Investigation of PD-L1 expression in circulating tumor cells isolated using the Parsortix system in metastatic lung and breast cancer patients

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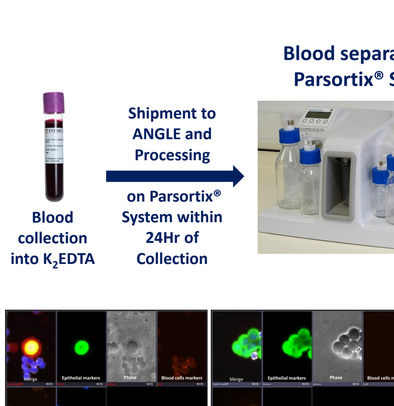
Introduction

Programmed death-ligand 1 (PD-L1) allows cancer cells to evade the host immune response when upregulated. PD-L1 antagonists are widely used as immunotherapies for treatment of cancer patients. The value of PD-L1 detection on tissue biopsies that may be out-of-date at the time of treatment is controversial. Measurement of PD-L1 expression in circulating tumor cells (CTCs) may enable repeat testing to provide up-to-date PD-L1 status and the potential to monitor patients on these therapies.

Figure 1. PD-1 and PD-L1 interaction on tumor cells and immune cells inhibits immune response. Figure taken from Akinleye *et al.*, J. Hematol. Oncol., 2019



In this study, we evaluated the performance of a newly developed research use only assay for the characterisation of PD-L1 expression on epithelial CTCs isolated using the Parsortix® system, a label-independent microfluidic device that isolates cells based on their size and compressibility .

Workflow

Performance of the assay was assessed using clinical samples on 17 healthy volunteers (HV), 17 metastatic breast cancer (MBC) patients, and 18 metastatic non-small cell lung cancer (NSCLC) patients as per the workflow below. CTCs were isolated using the Parsortix® system and stained using ANGLE's PD-L1 assay. Performance was defined as:

- CTC positivity rate = % of cancer patients with at least 1 CTC (PD-L1+/-)
- Specificity = 100 – (% healthy donors with at least 1 CTC)

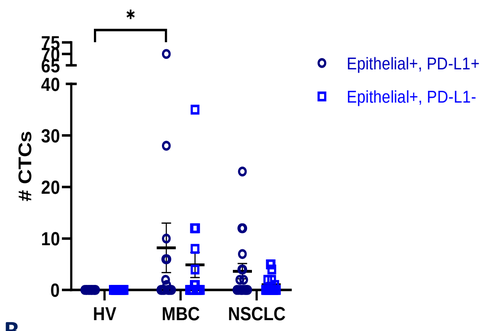
Figure 2. Schematic representation of the assay workflow. Peripheral blood (8 – 10mL) was drawn into K_2EDTA tubes from 17 HV, 17 MBC patients and 18 metastatic NSCLC patients and processed on Parsortix® systems within 24 hours from draw. Cells captured in the Parsortix® cassette due to their larger size and lower compressibility compared to other blood components were harvested, cytospun onto slides, and immunofluorescently stained for detection of CTCs and PD-L1 expression. Slides were imaged using a BioView AllegroPlus imaging system. CTCs were defined as FITC+, Cy5-, Cy3+/-, DAPI+ cells. Identification was based on morphological evaluation and thresholding techniques using fluorescence intensity of the markers established on control cancer cell lines (MCF7 as FITC+, Cy5-, Cy3-, DAPI+ and H226 as FITC+, Cy5-, Cy3+, DAPI+).

Results

- No CTCs were observed in the healthy volunteers (assay specificity of 100%).
- 70% of MBC patients and 55% of NSCLC patients had ≥1 CTC identified. Importantly, the CTC positivity rate observed in NSCLC patients was 2-fold higher compared to that in previously described studies using epithelial markers based epitope-dependent systems.
- MBC patients with CTCs had an average of 9 CTCs identified (range of 1 to 128). NSCLC patients with CTCs had an average of 11 CTCs identified (range of 1 to 23). CTC clusters (consisting of 3 to 45 cells per cluster) were observed in both patient groups.
- High heterogeneity of PD-L1 expression was observed. CTC positive patients were classified as: 1) all CTCs PD-L1+; 2) mixed population of PD-L1+/- CTCs; and 3) all CTCs PD-L1- (results shown in **Figure 3B**). Interestingly, these data are consistent with current publications on the subject (Janning *et al.*, 2019; Nicolazzo *et al.,* 2016; Khattak *et al.,* 2019, Po *et al.,* 2019), highlighting the reliability of the ANGLE's PD-L1 assay.

Figure 3. CTCs identification and phenotyping in MBC and NSCLC patients. (A) Dot plot shows number (error bars: mean ± SEM) of PD-L1+ and PD-L1- CTCs captured in each donor across the three cohorts (**P<0.05*, Two-way ANOVA followed by Tukey's multiple comparison test); (B) Table shows number of donors included in each cohort (N), percentage (%) donors with at least 1 CTC, range and mean of CTC captured across donors, range of number of CTC clusters per donor, range of number of CTCs found in clusters, % donors with only PD-L1+ CTCs, % donor with PD-L1+ and PD-L1- CTCs and % donors with only PD-L1- CTCs; (C) Representative images of PD-L1+ (top) and PD-L1- (bottom) CTCs captured, including clusters (left) and single cells (right). Merge colors: FITC (epithelial markers) in green, Cy3 (PD-L1) in red, Cy5 (blood lineage markers) in white, DAPI (nuclear staining) in blue.

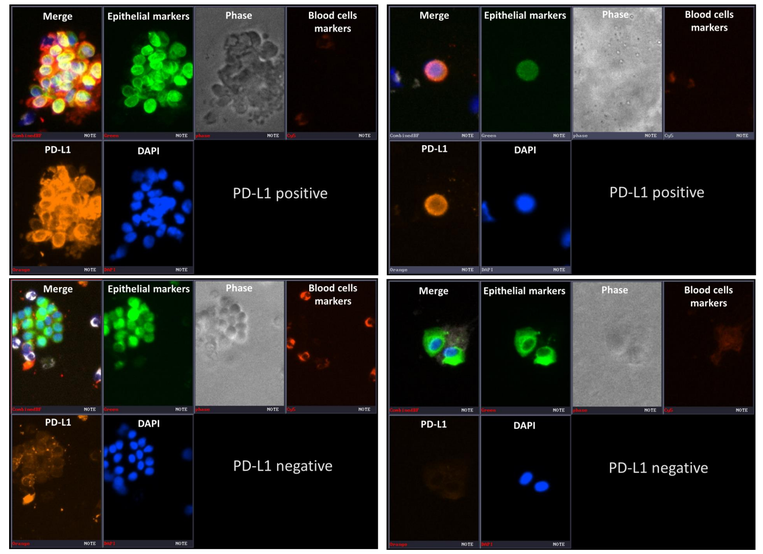
A



B

	HV	MBC	NSCLC
N	17	17	18
≥1 CTC	0%	70%	55%
CTC range	-	1-128	1-23
CTC mean	-	9	11
N Clusters per donor (range)	-	2-11	1-2
N CTCs per cluster (range)	-	3-45	4-22
All PD-L1+ CTC	-	36%	40%
≥1 PD-L1+ & ≥1 PD-L1- CTC	-	27%	40%
All PD-L1- CTC	-	36%	20%

C



Conclusions

- ANGLE's PD-L1 assay allowed for the determination of PD-L1 expression in a significant proportion of the MBC and NSCLC patients studied.
- The ability to isolate significant numbers of CTCs from peripheral blood lays the groundwork for the development of dynamic PD-L1 monitoring to support personalized patient management.