Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma. Justin Friedlander MD; Smith Institute for Urology, North Shore-Long Island Jewish Health System

Introduction

In 2010 alone, there were an estimated 70,530 new cases of bladder cancer diagnosed in the United States, with an estimated 14,680 dying from the disease[1]. Of the patients diagnosed with muscle invasive disease, 50% develop metastasis within 2 years and subsequently die of the disease[2]. Chemotherapy and surgical interventions are used to treat to patients with urothelial carcinoma (UC), however, there are no serum biomarkers for monitoring patients after treatment. Currently, patients are followed by imaging studies and cytopathological analysis of urine samples.

In reference to renal cell carcinoma (RCC) in the United States, there are approximately 32,000 new cases reported every year, with about 13,000 deaths annually[1]. While the majority of new cases of renal tumors are found as incidental radiographic findings[3], by the time a patient presents with symptoms of renal cancer such as hematuria, flank pain, and a palpable abdominal mass, they are often beyond a curative stage. Treatment options for renal masses consist of surgical removal of the lesion, percutaneous radiofrequency ablation, and, cryotherapy.

The presence of circulating tumor cells (CTCs) has been discussed in literature since 1869[4]. Over the past few decades, attempts have been made to isolate these rare cells largely with reverse transcriptase polymerase chain reaction (RT-PCR) assays[5]. The complexity and inconsistencies using this method resulted in difficulties for inter-laboratory comparisons of results[6]. Recent technological advances have made detection of CTCs feasible and consistently reproducible.

The CellSearch™ System (CSS) (Veridex, Inc.) is an automated system designed to identify and separate CTCs using multiple parameters. This has been extensively studied in metastatic breast cancer, and has been shown to be of prognostic value[7,8]. With regards to metastatic disease from all carcinomas, another group found the CSS to be an accurate and reproducible assay when comparing healthy donors to patients with metastatic disease[9].

More recently, CTCs have been investigated in the setting of advanced prostate cancer. Studies have shown that CTCs are present in a majority of patients with hormone-refractory prostate cancer and the number of CTCs correlates with age, serum PSA level and serum alkaline phosphatase. Furthermore, patients with lower levels of CTCs (less than or equal to 1.8 CTCs per mL) had significantly longer survival time than those with higher levels of CTCs ($p=0.02$)[10]. Other studies in prostate models are working to categorize molecular and genetic profiling of the CTCs found in patients using cell

cytology, FISH, and various markers with the hopes of developing molecular targeted therapy in the future[11].

There have been promising reports documenting the presence of CTCs found in patients with UC. The study led by Naoe found that it was possible to identify UC tumor cells in peripheral blood samples of patients with UC using the CSS assay. This study also found that there was a significant difference in the number of CTCs between those UC patients with non-metastatic disease and those with metastatic disease (0 vs. 9.2 respectively, $p=0.0004$)[12]. A study just recently published by Rink et al again used the CSS to detect and evaluate the biological significance of CTCs in patients with non-metastatic, advanced urothelial cancer. This group showed that CTC positive patients showed significantly worse overall survival, progression-free survival, and cancer-specific survival as compared to pre-operatively CTC-negative patients[13].

In regards to RCC, preliminary findings of CTC detection have been reported in Germany. Although this group did not specifically utilize the CellSearch system, they employed similar semi-automated immunomagnetic enrichment procedure termed Magnetic Activated Cell Sorting (MACS)[14]. In their study, 80 of 214 patients with renal cell carcinoma were found to have CTCs and more importantly, 62% of these patients subsequently developed progressive disease.

With emerging new technology, the study of CTCs in different cancer models will hopefully provide the medical community with crucial information as a therapeutic and prognostic biomarker. The purpose of this study is to determine the feasibility of using the CSS to detect CTCs in urothelial and renal cell carcinoma models. Secondary aims looked at a possible correlation of the number of CTCs with pathologic stage/grade in patients undergoing surgical intervention (radical cystectomy, radical nephrectomy, nephroureterectomy, etc.) for UC or RCC, correlation of the number of CTCs with chemotherapeutic response in patients with metastatic UC or metastatic RCC, and correlation of the number of CTCs associated with progression-free and overall survival in patients with advanced UC or RCC.

Materials and Methods

Over the period from June 2008 to January 2009 we enrolled and prospectively collected data for 26 patients presenting to our institution with newly diagnosed muscle invasive or metastatic UC and advanced RCC. Inclusion criteria included diagnosis with either urothelial carcinoma or renal cell carcinoma, clinical stage T2 disease or greater. Patients under the age of 18 years, or who had received previous chemotherapeutic or definitive surgical treatment for RCC or UC were excluded from this study. Patients who had only undergone prior cystoscopic transurethral resection of bladder tumor but not definitive therapy remained eligible.

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

The CellSearch System (Veridex, Inc.) is an automated system designed to identify and separate CTCs using multiple parameters. The system consists of a semiautomated sample preparation system and the CellSearch Epithelial Cell kit which immunomagnetically enriches cells expressing epithelial cell adhesion molecule (EpCAM). EpCAM is glycoprotein over expressed on most carcinomas, especially high grade and advanced UC[15]. It has only been verified in cells of epithelial origin[16], and hence it can be used as a marker for carcinoma cells[17].

The isolated cells are then fluorescently labeled with nucleic acid dye 4',6-diamidino-2-phenylindole (DAPI) and labeled monoclonal antibodies specific for leukocytes (CD45-APC) and epithelial cells (CK-PE). A semiautomated fluorescence microscopy system helps to reconstruct computer generated cellular images. CTCs are defined as nucleated cells negative for CD45 expression and positive for cytokeratin expression[9].

After determination of eligibility, and prior to initiation of chemotherapy or surgery, baseline 3 blood samples (7.5 ml each) were drawn. Between 6 and 12 weeks following treatment, chemotherapy or surgery, an additional blood sample (40 ml) was collected. Interim analysis was performed to ascertain correlation between number of circulating tumor cells before and after intervention and pathologic stage/grade. Institutional review board approval was obtained for this study protocol. All patients provided written informed consent prior to blood sampling.

Using the automated system (CellTracks® AutoPrep System, Veridex) as described above, epithelial cells were isolated from the blood samples and further characterized with antibodies tagged with a fluorescent dye. Isolated cells were distinguished and counted using the CellTracks® Analyzer II, a semi-automated fluorescence microscope. Two independent investigators reviewed CSS results, and results were blinded to patients.

Statistical analysis was carried out using SAS Software. Descriptive statistics were performed to evaluate the number of CTCs present in patients and the feasibility of using the CSS. Spearman correlation coefficients were computed to determine the degree of correlation between the number of CTCs with pathologic outcome, as well as the degree of correlation between the number of CTCs with chemotherapy response in tumor reduction. Among the patients with advanced UC or RCC, Kaplan-Meier survival analyses were used to analyze time-to survival metrics. Differences among groups were assessed using the pairwise log-rank test. $P<0.05$ was considered statistically significant.

Results

The baseline demographics of the entire study cohort can be seen in Table 1. Twenty-six patients were enrolled in the study, with a mean age of 63.7 years (range 44-90 years) for patients with RCC and a mean age of 70.5 years (range 46-88 years) for patients with

UC. The overall mean age of the entire study cohort was 68.9 years. Eleven of the patients had RCC, and 15 patients had UC. Patients were followed for a median of 16.8 months (mean 13.1 months) after intervention.

Of the patients with RCC, 4 were female and 7 were male. All eleven patients with RCC underwent radical nephrectomy subsequent to their diagnosis, and none of the patients received chemotherapy as the primary intervention. Staging of RCC was based on the American Joint Committee on Cancer (AJCC) system. The following breakdown was seen in patients with RCC: 1 patient with stage 0 disease, 4 patients had stage 2 disease, 2 patients had stage 3 disease, and 4 patients had stage 4 disease. Stage 0 indicated no tumor found on the pathological specimen.

Of the patients with UC, 4 were female and 11 were male. For primary intervention, four of the patients underwent radical nephroureterectomy, 8 of the patients underwent radical cystoprostatectomy, and 3 of the patients underwent chemotherapy as primary therapy. Staging of UC was based on the AJCC system. The following breakdown existed for patients with UC: one patient with stage 0 disease, 3 patients had stage 2 disease, 8 patients has stage 3 disease, and 3 patients with stage 4 disease.

As demonstrated in Table 2, CTCs were detectable in 88% (23/26) of patients of the combined cohort. Further breakdown demonstrated at least one CTC was detectable in 91% (10/11) patients with RCC and 87% (13/15) of patients with UC. When comparing those with non-organ confined to organ confined disease, 89% (8/9) patients with non-organ confined disease had at least 1 CTC, whereas 88% (15/17) of patients with organ confined disease has at least 1 CTC. Greater than 1 CTC per 7.5 ml blood sample was found in 50% of the combined cohort, 36% of the patients with RCC, and 60% of patients with UC. Greater than 2 CTCs per 7.5 ml blood sample was found in 27% of patients in the combined cohort, 18% of patients with RCC, and 33% of patients with UC. In terms of a maximum of > 2 CTC per 7.5 blood, this was seen in 38% of the combined cohort, 36% of patients with RCC, and 40% of patients with UC. No significant difference was found in the presence of CTCs between RCC and UC ($z = -0.6$).

Table 3 shows the average and maximum CTCs with 95% confidence intervals, broken down by stage, for RCC and UC. Analysis revealed no significant correlation between clinical stage ($p = 0.13$) or pathologic stage ($p = 0.2$). Figure 1, and Table 4 show the average CTC vs AJCC staging for RCC and UC, respectively. Although not statistically significant, patients with higher stage disease tended to have more CTC per 7.5 ml of blood.

No statistically significant difference was seen in progression-free survival for average CTCs <1 versus ≥ 1 for the combined cohort ($p=0.06$), RCC only ($p=0.06$), or UC only ($p=0.35$). Also no statistically significant difference was seen in cancer-free survival for average CTCs <1 versus ≥ 1 for the combined cohort ($p=0.9$), RCC only ($p=0.86$), or UC only ($p=0.08$). For overall survival, there was a statistically significant difference seen

for average CTCs <1 versus ≥ 1 for the combined cohort (p=0.01). This was not demonstrated for RCC only (p=0.2), just in UC (p=0.02). Figures 2,3, and 4 show the Kaplan-Meier plots for overall survival.

Discussion

Detection of CTCs has been demonstrated for patients with other cancers, such as breast, colorectal, and prostate cancer[7,11,18]. Specifically for breast cancer, one study reported that CTC burden may be superior, if not at least beneficially supplemental, to current imaging methods for metastatic disease[8]. Additionally, other studies have shown that the presence of CTCs was associated with disease progression and shorter overall survival[7,19]. For prostate cancer, the current studies show that higher CTC counts correlate poorly with survival[11,21].

At present there is no standard serum biomarker to monitor disease progression in RCC or UC. After surgical intervention or chemotherapy patients are followed with serial imaging studies, and adjuvant therapies are based on positive findings. Unfortunately imaging studies are not sufficient to detect the presence of micrometastatic disease, whereas a biomarker might be able to play this role. CTCs possibly represent not only a diagnostic modality, but also a dependable method to follow patients after treatment as well as predictive of outcomes in patients with both RCC and UC.

Prior CTC detection methods such as PCR-based protocols are not without limitation and controversy[6,20]. The CellSearch System (Veridex, Inc.) is designed to detect tumor cells in whole blood using immunomagnetic enrichment and immunocytochemical analysis[18]. This is an automated, standardized system that allows for reproducible quantitative results that are comparable between laboratories[9]. The system detects tumor cells on the basis of monoclonal antibodies against EpCAM and cytokeratins in order to classify cells as epithelial in origin[9].

Previous studies, such as the one by Brunner et al, demonstrated that UC expresses high levels of EpCAM, especially in high-grade disease[15]. Along this line, Naoe et al found that there was a significant difference in the number of CTCs found in patients with metastatic UC versus those without metastatic disease[12].

Using the CSS, we were able to demonstrate that detection of CTCs is feasible in patients with RCC and UC. We successfully detected CTCs in 88% (23/26) of patients in the combined cohort, 91% (10/11) of patients with RCC, and 87% (13/15) of patients with UC. Previously, Guzzo et al were able to detect CTCs in 9 of 43 patients (21%) prior to radical cystectomy[22]. Other studies have been able to describe CTCs in 45-100% of patients with metastatic UC[12,13,23]. Our study found CTCs in 91% (10/11) of patients with metastatic UC, which affirms that this technique is a reliable marker in this setting.

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Previous work has identified a pre-treatment threshold of > 2-5 CTCs per 7.5 ml blood as identified with worse prognosis and tumor progression[7,24,25]. Specifically in UC, there exists one case report that suggests that an increase in CTCs might indicate disease progression with ≥ 5 CTC[26]. We used a threshold of ≥ 1 CTC per 7.5 ml blood as positive for CTCs based on the work of Tibbe et al, who showed that for patients with metastatic disease, elimination of errors caused by variability between readers of CTC results might reduce the current threshold to 1 CTC per 7.5 ml blood[27]. By using two independent readers of CTC results we feel that this threshold is appropriate for our study population.

Unlike other studies[13,18,24], in our study an increasing CTC burden was not associated with higher stage disease, likely due to the small sample size. That patients with higher stage disease did tend to have more CTC per 7.5 ml of blood, even though not statistically significant, does suggest that patients with higher tumor burdens will have greater numbers of CTCs in the systemic circulation. Whether absolute number of CTC correlates with poorer outcomes is still an unanswered question.

When follow up data were analyzed, no significant differences were seen in progression-free survival or cancer-specific survival, for patients with and without CTCs, for the combined cohort, RCC only, or UC only. For overall survival, there was a significant difference seen in patients with and without CTCs, for the combined cohort (p=0.01). This held in patients with UC (p=0.02), but not RCC (p=0.2).

The study by Rink et al observed significant differences in overall (p=0.001) and progression-free survival (p<0.001) when comparing patients with and without proof of CTC in patients with non-metastatic bladder cancer[13]. This group also demonstrated that cancer-specific survival (p-0.003) was significantly worse in patients with UC without metastatic disease and proof of CTCs. It is the largest study to date (50 patients) on that subset of UC patients, and the findings suggest that the presence of CTCs may be predictive of early systemic disease. The majority of patients in our study had high-risk UC or advanced disease, and had detectable CTCs. These two findings are in agreement, and when both study results are considered together, speak to the potential of CTCs as an important biomarker in UC.

For RCC, multiple groups have used the MACS technique to detect CTCs[14], with one group finding a correlation between tumor cell number and high tumor grade, and an increased number of tumor cell positive patients with advanced tumor stage[28]. To date there are no studies using the CSS to assess for CTCs in patients with RCC. Our study was able to detect CTCs using CSS. The significance to disease progression and outcome remains unclear and additional studies are necessary.

The use of CTCs with the CSS is not without its limitations. Although rarely present in blood, normal epithelial cells or leukocytes expressing epithelial markers cannot be excluded by the current assay technology[29]. This creates the potential for false positive

results to bias studies. Also, as can be the case in advanced cancers, poorly differentiated tumor cells may lack the epithelial surface antigens that are detected by the CSS. This may lead to failure to detect CTCs in advanced, aggressive disease states. A third limitation, similar to the previous problem, is that tumor cells can undergo epithelial-to-mesenchymal transition (EMT), which can lead to the loss of epithelial marker expression[30,31]. If this occurs, it may result in an underestimation of the actual CTC number by CSS or any detection system centered on epithelial markers. Lastly, CSS lacks specificity for any particular carcinoma. It is possible that another epithelial tumor is the source of the CTCs detected. Additional molecular or phenotypical markers may be necessary to better discern cell-type of origin.

Specific limitations to our study include a small sample size, which limits the generalizability of our results. However, this study is intended as a feasibility study, and were we able to demonstrate that the CSS is able to detect CTCs in the patient cohorts selected. Our study also had a limited duration of follow up, which may have resulted in the inability to demonstrate significant results in all survival metrics.

Conclusion

We demonstrated that CTCs are detectible in the peripheral blood of patients with newly diagnosed advanced UC and RCC, prior to intervention, using the CSS. This pilot study suggests that although CTCs may not correlate with grade or stage of cancers, the presence of CTCs may predict worse overall survival, specifically in patients with UC. Larger studies are required to better determine the true significance of CTCs, as they could possibly represent a diagnostic modality, as well as a reliable and straightforward method to follow patients after treatment and predict outcomes in patients with both RCC and UC.

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Table 1 – Patient demographics

		RCC	UC
Patients		11	15
Mean age (range)		63.7 (44-90)	70.5 (46-88)
Gender	Female	4	4
	Male	7	11
Cancer stage	0	1	1
	2	4	3
	3	2	8
	4	4	3
Treatment			
	Surgery	11	12
	Chemotherapy	0	3

	Combined Cohort N=26	Non-Organ Confined N=9	Renal Cell Carcinoma N=11	Urothelial Carcinoma N=15
At Least 1 CTC detected	0.88	0.89	0.91	0.87
CTC/7.5mL >1	0.50	0.56	0.36	0.60
CTC/7.5 mL >2	0.27	0.44	0.18	0.33

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

CTC/7.5 mL Max >2	0.38	0.56	0.36	0.40

Table 2 – Results of CTC Enumeration

Table 3 – Mean and Maximum CTC burden with 95% Confidence Intervals for RCC and UC based on tumor stage

RCC Patients

AJCC

Stage	N	Mean CTC	P-Value	N	Max CTC	P-Value
0	1	0.67	0.9974	1	1.00	0.8972
2	4	1.50±1.93		4	2.50±3.11	
3	2	1.00±0.94		2	2.00±1.41	
4	4	1.25±1.13		4	2.50±1.91	

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

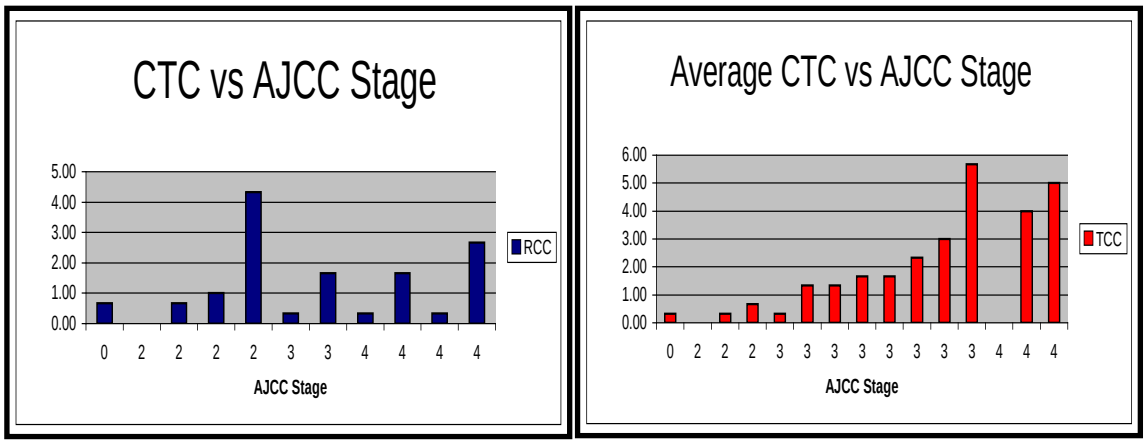
Category: Clinical Science Research

UC Patients

AJCC

Stage	N	Mean CTC	P-Value	N	Max CTC	P-Value
0	1	0.33	0.1951	1	1.00	0.2666
2	3	0.33±0.33		3	1.00±1.00	
3	8	2.17±1.61		8	3.63±2.67	
4	3	3.00±2.65		3	4.00±3.61	

Figure 1 – Average CTC vs AJCC Stage for RCC and UC



Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Table 4 - RCC and UC Combined Cohort, CTCs By Grade and Stage

Grade	N	Mean CTC	P value	N	Max CTC	P value
High	21	1.67±1.62	0.5548	21	2.86±2.37	0.3747
Low	4	1.42±1.99		4	2.25±3.20	

AJCC

Stage	N	Mean CTC	P value	N	Max CTC	P value
0,2	9	0.89±1.33	0.0762	9	1.67±2.12	0.0695
3,4	17	1.96±1.67		17	3.24±2.46	

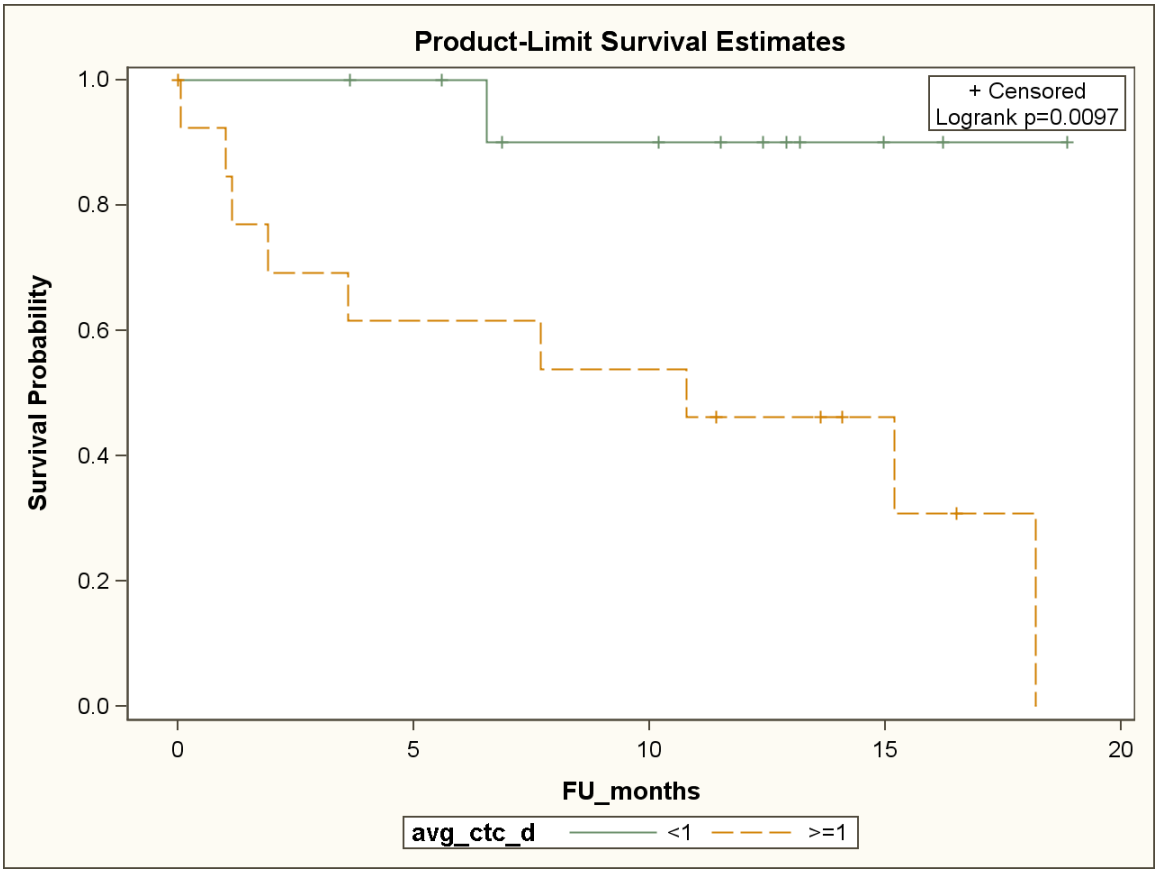
Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Figure 2 - Kaplan-Meier curve for overall survival for the combined cohort for average number <1 versus ≥ 1 CTCs

Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

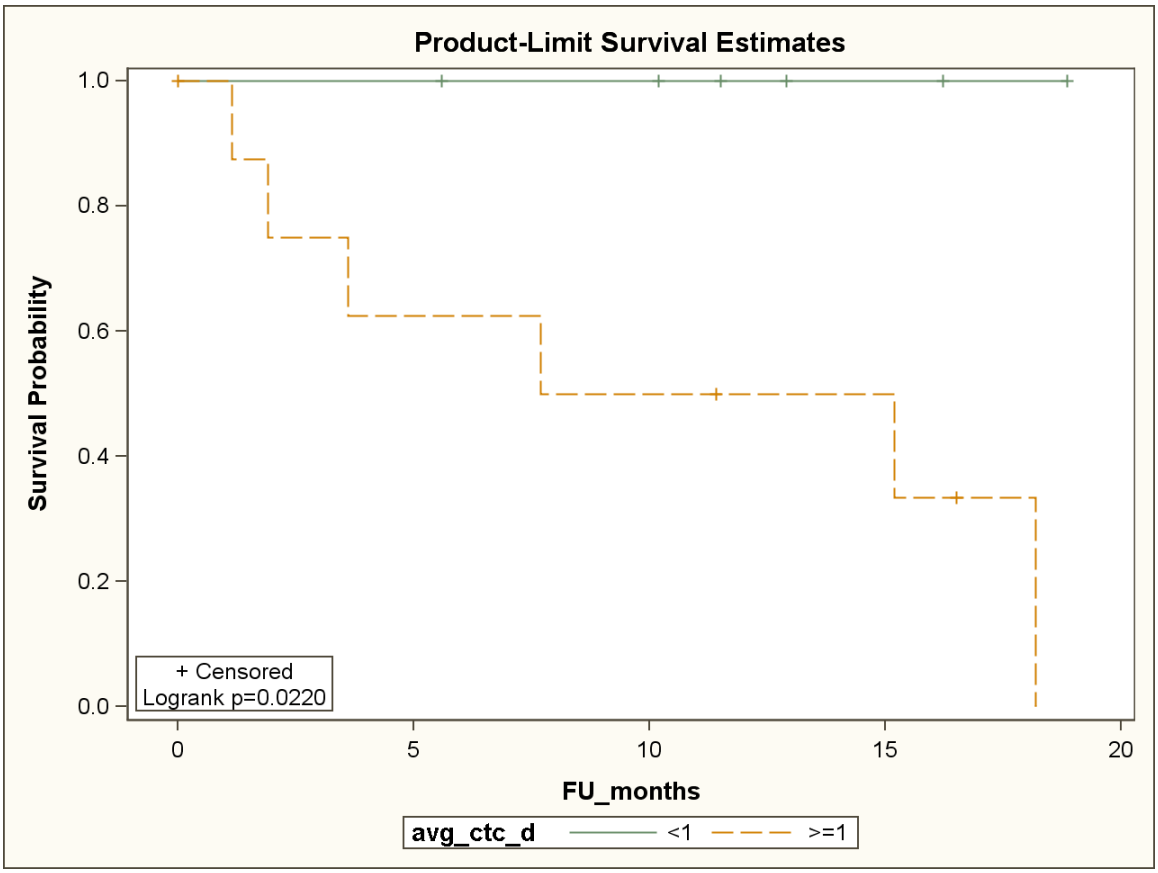
Category: Clinical Science Research



Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

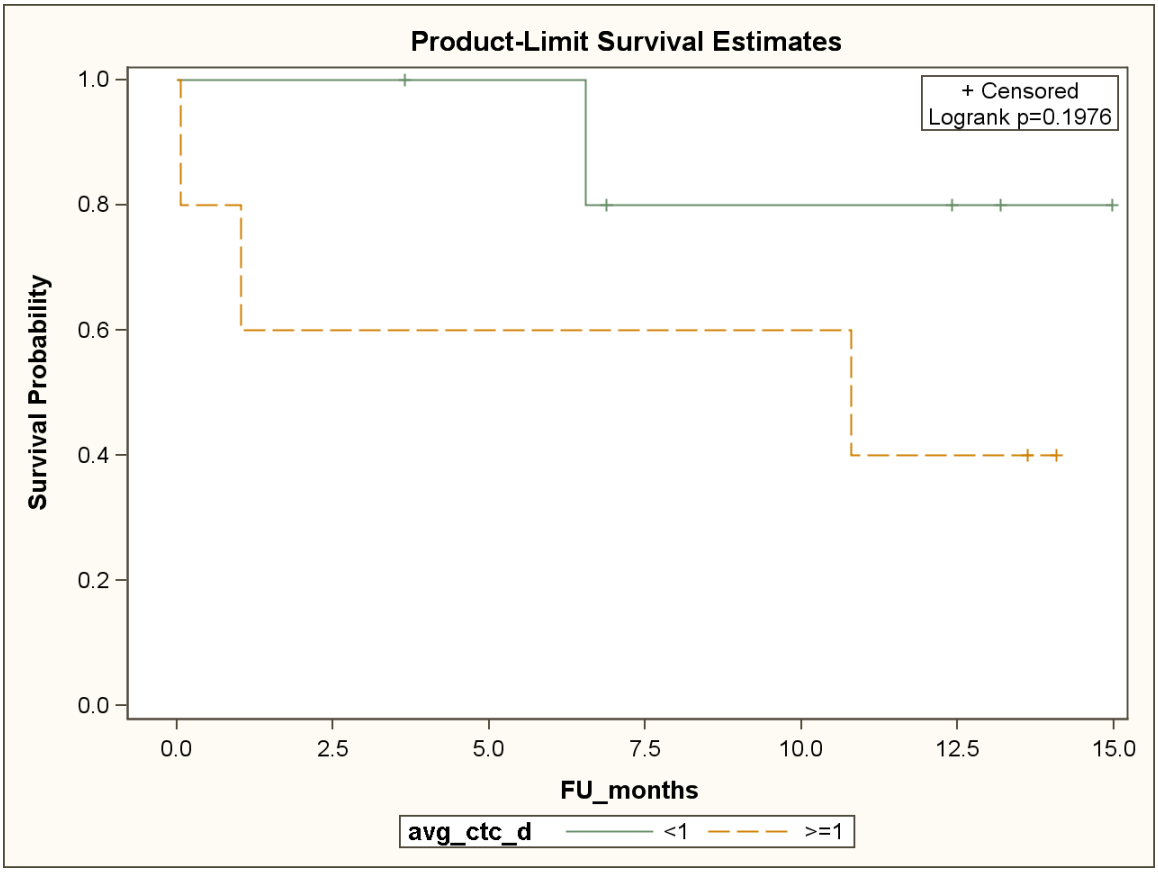
Figure 3 - Kaplan-Meier curve for overall survival for the UC cohort for average number <1 versus ≥1 CTCs



Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

Figure 4 - Kaplan-Meier curve for overall survival for the RCC cohort for average number <1 versus ≥1 CTCs



Title: Prognostic and Therapeutic Correlation of Circulating Tumor Cells in the Setting of Urothelial and Renal Cell Carcinoma

Category: Clinical Science Research

References

1 Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA Cancer J Clin 2010; 60 (5): 277-300

2 Stein JP, Lieskovsky G, Cote R, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol 2001; 19 (3): 666-75

3 Chen DY, Uzzo RG. Evaluation and management of the renal mass. Med Clin North Am 2011; 95 (1): 179-89

4 Weiss L. Metastasis of Cancer: a Conceptual history from antquity to the 1990s. Cancer Metastasis Rev 2000; 19 (3-4): I –XI, 193-383

5 Loberg RD, Fridman Y, Pienta BA, et al. Detection and isolation of circulating tumor cells in urologic cancers: a review. Neoplasia 2004; 6 (4): 302-9

6 Nezos A, Pissimisis N, Lembessis P, et al. Detection of circulating tumor cells in bladder cancer patients. Cancer Treat Rev 2009; 35 (3): 272-9

7 Cristofanilli M, Budd GT, Ellish MJ, et al. Circulating tumor cells, disease progression and survival in metastatic breast cancer. N Engl J Med 2004, August 351; 8: 781-791

8 Budd GT, Cristofanilli M, Ellis MJ, et al. Circulating tumor cells versus imaging – predicting overall survival in metastatic breast cancer. Clin Cancer Res 2006; 12 (21): 6403-6409

9 Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res 2004; 10: 6897-6904

10 Garcia JA, Rosenberg JE, Weinberg V, et al. Evaluation and significance of circulating epithelial cells in patients with hormone-refractory prostate cancer. BJU 2007. 99: 519-524

11 Shaffer DR, Leversha MA, Danila DC, et al. Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. Clin Cancer Res 2007; 13 (7): 2023-2029

12 Naoe M, Ogawa Y, Morita J, et al. Detection of circulating urothelial cancer cells in the blood using the CellSearch System. Cancer 2007; 109:1439-45

13 Rink M, Chun FK, Minner S, et al. Detection of circulating tumour cells in peripheral blood of patients with advanced non-metastatic bladder cancer. BJU Int 2010 Aug 24

14 Blumke K, Bilkenrother U, Schmidt U, et al. Detection of circulating tumor cells from renal carcinoma patients: experiences of a two-center study. Oncol Rep 2005 Oct; 14: 895-9

15 Brunner, A, Prelog M, Verdorfer I, et al. EpCAM is predominantly expressed in high grade and advanced stage urothelial carcinoma of the bladder. J Clin Pathol 2008; 61 (3): 307-310

16 Baeuerle, PA, Gires O. EpCAM (CD326) finding its role in cancer. Br J Cancer 2007; 96 (3): 417-423

17 Balzar, M, Winter MJ, de Boer CJ, et al. The biology of the 17-1A antigen (EpCAM). J Mol Med 1999; 77 (10): 699-712

18 Pantel K, Alix-Panabieres C, Riethdorf S. Cancer micrometastasis. Nat Rev Clin Oncol 2009; 6 (6): 339-51

19 Hayes, DF, Cristofanilli M, Budd GT, et al. Circulating tumor cells at each follow-up time point during therapy for metastatic breast cancer patients predict progression-free and overall survival. Clin Cancer Res 2006; 12 (14 pt 1): 4218-24

20 Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumor cells. Nat Rev Cancer 2008; 8 (58): 329-340.

21 de Bono JS, Scher HI, Montogmery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 2008; 14 (19): 6302-6309

22 Guzzo TJ, McNeil BK, Bivalacqua, et al. The presence of circulating tumor cells does not predict extravesical disease in bladder cancer patients prior to radical cystectomy. Urol Oncol 2009 [Epub ahead of print]

23 Gallagher DJ, Milowsky MI, Ishill N, et al. Detection of circulating tumor cells in patients with urothelial cancer. Ann Oncol 2009; 20 (2): 305-308

24 Sastre J, Maestro ML, Puente J, et al. Circulating tumor cells in colorectal cancer: correlation with clinical and pathological variables. Ann Oncol 2008; 19 (5): 935-8

25 Moreno JG, Miller MC, Gross S, et al. Circulating tumor cells predict survival in patients with metastatic prostate cancer. Urology 2005; 65 (4): 713-8

26 Naoe M, Ogawa Y, Takeshita K, et al. Use of CellSearch Circulating Tumor Cell Test for monitoring urothelial cancer: two case reports of metastatic urothelial cancer. South Med J 2008; 101 (4): 439-41

27 Tibbe AG, Miller MC, Terstappen LW. Statistical considerations for enumeration of circulating tumor cells. Cytometry A 2007; 71 (3): 154-62.

28 Bilkenroth U, Taubert H, Riemann D, et al. Detection and enrichment of disseminated renal carcinoma cells from peripheral blood by immunomagnetic cell separation. Int J Cancer 2001; 92 (4): 577-82.

29 Alix-Panabieres C, Riethdorf S, Pantel K. Circulating tumor cells and bone marrow micrometastatis. Clin Cancer Res 2008; 14 (16); 5013-21

30 Brabletz T, Jung A, Spaderna S, et al. Opinion: migrating cancer stem cells – an integrated concept of malignant tumor progression. Nat Rev Cancer 2005; 5 (9): 744-9

31 Christofori, G. New signals from the invasive front. Nature 2006; 441 (7092): 444-50