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Circulating tumor cells detection has independent prognostic impact in high-risk non-muscle invasive bladder cancer

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High-risk non-muscle invasive bladder cancer (NMIBC) progresses to metastatic disease in 10–15% of cases, suggesting that micrometastases may be present at first diagnosis. The prediction of risks of progression relies upon EORTC scoring systems, based on clinical and pathological parameters, which do not accurately identify which patients will progress. Aim of the study was to investigate whether the presence of CTC may improve prognostication in a large population of patients with Stage I bladder cancer who were all candidate to conservative surgery. A prospective single center trial was designed to correlate the presence of CTC to local recurrence and progression of disease in high-risk T1G3 bladder cancer. One hundred two patients were found eligible, all candidate to transurethral resection of the tumor followed by endovesical adjuvant immunotherapy with BCG. Median follow-up was 24.3 months (minimum–maximum: 4–36). The FDA-approved CellSearch System was used to enumerate CTC. Kaplan–Meier methods, log-rank test and multivariable Cox proportional hazard analysis was applied to establish the association of circulating tumor cells with time to first recurrence (TFR) and progression-free survival. CTC were detected in 20% of patients and predicted both decreased TFR (log-rank $p < 0.001$; multivariable adjusted hazard ratio [HR] 2.92 [95% confidence interval: 1.38–6.18], $p = 0.005$), and time to progression (log-rank $p < 0.001$; HR 7.17 [1.89–27.21], $p = 0.004$). The present findings provide evidence that CTC analyses can identify patients with Stage I bladder cancer who have already a systemic disease at diagnosis and might, therefore, potentially benefit from systemic treatment.

High-risk, non-muscle invasive bladder cancer (NMIBC) is defined as any transitional cell carcinoma (TCC) of the bladder that is high-grade, whether it is primary or recurrent. This high-risk group includes patients with high-grade papillary Stage Ta or T1 tumors and any patient with carcinoma *in situ* (CIS).[1] Following transurethral resection bladder (TURB) of the initial tumor with no additional therapy, there is a recurrence rate of 50–70% and a progression rate of 25–50%, leading to cancer death after bladder-sparing treatment within 5 years in about 16–23% of cases.[2] To date, the European Organization for Research and Treatment of Cancer (EORTC) risk tables is the best-established predictive tools to help decision making for patients with NMIBC. These tables group patients by risk (low, intermediate and high) with the sums of factors (number of tumors, tumor size, prior recurrence rate, T category, presence of CIS and grade) to enable easy prediction of the recurrence and progression of NMIBC.[3] CUETO tables were developed with a similar structure to the EORTC tables but were adapted for patients treated with Bacillus Calmette Guerin (BCG).[4]

Nevertheless, recent studies underline that both EORTC and CUETO risk tables have a low positive predictive value (PPV), thus not able to identify patients with higher risk to progress.[5]

Circulating tumor cells (CTCs) are associated to short survival in patients with some type of metastatic carcinomas. The milestone study by Cristofanilli *et al.* demonstrated that women affected by metastatic breast cancer (mBC) with five or more CTCs per 7.5 ml of peripheral blood before therapy have a statistically shorter overall survival (OS) and

Key words: circulating tumor cells, non-muscle invasive bladder cancer, disease progression, Kuhn's paradigm shift

Abbreviations: AJCC: American Joint Committee on Cancer; BCG: Bacillus Calmette Guerin; CTCs: circulating tumor cells; EORTC: European Organization for Research and Treatment of Cancer; FDA: Food and Drugs Administration; NMIBC: non-muscle invasive bladder cancer; TFR: time to first recurrence; TTP: time to progression; TURB: transurethral resection bladder

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What's new?
What if a cancer could reveal early on whether it was headed for metastasis? These authors tried to find out whether circulating tumor cells in bladder cancer patients could predict whether the cancer would recur and spread. They found circulating tumor cells in 20% of patients with stage I bladder cancer, and the presence of these cells predicted shorter time to recurrence and time to progression, suggesting that testing for CTCs could identify patients who would benefit from systemic treatment right from the start.

progression-free survival (PFS) than patients with less than five CTCs per 7.5 ml of blood.[6] Similar results were obtained in studies with patients with metastatic castrate-resistant prostate cancer (mCRPC) and metastatic colorectal cancer.[7] The prognostic significance of CTCs in non-metastatic tumors is currently under investigation. The interim results from SUCCESS trial, the largest clinical trial investigating the role of CTC in early breast cancer, demonstrate that CTC are significantly predictive of recurrence and death.[8] There is broad agreement that CTC as prognostic marker may be more beneficial in patients with early-staged cancers, particularly in those tumor types characterized by high recurrence rate and lack of prognostic markers. To this purpose, high-risk bladder cancer is an optimal model. Specifically, no biological marker exists, which may allow the prediction of cancer behavior and guide the decision-making process between conservative and aggressive treatment option. We recently demonstrated in a small group of patients with primary diagnosis of NMIBC that the presence of at least 1 CTC/7.5 ml was associated to a statistically significant shorter time to first recurrence (TFR).[9]

Aim of the study was to investigate whether the presence of CTC may improve prognostication in a large population of Stage I bladder cancer patients who were all candidate to conservative surgery. Thus, this cohort of patients allowed us to study the influence of CTC on the natural course of "early" bladder cancer.

Patients and Methods

Patients

The present study is a prospective single-center trial investigating the prognostic significance of CTC in 102 patients with primary diagnosis of high-risk NMIBC (T1G3) candidate to transurethral resection of the tumor followed by endovesical adjuvant immunotherapy with BCG.

The study was conducted after receiving protocol's approval from our institutional board committee and informed consent from each patient. One hundred thirty (130) patients were recruited. According to the population selection, all tumors were characterized by invasion of the sub-epithelial connective tissue (T1) and high grade (G3). All tumor samples were centrally reviewed for stage and grade. Blood draws were carried out in all patients at the first diagnosis, before performing TURB. Four weeks after the first TURB, all patients underwent RE-TURB with resection of the previous primary tumor site and collection of biopsy specimens from the suspect areas; in all patients the RE-TURB excluded the presence of residual or muscle invasive disease. Furthermore, our pathologists confirmed the presence of muscolaris mucosae in the resected tissue (always free of cancer infiltration). Adjuvant BCG immunotherapy scheme was started 2 weeks after the second TURB starting with induction and following with maintenance (instillation 1 weekly, for 6 weeks, followed by 3 years of maintenance instillation with 3 weekly instillation every 3 or 6 months). A strict follow-up program was established and applied to each patient with cystoscopy and urinary cytology every 3 months and a urological computed tomography every 12 months. Cystoscopy was carried out using a D-light system. All tumors and suspicious areas identified have been resected or biopsied.

Procedure of CTC count

About 7.5 ml of blood were drawn from each patient before performing TURB. Blood samples were drawn into evacuated blood draw tubes (CellSave, Veridex, Raritan, NJ), maintained at room temperature and processed within 96 hr of collection. CellSearch system (Veridex LLC, Raritan, NJ) was used for CTC enumeration. Technical details of the CellSearch system have been previously described.[10]

Statistical analysis

According to international guidelines, recurrence was defined as any tumor on biopsy or positive urine cytology during follow-up examinations; progression was defined as an increase in T stage to T2 or greater or lymph node (N+) disease or distant metastasis (M1).[1]

The primary objective was to assess the prognostic value of CTC in T1G3 patients in terms of TFR, time to progression (TTP) and occurrence of distant metastases. TFR was defined as the interval from the date of the CTC sample to the date of first local recurrence; TTP was determined from the date of CTC sample to the date of progression to muscle invasive disease or metastatic disease. Metastasis free interval (MFI) was defined as the time from the date of CTC sample to the date of occurrence of clinically evident distant metastases. Bivariate analyses were based on chi-squared and Fisher exact tests for categorical variables, and Student *t* and ANOVA tests for continuous variables. Survival curves were estimated using the Kaplan–Meier method and were

Table 1. Correlation between CTC status and outcome of patients

	CTC negative	CTC positive	Total	p^1
N	82	20	102	1.0
Local recurrence	26 (31.7%)	17 (85.0%)	43 (42.2%)	<0.001
Progression to muscle invasive disease	5 (6.1%)	16 (80.0%)	21 (20.6%)	<0.001
Distant metastases	0	6 (30.0%)	6 (5.9)	<0.001
Mean (95% CI) time to local recurrence (months)	19.2 (16.0–22.4)	6.3 (4.8–7.8)	16.4 (13.6–19.2)	<0.001
Mean (95% CI) time to progression (months)	28.7 (26.7–30.7)	12.9 (9.8–16.0)	23.6 (21.0–26.2)	<0.001
Mean (95% CI) time to MFI (months)	–	20.3 (17.2–23.4)	29.0 (27.5–30.6)	–

[1]At Student *t*, chi-squared, Fisher exact or log-rank test.

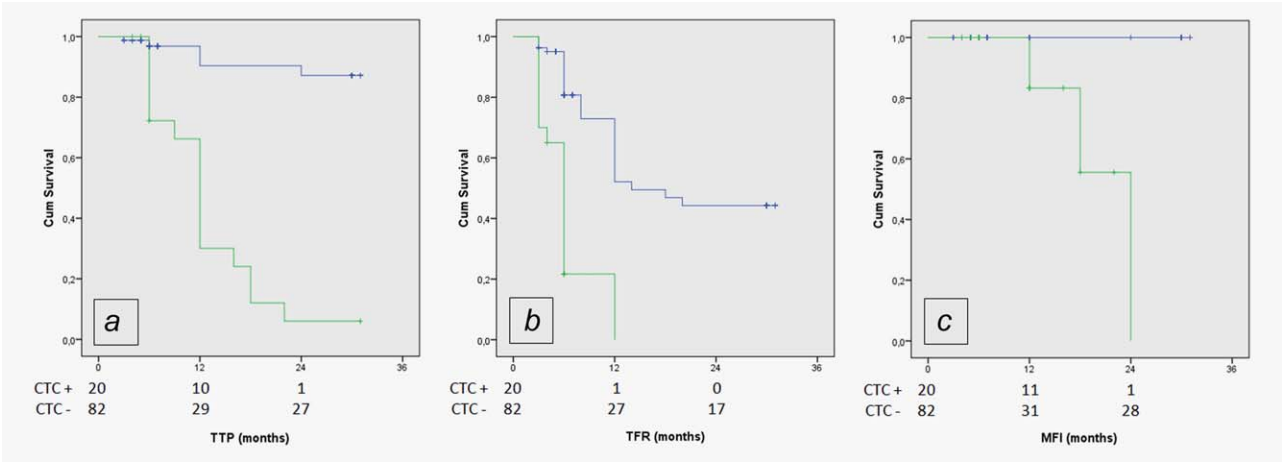

Figure 1. Panel (*a*): Kaplan–Meier curves of TTP of 102 patients with T1G3 bladder cancer according to the presence or absence of CTCs ($p < 0.001$ at log-rank test). Mean TTP was, respectively, 12.9 months (9.6–17.2) and 28.7 months (26.7–30.7). Panel (*b*): Kaplan–Meier curves of TFR of 102 patients with T1G3 bladder cancer according to the presence or absence of CTCs ($p < 0.001$ at log-rank test). Mean TFR was, respectively, 6.3 months (95% confidence interval 4.7–8.0) and 19.2 months (16.0–22.4). Panel (*c*): Kaplan–Meier curves of MFI of 102 patients with T1G3 bladder cancer according to the presence or absence of CTCs ($p < 0.001$ at log-rank test). Mean MFI was, respectively, 12.9 months (9.6–17.2) and 28.7 months (26.7–30.7). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

compared using log-rank tests. Multivariable Cox proportional hazard models were built using the presence of CTC, CIS, lymph vascular invasion, tumor size, age, gender and multiple lesions as covariates for TFR, TTP and distant metastases. Statistical analysis was performed with SPSS 20 (IBM, Armonk, NY). Statistical significance was set at the two-tailed 0.05 level, with *p* values unadjusted for multiplicity reported throughout. No attempt to define a pre-planned statistical power was performed.

Results

Patients

Within the enrolled population, 28 patients were excluded for inadequate samples or BCG intolerance. The 102 eligible patients were followed for a median follow-up period of 24.3 months (range 4–36). During the follow-up time, 43 patients (42%) had local recurrence, 21 patients (20%) experienced progression to muscle invasive disease, of whom 6 (29%) also progressed to metastatic disease (lymph node and bone metastases for all). Mean time to recurrence, mean TTP and mean MFI are shown in Table 1.

CTC detection rate at baseline and prognostic value

Before TURB, CTC were found in 20/102 (20%) patients. Median CTC number was 1 (range: 1–50). Among CTC positive patients, 16 (80%) had one CTC per 7.5·ml of blood, and 4 (20%) had more than one CTC per 7.5 ml of blood. CTC positive status was significantly associated with female gender (4 [20%] *vs.* 3 [4%] in CTC negative, $p = 0.026$), tumor size >3 cm (9 [45%] *vs.* 17 [21%], $p = 0.023$), CIS (12 [60%] *vs.* 9 [11%], $p < 0.001$), multifocality (20 [100%] *vs.* 58 [71%], $p = 0.003$), lymph vascular invasion (LVI) (10 [50%] *vs.* 6 [7%], $p < 0.001$) and appearance of distant metastases (6 [30%] *vs.* 0 [0%], $p < 0.001$. Identification of CTC through CellSearch strongly predicted progression, with a PPV of 75% and negative predictive value (NPV) of 93%.

At bivariate analysis, TFR (Fig. 1, Panel *a*), TTP (Fig. 1, Panel *b*) and MFI (Fig. 1, Panel *c*) were shorter in the group

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Table 2. Multivariable-adjusted Cox proportional hazard analysis exploiting a backward stepwise selection method

	Hazard ratio	95% confidence interval	*p*
Local recurrence			
Age	1.051	1.010–1.094	0.014
Female gender	–	–	0.377
Tumor size	–	–	0.646
CIS	–	–	0.596
LVI	0.355	0.165–0.764	0.008
CTC	2.922	1.382–6.175	0.005
Progression to muscle invasive disease			
Age	–	–	0.741
Female gender	–	–	0.241
Tumor size	0.341	0.131–0.888	0.028
CIS	0.369	0.117–1.166	0.090
LVI	–	–	0.804
CTC	7.169	1.889–27.210	0.004
Distant metastasis			
Age	0.875	0.763–0.998	0.047
Female gender	–		0.121
Tumor size	–		0.745
CIS	–		0.663
LVI	–		0.234

that had at least one or more CTC than in patients with no CTC ($p < 0.001$ for all).

At the multivariable Cox proportional hazards regression analysis, CTC positive status was found independent prognostic factor for TFR (hazard ratio [HR] 2.92 [95% confidence interval 1.38–6.18], $p = 0.005$) and TTP (HR 7.17 [1.89–27.21], $p = 0.004$). CTC presence was found the strongest independent predictor of disease progression to muscle invasive disease (Table 2).

Discussion

Our findings show that in a large and homogeneous population of patients with high-risk NMIBC, the presence of CTC is a strong predictor of progression, defined as an increase in T stage to T2 or greater or lymph node (N+) disease or distant metastasis (M1), according to international guidelines. Although any definitive conclusion can be drawn for the impact of CTC on distant metastases, since only six patients had progression to Stage IV disease, at the bivariate analysis CTC positive status was found significantly associated to the appearance of bone metastases and a shorter MFI was found in CTC positive compared to negative patients. To date in NMIBC, no marker can differentiate between indolent and aggressive tumors on individual level and the low PPV of the currently used risk scores makes them unsatisfactory to select patients at high risk to progress.[5] Although the identification of new molecular markers is desirable, to be translated into clinical practice the most crucial question is whether they may increase the predictive accuracy of classical prognostic models, thus improving the identification of true positive patients. The identification of micrometastatic disease in Stage I bladder tumors may suggest that the AJCC category cM0(i+), recently included for breast cancer staging as "no clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow or other non-regional nodal tissue that are $\leq$0.2 mm in a patient without symptoms or signs of metastases,"[11] may be suitable to be included in bladder cancer staging as well.

In bladder cancer, preliminary studies have suggested the potential prognostic value of CTC in patients with localized as well as metastatic disease.[12–14] According to these studies, the percentage of CTC positivity in patients with non-metastatic bladder cancer ranges from 18 to 30%. Although these studies were all limited by the small sample size and the high heterogeneity of patients population (stage of disease, grading, treatment), they agree that CTC in bladder cancer can be observed frequently enough to merit additional study. In 2012, the group by Rink *et al.* published a prospective study aimed to investigate the biologic and clinical significance of CTC in patients with clinically non-metastatic bladder cancer using the CellSearch. Authors found CTC in 23% of patients and demonstrated that the presence of even a single CTC conferred a worse prognosis in terms of disease recurrence, cancer-specific and overall mortality.[15] Our group recently reported a 18% detection rate of CTC in a small population of NMIBC (Ta, T1) patients.[9] In this report, the presence of $\geq$1 CTC/7.5 ml was found associated to a higher risk for local recurrence of disease. This seems not surprising since it has been recently demonstrated that CTC may recirculate from a distant site back to the primary site to give rise to recurrent tumors.[16] Evolving from these preliminary findings, we investigated whether CTC presence might predict a higher risk for disease progression in patients with NMIBC. At this purpose, a homogeneous population for T category, tumor grade and treatment was selected. We here report a 20% rate of CTC detection at baseline, consistently with data reported by Rink *et al.*, and a comparable prognostic significance of at least one CTC in terms of higher risk of disease progression. Nevertheless, the early clinical setting in which our analysis was conducted considerably differentiates our conclusions from those reached by Rink *et al.* in terms of clinical impact. Indeed, advances in bladder cancer control will be greatly aided by early detection of occult metastases, thereby facilitating treatment decision process in a pre-invasive state of disease. Our data confirmed that the $\geq$ 1 CTC/7.5 ml threshold has an independent prognostic value in high-risk NMIBC, consistently with previously published data in early setting of cancer.[8,17,18]

Although several studies have demonstrated the prognostic and predictive potential of CTC, to date their evaluation still does not guide changes in the treatment strategy.[19]To achieve an impact on the decision-making process, it is essential to demonstrate that CTC determination might answer to key clinical questions. In the metastatic setting, recently reported data from the SWOG S0500 trial failed to demonstrate the clinical utility of counting CTC to evaluate the effectiveness of frontline chemotherapy in patients with mBC.[20] Nevertheless, conclusive results from further ongoing interventional trials are awaited. Similarly, in non-metastatic setting, the role of CTC for prognostic stratification of patients is largely demonstrated, although data are still not mature to consider CTC as a reliable marker for treatment decision making. The scientific progress does not evolve in a linear manner, but with shifts between paradigms.[21] This paradigm shift theory was recently applied by Bajorin and Herr to muscle invasive bladder cancer in an Editorial published in *Journal of Clinical Oncology*,[22] starting from the reflection that the death rate in muscle invasive tumors after surgery alone is too high and a systemic approach is required. Translating this theory into the clinical practice, data from a recent large meta-analysis provide evidence of survival benefit in patients with muscle invasive bladder cancer receiving adjuvant chemotherapy after radical cystectomy.[23] Once confirmed in a larger cohort of patients, the prognostic value of at least 1 CTC in patients with high-risk NMIBC might indicate the need for a better stratification of patients for systemic therapy.

The prognostic significance of CTC presence in our series of patients is strong evidence that some patients suffering from NMIBC have already a systemic disease at diagnosis and are in need of a systemic treatment aimed to eradicate systemic tumor cell spread.

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