Programmed Death-1 Ligand (PD-L1) Expression on Circulating CD45(-) Cells is an Independent Prognostic Factor for Overall Survival in Patients with Lung Cancer

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Background

Lung cancer patients currently require invasive tissue biopsies to diagnose and profile tumors prior to treatment. There is a need for tumor profiling from circulating biomarkers to avoid invasive and complicated procedures. 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.

PD-L1 expression on lung cancer biopsy specimens correlates with efficacy of immune checkpoint therapy. However, the expression of PD-L1 on circulating cells has not been fully characterized, limiting their ability to guide therapy. Using the Epic Sciences PD-L1 assay, we sought to correlate patient outcome with PD-L1(+) circulating CD45(-) cells, as a prerequisite to prospective evaluation as a predictive biomarker in a prospective, multicenter cohort.

Methods

145 peripheral blood samples were collected from suspected lung cancer patients at three clinical sites prior to diagnostic biopsy (baseline), or at follow-up 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 stained and characterized with the CST antibody PD-L1 (E1L3N®, XP® Rabbit mAb #13684) to assess protein expression.

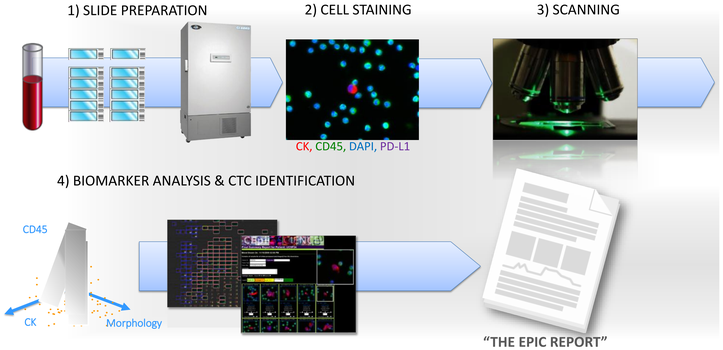


Figure 1: Schematic of Epic Sciences' CTC Detection Platform: 1) Nucleated cells from a blood sample are plated onto slides and stored in the -80ºC biorepository. **2)** Slides are stained with CK, CD45, DAPI and PD-L1. **3)** Slides are scanned, and multi-parametric digital pathology algorithms are 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

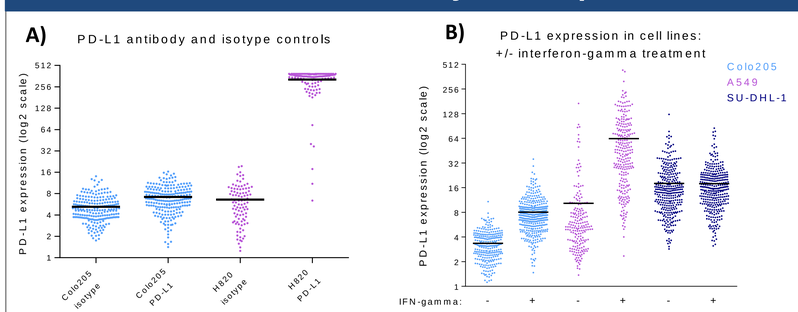


Figure 2: Demonstration of PD-L1 Assay Specificity: (A) PD-L1-specific antibody (CST E1L3N®, XP® Rabbit mAb #13684) and species-matched isotype control were tested in negative (Colo205) and high (H820) PD-L1-expressing cell lines. No specific staining was seen in negative control cell lines or with isotype 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 up-regulation of cytokine signaling suppressor genes in this particular cell line.

PD-L1 Protein Assay Development (cont.)

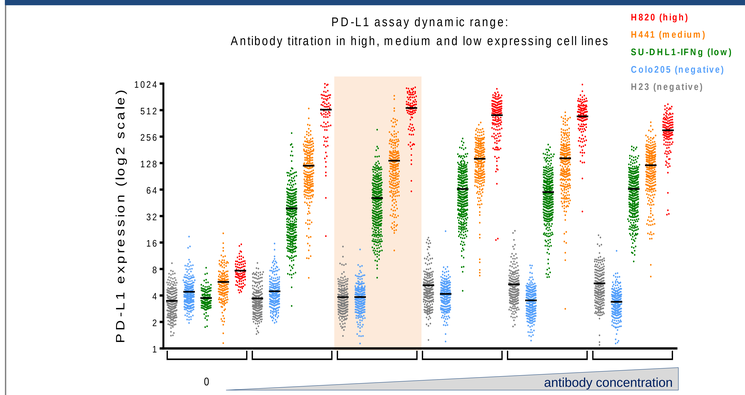


Figure 3: Assessment of PD-L1 Assay Sensitivity and Dynamic Range: PD-L1 antibody concentration was titrated in high (H820), medium (H441), low (SU-DHL-1) and negative (Colo205, H23) PD-L1-expressing cell lines to determine assay sensitivity and specificity. At the proprietary optimal antibody concentration (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.

Patient Demographics

By Histology	Patients (n = 118)	Baseline Samples (n = 118)	Follow-Up Samples (n = 27)	Total Samples (n = 145)
Adenocarcinoma	80	80	21	101
Squamous Cell Carcinoma	19	19	5	24
Small Cell Carcinoma	6	6	1	7
Other Malignant Histology	13	13	0	13
By AJCC Stage	**Patients (n = 118)**	**Baseline Samples (n = 118)**	**Follow-Up Samples (n = 27)**	**Total Samples (n = 145)**
I	51	51	7	58
II	17	17	4	21
III	28	28	8	36
IV	22	22	8	30

Table 1 & 2: Patient Demographics by Histology and AJCC Staging. Lung cancer patients with diverse histology were selected for interim analysis of PD-L1 expression.

Histologic PD-L1(+) Rare Cell Subtypes

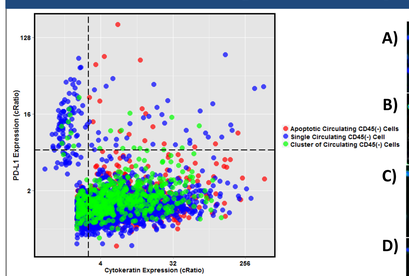


Figure 4: Scatter Plot of Circulating CD45- Cells. Scatter plot of all CD45(-), morphologically distinct cells from the cohort (n = 3040) showing quantified PD-L1 expression in relation to quantified cytokeratin expression, with horizontal and vertical dashed lines reflecting analytical cutoffs for PD-L1 and cytokeratin positivity, respectively.

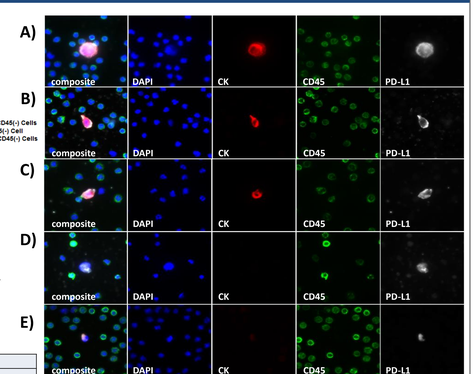


Figure 5: Rare CD45(-)PD-L1(+) Cell Phenotype. Panels include DAPI (blue), Pan-Cytokeratin (red), CD45 (green), and PD-L1 (white). Example CK(+)/PD-L1(+) CTCs (A-C) and CK(-)/PD-L1(+) cells (D-E) are shown.

	Total Cells
CK(+)PDL1(+)	63
CK(-)PDL1(+)	86
PDL1(-)	2891
Total	3040
	Screening Sample-level Positivity
CK(+)PDL1(+) cells but not CK(-)PD-L1(+) cells	6
CK(-)PDL1(+) cells but not CK(+)PD-L1(+) cells	2
Both CK(-)PDL1(+) cells and CK(+)PD-L1(+) cells	14
No PD-L1(+) cells present	96
Total Patients	118

Table 3: Detection of PD-L1 Expression in Circulating CD45- Cell Populations of Patient Samples. The ability to detect PD-L1 expression of both CK-/CD45- and CK+/CD45- circulating cells on the Epic Sciences platform, allows for increased prognostic power in identifying PD-L1+ patient samples irrespective of cytokeratin expression.

Rare Cell Enumeration by Histology and Stage

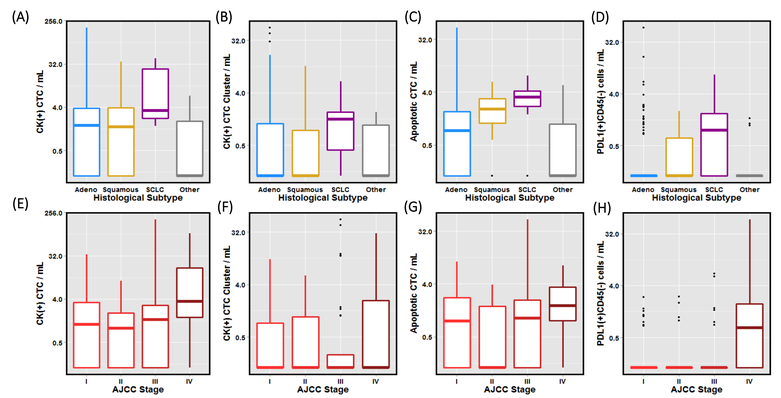
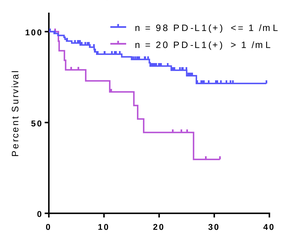


Figure 6: Rare Cell Enumeration by Histology and Stage Enumeration comparisons by histology of (A) CTCs, (B) CK+ CTC Clusters, (C) Apoptotic CTCs,. and (D) PD-L1(+)CD45(-) cells. Enumeration comparisons by AJCC Stage of (F) CTCs, (G) CK+ CTC Clusters, (H) Apoptotic CTCs, and (I) PD-L1(+)CD45(-) cells

PD-L1 Expression is a Prognostic Factor for Overall Survival at Diagnosis and Follow-Up

(A)

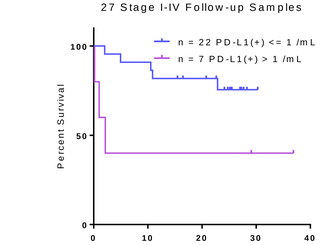


(B)

Univariate Kaplan-Meier Analysis	
Threshold	>1.0 PD-L1(+) /mL
Baseline Samples Positive	20 of 118 (17%)
Median Survival	17.2 Months vs Not Reached
% Survival at 2 Years	45% vs. 79%
Logrank Hazard Ratio	3.38 (2.06 to 17.1)
Logrank p-value	0.0010

Variable	HR	Multivariate p-Value
AJCC Staging (IV vs. I-III)	2.84	0.0108
PD-L1(+) cell burden (high vs. low)	2.40	0.0382

(C)



(D)

Univariate Kaplan-Meier Analysis	
Threshold	>1.0 PD-L1(+) /mL
Follow-up Samples Positive	5 of 27 (19%)
Median Survival	2.1 Months vs Not Reached
% Survival at 2 Years	40% vs. 76%
Logrank Hazard Ratio	4.24 (1.2 to 85)
Logrank p-value	0.0302

Variable	HR	Multivariate p-Value
AJCC Staging (IV vs. III)	8.57	0.0131
PD-L1(+) cell burden (high vs. low)	1.78	0.459

Figure 7: PD-L1(+) CTCs is a Prognostic Factor for Overall Survival at Diagnosis and Follow-Up. Baseline Kaplan-Meier estimates (A) of overall survival from baseline draw and multivariate estimate comparison to AJCC staging (B). Follow-up Kaplan-Meier estimates (C) of overall survival from baseline draw and multivariate estimate comparison to AJCC staging (D).

Conclusions

- In a prospectively enrolled, multicenter cohort, PD-L1(+) cell burden prognosticated OS in univariate and multivariate settings, independent to AJCC staging in pre-biopsy and follow-up samples.
- This lays the groundwork for PD-L1(+) circulating cells to be prospectively assessed as a predictive biomarker to immune checkpoint inhibitors in lung cancer.