Clinical Cancer Research

Improved Risk Stratification by Circulating Tumor Cell Counts in Pancreatic Cancer

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Abstract

Purpose: Pancreatic cancer is one of the most devastating diseases with a 5-year survival rate of 3% to 5%. Here, we investigated whether circulating tumor cells (CTC) may predict metastatic spread and survival in pancreatic cancer patients.

Experimental Design: In a prospective study, we enrolled 69 pancreatic cancer patients. In peripheral blood, CTCs were identified by MACS enrichment (anti-cytokeratin/anti-EpCam) and subsequent automated analysis after combined anti-cytokeratin/anti-CD45/DAPI staining. CTC results were correlated to established clinicopathologic risk factors, detection of disseminated tumor cells (DTC) in bone marrow, and clinical outcome (follow-up time: 48 months).

Results: Median patient survival was 11 months (0–48 months). Thirty-eight patients were male and 31 were female, and the majority received gemcitabine (58/69). CTCs were present in 23 of 69 patients (33.3%) ranging from 1 to 19 cells (17 with >1 CTC). Although clinicopathologic parameters and DTC status did not correlate with CTC incidence, progression-free survival (PFS) and overall survival (OS) were significantly reduced in CTC-positive patients in univariate ($P = 0.009$, PFS; $P = 0.030$, OS, both log rank) and multivariate analysis [HR = 4.543; confidence interval (CI), 1.549–13.329; $P = 0.006$, PFS; HR = 2.093; CI, 1.081–4.050; $P = 0.028$, OS, both Cox regression). Also within patients receiving chemotherapy, PFS was significantly reduced in CTC-positive patients in univariate ($P = 0.013$) and multivariate (HR = 4.203; CI, 1.416–12.471; $P = 0.010$) analysis.

Conclusions: CTCs affect the outcome of patients with pancreatic cancer independent from other risk factors, including patients receiving (adjuvant) cytotoxic therapy. CTC stratification may allow a better upfront identification of patients with a longer lifespan who might profit from new adjuvant therapies. *Clin Cancer Res; 24(12); 2844–50. ©2018 AACR.*

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Introduction

Malignant tumors of the pancreas are the fourth leading cause of cancer-related mortality in Germany and the United States. They are characterized by rapid progression, early metastasis, and low sensitivity for radiation and chemotherapy with a 5-year survival rate of only 3% to 5%. Despite the very short median survival time, there is a subset that survives significantly longer. Because of the lack of symptoms in early stages, 75% of the patients are diagnosed in a metastatic stage of disease and only 10% to 20% of patients can get surgical resection with curable intention. Only tumor detection as early as possible resulting in complete surgical resection may yet improve the survival rates of patients.

One reason for its fast progression lies in the presence of micrometastasis or single disseminated tumor cells at the time of diagnosis, which contribute to initiate the metastatic cascade. Disseminated tumor cells (DTC) in bone marrow have been proven to be a prognosticator for disease-free and overall survival in pancreatic cancer (1), suggesting that the bone marrow might be a reservoir for disseminating pancreatic cancer cells. However, bone marrow aspiration is an invasive procedure not accepted in clinical practice for solid tumor patients. Moreover, DTCs might be also present in other organs, most likely in the liver as one of the most prominent sites of metastasis in pancreatic cancer. Thus, the detection and characterization of CTCs already in existence has been integrated into the definition of a "liquid biopsy" 7 years ago (2) and received enormous attention over the past years including other tumor-derived biomarkers such as circulating tumor DNA (3).

So far, published CTC data for pancreatic cancer mostly rely on small patient cohorts of different disease stages using various CTC techniques, and they show contradictory results (4, 5). For other carcinomas, for example, metastatic breast cancer, colorectal, esophageal, or prostate cancer, the prognostic value of CTCs has been reported (6–9). In the current study, we evaluated CTCs in the peripheral blood obtained from patients with pancreatic ductal adenocarcinoma (PDAC) by using an immunomagnetic procedure for CTC capture.

Our goal was to investigate the clinical significance of CTCs regarding progression-free (PFS) and overall survival (OS) in

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Translational Relevance

Diagnosis of pancreatic cancer still implies fast progress and death in most of the cases. Prognostic biomarkers for patient survival stratification are missing yet. For the first time, we could show that the circulating tumor cell (CTC) status can discriminate between patients with longer and shorter progression-free as well as overall survival independent of the other relevant clinical risk parameters used for prognostic staging so far. Improved staging can lead to early identification of patients with longer survival times who might benefit from novel therapies such as immune checkpoint inhibition. The detection and molecular characterization of CTCs opens new avenues for the identification of therapeutic targets and resistance mechanisms, which might lead to a better understanding of the molecular progression of pancreatic cancer.

special consideration of the small subgroup of long-term survivors who have become the focus of immunotherapeutic interventions (10). Better risk stratification of pancreatic cancer patients might therefore help to identify a subset of patients that might profit more from adjuvant therapy than the overall population of patients.

Materials and Methods

Study design and patients

Ninety-two patients who underwent surgery of the pancreas in suspect of resectable PDAC at the University Medical Center Hamburg-Eppendorf (Hamburg, Germany) between 2009 and 2012 were enrolled for this prospective study. Sixty-nine patients with assured pathologic PDAC fulfilled the inclusion criteria (written informed consent, blood draw before surgery, assured diagnosis of PDAC). Informed consent was obtained from all patients. The study was approved by the medical ethics committee of the Chamber of Physicians of Hamburg. Our report adheres to the REMARK criteria (11).

Twenty-two patients (31.9%) were completely resected, whereas 43 (62.3%) were not due to extended metastatic disease (missing resection status in case of 4 patients).

Histopathologic staging included tumor type, stage, and grade, as determined according to the seventh edition of the TNM classification (12).

Peripheral blood samples for CTC analysis were collected immediately before surgery. Five patients died within 30 days after surgery and were excluded from survival analysis. The follow-up time ranged from 0 to 48 months. OS was the time from surgery to death or last follow-up, and PFS was defined as the time from surgery to diagnosis of local recurrence, distant metastasis, or death, whichever occurred first.

In addition to the patient recruitment described above, blood specimens were also collected from 18 healthy donors and from 9 patients with nonmalignant pancreatic disease, including PanIN 1a (2), chronic pancreatitis (3), chronic fibrotic pancreatitis (2), benign pancreatic cyst (1), and chronic fibrotic pancreatitis plus PanIN 1a (1).

CTC enrichment

CTC analysis was performed using the MACS Technology (Miltenyi Biotech GmbH) and subsequent immunocytochemical staining as previously described in detail (13). Briefly, blood samples (7.5 mL) were collected in CellSave Preservative Tubes (Menarini Diagnostics). They were processed within 48 hours of storage at room temperature. After red blood cell lysis, the cell pellet was dissolved with 1× Dilution Buffer (Miltenyi Biotech GmbH). FCR Blocking Reagent (100 μL; Miltenyi Biotech GmbH) was added followed by cell permeation using digitonin. Cells were fixed using 4% paraformaldehyde, and potential CTCs were enriched by a mixture of anti-cytokeratin (CK 7,8) and anti-EpCAM microbeads (100 μL each, Miltenyi Biotech GmbH) for 45 minutes at room temperature. Dilution Buffer was added (ad 10 mL), and the suspension was centrifuged at 300 × *g* for 10 minutes. The supernatant was discarded and cells were resuspended in 1.5 mL 1× Dilution Buffer. Three MS columns were prepared with 1× Dilution Buffer in an OctoMACS Separator (all Miltenyi Biotech GmbH), and 0.5 mL of the cell suspension was slowly added to each column. Before the total liquid went through, columns were taken out of the magnet and placed over cytospin funnels (1 cm diameter) adjusted to poly-prep slides (Sigma Aldrich, Inc.). After two washings with 0.5 mL 1× Dilution Buffer, the stamp was pressed down the column and the cell suspension containing potential CTCs was spun onto the slides for 3 minutes at 100 × *g* in a Hettich cyto-centrifuge. The supernatant was carefully discarded and slides were centrifuged once more (150 × *g*, 1 minute) before air drying.

CTC staining and analysis

Glass slides with deposited cells were fixed for 10 minutes in 100% acetone at −20°C and air dried for 30 minutes at room temperature. After 3 washing steps with 1− PBS, cytospins were covered with warm Image-iT FX Signal Enhancer (Thermo Fisher Scientific Inc.) for 30 minutes in the dark. The supernatant was discarded and the antibody cocktail containing anti-pan-cytokeratin (AE1/AE3) Alexa Fluor 488 (1:300; eBioscience, Thermo Fisher Scientific Inc.), anti–pan-keratin (C11) Alexa Fluor 488 Conjugate (1:300; Cell Signaling Technology, Inc.), and anti-CD45 Alexa Fluor 647 antibody (1:50; BioLegend) was added thereafter and incubated for 45 minutes. After washing with 1× PBS, slides were mounted with Vectashield Mounting medium containing DAPI (Vector Laboratories) and covered. Analysis followed one hour later.

To identify CTC candidates, slides were scanned using the semiautomated fluorescent scanning system Ariol SL-50 (Leica Biosystems) with a special CTC scanning software. Slides of each patient were calibrated manually to reflect interpatient background variability before automated scanning. Potential CTC candidates were presented by the software as well as all stained events per slide. Cells were relocated and analyzed through all fluorescent channels plus brightfield by two independent trained scientists (K.E. Effenberger and C. Eulenburg).

Presence of a nucleus, cytokeratin expression, round or oval cell morphology, and absent CD45 expression were the criteria for CTCs. Cut-off value for CTC positivity was one CTC.

CellSearch method

CTC analysis was performed using CellSearch as described previously (14). Blood samples (7.5 mL) were collected in CellSave preservative tubes, stored at room temperature, and processed within 48 hours of collection, according to the manufacturer's instructions. Presence of a nucleus, cytokeratin expression,

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round or oval cell morphology, and absent CD45 expression were the criteria for CTCs (14).

DTC analysis

Tumor cell detection in the bone marrow has been extensively described before (1).

Statistical analysis

IBM SPSS Statistics 23 software (IBM Deutschland GmbH) was used. Histologic characteristics were displayed by descriptive statistics. Missing values were imputed in categorical variables to define separate categories. We applied the χ^2 test to evaluate a potential association between the CTC status and histopathologic parameters.

Survival curves for patient OS and PFS were plotted using the Kaplan–Meier method and analyzed by the log-rank test. Results are presented as median survival in months with 95% confidence interval (CI) and number of patients at risk. Mean values are presented and specifically indicated in case the median survival was not reached. The OS was computed at the time period from the date of surgery to either the date of death, or last follow-up, whichever occurred first. The PFS was defined at the time period from the date of surgery to the date of recurrence, last follow-up, or date of death, whichever occurred first. The Cox regression model was used for multivariate analysis to assess the independent influence of CTCs and other covariates on tumor recurrence and OS. Results are presented as HR with 95% CI. Significant statements refer to *P* values of two-tailed tests that were <0.05. Kaplan–Meier curves were computed using Stata 11.0 (StataCorp LP).

The χ^2 test was used to investigate the association between CTCs and histopathologic parameters. Univariate survival analysis was plotted by the Kaplan–Meier method and analyzed using the log-rank test. The results were presented as the median survival in months with the 95% CI and number of patients at risk. For the multivariate analysis, the Cox regression model was used. The results were presented as HR with 95% CI. Significance was indicated by *P* values of two-tailed tests <0.05.

Results

Patient characteristics and CTC incidence

Sixty-nine patients were included in this study. Thirty-one were female (44.9%) and 38 (55.2%) were male with UICC stages ranging from UICC I ($n = 2$) to IV ($n = 27$; Table 1). Median age at diagnosis was 69 years ranging from 39 to 83. The CTC positivity rate was 33.3% (23/69) ranging from 1 to 19 CTCs per patient; 17 (24.6%) CTC-positive patients had >1 CTC, and 13 (18.8%) >2 CTC. Chemotherapy with gemcitabine was applied in 58 cases (84.1%; 6 cases unknown), neoadjuvant chemo in 3 (4.3%; one unknown), and radiation in 4 cases (5.8%; one unknown).

Within the group of patients who received chemotherapy ($n = 58$), almost one third (29.3%) displayed CTCs before onset of therapy.

As 27 patients (39.1%) presented with distant metastasis at the time of primary diagnosis, 23 underwent palliative surgery, while 33 patients received a Whipple (13 patients with other or combined surgical techniques: 3 subtotal spleen pancreatectomies, 10 total spleen pancreatectomies).

When correlating histopathologic characteristics with the CTC status, the only significant correlation found was between CTC status and application of chemotherapy ($P = 0.039$; Table 1).

Table 1. Histopathologic characteristics and CTC status

Characteristics	Total number of patients	Number or CTC+ (%)	*P*
All	69	23 (33.3%)	
Sex			0.610
Male	38	14 (36.8%)	
Female	31	9 (29.0%)	
Tumor size			0.135
pT1	0	0	
pT2	2	0	
pT3/cT3	40	11 (27.5%)	
pT4/cT4	24	12 (50%)	
pTx	3	0	
Nodal status			0.474
pN0	14	3 (21.4%)	
pN1	33	11 (33.3%)	
pNx	22	9 (40.9%)	
Metastatic stage			0.190
M0	42	11 (26.2%)	
M1	27	12 (44.4%)	
UICC stage			0.290
I	2	0	
II	30	7 (23.3%)	
III	10	4 (40.0%)	
IV	27	12 (44.4%)	
Tumor grade			0.654
G1–2/G2	34	10 (29.4%)	
G2–3/G3	11	5 (45.5%)	
Gx	24	8 (33.3%)	
Chemotherapy			0.039
Yes	58	17 (29.3%)	
No	5	4 (80.0%)	
Missing	6	2	
Surgery			0.668
Whipple	33	11 (33.3%)	
Palliative	23	9 (39.1%)	
Other	13	3 (23.1%)	
Resection margins			0.311
R0	22	7 (31.8%)	
R1	23	6 (26.1%)	
R2	20	7 (35.0%)	
Rx	4	3 (75.0%)	
Bone marrow status			1.000
Positive	8	3 (37.5%)	
Negative	40	14 (35.0%)	
Not evaluable	1	0	

The specificity of our methodology was tested on the blood of 18 healthy donors who did not suffer from tumorigenic disease in the past and were used as a control group. In addition, we included 9 blood specimens from patients with nonmalignant pancreatic disease to test the robustness of our technique. Applying our methodology, none of these control patients showed CTCs.

In addition, we analyzed 49 bone marrow samples in parallel to peripheral blood, both drawn at the same time point. We found DTC in 8 of 49 (16.3%) samples (one was not evaluable; Table 1) and CTC in 17 of 49 (34.7%). There was a 59.2% overlap between CTC and DTC results, of which 3 cases were double positive (CTC and DTC detected). In total, DTC- and/or CTC prevalence was 44.9% (22/49). DTCs did not correlate with survival in this cohort.

Comparison with CellSearch

Twenty patient samples were analyzed with the CellSearch system in parallel. CTC rates were 5/20 (25%) by CellSearch and 10/20 (50%) by MACS enrichment/Ariol. Three cases were not

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evaluable by CellSearch due to technical issues, resulting in 17 comparable blood specimens. CellSearch detected CTC in 5 of 17 cases, whereas 8 of 17 were CTC+ after MACS enrichment/Ariol. Results overlapped in 10 of 17 cases (58.8%), of which 3 cases were commonly CTC+ by both techniques. These 3 "double positive" patients presented with distant metastases (UICC IV).

Univariate survival analysis

Median survival of the whole cohort was 11 months (range, 0–48 months), 8 months (range, 0–24) in the group of CTC+, and 12 months (range, 0–48) in the group of CTC− patients, respectively. Within the CTC+ group ($n = 23$), only one patient was alive at 24 months, whereas within the CTC− patients ($n = 46$), 24% were still alive at 24 months, and one patient at 48 months.

During the observation time, 76.8% (53/69) died while 15.9% (11/69) survived, and 5 patients were lost from follow-up. Five patients died within 4 weeks after surgery and were excluded from all survival analyses. Twenty-nine patients (42.0%) presented with relapse (data of 32 patients were not obtainable).

Furthermore, we investigated whether the CTC status may aid to predict long-time survivors in pancreatic cancer. Indeed, among CTC− patients 37 patients were still alive after 20 months, whereas in CTC+ patients, only two were alive after this period.

PFA was significantly reduced in CTC+ compared with CTC− patients ($P = 0.009$, $n = 33$; Fig. 1A). The same was true for the overall patient survival (OS; $P = 0.030$; $n = 59$; Fig. 1B). Other histopathologic parameters were not affecting PFS in univariate analysis, whereas OS was significantly influenced by UICC status, resection status, and mode of surgery (data not shown).

Within the 58 patients receiving chemotherapy, PFS was significantly reduced for patients harboring CTCs compared with CTC− patients ($P = 0.013$; Fig. 1C), while the effect on OS merely showed a trend in the same direction as well ($P = 0.090$; Fig. 1D).

Multivariate survival analysis

Histopathologic factors that turned out to be significant in univariate survival analysis were included into the multivariate analysis (UICC stage, resection status, mode of surgery). As there were no significant parameters for PFS, those factors being significant for OS were adopted.

Independent of these clinical risk factors, the presence of CTCs elevated the risk of a reduced PFS more than 4-fold (HR = 4.543; CI, 1.549–13.329; $P = 0.006$), and the risk of a shortened OS more than 2-fold (HR = 2.093; CI, 1.081–4.050; $P = 0.028$; Table 2). Other independent risk parameters were for PFS: the resection status (R2 vs. R0) and the mode of surgery (Whipple vs. palliative, and subtotal spleen pancreatectomy vs. palliative), while no other was significant for OS.

Also within chemo-receiving patients, the CTC status stratified patients regarding their PFS time. Presence of CTCs increased the risk of a shorter PFS more than 4-fold compared with absence of CTCs in patient blood (HR = 4.203; CI, 1.416–12.471; $P = 0.010$; Supplementary Table S1). As for the whole patient cohort, resection status and mode of surgery influenced PFS in multivariate analysis, respectively.

Discussion

The diagnosis of pancreatic cancer implies a devastating outcome for patients. For the first time, we were able to show that the CTC status can stratify PDAC patients in terms of their PFS and OS independent of other relevant clinical risk parameters. CTCs can serve as a liquid biopsy and give insight into the actual disseminated tumor load in cancer patients.

Using a combined immunomagnetic CTC enrichment approach, we discovered CTCs in 33% of the patients throughout all UICC stages. To our knowledge, there is only one other study reporting on 105 stage I–IV patients and immunocytochemical CTC detection using keratins with a CTC rate of 26% (4). Another large cohort of 154 patients (stage I–IV) detected $CK20^+$ CTCs by RT-PCR obtaining a CTC rate in line with ours of 34% (15). Using the same setup, Zhang and colleagues report 57% positive cases in 40 stage II + III patients (16), while Hoffmann and colleagues even found CTCs in 65% by RT-PCR for CK19 within 37 patients of mainly stages III and IV (17). The novel finding of our study is that those 33% of the stage I–IV patients with a positive CTC status have a significant higher risk of early relapse (HR = 4.2) as well as death (HR = 2.1) independent of other clinical risk factors.

Results obtained by CellSearch analysis seem controversial and range from 5% (UICC III cohort) to 42% (mainly stage IV; refs. 5, 18–20). Even in metastatic patients, Dotan and colleagues reported 48% positive cases only (21). In our study, analysis of a subset of 20 patients by CellSearch and MACS enrichment in parallel indicates that in case of PDAC, CellSearch may not be the choice method for CTC identification as with MACS, the CTC rate was twice as high. Consequently, a combined cytokeratin/EpCAM enrichment should be preferred for CTC isolation in PDAC.

A couple of other technologies [size-based filtration (ISET), CTC chip, HD-CTC] were applied for PDAC patients with small cohort sizes reporting CTC rates of more than 50% in locally advanced and metastatic settings (22–24).

Nine publications based on either of the aforementioned techniques were combined in a meta-analysis resulting in 623 patients with a CTC rate of 43% (25). The distribution of UICC stages of these patients although was not described. Their pooled analysis identified a reduced OS for CTC+ patients in univariate analysis. These results may deliver a trend if at all as covariates were not regarded. In addition, critical comments on study selection and statistical implementation advice caution though when interpreting this analysis (26).

Bidard and colleagues showed that the CTC status (5% CTC+) evaluated with CellSearch in a cohort of 75 UICC III patients in a follow-up treatment study was an independent prognosticator of OS (RR = 2.5) under certain conditions (time points of blood draw were pooled and treatment not included as risk factor) but not for PFS (18). CellSearch was also recently applied by another group investigating CTCs in 65 stage III and mainly IV patients (20). Reporting presence of CTCs in 32% of the patients, they found a significant correlation between CTC status and OS with an HR of 1.92 for the 56 M1-patients (other risk factors included were pN, locoregional dissemination and M). CTC rate and the significance for OS were in line with our results, although we found this influence through all stages, included more risk factors and observed a significant meaning of CTCs for PFS as well. To our knowledge, all other studies reported univariate survival models only, which is clinically not relevant as other important risk factors were disregarded.

Our results underline that an individual, tumor entity-specific CTC enrichment approach identifies CTCs in PDAC patients' blood very reliably and represents a prognostic biomarker.

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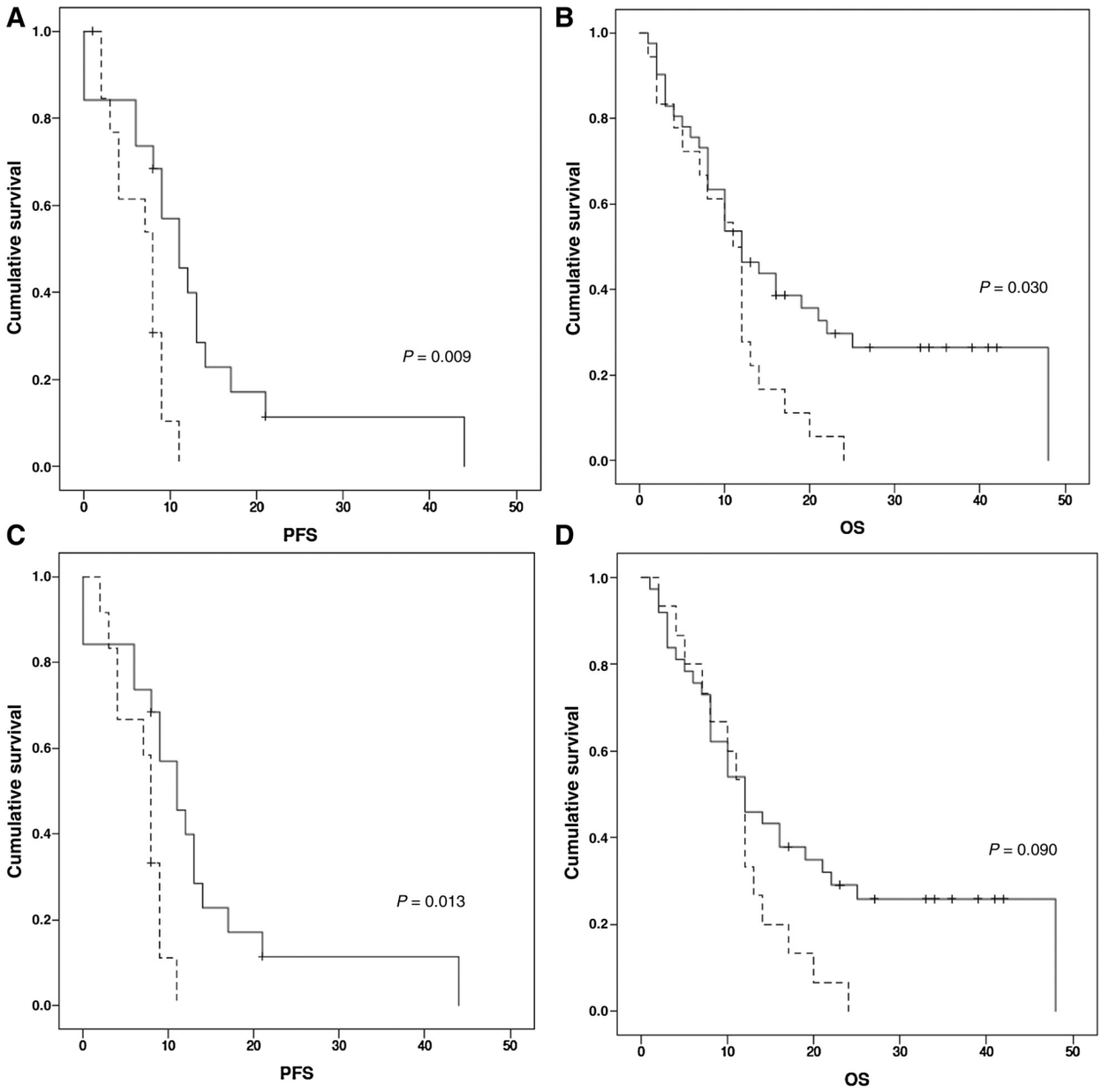

Figure 1.
Univariate survival analysis. **A,** PFS and CTC status of all patients [$n = 33$; 19 CTC− (continuous black), 14 CTC+ (broken black)]. **B,** OS and CTC status of all patients [$n = 59$; 41 CTC− (continuous black), 18 CTC+ (broken black)]. **C,** PFS and CTC status of patients treated with chemotherapy [$n = 31$; 19 CTC− (continuous black), 12 CTC+ (broken black)]. **D,** OS and CTC status of patients treated with chemotherapy [$n = 52$; 37 CTC− (continuous black), 15 CTC+ (broken black)].

In the past, we showed that the DTC status in PDAC patients' bone marrow was a significant independent prognosticator for OS and PFS (1). Here, we analyzed bone marrow and blood in parallel in a subgroup of 49 patients and found an overlap of 59.2% of the cases, which has similarly been reported by Soeth and colleagues with a 61.6% overlap in 117 cases (15). Congruence of positive CTC/DTC findings was quite low in both studies with 6.1% and 13.7%, suggesting that tumor cells in blood and bone marrow represent different classes of minimal residual tumor cells. Although CTCs merely have a half-life of less than 2.5 hours in peripheral blood, DTCs can survive in the bone marrow niche in a dormant state potentially for several years, from where they might enter the blood stream again (recirculation) and potentially may contribute to locoregional relapse or distant metastasis (27–30).

Premalignant lesions within the pancreas are likely to progress to PDAC. In a functional study, Rhim and colleagues were able to show that CTCs maintaining a mesenchymal phenotype escaped

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Table 2. Multivariate analyses of PFS and OS ($n = 64$)

	PFS		OS	
Variables	**HR (95% CI)**	**P^a**	**HR (95% CI)**	**P^a**
Circulating tumor cells				
Neg. vs. pos.	4.543 (1.549–13.329)	0.006	2.093 (1.081–4.050)	0.028
Resection margins				
R1 vs. R0	1.132 (0.430–2.979)	0.801	0.652 (0.270–1.574)	0.314
R2 vs. R0	0.009 (0.000–0.181)	0.002	1.079 (0.086–13.475)	0.953
Rx vs.R0	0.032 (0.001–1.415)	0.075	0.258 (0.015–4.541)	0.354
UICC stage				
UICC I vs. UICC II	0.986 (0.112–8.686)	0.990	0.252 (0.027–2.341)	0.226
UICC III vs. UICC II	1.719 (0.271–10.890)	0.565	2.500 (0.630–9.923)	0.193
UICC IV vs. UICC II	1.714 (0.578–5.084)	0.331	1.969 (0.668–5.802)	0.219
Mode of surgery				
Whipple vs. palliative	0.017 (0.001–0.251)	0.003	0.823 (0.101–6.740)	0.856
SSP[§] vs. palliative	0.042 (0.003–0.630)	0.022	2.241 (0.112–44.736)	0.597
TSP[§§] vs. palliative	–		2.431 (0.265–22.281)	0.432

Abbreviations: SSP, subtotal spleen pancreatectomy; TSP, total spleen pancreatectomy.
[a]Indicates significance according to Cox regression analysis comparing the specified variables.

from pancreatic neoplasia even before PDAC was detectable (31). Also in a clinical study including 61 patients with pancreatic disease (22 PDAC, 9 nonmalignant, 30 healthy individuals), CTCs were detected in a benign pancreatic tumor, in two borderline solid false papillomas and in two healthy control patients (32). These CTCs were CK^- and $CD45^-$ but expressed more than 2 CEP8 hybridization signals, that is, hyperdiploid cells.

The occurrence of potential CTCs in premalignant lesions and a higher CTC rate in PDAC arises the question whether the UICC stage correlates with the presence of CTCs. The answer to this seems controversial. Although we and other groups did not discover significant associations (17, 19, 22, 33–35), others reported correlations between CTCs and UICC (4, 15, 16, 32, 36, 37), nodal (16) or metastatic status (20), or tumor grade (15, 16, 18), regardless of the cohort size or CTC detection method applied in the different studies. The anatomic locus of the pancreas in close proximity to kidney and spleen as well as large blood vessels like large inferior vena cava and portal vein may be responsible for an early CTC dissemination even in low-stage PDAC and consequently for the rapid metastatic tumor spread. In summary, the CTC status may assist to a broader and more precise tumor staging for PDAC.

Median survival of PDAC patients is low regardless of therapy. A very small percentage of patients though are classified as "long-term survivors" (>36 months after surgery) presumably influenced by a low number of lymph node metastases, low preoperative serum CA19-9, and R0-resection (38). The CTC status may represent another influencing factor: In our study, 4 patients were still alive at 36 months, and these were all CTC−. Recently, a 25-gene classifier was published, which identified two survival classes of patients (short and long term) with a 25% and 48% 2-year OS, respectively (39). Prospective clinical validation is still under investigation, and future studies will show whether CTC assessment may provide complementary information. As CTCs can reliably be isolated from peripheral blood, they open new avenues for the assessment of therapy targets by single-cell downstream analysis (3, 40), potentially with emphasis on mechanisms of immune defense, which seem to be important for long-term survival of PDAC patients (10). The current results need to be validated in a larger prospective study.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.E. Effenberger, S. Wolter, J.R. Izbicki, K. Pantel, M. Bockhorn
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Writing, review, and/or revision of the manuscript: K.E. Effenberger, C. Schroeder, C. Eulenburg, J.R. Izbicki, K. Pantel, M. Bockhorn
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.E. Effenberger, C. Schroeder, A. Hanssen, S. Wolter, M. Tachezy, F. Gebauer, J.R. Izbicki, M. Bockhorn
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