

## “PD-L1ing to the C (CTCs)”: Developing a new Assay for Bladder Cancer Circulating Tumor Cells

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**Objective:** Bladder cancer patients being considered for immune checkpoint blockade are often judged on immunohistochemical staining for the checkpoint target protein PD-L1 in the original surgery or biopsy sample. However, sampling error or the clinical evolution of most patients' cancer can render the original PD-L1 assessment no longer accurate. In contrast, circulating tumor cells (CTCs) allow serial noninvasive sampling of the current tumor status throughout a patient's clinical course including those with the highest metastatic potential. We therefore sought to develop a method for quantifying PD-L1 expression in CTCs towards addressing inherent limitations of current UC management.

**Methods:** This work utilizes both cancer cell lines as well as patient samples. Positive and negative control cancer cell lines were assessed via “industry standard” antibodies for PD-L1 expression via Western Blots and immunofluorescence, and a threshold-based method was developed for reliable quantification. PDL-1 expression was additionally verified via interferon-mediated up-regulation. CTCs isolated from bladder cancer patient samples via a microfluidic-based isolation method (see the TIPSTer abstract in this same RBI Retreat) were then assessed for PD-L1 via the same antibodies.

**Results/ Conclusion:** We will show preliminary preclinical and clinical data that validates the sensitivity and specificity of our assay. A case study will be presented that illustrate the potential useful of the novel approach we describe and which should be complementary to current clinical practices. In a patient with metastatic bladder cancer, this method effectively detected the PD-L1 expression in CTCs taken at a time coincident to when the patient derived an excellent response to the PD-L1 checkpoint inhibitor Pembrolizumab.