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 capture 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.

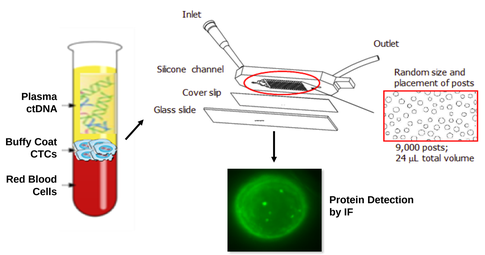

Fig. 1: Biocept platform for CTC capture and staining. CTCs are captured in transparent microfluidic channels and can be viewed *in situ* by fluorescent microscopy. CTCs can be analyzed via immunofluorescence (IF)

Results

PD-L1 Expression in Lung Cancer Cell Lines

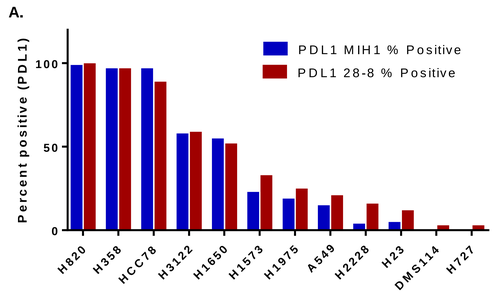

Fig. 2: Identification of PD-L1 expressing lung cell lines by flow cytometry. PD-L1 epitopes were detected with either the MIH1 or 28-8 clone and presented as A) percent positives or B) mean fluorescent intensity (MFI)

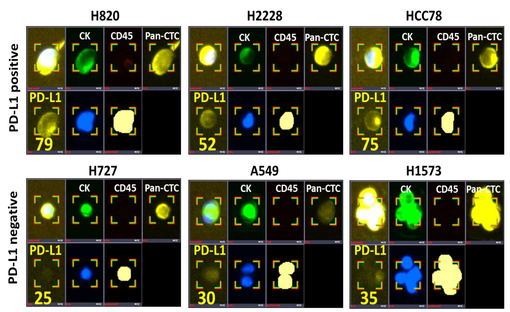

Fig. 3: PD-L1 stain on microfluidic enriched lung cancer cells spiked into blood. Antibody stains are presented in following order:
UL: combined; cytokeratin (green); CD45 (red); CEE-Enhanced (yellow);
LL: PDL1 (yellow) (***number indicates average intensity***); DAPI (blue); synthetic combined

Results

PD-L1 Expression in Lung Cancer Cell Lines

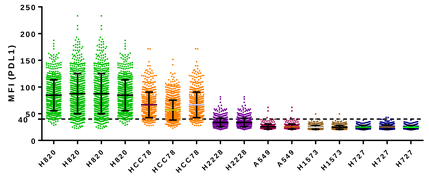

Fig. 4: This scatter plot (mean with SD) shows the mean fluorescent intensity (MFI) measurement of PD-L1 protein in various blood spiked lung cancer cell lines enriched and quantified in the Biocept microfluidic device. (Cut-off value at 40 MFI is depicted by the dotted line.)

Analytical Validation

A cut-off value (average fluorescence intensity value) was established to yield 100% concordance between the result of the PD-L1 test and the identity of the introduced cell lines. The negative cut-off of 3.9% is based on a BETA inverse of the 95% confidence level. In our microfluidic PD-L1 assay we demonstrate H727 and BT474 cells to be negative for PD-L1, while H358 cells have low-medium and HCC78 and H820 cells high PD-L1 expression.

PD-L1 Accuracy Study (BioView "40" orange value cut-off)					
Cell line tested	Total CTC	# cells PDL+	CTC PDL1 Status	Cell Line PDL1 status	Concordance
H727	400	2	Not Detected	Not Detected	Concordant
H727	400	2	Not Detected	Not Detected	Concordant
H727	400	3	Not Detected	Not Detected	Concordant
H727	400	9	Not Detected	Not Detected	Concordant
H727	400	4	Not Detected	Not Detected	Concordant
H727	400	2	Not Detected	Not Detected	Concordant
H727	400	5	Not Detected	Not Detected	Concordant
BT474	400	3	Not Detected	Not Detected	Concordant
BT474	400	7	Not Detected	Not Detected	Concordant
BT474	400	2	Not Detected	Not Detected	Concordant
BT474	400	8	Not Detected	Not Detected	Concordant
BT474	400	7	Not Detected	Not Detected	Concordant
BT474	400	17	Not Detected	Not Detected	Concordant
BT474	400	42	Not Detected	Not Detected	Concordant
BT474	400	19	Not Detected	Not Detected	Concordant
BT474	400	10	Not Detected	Not Detected	Concordant
BT474	400	13	Not Detected	Not Detected	Concordant
HCC78	400	364	Detected	Detected	Concordant
HCC78	400	335	Detected	Detected	Concordant
HCC78	400	317	Detected	Detected	Concordant
HCC78	400	387	Detected	Detected	Concordant
HCC78	400	280	Detected	Detected	Concordant
HCC78	400	287	Detected	Detected	Concordant
HCC78	400	239	Detected	Detected	Concordant
HCC78	400	385	Detected	Detected	Concordant
H820	400	391	Detected	Detected	Concordant
H820	400	371	Detected	Detected	Concordant
H358	400	260	Detected	Detected	Concordant
H358	400	254	Detected	Detected	Concordant
H358	400	304	Detected	Detected	Concordant
H358	400	286	Detected	Detected	Concordant
H358	400	240	Detected	Detected	Concordant
H358	400	319	Detected	Detected	Concordant
H358	400	328	Detected	Detected	Concordant
H358	400	329	Detected	Detected	Concordant
H358	400	301	Detected	Detected	Concordant
H358	400	304	Detected	Detected	Concordant

Table 1: Analytical performance of PDL1 assay. Negative control cell lines and cell lines expressing high, medium, and low levels of PD-L1 are depicted

Results

Reproducibility

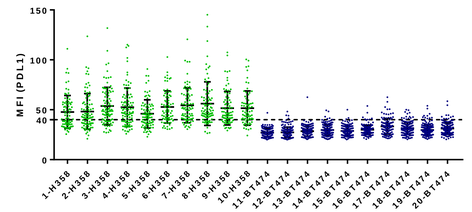

Fig. 5: PD-L1 reproducibility. Replicate measurements of negative and positive PD-L1 expressing cells, spiked into blood, and enriched in our microfluidic device are presented as MFI.

PD-L1 Expression in Clinical Samples

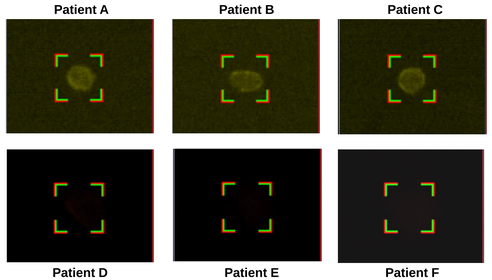

Fig. 6: Clinical examples. Six cancer patient cases are shown where the Biocept platform was used to capture CTCs and the PD-L1 assay identified cells expressing PD-L1 protein. PD-L1 positive CTCs were identified in patents A-C, while PD-L1 negative CTCs were identified in patents D-F.

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 expressing CTCs**
- **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

➢ **Phillips T et al, Development of an automated PD-L1 immunohistochemistry (IHC) assay for non-small cell lung cancer. Appl Imm Mol Morphol. 2015 PMID: 26317305**