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# Characterization of Programmed Cell Death 1 Ligand 1 (PD-L1) Expression in Circulating Tumor Cells (CTCs) of Lung Cancer

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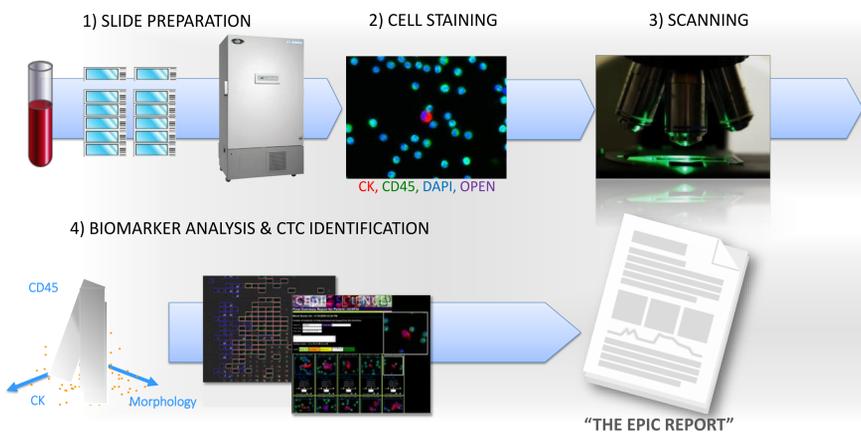
## Background

Programmed cell death 1 ligand 1 (PD-L1) is an immuno-suppressor 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 lung cancer is associated with increased tumor invasiveness and worse overall survival.

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, lung cancer patients may have insufficient tumor sample or have high co-morbidities, preventing the IHC evaluation of PD-L1 expression that could potentially direct treatment toward novel PD-1/PD-L1 immunotherapies. **Here, we present the development of a PD-L1 protein assay for the enumeration and molecular characterization of CTCs and CTC subpopulations in newly diagnosed lung cancer patients.**

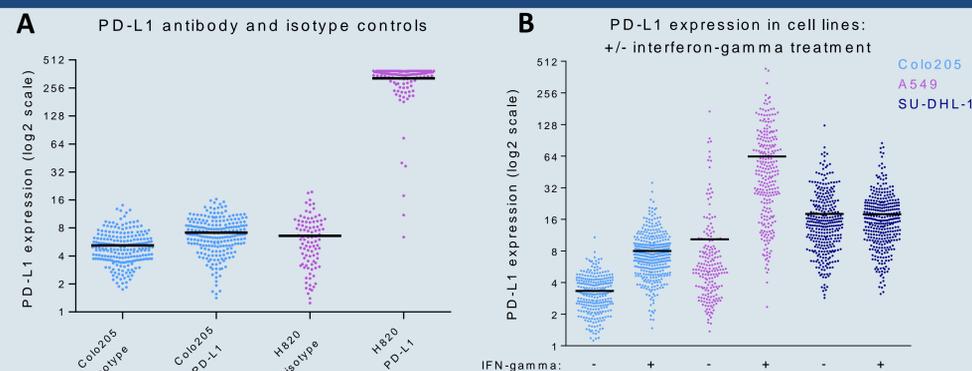
## Methods

79 blood specimens from newly diagnosed lung cancer 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.



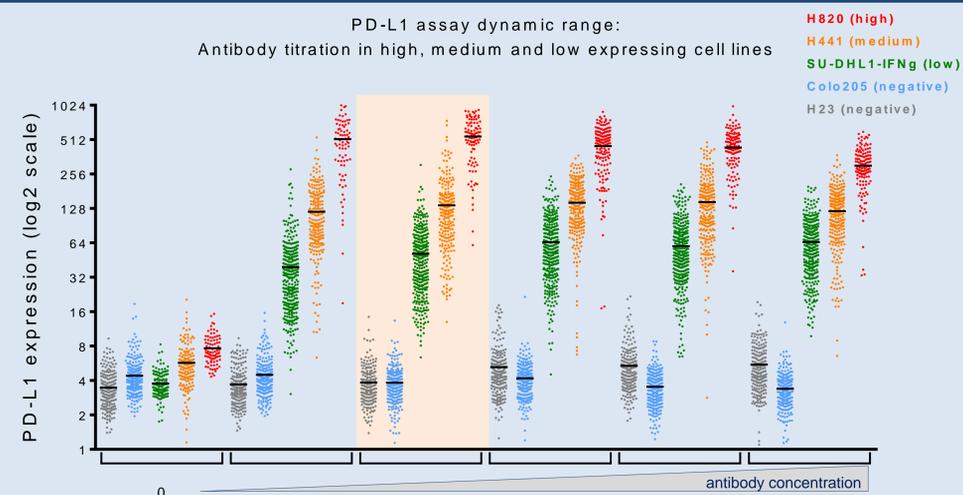
**Figure 1: Schematic of Epic's CTC collection and detection process:** 1) Nucleated cells from a blood sample are plated onto slides and stored in the -80°C biorepository; 2) Slides are stained with CK, CD45, DAPI and PD-L1; 3) Slides are scanned, and multi-parametric digital pathology algorithms are 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



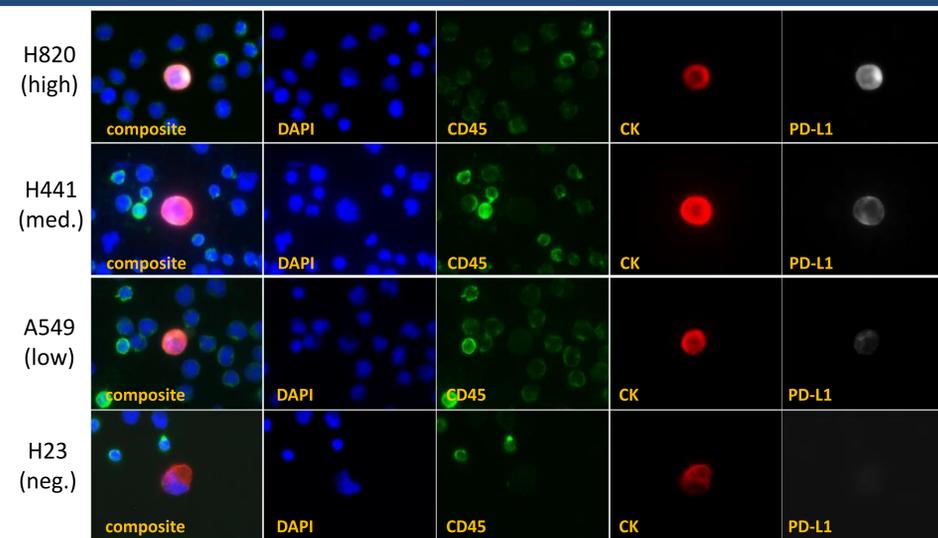
**Figure 2: Demonstration of PD-L1 assay specificity:** (A) PD-L1-specific antibody and species-matched isotype control were tested in negative (Colo205) and high (H820) PD-L1-expressing cell lines. No specific staining was seen in negative control cell lines or with isotype control antibody. (B) Interferon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



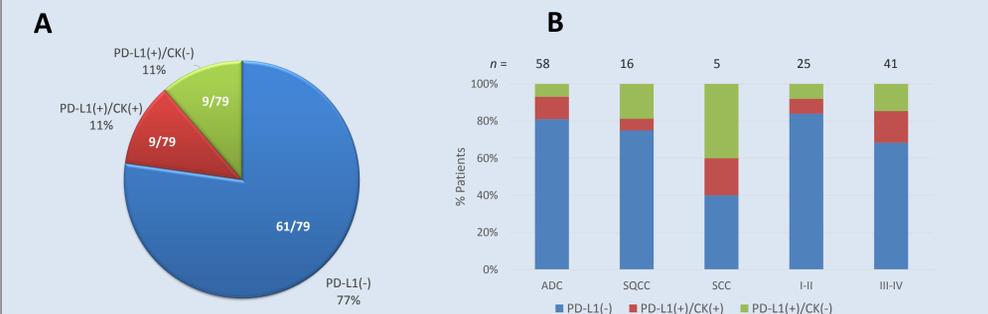
## Clinical Feasibility in Lung Cancer Samples

79 lung cancer patient samples consisting of 58 adenocarcinoma (ADC), 16 squamous cell carcinoma (SQCC) and 5 small cell carcinoma (SCC) cases of various staging were stained with the Epic PD-L1 assay. Results are summarized in Table 1. 18/79 (22.8%) patients had detectable PD-L1+ CTCs: 19% of ADC patients and 25% of SQCC patients. 4/25 (16%) patients with stage I-II at time of draw had PD-L1+ CTCs; 13/41 (31.7%) stage III-IV patients had PD-L1+ CTCs. The greater incidence of PD-L1 positivity in late stage cancer patient is consistent with previous reports examining primary tumor tissue.

Stage	ADC	SQCC	SCC	Total
I-II	3/19 (15.8%)	1/6 (16.7%)	0	4/25 (16%)
III-IV	7/28 (25%)	3/8 (37.5%)	3/5 (60%)	13/41 (31.7%)
Unknown	1/11 (9.1%)	0/2 (0%)	0	1/13 (7.7%)
<b>Total</b>	<b>11/58 (19.0%)</b>	<b>4/16 (25%)</b>	<b>3/5 (60%)</b>	<b>18/79 (22.8%)</b>

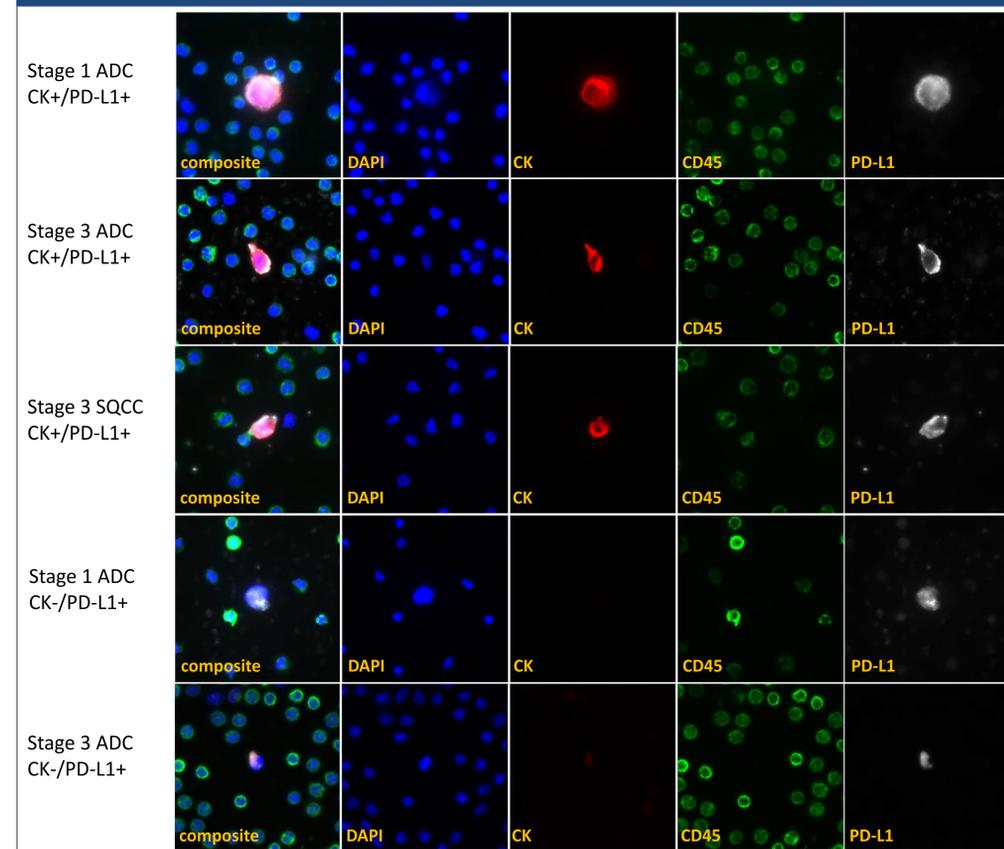
**Table 1: % Patients with PD-L1+ CTCs (# PD-L1+ patients/# total patients)**

## Clinical Feasibility in Lung Cancer Samples (cont.)



**Figure 4: PD-L1+ CTCs demonstrate epithelial plasticity:** (A) Of the 18 total patients with PD-L1+ CTCs, 9 had predominately traditional CK+/PD-L1+ CTCs. However, 9 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 in CTC subpopulations from lung cancer patients at diagnosis is feasible on the Epic CTC platform.** The test demonstrates analytical sensitivity and specificity and may aid in the identification of patients suitable for clinical trial studies with novel anti-PD-1 or anti-PD-L1 therapies. The assessment of PD-L1 expression from a circulating marker could enable longitudinal and pharmacodynamic analysis in response to PD-1 axis blockade in patients.