Background
The prognosis for patients with muscle invasive (MIBC) and metastatic (M0C) bladder cancer is poor, with median survival of 10-20 months despite systemic chemotherapy. While bladder cancer has been found to express the marker programmed death 1 (PD-L1) and programmed death 1 ligand 1 (PD-L1) on their cell surfaces. Expression of PD-L1 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-L1 and PD-L1 checkpoint immunotherapies.

While programmed death 1 (PD-L1) and programmed death 1 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-L1 or PD-L1 expression on tumor cells or tumor-infiltrating lymphocytes has been correlated with an increased likelihood of response to immunotherapy, 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 cells (CTC) isolation and analysis from peripheral blood samples provides a non-invasive approach to identify biomarkers and derive clinical information.

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

Methods
Blood from 21 muscle invasive and metastatic bladder cancer patients naive to anti-PD-L1 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 analysis workflow:
1. Nucleated cells from blood sample placed onto slides and stained at ASC.
2. Slides stained with CK, CD45, DAPI and a biomarker of interest (PD-L1).
3. Slides scanned.
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 WBs are scored to assess normal.
9. CTCs identified and scored.

Study Population

Baseline Characteristics

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<tr>
<td>Median Age (range)</td>
<td>62 yrs (43 - 89)</td>
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<td>Gender</td>
<td>Male 14, Female 7</td>
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<td>Extent of Disease</td>
<td>MIBC 4, Metastatic 17</td>
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<td>Patients with prior chemotherapy</td>
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Follow-up

<table>
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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-L1 therapy prior to draw

A) Left: A monoclonal antibody against PD-L1 was tested using high (H2820), medium (H441), low (SU-DHL1) and negative (Colo205) PD-L1 expressing cell lines to determine assay sensitivity and specificity. At the optimal antibody concentration, mean H2820 PD-L1 expression was determined to be 142-fold higher than mean background 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. 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).

B) 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 positively exclusive to CR+ CTCs.

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 shallower 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 on 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 PO-1 and PD-L1 inhibitors in bladder cancer.