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

Archana Anantharaman[1], Terence Friedlander[1], David Lu[2], Rachel Krupa[2], Gayatri Premasekharan[3], Jeff Hough[1], Matthew Edwards[1], Ryon Graf[2], Adam Jendrisak[2], Jessica Louw[2], Lyndsey Dugan[2], Dena Marrinucci[2], Ryan Dittamore[2], and Pamela L. Paris[1,3]

1. Division of Hematology-Oncology, Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA.
2. Epic Sciences. San Diego, CA
3. Department of Urology, Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA.

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Background

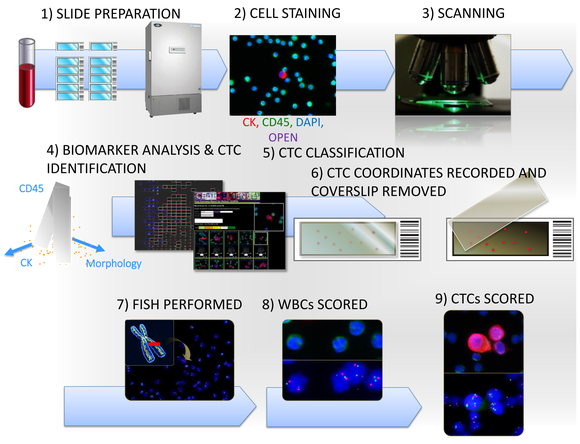
The prognosis for patients with muscle invasive (MIBC) and metastatic (mBCa) bladder cancer is poor, with median survival with cisplatin-based chemotherapy averaging 14 months in the metastatic setting. Urothelial bladder cancers have been found to express the markers programmed death-1 (PD-1) and programmed death ligand 1 (PD-L1) on their cells. Expression of PD-1 and PD-L1 on cancer cells is hypothesized to allow cancers to evade immune surveillance and eradication, and this understanding has provided the rationale for the development of PD-1 and PD-L1 checkpoint immunotherapy.

While programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) checkpoint inhibitors have activity in a significant proportion of patients with advanced bladder cancer, predictive and prognostic biomarkers are still lacking. While higher PD-1 or PD-L1 expression on tumor cells or tumor-infiltrating lymphocytes has been correlated with an increased likelihood of response, the positive and negative predictive value of these assays remains modest. Obtaining solid tumor tissue biopsy specimens involves an invasive, technically challenging procedure posing risks to the patient. Instead, circulating tumor cell (CTC) isolation and analysis from peripheral blood samples may provide a non-invasive approach to identify biomarkers and serially monitor treatment effect.

We have developed an assay for quantification of PD-L1 expression on circulating tumor cells (CTCs) detected on the Epic CTC Platform. Here, we present data on the incidence, morphology, and PD-L1 expression of CTCs detected from the venous circulation of 21 muscle invasive and metastatic bladder cancer naïve to anti-PD-1 axis therapy.

Methods

Blood from 21 muscle invasive and metastatic bladder cancer patients naive to anti-PD-1 axis therapy was sent to Epic Sciences and processed with the Epic CTC Platform 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 at -80C
2) Slides stained with CK, CD45, DAPI and a biomarker of interest (PD-L1)
3) Slides scanned
4) Multi-parametric digital pathology algorithms run
5) Software and human reader confirmation of CTCs & quantitation of biomarker expression
6) For FISH, coordinates are recorded and coverslip removed
7) FISH assay is performed
8) Regional WBCs are scored to assess normal
9) CTCs relocated and scored

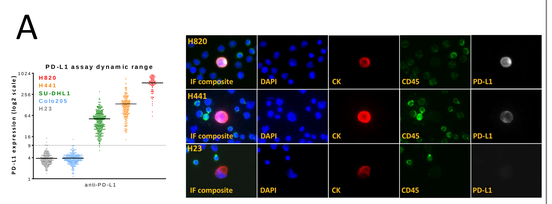
Study Population

Baseline Characteristics		
Median Age (range)	67 years (43 – 89)	
Gender	Male	14
	Female	7
Extent of Disease	MIBC	4
	Metastatic	17
Patients with prior chemotherapy	13	
Follow-up	Deceased	9
	Alive	6
	No follow-up	6
Median survival after CTC draw	108.5 days (40 – 414 days)	

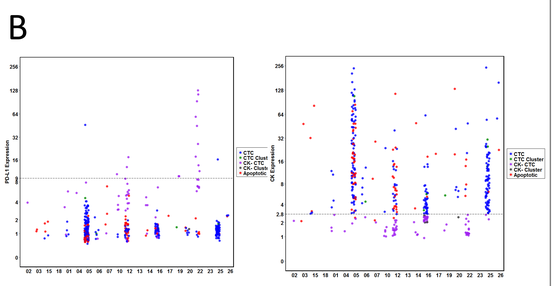
- Blood drawn from 21 patients between May 2014 – September 2015
- 14 men, 7 women
- 17 metastatic bladder cancer, 4 muscle-invasive
- 13 had prior chemotherapy
  - 10 had at least one cisplatin – based regimen
- 1 patient subsequently developed secondary malignancy (breast cancer)
- 1 patient received PD-1 therapy prior to draw

Bladder Cancer CTC Subtype Heterogeneity

A) 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.

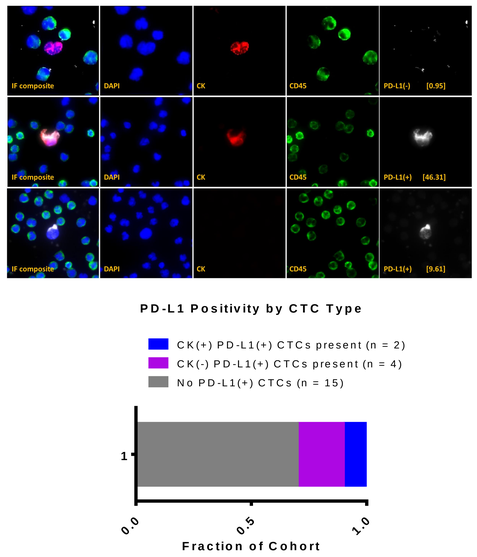


B) Dot plot of patient CTC PD-L1 (left) and CK (right) expression as assessed by protein IF for every CTC detected (2 slides stained per patient). Each dot indicates one CTC detected; the color indicates CTC sub-population membership. Thresholds for PD-L1 and CK assay positivity were determined using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

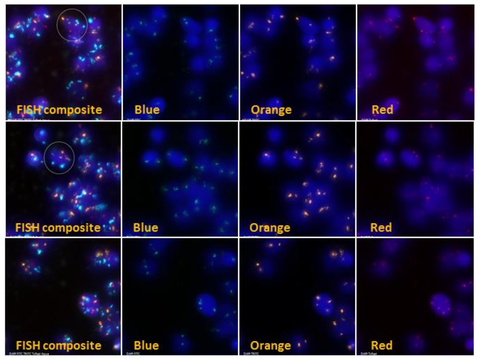


PD-L1+ Bladder Cancer CTCs are Frequently CK-

- CTCs are detected via:
  - Cell morphological parameters
  - Cytokeratin expression
  - Absence of CD45 (pan-leukocyte) staining
  - Cancer-specific marker
  - Combination of the above
- Both CK+ and CK- CTCs were detectable in a significant fraction of patients
- PD-L1 signal distinctly localized to cell surface, consistent with established PD-L1 cell biology.
  - Thirty two percent of patients had PD-L1+ CTCs (7/22)
  - Four of seven of these patients had CK-/PD-L1+ CTCs
- **Bottom:** A summary of patient PD-L1 biomarker status in study cohort. Patients were categorized as positive if a concentration of >1 PD-L1(+) CTC/mL was detected. Several PD-L1(+) patients had PD-L1 positivity exclusive to CK(-) CTCs



CK- PDL1+ CTCs Have Gross Genomic Abnormalities



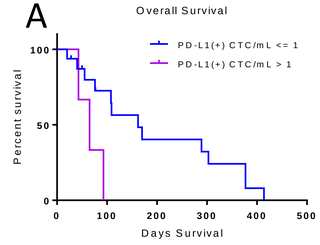
	# CTCs	%
CTCs w/ no abnormalities	65	95.5%
CTCs w/ at least 1 abnormality	3	4.5%
CTCs w/ all abnormalities	3	4.5%
Total CK(-)/PD-L1(+) CTCs scored	68	100%

CK+ PDL1+ CTCs were tested for abnormal ploidy via FISH. The presence of gross genomic abnormalities is a secondary confirmation of malignant origin; adjacent WBCs serve as controls.

FISH probes:
CEP10: green (seen in composite)
CEP7: orange
CEP3: blue
5p15: red

PD-L1 Expression on CTCs as Prognostic Indicator

A



B

High PD-L1(+) Median Survival (n = 3)	65 Days
Low PD-L1(+) Median Survival (n = 14)	162 Days
Logrank Test	p = 0.0302
Hazard Ratio (logrank)	3.46
95% CI of Hazard Ratio	1.39 to 79.4

(A) Survival curves of patients with high (n = 3) and low (n = 14) PD-L1(+) CTC burden (high burden >= 1 PD-L1(+) CTC/mL).

(B) Overall survival comparison of high and low PD-L1(+) CTC burden

Survival follow-up data was available for 16/21 patients in the study. Of the 16 patients, those with the highest burden of PD-L1(+) CTCs (> 1/mL, n = 3) were compared to the rest of the cohort (n = 14). High circulating PD-L1(+) CTC burden was associated with shorter overall survival (median 65 vs. 162 days) and a higher log-rank hazard ratio (3.46, p = 0.0302)

Discussion

- The Epic CTC Platform can detect PD-L1+ CTCs in patients with metastatic and locally advanced bladder cancer. Bladder cancer CTCs expressing PD-L1 are frequently CK-, and show gross genomic abnormalities consistent with malignant origin.
- While these patient samples represent a small, cross-sectional cohort rather than a prospective controlled trial, it is worth noting that those with the highest PD-L1(+) CTC burden (> 1/mL) had shorter overall survival from the time of the CTC draw, consistent with previous reports in bladder cancer and other solid tumors using solid tissue biopsy staining.
- Biomarkers predicting response to PD-1 axis inhibitors are needed to help select patients for therapy. Inclusion of patients onto current PD-1 axis therapy trials typically requires a biopsy, for which sampling can be difficult in bladder cancer and other indications.
- Future work will focus on the relationship between PD-L1 expression on CTCs and response to PD-1 and PD-L1 inhibitors in bladder cancer.