Circulating Tumor Cells as Potential Biomarkers in Bladder Cancer

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Abbreviations and Acronyms

CTC = circulating tumor cell

MVAC = methotrexate, vinblastine, doxorubicin and cisplatin

NGS = next generation sequencing

pStage = pathological stage at cystectomy

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Study received University of Michigan and University of California-San Francisco institutional review board approval.

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Purpose: We explored the diagnostic use of circulating tumor cells in patients with neoadjuvant bladder cancer using enumeration and next generation sequencing.

Materials and Methods: A total of 20 patients with bladder cancer who were eligible for cisplatin based neoadjuvant chemotherapy were enrolled in an institutional review board approved study. Subjects underwent blood draws at baseline and after 1 cycle of chemotherapy. A total of 11 patients with metastatic bladder cancer and 13 healthy donors were analyzed for comparison. Samples were enriched for circulating tumor cells using the novel IsoFlux™ System microfluidic collection device. Circulating tumor cell counts were analyzed for repeatability and compared with Food and Drug Administration cleared circulating tumor cells. Circulating tumor cells were also analyzed for mutational status using next generation sequencing.

Results: Median circulating tumor cell counts were 13 at baseline and 5 at followup in the neoadjuvant group, 29 in the metastatic group and 2 in the healthy group. The concordance of circulating tumor cell levels, defined as low—fewer than 10, medium—11 to 30 and high—greater than 30, across replicate tubes was 100% in 15 preparations. In matched samples the IsoFlux test showed 10 or more circulating tumor cells in 4 of 9 samples (44%) while CellSearch® showed 0 of 9 (0%). At cystectomy 4 months after baseline all 3 patients (100%) with medium/high circulating tumor cell levels at baseline and followup had unfavorable pathological stage disease (T1-T4 or N+). Next generation sequencing analysis showed somatic variant detection in 4 of 8 patients using a targeted cancer panel. All 8 cases (100%) had a medium/high circulating tumor cell level with a circulating tumor cell fraction of greater than 5% purity.

Conclusions: This study demonstrates a potential role for circulating tumor cell assays in the management of bladder cancer. The IsoFlux method of circulating tumor cell detection shows increased sensitivity compared with CellSearch. A next generation sequencing assay is presented with sufficient sensitivity to detect genomic alterations in circulating tumor cells.

Key Words: urinary bladder neoplasms; neoplastic cells, circulating; neoadjuvant therapy; pathology, molecular; genomics

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Bladder cancer accounts for 15,500 deaths annually.[1] In the absence of metastases radical cystectomy offers the possibility of cure but with limited success.[2] Pathological stage, lymphovascular invasion and histological grade are prognostic factors for survival. pStage is associated with excellent 5-year disease-free survival (85% to 90%).[2–4]

Perioperative chemotherapy has been investigated to improve survival in bladder cancer. Adjuvant cisplatin based chemotherapy is only applicable to 20% of patients due to the high incidence of renal insufficiency and perioperative complications. Patients generally have better performance status and organ function before cystectomy, providing the rationale for neoadjuvant therapy. Neoadjuvant chemotherapy prior to cystectomy results in pathological down-staging at surgery with a 38% frequency of pT0 compared to 15% in control patients who did not receive neoadjuvant chemotherapy in a randomized phase III trial.[3] It also provides an overall survival benefit.[2,5–7] Unfortunately cisplatin based chemotherapy is only feasible in 50% of patients with bladder cancer due to inadequate renal, cardiac and neurological function.[8] Cisplatin also carries a risk of kidney damage and neuropathy. Overall survival with cisplatin based neoadjuvant chemotherapy is only 5% at 5 years. The critical unmet need in the management of muscle invasive bladder cancer is the identification of minimally invasive biomarkers to stratify patients who would benefit from neoadjuvant chemotherapy while sparing others needless toxicity.[9]

CTCs are detectable in most epithelial cancers and may enable early assessment for neoadjuvant chemotherapy in bladder cancer.[10–13] CTC counts predict progression-free and overall survival in metastatic breast cancer,[14] and overall survival in colon cancer and castration resistant prostate cancer.[15,16] CTCs were shown to be predictive biomarkers in patients with castration resistant prostate cancer treated with enzalutamide.[17,18]

The CellSearch CTC Test™ detects CTCs in nonmetastatic and metastatic bladder cancer.[19–22] CellSearch has low sensitivity for localized bladder cancer, demonstrating CTCs in only 17% to 23% of patients before cystectomy.[19,22] Nevertheless, the presence of CTCs preoperatively was an independent adverse prognostic factor for cancer-free survival in patients undergoing cystectomy. One recent study showed that CTCs remained an independent predictor of cancer specific mortality in patients treated with cystectomy without chemotherapy.[23]

The low sensitivity of CellSearch limits its usefulness for bladder cancer. The IsoFlux System has previously been shown to increase sensitivity for CTC detection and molecular profiling in prostate and colorectal cancer.[24] This platform uses immunomagnetic isolation of CTCs in a microfluidic environment to enhance CTC capture and minimize white blood cell carryover. The magnetic beads can be functionalized with multiple antibodies, although EpCAM alone was used in this study for a direct comparison with CellSearch. The resulting CTC samples are suitable for enumeration and NGS.

This study explored the potential role of CTCs in identifying patients who might be better served by moving directly to cystectomy to avoid additional chemotherapy.

MATERIALS AND METHODS

Study Aims

1) We explored the use of CTCs as a bladder cancer biomarker. 2) We compared assay performance of the IsoFlux System to that of CellSearch. 3) We tested the repeatability of the IsoFlux assay. 4) We established the feasibility of using CTCs for NGS (fig. 1).

Patient Selection

Two prospective pilot studies in a total of 30 patients with bladder cancer received institutional review board approval at University of Michigan and University of California-San Francisco. One cohort of 20 patients had locally advanced, resectable bladder cancer. These patients had blood collections before and during neoadjuvant chemotherapy. Eligible patients met certain criteria, including 1) histological evidence of urothelial carcinoma invading the muscularis propria, 2) no radiological evidence of metastases, 3) candidacy for cisplatin based chemotherapy and 4) candidacy for radical cystectomy with curative intent. Two patients were lost to followup. Of the patients 16 underwent pathological staging at cystectomy. A second cohort of 11 patients with metastasis was used for comparison and 13 healthy donor samples (AllCells®) were collected to serve as controls.

Sample Collection

Neoadjuvant patient blood samples were collected at baseline and after the first cycle of chemotherapy. The second blood draw was taken 3 or 4 weeks after baseline in those on the dose dense MVAC, MVAC or classic cisplatin-gemcitabine regimen. In the metastatic and healthy groups only 1 blood draw was taken. Samples were collected in 10 ml BD Vacutainer® ethylenediaminetetraacetic acid tubes and shipped overnight to Fluxion Biosciences for processing within 36 hours. Actual blood volume was 5 to 10 ml for IsoFlux enumeration tests. CellSearch CTC Test samples were collected in CellSearch CellSave tubes and processed at University of Michigan within 36 hours. All results were normalized to 7.5 ml.

CTC Enrichment and Enumeration

IsoFlux System samples were enriched using the IsoFlux CTC Enrichment Kit. CTCs were defined as morphologically intact, cytokeratin positive, CD45 negative and nucleated according to the IsoFlux CTC Enumeration Kit.