Programmed death-ligand 1 (PD-L1) Characterization of Circulating Tumor Cells (CTCs) and White Blood Cells in Muscle Invasive and Metastatic Bladder Cancer Patients

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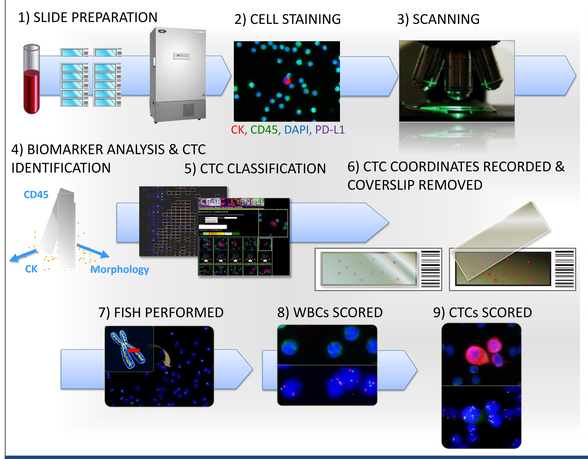
Background

PD-1 immune checkpoint therapies have demonstrated durable responses in a subset of metastatic cancer patients including those with melanoma, non-small cell lung cancer (NSCLC), and bladder cancer. Recent clinical studies have observed improved progression free survival (PFS) after treatment with PD-1 directed therapy in patients with higher expression of PD-L1 protein in tumor tissue sections. Additionally, the magnitude of PD-L1 expression on tumor infiltrating T-cells is being investigated as a potential biomarker. Currently, fresh solid tumor biopsy is required for these assays, which can be difficult to access, lead to patient morbidity, and miss relevant subpopulations reflective of tumor heterogeneity.

We have developed an assay to quantify PD-L1 expression on circulating tumor cells (CTCs) and white blood cells (WBCs) detected using the Epic CTC Platform. Here, we present data on the incidence, morphology, and PD-L1 expression of CTCs and WBCs detected from the venous circulation of 17 muscle invasive and metastatic bladder cancer patients prior to initiation of PD-1 immune checkpoint therapy.

Methods

Blood was drawn from 17 muscle invasive and metastatic bladder cancer patients, just prior to PD-1 immune checkpoint therapy, and sent to Epic Sciences for processing with the Epic CTC PD-L1 assay.



Schematic of Epic CTC Platform CTC enumeration, morphology, protein, & FISH analyses workflow:

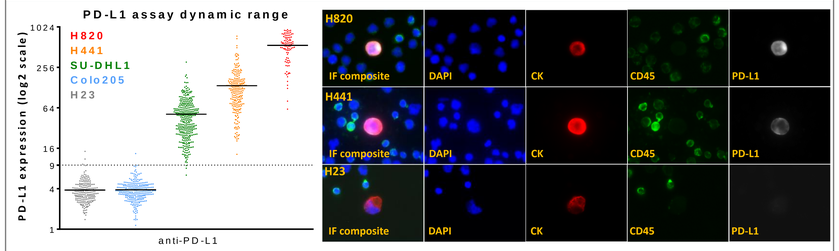
1) Nucleated cells from blood sample placed onto slides and stored in a -80˚C biorepository
2) Slides stained with cytokeratin (CK), CD45, DAPI and a biomarker of interest (PD-L1)
3) Slides scanned
4) CTC candidates are detected by a multi-parametric digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression
6) For fluorescence in situ hybridization (FISH), coordinates are recorded and coverslip is removed
7) FISH assay is performed
8) Regional WBCs are scored as controls
9) CTCs relocated and scored

Bladder Cancer Patient Demographics

Patient ID	Age/Gender	Disease state	Previous chemotherapy		Cytokeratin(+)		Cytokeratin(-)	
				All CTC/mL	PD-L1(+)/mL	PD-L1(-)/mL	PD-L1(+)/mL	PD-L1(-)/mL
2	88/M	MIBC	No	**1**	0	0	0	**1**
3	59/M	MIBC	No	0	0	0	0	0
4	77/F	MIBC	Yes	**5**	0	0	0	**5**
15	77/M	MIBC	Yes	0	0	0	0	0
18	46/M	MIBC	Yes	0	0	0	0	0
1	78/M	metastatic	No	**6**	0	**3**	0	**3**
5	67/F	metastatic	Yes	**41**	**1**	**39**	0	**1**
6	61/M	metastatic	Yes	**11**	0	**9**	0	**2**
7	84/F	metastatic	No	**1**	0	0	0	**1**
10	75/M	metastatic	Yes	**6**	0	**2**	**1**	**3**
11	81/M	metastatic	No	**2173**	**30**	**130**	**930**	**1083**
12	79/M	metastatic	Yes	**13**	0	**8**	**1**	**4**
13	43/M	metastatic	Yes	0	0	0	0	0
14	66/F	metastatic	Yes	**3**	0	0	0	**3**
16	65/F	metastatic	Yes	**16**	0	**9**	0	**7**
17	54/M	metastatic	No	0	0	0	0	0
19	55/F	metastatic	No	**2**	0	**1**	**1**	0

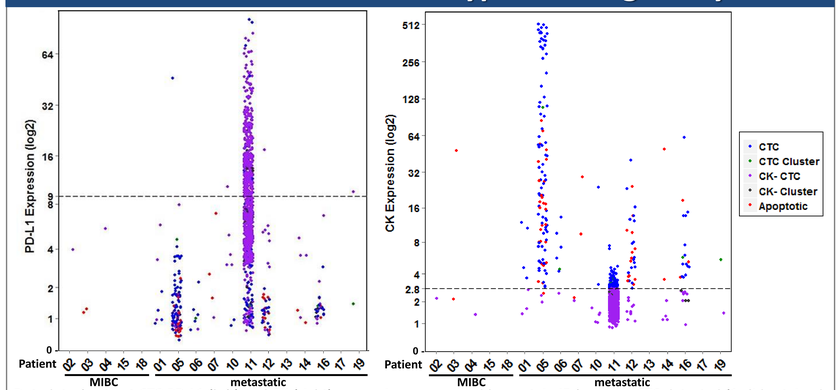
Patient demographics and summary of total non-apoptotic CTCs and CTC subpopulations detected per mL of blood in 17 bladder cancer patients. MIBC: muscle invasive bladder cancer.

CTC PD-L1 Assay Development

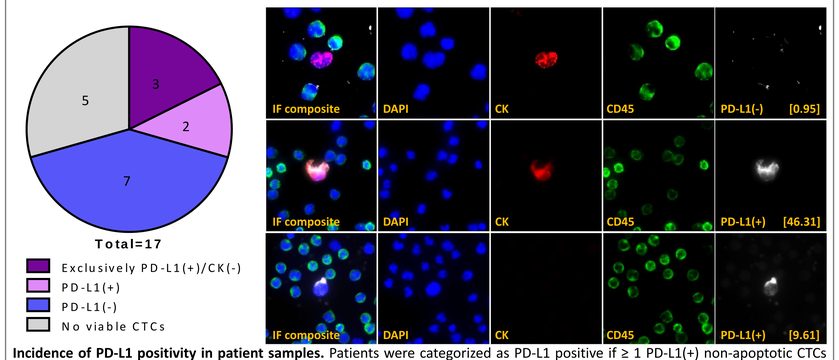


Assessment of PD-L1 assay sensitivity and specificity. Left: A monoclonal antibody against PD-L1 was tested using high (H820), medium (H441), low (SU-DHL1) and negative (Colo205, H23) PD-L1-expressing cell lines to determine assay sensitivity and specificity. At the optimal antibody concentration, mean H820 PD-L1 expression was determined to be 142-fold higher than mean background staining in negative controls. **Right:** Representative images.

Bladder Cancer CTC Subtype Heterogeneity

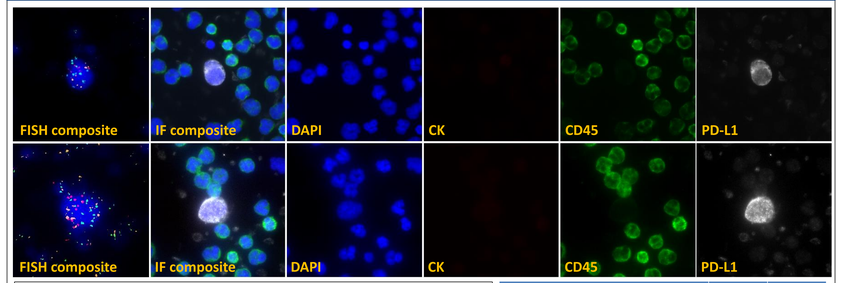


Dot plot of patient CTC PD-L1 (left) and CK (right) expression as assessed by protein IF for every CTC detected (1 slide stained for patient #11, 2 slides stained for all others). Each dot indicates one CTC detected; the color indicates CTC sub-population membership. Thresholds for PD-L1 and CK assay positivity were determined using respective positive and negative control cell lines. No CTCs were detected in each of 5 healthy donor samples tested (not shown).



Incidence of PD-L1 positivity in patient samples. Patients were categorized as PD-L1 positive if ≥ 1 PD-L1(+) non-apoptotic CTCs were detected **(left)**. 2 slides per patient (with the exception of patient #11) corresponding to ~1 mL of blood were analyzed. 7/17 (41%) patients had exclusively PD-L1(-) CTCs, while 5/17 (29%) had PD-L1(+) CTCs. Of the 5 patients with PD-L1(+) CTCs, 3 had exclusively CK(-)/PD-L1(+) CTCs, suggesting epithelial plasticity. CTC PD-L1 expression shows distinct membranous localization, consistent with established PD-L1 biology. Representative images **(right)** show PD-L1(-) and PD-L1(+) traditional (CK+) CTCs as well as CK(-)/PD-L1(+) CTCs. Bracketed numbers denote relative PD-L1 signal intensity as fold over background.

CK(-)/PD-L1(+) CTCs have Genetic Abnormalities

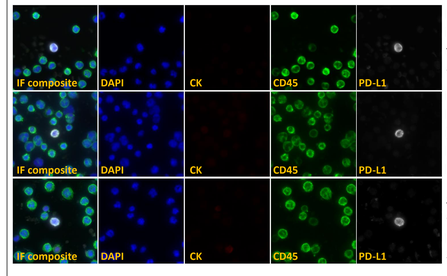


FISH probes: CEP3: aqua; CEP7: orange; CEP10: green; 5p15: red

33 CK(-)/PD-L1(+) CTCs detected in metastatic bladder cancer patient #11 were assessed by FISH for genetic alterations commonly associated with bladder cancer. The presence of these genetic abnormalities in CTCs is a confirmation of their malignant origin.

	# CTCs	%
CTCs w/ no abnormalities	4	12%
CTCs w/ at least 1 abnormality	29	88%
CTCs w/ all abnormalities	17	52%
Total CK(-)/PD-L1(+) CTCs scored	33	100%

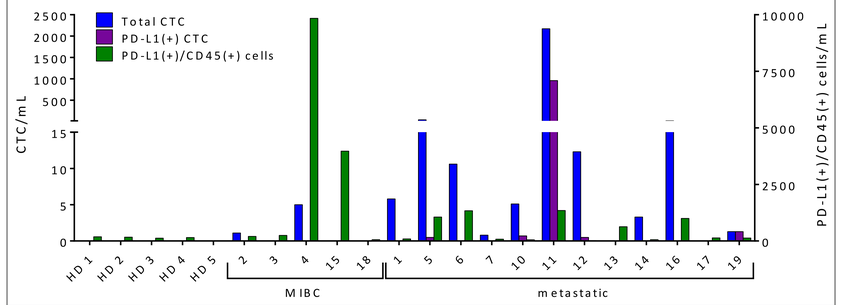
PD-L1(+)/CD45(+) Cells Associated with Bladder Cancer



Recent studies indicate that the presence of PD-L1(+) WBCs in solid tumors is a potential PD-1 immune checkpoint therapy response biomarker, both as a univariate biomarker or as part of a multivariate panel.

Bladder cancer patients frequently have elevated PD-L1(+)/CD45(+) WBC counts compared to healthy donors (HD). **Left**: representative images of PD-L1(+) WBCs.
Below: Graph depicting total CTCs, PD-L1(+) CTCs, and PD-L1(+)/CD45(+) WBCs per mL patient blood or HD control.

A combination of PD-L1(+)/CD45(+) cell count, PD-L1 expression on CTCs, and other CTC morphological parameters could be pursued as a possible response signature to PD-1 immune checkpoint therapies.



Conclusions

- The Epic CTC Platform can detect PD-L1(+) CTCs in bladder cancer patients
- Bladder cancer CTCs expressing PD-L1 are frequently CK(-) and show genetic abnormalities consistent with malignant origin and PD-L1 localization consistent with established literature
- Bladder cancer patients frequently have higher PD-L1(+)/CD45(+) WBC counts
- Correlation with treatment response is necessary to determine if the presence of PD-L1(+) CTCs or high PD-L1(+) WBC counts may be used for patient selection and as early pharmacodynamic biomarkers of response to PD-1/PD-L1 directed therapy