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Prognostic Significance of MUC-1 in Circulating Tumor Cells in Patients With Metastatic Pancreatic Adenocarcinoma

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Abstract

Objectives—Development of targeted therapies for pancreatic cancer could be enhanced by a reliable method for noninvasive tumor cell assessment. In this pilot study we isolated and phenotypically characterized circulating tumor cells (CTC) from patients with metastatic pancreatic cancer and explored their relationship to clinical outcome.

Methods—Peripheral blood from 50 patients was collected at treatment initiation and first disease evaluation for CTC enumeration and phenotyping by CellSearch® system. Expression of human mucin 1 (MUC-1) was performed.

Results—48 and 37 patients had evaluable samples at baseline and first disease evaluation, respectively. The cohort was 62% male, with a median age of 63 years. At least 1 CTC/7.5mL was detected in 23 patients (48%) pre-treatment and 11 patients (30%) at first disease evaluation. No difference was seen in overall survival between patients with 1 CTC vs no CTC at baseline ($p=0.14$). Patients with MUC-1 expressing CTC ($n=10$) had shorter median overall survival compared to those with MUC-1 negative CTC ($n=13$) (2.7 vs. 9.6m; $p=0.044$).

Conclusions—CTC enumeration and phenotypic characterization from metastatic pancreatic cancer patients is feasible. No correlation was found between CTC isolation and survival. However, the presence of MUC-1 expressing CTC demonstrated a trend toward inferior survival.

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Keywords

Pancreatic Cancer; Circulating Tumor Cells; MUC-1; Mucin-1

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States, with approximately 40,000 new cases and approximately the same number of deaths reported yearly¹. Systemic chemotherapy is the mainstay of treatment for locally advanced and metastatic pancreatic cancer (mPC)². Despite the most aggressive chemotherapy regimens available today, median survival remains less than 1 year^{3,4}. Thus, there is an urgent need for advances in the management of these patients through better systemic therapy. The development of effective therapies in pancreatic cancer is impeded by the relative inaccessibility of tumor for repeat biopsy to document therapeutic effect or evaluate tumor evolution through various lines of therapy. The capture of circulating tumor cells (CTCs) from patients is one potential strategy for in vivo pharmacodynamic assessment of pancreatic cancer cells.

CTCs studied in multiple cancers including breast, prostate, and colorectal cancers correlate with underlying tumor biology including genetic alterations, biomarker expression, and prognosis^{5–8}. Our group previously demonstrated the prognostic significance of CTC enumeration in the peripheral blood of patients with metastatic colon cancer⁹. The presence of CTC at treatment initiation and during therapy conferred a poorer prognosis regardless of the treatment type^{10,11}. The utility of CTC enumeration in pancreatic cancer patients has not been clearly defined.

MUC-1 is a large trans-membrane glycoprotein highly expressed in a subset of pancreatic adenocarcinomas compared to non-cancerous pancreatic tissue¹². Increased expression of MUC-1 has been associated with increased metastatic properties of pancreatic cancer cells, possibly by induction of epithelial to mesenchymal transition¹³. Increased MUC-1 expression in a tumor correlates with higher stage and poorer outcome¹⁴. In addition, the presence of circulating anti-MUC-1 IgG antibodies is associated with improved survival among pancreatic cancer patients irrespective of stage¹⁵. Agents targeting MUC-1 are now being evaluated in clinical trials in pancreatic cancer^{16–19}.

We conducted a pilot study to prospectively evaluate CTC yield using the CellSearch® system and to assess their relationship to clinical outcome in patients with metastatic pancreatic cancer (mPC). We further assessed expression of MUC-1 on captured CTCs and its relationship to clinical outcome.

Patients and Methods

Study Design

Patients 18 years or older with metastatic adenocarcinoma of the pancreas were enrolled to this study. Enrollment occurred shortly after diagnosis of mPC and prior to the initiation of front line systemic therapy. Patient demographics, tumor characteristics, and treatment

regimens were recorded. Patients provided written informed consent according to institutional and federal requirements, following the approval of the protocol by the institutional review board of Fox Chase Cancer Center.

Fifty milliliters (mL) of peripheral blood were obtained from patients at study entry and at first disease evaluation (6–10 weeks after treatment initiation). Levels of the tumor marker CA-19-9 were recorded at the same timepoints. Overall survival (OS) was defined as the time between date of study enrollment and death.

Specimen Analysis

CTC evaluation was performed using the CellSearch® assay (Janssen Diagnostics LLC) as previously described²⁰. Peripheral blood samples for CTC were collected in two 10 mL CellSave Preservative tubes™, and processed using the CellSearch™ Epithelial Cell kits. All automated enrichments were performed on the CellTracks™ AutoPrep System. Data was collected and analyzed on the CellTracks™ Analyzer II. Immunomagnetic enrichment of CTC using the Cell Tracks AutoPrep System has been described in detail by Kagan et al.²¹. The assay was performed on a 7.5mL of blood sample from each patient at each timepoint. Cells were fluorescently labeled with anti-CD45APC, anti-pan cytokeratin-PE (CK8, 18, 19), DAPI, and anti- MUC-1-FITC (provided by Janssen LLC Huntington Valley). Images for analyses were sorted by computer-assisted software selecting events based on the parameters of CD45 negative, cytokeratin positive and DAPI positive. Selected CTCs were assessed for MUC-1 expression.

Statistical Methods

A sample size of 50 was selected as a pilot cohort for evaluation of CTCs in metastatic pancreatic cancer patients. The number of CTCs at different time points was summarized. Based on the distribution of CTC category, we dichotomized them into two categories (0 vs.

1). Differences in demographic and treatment characteristics by CTC count were assessed using Chi-square, Fisher's exact, or Wilcoxon rank sum tests as appropriate. OS was visually examined using Kaplan-Meier curves and median survival estimated based on CTC counts and MUC-1 expression. Wilcoxon tests were used to test any difference in survival. The relation between CA19-9 and CTC number was examined. Spearman rank correlation was computed and tested, since both distributions of CA19-9 and CTC counts were skewed.. Analyses were performed using SAS statistical software ver. 9.3 (Cary, NC). All tests were considered significant using p-value <0.05.

Results

Patient Characteristics

Fifty patients were enrolled in this study between March 2007 and March of 2011. Patient characteristics for the entire cohort are shown in Table 1. Patient and treatment characteristics were balanced between the group of patients with CTC = 1 versus the group with CTC=0. Median age was 61 years (range: 39–86 years), with the majority being Caucasian (84%) males (62%). The majority of patients received gemcitabine-based therapy

(96%). Thirteen patients (26%) had received chemotherapy for localized disease prior to study enrollment, of which 12 received radiation and 2 underwent surgical resection.

Circulating Tumor Cell Yield

Forty-eight patients provided samples that were evaluable for CTC enumeration at baseline, and 37 provided evaluable samples at first disease evaluation. Figure 1 demonstrates CTC yield at baseline and first disease evaluation. At baseline, 23 of 48 patients (48%) had 1 CTC/7.5mL peripheral blood. Thirty percent (11 of 37 patients) had 1 CTC/7.5mL peripheral blood at first disease evaluation. Isolation of 2 CTC/7.5mL was rare, and was noted in only 6 patients (13%) pre-treatment and 3 patients (8%) at first disease evaluation.

Among the 37 patients who submitted samples at the two time points, we evaluated the change in CTC count between the two samples. Of the patients who presented with 0 CTC, 84% (19/22) remained in this category at first disease evaluation. For patients with 1 CTC at presentation, approximately half had no CTCs detected at first disease evaluation (47%, 7/15 patients). No association was seen between treatment response (partial response, stable disease, or progressive disease) and CTC level at baseline ($p=0.66$), or at first disease evaluation ($p=0.90$) (Table 2). No correlation was found between CTC enumeration and absolute CA19-9 levels pre-treatment or at first disease evaluation (Spearman correlation coefficients 0.04 and -0.214, respectively; $p>0.20$ data not shown).

Relation of circulating tumor cell yield to overall survival

With a median follow up of 7.5 months (range – 1–24months), the median OS of our cohort was 7.5 months (95% CI: 5.3–9.9 months). Patients who did not have CTC detected at baseline had longer median survival compared to those with 1 CTC, but this difference was not statistically significant (8.8 months; 95% CI: 6.8–10.9 vs. 6.3 months; 95%CI: 2.6–9.7; $p=0.14$) (Figure 2A). At first disease evaluation, 37 patients provided follow-up samples for CTC enumeration. A similar non-significant difference was noted in the median OS of patients without CTC and patients with 1 CTC/7.5mL at first disease evaluation [9.6 months (95% CI: 7.1–10.9 months) vs. 8.8 months (95%CI: 2.9–13.8) respectively; $p=0.25$] (Figure 2B).

Relation of MUC-1 expression in circulating tumor cells to overall survival

MUC-1 expression was found in 10 samples (43%) of the 23 patients with 1 CTC at baseline. Patients with MUC-1 positive CTC had shorter median OS [2.7 months (95%CI: 0.1–7.6)] compared to patients with MUC-1 negative CTC [9.6 months (95%CI: 3.9–12.8); $p=0.044$] or no isolated CTC [8.8 months (95%CI: 6.0–10.9) $p=0.014$] (Figure 3).

Discussion

The range of CTC detection among pancreatic cancer patients is between 30–100% across different studies using various methods^{22–26}. The initial studies evaluating CTC detection using the CellSearch® system demonstrated that up to 40% of pancreatic cancer patients with various disease stages had detectable CTC^{20,27}. Available data regarding the correlation between CTC number and clinical outcomes is conflicting. Soeth et al.

demonstrated a statistically significant correlation between preoperative CTC detection and decreased OS among early stage pancreatic cancer patients²³. Similarly Kurihara et al. reported worse survival among advanced pancreatic cancer patients with ≥ 1 CTC²⁷. In a recent meta-analysis of 9 cohort studies with a total of 623 pancreatic cancer patients of various disease stages, CTC positivity was associated with poorer progression-free survival (HR=1.89, 95 %CI: 1.25–4.0, $p<0.001$), and worse OS (HR=1.23, 95%CI: 0.88–2.08, $p<0.001$)²⁸. Other groups failed to show any correlation between clinical outcomes and CTC enumeration in the locally advanced or metastatic setting^{29, 30}.

In this pilot study we prospectively evaluated the ability to characterize CTC enriched by the CellSearch® system from the peripheral blood of mPC patients and assess a correlation with clinical outcomes. Although data from the original cohort of patients evaluated by this assay found 6 of 16 patients with pancreatic cancer with ≥ 3.5 CTC/7.5ml of peripheral blood, our results demonstrate a lower rate of CTC enumeration²⁰. For the current study, we utilized a threshold of ≥ 1 CTC to declare a sample positive. In our cohort, 48% had ≥ 1 CTC/7.5ml peripheral blood and very few patients had ≥ 2 CTC/7.5ml. This yield is less than that noted in other epithelial malignancies such as metastatic breast, colon and prostate with 30–65 percent of patient having ≥ 5 CTC/7.5 ml peripheral blood^{6,8,10}. Differences in biology between pancreatic cancer and other epithelial malignancies likely play an important role in CTC recovery. Specifically, the lower rate of hematogenous spread of metastatic disease in pancreatic cancer as compared to other cancers^{31,32}. The lower percentage of mPC patients with detectable CTC mirrors data from other groups studying CTC in pancreatic cancer^{10,25,26,33}.

The threshold of ≥ 1 CTC has been used in previous studies evaluating CTC in pancreatic cancer patients. Bidard et al analyzed a cohort of 79 patients with locally advanced pancreatic cancer for the presence of ≥ 1 CTC pre-treatment using the CellSearch® system and detected CTC in 11% of patients overall (5% at presentation and 9% after 2 months of therapy). CTC positivity was associated with poor differentiated and shorter OS³³. Similarly, in our study, patients with ≥ 1 CTC demonstrated a trend towards worse OS compared to patients with no CTC isolated. This trend is most clearly seen in the first 3 months of follow up (Figure 3), yet it did not translate into a survival benefit. The small sample size may have limited our ability to detect a survival benefit in this pilot study. Furthermore, the results may change if tested on a cohort of mPC patients treated with novel therapeutic regimens.

The isolation of CTC represents a potential platform for drug development and molecular analysis of cancers, and provides a non-invasive method to study the tumor *in-vivo*³⁴. Our study evaluated the prognostic value of MUC-1 expression on isolated CTC, which was found in 43% of patients. We demonstrated a statistically significant difference in OS between patients with MUC-1 positive versus MUC-1 negative CTC. This finding is supported by other biologic observations, as MUC-1 is thought to enhance metastatic properties of pancreatic cancer cells and promote mesenchymal transition. MUC-1 expression was shown to correlate with worst outcomes^{13,14}. We could not perform an analysis of MUC-1 expression on corresponding tumor tissue due to lack of available specimens, as most patients had limited material from a diagnostic fine needle aspiration. As there are several investigational agents targeting MUC-1 in clinical trials, the ability to test

the expression of this protein on CTC as a “liquid biopsy” could be utilized in the future to select treatments for patients or measure treatment response^{16,19,35}.

Our study has several limitations. As a pilot study, it is by its nature limited from drawing firm conclusions due to the small sample size and the heterogeneity of delivered chemotherapy. Given the trend of an association between CTC and OS, a larger cohort of patients would be required to reach a definitive conclusion. The low CTC yield in our study raises potential questions regarding the sensitivity of the assay. Utilizing a lower threshold of 1 CTC increases the rate of “positivity” and the risk of error. Given the potential utility of CTC evaluation in this disease, in which abundant tissue availability is often limited, additional studies are needed to develop more sensitive platforms and novel techniques for CTC isolation in pancreatic cancer^{36,37}.

In summary, this pilot study demonstrates the feasibility of capturing and characterizing CTC with the CellSearch® system in patients with mPC. The sample size was too small to make definitive conclusions regarding the association of CTC number and survival. However, our data suggests that patients with MUC-1 positive CTC may have shorter survival than those with MUC-1 negative CTC. Further studies to test the prognostic impact of CTCs and their potential as a pharmacodynamic marker for MUC-1 targeted therapies in pancreatic cancer are warranted.

Acknowledgments

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Figure 1. CTC yield among mPC patients pre-treatment and at first disease evaluation
Peripheral blood CTC yield among 48 patients with available samples for enumeration pre-treatment and 37 patients with available samples for enumeration at first disease evaluation.

Figure 2. Overall survival by CTC level pre-treatment

OS based on CTC count at baseline **(A)** and first disease evaluation **(B)**. **(A)** Kaplan-Meier curve estimates of overall survival based on CTC enumeration pre-treatment (0 vs. 1); N=48; median OS 8.8 months vs. 6.3 months, respectively; p=0.139. **(B)** Kaplan-Meier curve estimates of OS based on CTC enumeration at first disease evaluation (0 vs. 1); N=37; Median OS 9.6 months vs 8.8 months; respectively; p=0.25.

Figure 3. Overall survival by MUC-1 expression at baseline

OS based on MUC-1 expression. Kaplan-Meier curve estimates of OS based on CTC enumeration and MUC-1 expression pre-treatment (CTC=0 (n=25) – mOS=8.8 months; MUC-1 positive CTC (n=10)- mOS=2.7 months; MUC-1 negative CTC (n=13) – mOS=9.6 months; p=0.014). Median follow-up 7.5 m (range 1–24 months).

Table 1

Patient Demographics and Treatment Characteristics

	All patients N=50* n (%)	Baseline CTC		p-value
		CTC=0 N=25 n (%)	CTC 1 N=23 n (%)	
Sex				
Female	19 (38.0%)	12 (48.0%)	7 (30.4%)	0.21
Male	31 (62.0%)	13 (52.0%)	16 (69.6%)	
Median Age at diagnosis				
years (range)	61.5 (38–86)	61 (49–84)	62 (38–86)	0.80
Race				
Caucasian	42 (84.0%)	21 (84.0%)	20 (87.0%)	0.77
African American	7 (14.0%)	4 (16.0%)	3 (13.0%)	
American Indian	1 (2.0%)			
CA 19-9 (U/ML)				
Median CA 19-9 at baseline	1810	511	2090	0.93
<37	9 (18.0%)	3 (12.0%)	6 (26.1%)	0.080
37–1000	13 (26.0%)	10 (40.0%)	3 (13.0%)	
>1000	28 (56.0%)	12 (48.0%)	14 (60.9%)	
Metastatic sites				
Liver	33 (66.0%)	17 (68.0%)	16 (69.6%)	0.91
Lung	8 (16.0%)	3 (12.0%)	4 (17.4%)	0.70
Peritoneum	18 (36.0%)	8 (32.0%)	7 (30.0%)	0.82
Lymph nodes	6 (12.0%)	3 (12.0%)	2 (8.7%)	1.00
Bone	2 (4.0%)	1 (4.0%)	1 (4.0%)	1.00
Therapy**				
Gemcitabine	46 (96.0%)	23 (92.0%)	23 (100%)	0.17
FOLFIRINOX	3 (6.0%)	2 (8.0%)	1 (4.3%)	0.60
Gemcitabine/nab-paclitaxel	1 (2.0%)	0 (0%)	1 (4.3%)	0.29
Any Imexon, inj. ***	21 (42.0%)	7 (28.0%)	12 (52.2%)	0.087

* 50 patients were enrolled in the study. Only 48 patients had samples available for CTC evaluation pre-treatment.

** Some patients in this cohort received treatment with multiple agents.

*** 21 untreated mPC patients were enrolled in a Phase Ib study of imexon injection plus gemcitabine³⁸.

Table 2

Association Between Overall Response and CTC Level Pre-treatment (A) and First Disease Evaluation (B)

Response	Pre-treatment				1 st disease evaluation			
	CTC = 0 (n = 25)	CTC 1 (n = 23)	CTC = 0** (n = 26)	CTC 1*** (n = 11)				
	N	%	N	%	N	%	N	%
<i>PR</i>	5	20.0	3	13.0	6	23.1	2	18.2
<i>SD</i>	11	44.0	9	39.1	13	50.0	5	45.4
<i>PD</i>	6	24.0	5	21.7	7	26.9	4	36.4
<i>Not evaluable</i>	3	12.0	6	26.1	-	-	-	-
<i>P Value</i>	P = 0.66				P = 0.90			

PR-partial response, SD- stable disease, PD-progressive disease

* Patients that were not evaluable and those that did not have CTC enumeration at first disease evaluation were excluded.