Editorial

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Circulating tumor cells in bladder cancer: a new horizon of liquid biopsy for precision medicine

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Clinical management of bladder cancer (BC) patients offers several challenges such as poor outcome because of elevated recurrence rates and lack of response to chemotherapy [1]. So, there is a need of noninvasive prognostic and predictive tools able to allow risk category assessment and real-time supervision of drug response [2]. Recently, circulating tumor cells (CTCs) have been proposed as prognostic tool able to improve cancer patients' clinical management [3–6]. CTCs detached from the primary tumor, enter the bloodstream and colonize distant organ, promoting cancer dissemination [7]. Emerging technologies are available to isolate CTC from patient's blood to provide a "liquid biopsy". Such a tool provides a molecular picture of the metastatic disease, useful to assess the cause of drug resistance onset [3, 6, 8–14]. CTC are very scarce in the blood, so robust methods are still needed for their routine use in laboratory practice [3, 11]. Several technologies have been developed in the last few years [11, 12] and several studies have been performed on the potential use of CTCs in bladder cancer patient clinical management.

CTCs, derived from both primary or metastatic tumors by passive shedding or dynamic stromal invasion, are considered responsible of disease dissemination. Once in the circulatory system, CTC survive to the sheer stress and escape to the immune system to reach distant organs. The microenvironment of the reached site will influence their state of quiescent or proliferation developing metastatic foci. Isolation from blood is challenging because CTC account for less than 0.004% of all mononucleated blood cells. For this reason, various methods of isolation and count assessment including immunoaffinity, biophysical and direct molecular approaches, have been developed to enrich CTCs from many millions of normal blood cells. CellSearch (Janssen Diagnostics, USA) has been the most used platform for CTC-based studies in BC. This is the only FDA-approved method for monitoring CTC in patients with metastatic breast, colorectal, and prostate cancer, but not yet for BC. This assay is based on the characteristic of CTC that are nucleated EpCAM+/CK+/CD45 − cells, different from healthy EpCAM-/CK-/CD45+ blood cells. This test is based on the positive selection of CTCs using antibodies against the epithelial cell adhesion molecule (EpCAM) antigen. Thus, blood samples are first incubated with ferroparticles coated in antiEpCAM antibodies. Then ferroparticle bound cells are then captured in a magnetic field, following the flow cytometry analysis of cells fluorescently labelled with antibodies against CK8, CK18, CK19, and CD45 and cytokeratins 8, 18, and 19. By using CellSearch assay, a CTC is defined as any nucleated cell that is positive for cytokeratin expression, but negative for CD45 typically expressed on leukocytes.

Currently, several studies have evaluated the CTCs by using CellSearch system, in nonmetastatic BC patients. Regarding the studies enrolling nonmetastatic patient's cohorts, eight were able to detect CTC in 17–30% of patients [15–20], whereas the remaining two were unable to detect CTC in the blood [21, 22]. Such low yields could be associated with the lack of sensitivity and specificity of the assay for diagnostic use. Several studies showed that CTCs were associated with staging, grading, and metastasis and CTC-positive patients have poorer progression-free, cancer-specific, and overall-survival compared to CTC-negative subjects [18, 23–26]. Worth of attention is that the detection of a single CTC was significantly associated with a decreased survival in patients with BC [27]. Moreover, some authors demonstrated the relevance of the molecular characterization of CTCs. Osman et al. reported that patients presenting *uroplakin/EGFR*-positive CTCs after radical cystectomy had a higher risk of recurrence [28]. Gudemann et al. found that the expression of CK20 on CTCs

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was significantly associated with staging and tumor burden [29]. Anantharaman et al. [30] showed that programed death-ligand 1 (PDL-1) expression was significantly associated with reduced overall survival. Predicting which individuals will respond to a specific treatment is still challenging for BC clinical management. Alvea et al. [31] collected blood samples from 20 patients with BC who were eligible for cisplatin based neoadjuvant chemotherapy. CTCs were detected by using IsoFlux method and compared to CellSearch, showing an improvement in the CTCs identification capacity. It was demonstrated that high (>30 CTCs) counts prior to neoadjuvant chemotherapy are associated with unfavorable tumor features at radical cystectomy. In the adjuvant chemotherapy (AC) setting a meta-analysis including 945 patients found an overall survival and disease-free survival benefit in patients with muscle-invasive BC (MIBC) receiving adjuvant cisplatin-based chemotherapy after radical cystectomy [32]. In a prospective study including 185 patients, Soave et al. investigated the potential role of the CTC status regarding decision making on AC administration in BC patients, following RC. In this study, the presence of CTC was associated with inferior outcomes in patients without administration of AC, but not in patients with administration of AC [19]. The authors concluded that CTC may be useful for decision-making pro or contra AC. The in SWOG S1314, a randomized phase II trial testing a gene panel derived by coexpression extrapolation (COXEN) [33], was initiated to evaluate prospectively the potential benefit of AC for localized MIBC. If successful, COXEN score derived from CTC RNA can predict treatment response to AC as RNA from bladder tumor biopsy.

Based on currently available data, the detection of circulating tumor cells in BC patients represents a promising noninvasive tool for prognostic assessment and to guide personalized treatment (Figure 1). Further studies are encouraged to better clarify the clinical impact of CTCs enumeration and molecular characterization in clinical practice.

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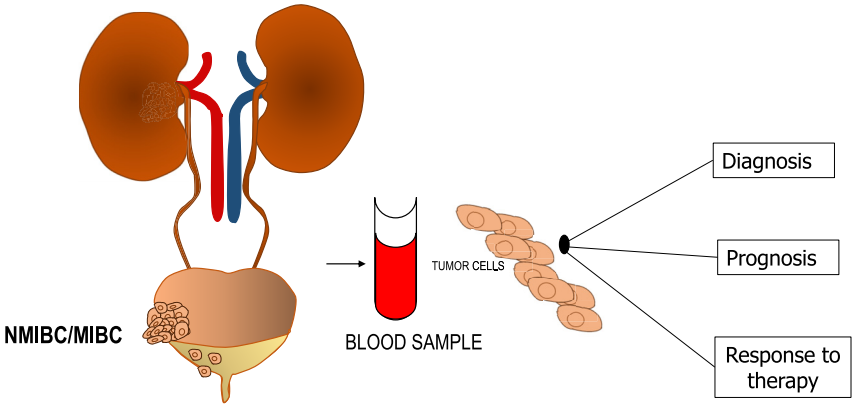

Figure 1: Potential of circulating tumor cells in bladder cancer patient clinical management.

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