to produce the final Epic Report. Downstream FISH and genetic analysis of CTCs can also be conducted, where applicable.

PD-L1 Protein Assay Development

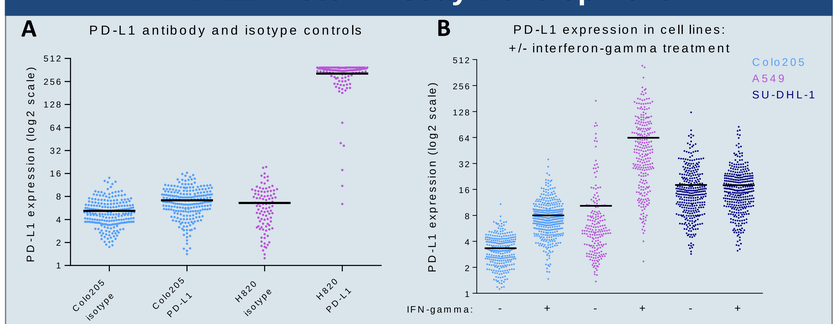


Figure 2: Demonstration of PD-L1 assay specificity: (A) PD-L1-specific antibody and species-matched isotype control were tested in negative (Colo205) and high (H820) PD-L1-expressing cell lines. No specific staining was seen in negative control cell lines or with isotype control antibody. **(B)** Interferon (IFN)-gamma treatment increases PD-L1 expression in Colo205 and A549 cell line cells. PD-L1 expression in IFN-gamma-treated SU-DHL-1 cells remain unchanged, likely due to the upregulation of cytokine signaling suppressor genes in this particular cell line.

PD-L1 Protein Assay Development (cont.)

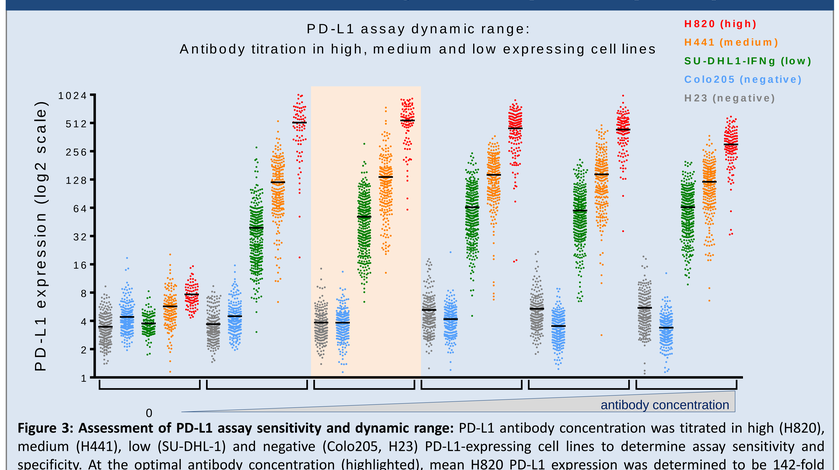
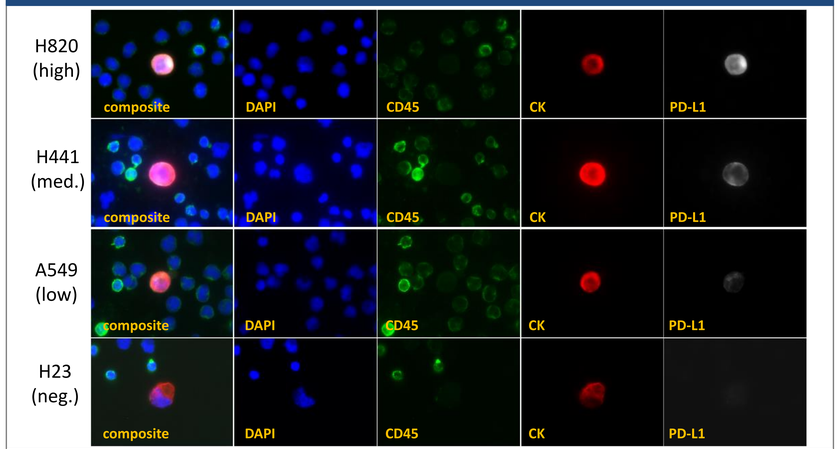


Figure 3: Assessment of PD-L1 assay sensitivity and dynamic range: PD-L1 antibody concentration was titrated in high (H820), medium (H441), low (SU-DHL-1) and negative (Colo205, H23) PD-L1-expressing cell lines to determine assay sensitivity and specificity. At the optimal antibody concentration (highlighted), mean H820 PD-L1 expression was determined to be 142-fold higher than mean background staining in negative controls. For subsequent patient stains, thresholds for CTC PD-L1 positivity were set based on positive and negative control cell lines allowing for maximum sensitivity with >90% specificity.

Representative Cell Line Cell Images



Clinical Feasibility in NSCLC Patient Samples

25 NSCLC patient samples consisting of 15 adenocarcinoma (ADC) and 10 squamous cell carcinoma (SQCC) cases of various staging were stained with the Epic PD-L1 assay. Results are summarized in **Table 1.** 9/25 (36%) patients had detectable PD-L1+ CTCs: 46.7% of ADC patients and 20% of SQCC patients. The greater incidence of PD-L1 positivity in ADC-derived CTCs is consistent with previous reports examining primary tumor tissue. 4/17 (23.5%) patients with stage I-II NSCLC at time of draw had PD-L1+ CTCs; 3/6 (50%) stage III-IV NSCLC patients had PD-L1+ CTCs.

Stage	ADC	SQCC	Total
I-II	3/8 (37.5%)	1/9 (11.1%)	4/17 (23.5%)
III-IV	2/5 (40%)	1/1 (100%)	3/6 (50%)
Unknown	2/2 (100%)	0	2/2 (100%)
Total	7/15 (46.7%)	2/10 (20%)	9/25 (36%)

Table 1: % Patients with PD-L1+ CTCs (# PD-L1+ patients/# total patients)

Clinical Feasibility in NSCLC Patient Samples (cont.)

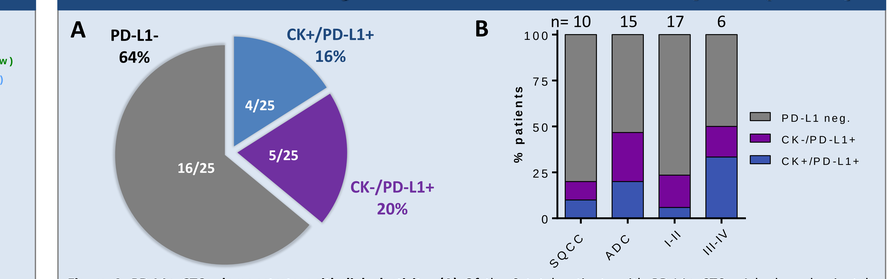
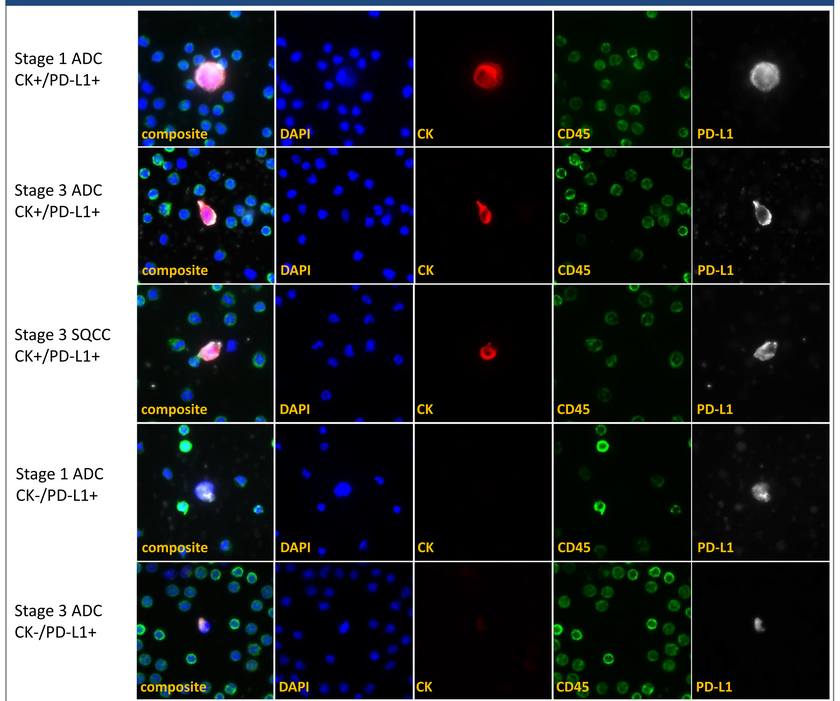


Figure 4: PD-L1+ CTCs demonstrate epithelial plasticity: (A) Of the 9 total patients with PD-L1+ CTCs, 4 had predominately traditional CK+/PD-L1+ CTCs. However, 5 patients had CTCs that were exclusively CK-/PD-L1+, suggestive of epithelial plasticity (i.e., loss of cytokeratin and likely, EpCAM expression). **(B)** Further breakdown of CK+/PD-L1+ and CK-/PD-L1+ CTCs detected by tumor subtype and staging indicates that inclusion of CK-negative CTCs substantially increased sensitivity of PD-L1+ CTC detection across all groups.

Representative Patient CTC Images



Conclusions

PD-L1 protein assessment of CTCs and CTC subpopulations from NSCLC patients at diagnosis is feasible on the Epic platform. This test demonstrates sensitivity and specificity and may aid in the identification of patients suitable for clinical trial studies with novel PD-1 or PD-L1 therapies. The identification of PD-L1 positive CTC subpopulations identifies unique tumor cell morphology and suggests evidence of epithelial plasticity. Enablement of PD-L1 characterization in cytokeratin-negative CTCs substantially increases sensitivity. Further pharmacodynamic analysis of CTCs and PD-L1 expression on CTCs in the setting of PD-1/PD-L1 therapies is warranted in NSCLC and in cancers of other tissues.