Simultaneous assessment of PD-L1 and IRF1 expression on breast cancer circulating tumor cells

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Background
There is a need for non-invasive predictive biomarkers of response to anti-PD-1/PD-L1 therapies. Assessment of circulating tumor cells (CTCs) is a rational approach to non-invasive sampling of tumors to understand the potential response or non-response to anti-PD-1/PD-L1 therapies. IFN-gamma signals through the JAK/STAT cascade to induce PD-L1 via the Interferon Regulatory Factor-1 (IRF1) transcription factor, and is a potent inducer of PD-L1 expression in tumor cells. Recent studies have shown that low or absent IRF1 expression can identify melanomas that lack IFN-gamma responsiveness, and that IRF1 can have a higher predictive value of response to anti-PD-1/PD-L1 therapy than PD-L1 itself.

Methods
Peripheral blood from normal donors or cancer patients under an IRB-approved protocol was collected into RareCyte blood collection tubes. PD-L1(+) and PD-L1(-) CTC models were created by culturing A549 overnight with or without 10ng/ml INF-gamma. The A549 cells were spiked into normal donor blood and buffy coats isolated from 7.5ml of blood by AccuCyte® separation and spread onto slides. MDA-MB-231 cells were used as a model to represent breast cancer cells that express PD-L1 in the absence of IFN-gamma signaling. Slides were stained with a 6-marker panel that included antibodies to pan-cytokeratin (CK), EpCAM, CD45, PDL1, IRF1, and a nuclear dye on the Ventana Discovery Ultra auto-stainer. Slides were scanned with CyteFinder® and CTCs identified by CK and/or EPcam positivity and negative CD45 staining. Nuclear IRF1 and PDL1 expression was determined by mean fluorescence intensity in computationally estimated cell compartments. Currently, estimation of the nuclear compartment has a 5% failure rate, these data points were excluded from this analysis.

Results
Nuclear expression of IRF1 correlated with PD-L1 expression in IFN-gamma stimulated A549 cells. Unstimulated MDA-MB-231 cells expressed high levels of PD-L1, but this did not correlate with nuclear IRF1 expression. Breast cancer patient samples were identified having CTCs that expressed PD-L1. A PD-L1 mean fluorescence intensity (MFI) threshold was set at the upper 95% CI of unstimulated A549 cells to define “high” and “low” PD-L1 expression in the patient samples. The population of high-PD-L1 CTCs had higher nuclear IRF1 MFI than low-PD-L1 CTCs, with mean fold-increase of 2.7 (range from 1.4 to 3.3).

Conclusions
• In unstimulated A549s, PD-L1 staining and nuclear IRF1 staining were absent. In contrast, IFN-gamma-stimulated A549s demonstrated PD-L1 staining and strong nuclear staining for IRF1.
• MDA-MB-231 had strong PD-L1 staining but low to absent nuclear IRF1 staining. This staining pattern may identify cancer cells with PD-L1 expression not due to IFN-gamma signaling.
• Identifying PD-L1 and IRF1 biomarkers on CTCs is feasible for non-invasive assessment. The cellular distribution of IRF1 together with PD-L1 expression may contribute additional predictive value to the assay.

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