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 The value of PD-L1 detection on tissue biopsies that may be out-of-date at the time of treatment is controversial.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.

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)

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; Nicozzolo et al., 2016; Khattak et al., 2019), highlighting the reliability of the ANGLE’s PD-L1 assay.

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.