Validation of PD-L1 Expression on Circulating Tumor Cells in Lung Cancer

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Background

The human immune system recognizes and eliminates certain types of tumor cells, whereas other malignancies are capable of suppressing immune function. A number of cancer cell types express programmed cell death ligand 1 (PD-L1), which binds to its receptor PD-1 on T cells to prevent their activation. High levels of PD-L1 expression are typically associated with poor patient prognosis. Researchers have developed immunotherapies (e.g., inhibitors of the PD-1/PD-L1 pathway) to stimulate the immune system, allowing the body’s natural defenses to combat the tumor. To determine which patients are suitable candidates for receiving immunotherapy, levels of PD-L1 expression are often determined from tumor biopsies. However, tumor heterogeneity can confound these results and obtaining tumor tissue is often not feasible. To enable non-invasive detection and sequential monitoring of tumor-associated PD-L1 expression we have developed a highly sensitive method of detecting PD-L1 levels in circulating tumor cells (CTCs). This work describes the analytical validation of the Biocept PD-L1 assay in CTCs.

Methods

PD-L1 expression levels on carcinoma cell lines were identified by flow cytometry. For analytical validation, H727, BT474 H358, HCC78 and H820 cells were spiked into whole blood into CEE-Sure™ blood collection tubes. Samples were prepared in replicate and on different days, incubated overnight and then processed. The leukocyte fraction was incubated with our pan-CTC antibody cocktail labeled with biotinylated secondary antibody, followed by enrichment in our streptavidin coated microfluidic channels. Enriched cells were stained for DAPI, cytokeratin, CD45, PD-L1 (clone 28-8) and CEE-Enhanced (pan-CTC stain). After automated fluorescence scanning, spiked tumor cells within the microchannel were identified and average PD-L1 intensities were quantified for each cell. Cut-off criteria were determined.

Results

PD-L1 Expression in Lung Cancer Cell Lines

Fig. 2: Identification of PD-L1 expressing lung cells by flow cytometry. PD-L1 epitopes were detected with either the M9H1 or 28-8 clone and presented as A) percent positive or B) mean fluorescent intensity (MFI).

Fig. 3: PD-L1 stain on microfluidic enriched lung cancer cells spiked into blood. Antibody staining patterns are presented on a representative 2-D scatter plot: H820 (red); H727 (blue); H358 (green); HCC78 (magenta). The CEE-Enhanced (yellow) and PD-L1 (clone 28-8) stain were performed simultaneously.

Fig. 4: This scatter plot (mean with SD) shows the mean fluorescent intensity (MFI) measurement of PD-L1 protein in various blood spiked lung cancer cell lines enriched and quantified in the Biocept microfluidic device. (Cut-off value at 40 MFI is depicted by the dotted line.)

Fig. 5: PD-L1 reproducibility. Replicate measurements of negative and positive PD-L1 expressing cells, spiked into blood, and enriched in our microfluidic device are presented as MFI.

Table 1: Analytical performance of PD-L1 assay. Negative control cell lines and cell lines expressing high, medium, and low levels of PD-L1 are depicted.

Conclusions

- The Biocept PD-L1 assay can accurately detect PD-L1 expressing cells spiked into whole blood samples.
- The Biocept platform is able to capture CTCs from cancer patient blood samples and identify the cells expressing PD-L1.
- The ability to detect PD-L1 expressing CTCs in blood affords a way to identify patients likely to benefit from immune therapy, as well as monitor the efficacy of such treatments.

References