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

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Background
Programmed death-1 (PD-1) is an immune checkpoint receptor that is upregulated in response to chronic inflammation and is constitutively expressed on T cells, B cells, and other cell populations. PD-1 interaction with its ligand PD-L1 is associated with decreased overall survival and increased tumor invasiveness. 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-L1/PD-1 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 (CD45-, CD145+ with intact and morphologically distinct DAPI/nucleated), apparent (CD45-, DAPI-, intact nucleated) and CD45+ (CD45-, intact and distinct nucleated) were identified. Samples were characterized with PD-L1 IHC to assess protein expression.

PD-L1 Protein Assay Development

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 4°C biorepository; 2) Slides are stained with CD, 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 (cont.)

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 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 Colo205 cells remains unchanged, likely due to the upregulation of cytokine signaling suppressor genes in this particular cell line.

Clinical Feasibility in NSCLC Patient Samples

Figure 3: Assessment of PD-L1 assay sensitivity and dynamic range: PD-L1 antibody concentration was titrated in high (H820), medium (H441), low (SU-DHL1) 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 140-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 100% specificity.

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., lack of cytokeratin and high, EpCAM expression). (B) Further breakdown of CK+/PD-L1+ or 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

Table 1: CTCs: NSCLC patient analysis consisting of 25 immunofluorescence (IF) and 32 sequence- and cytometry (SCYA) slides of various stages 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-defined CTCs is consistent with previous reports examining primary tumor tissue. 4/17 (23%) patients with stage III NSCLC at time of draw had PD-L1+ CTCs: 3/8 (38%) stage IV NSCLC patients had PD-L1+ CTCs.

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. Endowment 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.