Persistent Circulating Tumor Cells at 1 Year After Oncologic Resection Predict Late Recurrence in Pancreatic Cancer

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Objective: The aim of the study was to assess the association between persistent circulating tumor cells (CTCs) and subsequent recurrence in patients who were clinically recurrence free ~12 months postoperatively.

Background: Circulating tumor cells have been proposed as biomarkers to predict survival in pancreatic cancer. Some patients demonstrate persistent CTCs postoperatively, which could represent minimal residual disease.

Methods: Patients from previously published prospective circulating tumor cell in pancreatic cancer trial without clinical evidence of recurrence 12 months postoperatively and CTC testing performed 9 to 15 months postoperatively were included. The presence of epithelial and transitional CTCs (trCTCs) was evaluated as predictor of recurrence. Kaplan-Meier curve, log-rank test, and Cox model were used for survival analysis.

Results: Thirty-three of 129 eligible patients (circulating tumor cell in pancreatic cancer trial) were included. The trCTC-positive and negative patients were well balanced in clinicopathologic features. Patients with trCTCs had a recurrence rate per-person-month of 10.3% compared with 3.1% in trCTCs-negative patients with a median time to recurrence of 3.9 versus 27.1 months, respectively. On multivariable analysis, trCTCs positivity was associated with higher risk of late recurrence (hazard ratio: 4.7, 95% CI, 1.2–18.3, $P = 0.024$). Fourteen (42.4%) patients recurred during the second postoperative year. One-year postoperative trCTCs positivity was associated with a higher rate of recurrence during the second year (odds ratio:13.1, 95% CI, 1.6–1953.4, $P = 0.028$, area under curve = 0.72). Integrating clinicopathologic features with trCTCs increased the area under curve to 0.80. A majority of trCTCs-positive patients (N = 5, 62.5%) had multisite recurrence, followed by local-only (N = 2, 25.0%) and liver-only (N = 1, 12.5%) recurrence. This was in striking contrast to trCTCs-negative patients, where a majority (N = 6, 66.7%) had a local-only recurrence, followed by liver-only (N = 2, 22.2%) and multisite (N = 1, 11.1%) recurrence.

Conclusions: In patients deemed to be clinically disease-free 12 months postoperatively, trCTCs positivity is associated with higher rates of subsequent recurrence with distinct patterns of recurrence. CTCs could be used a putative biomarker to guide patient prognostication and management in pancreatic cancer.

Keywords: biomarkers, circulating tumor cells, EMT, pancreatic ductal adenocarcinoma, pancreatic neoplasms, precision therapy, transitional CTCs

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It is estimated that, at most, one third of patients newly diagnosed with pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC) is a candidate for surgical resection. The only chance for cure or long-term survival of PDAC is through an oncologic resection in patients with clinically localized disease. Optimal survival in this cohort is achieved when resection is combined with multimodal therapies such as cytotoxic systemic agents. However, even among aggressively treated patients with clinically localized disease, most will still develop systemic relapse and die.[1–3]

Pathologic features have been identified that predict relative aggressive behavior and early recurrence for resected PDAC.[4,5] These attributes described on surgical pathology, such as primary tumor size,[6] positive lymph nodes,[7] and resection margin status,[8] are widely accepted as prognostic features but tend to lose their predictive power over time.[9] Moreover, these features are static and represent the disease status at one point in time—that is, at the time of surgical resection. Very few biomarkers exist for PDAC biology that predict the disease status over the treatment cycle and follow-up of patients. Liquid biopsy based on body fluids, particularly blood, serum, and plasma, has the best potential to fulfill this role of a dynamic biomarker.[10–12] For example, in the initial report of our prospective CLUSTER study (circulating tumor cells in pancreatic cancer [NCT02974764]), we have demonstrated that circulating tumor cells (CTCs) obtained from a peripheral blood draw before surgery can predict early recurrence of disease.[13] Similarly, CTCs obtained during adjuvant therapy predict response to therapy (Javed et al. 2020, unpublished data). Other blood biomarkers, such as plasma cell-free tumor DNA (ctDNA), protein biomarkers, extracellular vesicles, and microRNA also hold potential. Our group has developed a Clinical Laboratory Improvement Amendment–approved blood test to measure circulating mutated *k-ras* ctDNA using digital droplet polymerase chain reaction methodology that reliably predicts survival outcome and mirrors disease progression over time.[14]

We have reported that 76% of the recurrences after a potentially curative pancreatectomy for PDAC are metastatic,[15] with recurrence rates of 51.5%, 79.5%, and 97% within 1, 2, and 5 years postsurgery. Recurrence beyond 1 year after surgery, which

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is defined as late recurrence in PDAC, is associated with better overall survival[16] compared with early recurrence (within 1 year after surgery). However, this subgroup of patients is still at risk of recurring at a later date. Identifying subgroups with a high ongoing risk of late recurrence remains a high priority, as interventions such as maintenance chemotherapy may uniquely benefit this group.

In the previous work from our group focusing on early recurrence as the outcome,[13] heterogeneity within CTC populations has been identified and shown to have predictive implications. A group of CTCs expressing a mixed epithelial/mesenchymal phenotype (transitional CTC, trCTCs) was identified that is highly predictive of survival, in contrast to the epithelial phenotype (epithelial CTCs). The trCTCs subgroup is presumed to be in the state of epithelial-to-mesenchymal transition and is important in mediating cancer metastasis.[17–19] In the postsurgery follow-up CTC assays, it was observed that in some patients, trCTCs disappeared from circulation in the immediate postoperative period, whereas in others trCTC persisted, albeit at lower levels. Interestingly, in some patients who made it to 1 year without recurrence, residual trCTCs continued to be detectable.

Given that the estimated half-life of CTC is on the order of minutes,[20,21] we hypothesized that residual trCTCs are a manifestation of dormant micrometastatic disease, and further, that trCTC are predictive of late recurrence. In the current study, we tested this hypothesis using patients enrolled in the ongoing CLUSTER study by assessing the ability of consistent trCTC to predict late recurrence in patients who were disease free at 1 year. We demonstrate that patients who are disease free at 1-year postsurgery but with consistent trCTCs have a higher risk of late recurrence comparing with those without consistent trCTCs. To our knowledge, this is the first study to use follow-up trCTCs at 1-year postsurgery to predict late recurrence in PDAC patients.

METHOD

Patient Cohort

The study population was retrospectively selected from patients enrolled in the prospective CLUSTER study, which completed accrual of 200 patients in March 2018. In this protocol, patients undergo intermittent blood draws for CTC analysis and are followed for 5 years or until death.[13] The inclusion criteria used for the late recurrence cohort were (1) patients who went through successful resection with localized PDAC (with or without neoadjuvant therapy); (2) late recurrence patients who were clinically recurrence free within 1 year after surgery (with or without adjuvant therapy); and (3) patients with blood samples available for CTCs assay within the intended prediction period of 9 to 15 months after surgery. Exclusion criteria include patients diagnosed with recurrence within 30 days after the CTCs assay date in (3).

Data Collection

All patients enrolled were scheduled for postsurgery longitudinal blood collection for CTCs assay every 3 to 6 months in the first 2 years and yearly after that, often in conjunction with scheduled follow-up or treatment unless a patient refused further blood draws, died, or was lost to follow-up. The CTCs assay result within 9 to 15 months after surgery was used as the trCTCs value to predict late recurrence. The result of trCTCs was categorized as either positive (+) or negative (−). If there were more than 1 CTCs assays in the period, the value closest to 15 months was used.

Demographic and clinical information was collected from a prospectively maintained institutional data registry. The presence of malignant cells within 1 mm ($\leq$1 mm) from the surgical margin was defined as R1. Postoperative imaging for recurrence, including abdominal/pelvic and thoracic computed tomography. Clinical recurrence was recorded when there was image evidence of local progression or distant metastasis.

CTCs Enumeration and Characteristics

Each CTCs assay in this study required 10 mL of peripheral blood. Reported results were in CTCs per milliliter of blood. First, for isolation, blood samples were processed within 4 hours with Isolation by Size of Epithelial Tumor Cell assay described previously.[22] Second, immunofluorescent staining was used for CTCs enumeration and phenotype characteristics. A combination of pancytokeratin and vimentin antibodies were utilized to assess epithelial and mesenchymal cell stains, and CD45 was utilized to exclude myeloid-derived cells. CTCs were stratified as epithelial CTCs (pan-cytokeratin+, vimentin−, CD45−) and trCTCs (pan-cytokeratin+, vimentin+, CD45−).[10] Cells with the purely mesenchymal phenotype (pan-cytokeratin−, vimentin+, CD45−) were not recognized in any patient samples. Details of the method were previously described.[13]

Statistical Evaluation

The primary outcome was time to recurrence, defined as from the day of trCTCs assay around 1-year postsurgery to the day of the first recorded clinical recurrence (local and/or distant). Patients without evidence of recurrence were censored at last image recording no detectable recurrence. The secondary outcome was time to death, defined as the day of trCTC assay to the day of death, or censored at last day with contact.[23,24] Kaplan-Meier curve and log-rank test were used to estimate survival distribution and comparisons. Cox proportional-hazard model was used for hazard ratio estimation and controlling for confounders. Variables with >10% missing data were not included in the multivariate analysis. Median follow-up was calculated after excluding patients with death records. For continuous variables, the median and interquartile range were used for description, and t or Wilcoxon test was used for comparisons as appropriate. For categorical variables, frequency and percentage were used for description, and Fisher exact or χ^2 test was used for comparisons as appropriate. All P values were 2 sided. P value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 33 of 129 evaluable patients from the CLUSTER trial met the selection criteria and were included in the study. The details of the patient selection are shown in Figure 1. Comparison of demographic and clinicopathologic features between the study cohort and the overall CLUSTER study population did not show any statistically significant differences (Table S1, Supplemental Digital Content 1, http://links.lww.com/SLA/E259). The median age of the late recurrence cohort was 66 years (IQR [58, 73]) and 42.4% were male. Among these 33 patients, 97.0% demonstrated the presence of CTC of any type. trCTCs were found in 11 (33.3%) patients, with 7 (63.6%), 2 (18.2%), and 2 (18.2%) patients having 1, 2, and 3 trCTCs, respectively. There was no significant difference in demographic and clinicopathologic features between trCTCs-positive and negative groups, as summarized in Table 1. For trCTCs-positive patients, 7 (63.6%) tumors were larger than 2 cm in diameter, 5 (45.6%) had lymph node involvement, 5 (45.5%) patients received neoadjuvant chemotherapy, and 10 (90.9%) who

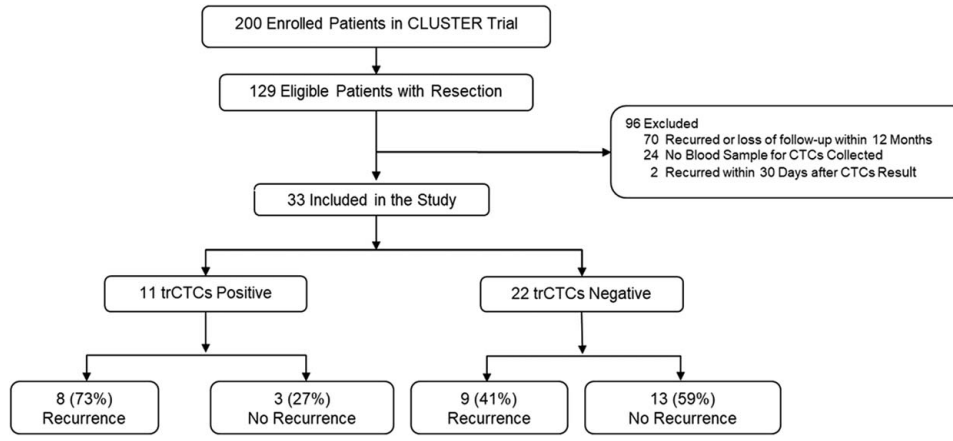

FIGURE 1. Patient cohort selection flow.

had adjuvant chemotherapy (4 patients had neoadjuvant chemotherapy, 6 patients were surgery-first). In addition, a majority of patients (9, 81.8%) had an R0 resection margin.

trCTCs and Prediction of Late Recurrence Risk

The median follow-up for patients from time of the trCTCs assay was 19.7 months (IQR: 14.7–26.1 mo), and from the time of surgical resection was 31.9 months (IQR: 25.8–38.1 mo). Patients with trCTCs had a 10.3% recurrence rate per-person-month (8 recurrences per 77.6 person-month) comparing with 3.1% (9 recurrences per 291.1 person-month) in patients without trCTCs. The median time from surgery to time of trCTC were 12.6 months (IQR: 10.6–13.4 mo) for trCTC positive and 11.4 (IQR: 10.3–12.5 mo) for trCTC-negative patients ($P = 0.43$). The Kaplan-Meier curves comparing the time to recurrence distributions of trCTCs-positive and negative patients are shown in Figure 2. The median time to recurrence for the trCTCs-positive group was 3.9 versus 27.1 months in the trCTCs-negative group. Patients with trCTCs had a 3.0-fold higher risk of late recurrence than those without trCTCs (hazard ratio 3.0 [95% CI, 1.1–8.0], $P = 0.026$). Using difference cutoffs for trCTCs, patients with trCTCs > 1 had a 17.7-fold higher risk of late recurrence than those with trCTCs ≤ 1 (no. 4 vs 29, median: 2.7 vs 27.1 months, hazard ratio: 17.7 [95% CI, 3.8–82.5], $P < 0.001$, Fig. S2, Supplemental Digital Content 1, http://links.lww.com/SLA/E259). Patients with trCTCs > 2 had a 10.0-fold higher risk of late recurrence than those with trCTCs ≤ 2 (no. 2 vs 31, median: 2.7 vs 22.0 mo, hazard ratio: 10.0 [95% CI, 1.9–51.9], $P = 0.006$). Using trCTC count as continuous variable, every increase in trCTC had a 2.7-fold higher risk of late recurrence (hazard ratio: 2.7 [95% CI, 1.5–4.6], $P < 0.001$). As only 2 (6.1%) and 2 (6.1%) patients having 2 and 3 trCTCs, patients with trCTCs $= 0$ (positive) or trCTCs > 0 (positive) was used as the final categorical groups.

After controlling for important clinical and pathologic features in a multivariable model, the risk of late recurrence was 4.7 times higher in patients with trCTCs than those without trCTCs (hazard ratio: 4.7 [95% CI, 1.2–18.3, $P = 0.029$]). The results of univariate and multivariate Cox model are summarized in Table 2.

Fourteen (42.4%) of the 33 patients included in this study presented with recurrence within the second after surgery. We further sought to use the available trCTCs around 1-year postsurgery to predict disease recurrence within the second year after surgery. Positive trCTCs at 1-year postsurgery were associated with a higher recurrence rate in the second year (odds ratio 9.7 [95% CI, 1.6–70.5], $P = 0.02$). The area under curve of trCTCs for prediction of second-year recurrence was 0.72. The area under curve of the multivariate logistic prediction model with clinical and pathologic features and trCTCs was 0.80 (Fig. 3). The univariate and multivariate results of the logistic prediction model are summarized in Table 3.

TABLE 1. Comparisons of Demographic and Clinicopathologic Features Between trCTC-positive and Negative Patients

Features	trCTC Negative N = 22	trCTC Positive N = 11	*P*
Sex, female (%)	10 (45.5)	4 (36.4)	0.901
Age [median (IQR)]	67.00 (59.50, 71.00)	63.00 [53.50, 81.50]	0.924
Neoadjuvant chemotherapy = yes (%)	11 (50.0)	5 (45.5)	1.000
Surgery type, n (%)	—	—	0.153
DP	6 (27.3)	0	—
TP	1 (4.5)	1 (9.1)	—
Whipple	15 (68.2)	10 (90.9)	—
Tumor size, cm, n (%)	—	—	0.741
< 2	11 (50.0)	4 (36.4)	—
2-4	9 (40.9)	6 (54.5)	—
> 4	2 (9.1)	1 (9.1)	—
Positive lymph nodes, n (%)	—	—	0.769
0	10 (45.5)	6 (54.5)	—
1-3	8 (36.4)	4 (36.4)	—
> 3	4 (18.2)	1 (9.1)	—
Grade, n (%)	—	—	1.000
Well/moderate	15 (73.3)	8 (80.0)	—
Poor	3 (16.7)	2 (20.0)	—
Margin, positive, n (%)	1 (4.5)	2 (18.2)	0.521
Perineural invasion, present, n (%)	12 (54.5)	9 (81.8)	0.250
Lymphovascular invasion, present, n (%)	6 (30.0)	4 (44.4)	0.738
Adjuvant chemotherapy, administered, n (%)	19 (86.4)	10 (90.9)	1.000

DP indicates distal pancreatectomy; TP, total pancreatectomy.

CTCs Longitudinal Dynamics and Prediction of Late Recurrence Risk

In priori CLUSTER study, we showed the importance of the evolution in presurgery, postsurgery, and 3- to 6-month

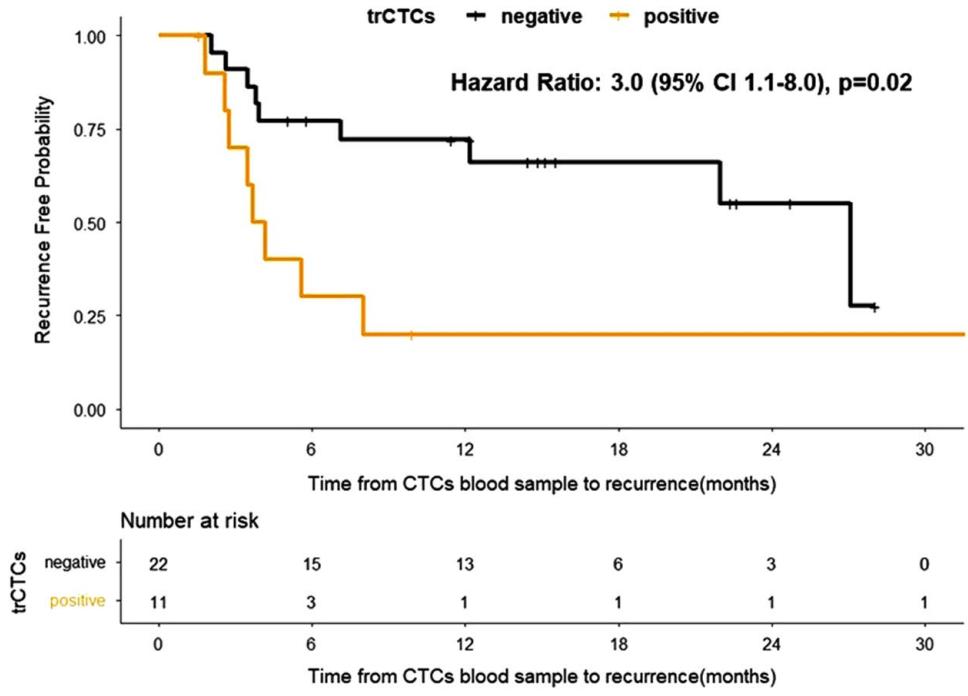

FIGURE 2. Kaplan-Meier curves of time to recurrence for patients with or without trCTCs.

postsurgery trCTC counts in predicting early recurrence. In this study, we also analyzed the change between trCTC around 1 year (9–15 mo postsurgery) and the trCTC before this period. Within all 33 patients in the study, we found 8 (24.2%), 12 (36.4%), and 13 (39.4%) patients with the most recent trCTC assay within 0- to 2-month, 3- to 5-month, and 6- to 8-month postsurgery. The median time between most recent trCTC and trCTC within 9 to 15 months was 6.0 months (IQR [5.3, 9.2]). The evolution in trCTC was correlated with trCTC around 9 to 15 months (0.85, $P < 0.001$). In univariable model, every increase in sequential trCTC had a 2.1-fold higher risk of late recurrence (hazard ratio: 2.1 [95% CI, 1.1–4.1], $P = 0.028$). In multivariable model after controlling for important clinical and pathologic features, the hazard ratio for late recurrence with each increase in sequential trCTC was 2.3 ([95% CI, 1.1–5.7], $P = 0.05$). The univariate and multivariate results of the logistic prediction model are summarized in Table 4.

trCTCs and Late Recurrence Pattern

Seventeen (51.5%) patients had recurrences between the time of trCTCs assay around 1-year postsurgery to the time of data analysis, with a systemic recurrence rate of 52.9% (n = 9). Eight of 11 (72.7%) patients with trCTCs had a recurrence compared with nine of 22 (40.9%) patients without trCTCs. For patients with trCTCs, 5 (62.5%) had multiple-site metastases, followed by 1 (12.5%) with liver-only and 2 (25.0%) with local-only recurrence. This is in striking contrast to the pattern of recurrence in trCTCs-negative patients. In this cohort, the majority (6, 66.7%) had a local-only recurrence, followed by 2 (22.2%) with liver-only and 1 (11.1%) multiple-site metastases. Patients with trCTCs had a higher rate of systemic metastases than those without trCTCs (75.0% vs 33.3%, $P = 0.15$). Although not statistically significant, the result still showed a propensity for systemic recurrence in trCTCs-positive patients.

DISCUSSION

In this study, we identified a group of patients who were recurrence free using standard clinical metrics at 1 year after surgery but had persistent trCTCs. We demonstrated that trCTCs are independently associated with significant higher risk of late recurrence. Moreover, in this group, patients with consistent trCTCs at 1-year postsurgery are more likely to have a systematic recurrence.

TABLE 2. Univariate and Multivariate Analyses of trCTCs, Clinicopathologic Features' Associations With Late Recurrence

	Univariate		Multivariate	
Features	**Hazard Ratio**	***P***	**Hazard Ratio**	***P***
trCTC: positive vs negative	3 (1.1-8)	0.026	4.7 (1.2-18.3)	0.024
Age: 66 or above vs younger than 66 y	1.2 (0.5-3.2)	0.692	1.7 (0.5-5.9)	0.428
Neoadjuvant chemotherapy: yes vs no	1.2 (0.4-3)	0.751	1.4 (0.4-5.1)	0.599
Adjuvant chemotherapy: yes vs no	1.3 (0.2-10.1)	0.786	0.6 (0.1-6.6)	0.711
Tumor size (cm): >2 to ≤ 4 vs ≤ 2	1.6 (0.6-4.4)	0.398	2 (0.4-9.7)	0.377
Tumor size (cm): >4 vs ≤ 2	1.4 (0.3-7.1)	0.683	3.4 (0.3-33.1)	0.299
Positive lymph nodes: >0 to ≤ 3 vs 0	0.7 (0.2-2.3)	0.612	0.6 (0.1-4.2)	0.573
Positive lymph nodes: >3 vs 0	1.6 (0.5-5.5)	0.438	1.5 (0.2-10.1)	0.675
Margin: R1 vs R0	1.5 (0.3-6.8)	0.615	0.9 (0.1-7.2)	0.882

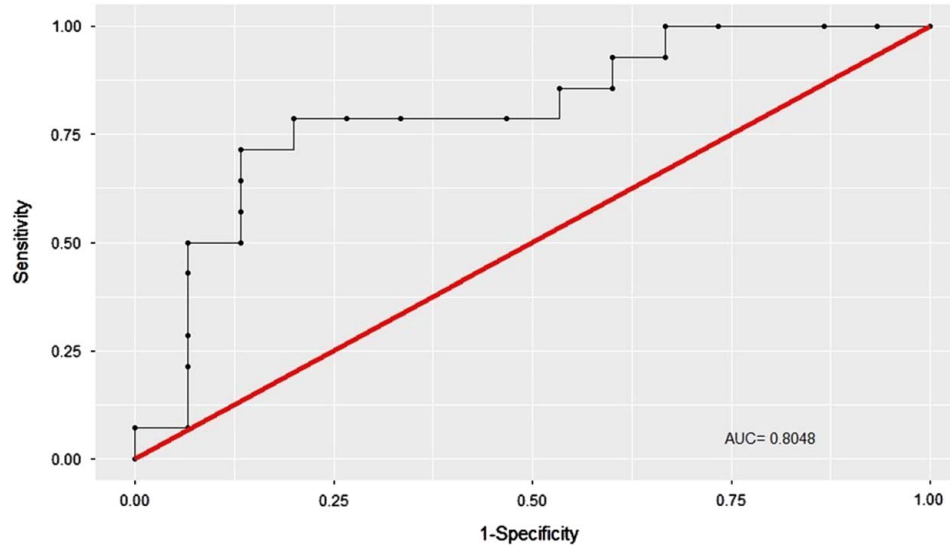

FIGURE 3. ROC curve of trCTCs and clinicalpathologic features to predictive late recurrence within or later than the second year. ROC indicates receiver operating characteristic.

To our knowledge, this is the first report of the association between dynamic trCTCs at 1-year postsurgery and late recurrence risk for PDAC patients. The significant difference observed herein makes trCTCs at 1-year postsurgery a promising biomarker to stratify patients with a higher risk for late recurrence. Although there is still a gap to clinical use, trCTCs dynamics provide important indications for further clinical trial designs, particularly follow-up schedules, adjuvant chemotherapy plans, or early interventions.[25,26]

Longitudinal CTCs assays can provide a window to look into the residual disease and follow disease resurgence dynamically. Persistent CTCs during the following are associated with recurrence, and distant associations of post-surgery persistent CDCs with recurrence and overall survival have been reported in breast and colorectal cancer before.[27,28] In our study, among the 11 patients with trCTCs at 1-year postsurgery, 3 (22.3%) had both negative presurgery and postsurgery trCTCs. Previous work has demonstrated that CTCs mediate micrometastases in local or distant organs, and this phenomenon has been observed at an early stage of tumor genesis, even before overt primary tumor detection.[29,30] Of note, 6 (54.5%) out of the 11 patients had positive presurgery but negative postsurgery trCTCs, and only 2 (18.2%) had both positive presurgery and postsurgery trCTCs. The majority (9, 81.8%) with undetectable postsurgery and relapsed trCTCs at 1 year after surgery may go through the dormant and proliferative status, which can be heterogeneous among patients.[31–33] Those disseminated tumor cells could enter a dormant state while maintaining the ability to generate late recurrence in specific microenvironments.[34] The organ sites where disseminated or dormant tumor cells hide might be liver or bone marrow, as suggested in previous studies,[35,36] which will need further investigations to describe the mechanisms.

Dynamic trCTCs at 1-year postsurgery take over as an independent predictor for late recurrence when presurgery trCTCs fail to further stratify higher recurrence risk in the late recurrence group ($P = 0.32$). The reasons that dynamic trCTCs status is a better predictor for late recurrence maybe (1) after resection, the change of tumor burden, response of the immune system, and surgery itself all contribute to the dynamic trCTCs level; (2) trCTCs level around 1 year is the updated biomarker that could better reflect the activity level of minimal residual disease or micrometastases before turning into overt recurrent neoplasms; (3) the intermediate epithelial-mesenchymal transition (EMT) could be a complex system with heterogeneity among trCTCs. Each trCTC could represent a unique clone and dominate clones and their expression profiles might change over time during the cascade of metastasis. For example, adjuvant chemotherapy and the immune microenvironment may influence the clone selection or expression profile regulation. Consequently, longitudinal CTC analysis allows for the observation of dynamic changes over time.

TABLE 3. Univariate and Multivariate Analyses of trCTCs, Clinicopathologic Features' Associations With Recurrence Within the Second Year After Surgery for Late Recurrence Patients

	Univariate		Multivariate	
Features	**Odds Ratio**	***P***	**Odds Ratio**	***P***
trCTC: positive vs negative	8.7 (1.6-195.4)	0.02	13.1 (1.6-195.4)	0.028
Age: 66 or above vs younger than 66 y	0.9 (1.6-195.4)	0.876	1.2 (0.2-9.6)	0.882
Neoadjuvant chemotherapy: yes vs no	1.5 (1.6-195.4)	0.589	1.1 (0.1-9.7)	0.959
Adjuvant chemotherapy: yes vs no	0.9 (1.6-195.4)	0.96	0.6 (0-52.5)	0.81
Tumor size (cm): >2 to ≤ 4 vs ≤ 2	1 (0.2-4.8)	1	0.6 (0.1-6.2)	0.67
Tumor size (cm): >4 vs ≤ 2	0.5 (0-6.7)	0.609	0.6 (0-40.4)	0.794
Positive lymph nodes: >0 to ≤ 3 vs 0	0.4 (0.1-2.3)	0.345	0.6 (0-10.6)	0.693
Positive lymph nodes: >3 vs 0	1.3 (0.2-12.3)	0.796	3.3 (0.2-65.5)	0.388
Margin: R1 vs R0	2.3 (1.6-195.4)	0.51	1.4 (0-68.2)	0.85

TABLE 4. Univariate and Multivariate Analyses of trCTCs Change Around 1 Year, Clinicopathologic Features' Associations With Late Recurrence

	Univariable		Multivariable	
Features	**Hazard Ratio**	***P***	**Hazard Ratio**	***P***
trCTC change	2.1 (1.1-4.1)	0.026	2.4 (1.0-5.7)	0.050
Age: 66 or above vs younger than 66 y	1.2 (0.5-3.2)	0.692	1.1 (0.3-3.9)	0.824
Neoadjuvant chemotherapy: yes vs no	1.2 (0.4-3)	0.751	1.9 (0.5-7.0)	0.341
Adjuvant chemotherapy: yes vs no	1.3 (0.2-10.1)	0.786	1.1 (0.1-9.7)	0.949
Tumor size (cm): >2 to ≤ 4 vs ≤ 2	1.6 (0.6-4.4)	0.398	2.4 (0.5-11.4)	0.281
Tumor size (cm): >4 vs ≤ 2	1.4 (0.3-7.1)	0.683	2.5 (0.3-24.5)	0.434
Positive lymph nodes: >0 to ≤ 3 vs 0	0.7 (0.2-2.3)	0.612	0.7 (0.1-5.1)	0.718
Positive lymph nodes: >3 vs 0	1.6 (0.5-5.5)	0.438	0.9 (0.2-5.5)	0.937
Margin: R1 vs R0	1.5 (0.3-6.8)	0.615	0.8 (0.1-7.4)	0.813

Dynamic trCTCs were not correlated with major pathologic features, including tumor size, positive lymph nodes, margin status, histologic grade, or perineural invasion. Similar results were reported in a recent study of 69 patients.[37] Compared with the pathologic features, which were tumor-centric, trCTCs at 1-year postsurgery were more likely seeded from micrometastases, which can happen at any time, even an early stage before overt primary tumor development. Consequently, dynamic trCTCs may not reflect the primary tumor's characteristics at resection. Rather, trCTCs may be a more direct assessment of the driving source of recurrence.

The trCTCs coexpress mesenchymal and epithelial markers, which indicates this cell population is in the intermediate stage of EMT, and are believed to be the phenotype with the most plasticity in terms of extravasation and colonization.[38–40] Except for PDAC patients in our study, CTCs with mixed epithelial and mesenchymal markers have been previously reported as an independent predictor of poor outcomes in other solid tumors.[41] EMT has been extensively studied to promote cancer metastasis. Contrary to cells with exclusively epithelial phenotypes, which are showed to have no capacity to form solid metastases in a new microenvironment,[42–44] the mesenchymal phenotype confers the capacity to invade, disseminate, and metastasize.[45,46] Of note, the complete switch to a purely mesenchymal phenotype is rarely reported,[47] and a complete inexpression of all epithelial markers in human carcinoma is probably less frequent than expected.[48]

As for the recurrence pattern, a trend of higher late systemic recurrence was observed in patients with persistent trCTCs. It is consistent with our hypothesis that trCTCs are important sources for distant or multiorgan micrometastases. The association of persistence of CTCs during follow-up and distant recurrence has also been reported in stage I non–small cell lung cancer after radiation.[49] On the other hand, local recurrence could also develop from disseminated tumor cells from distant micrometastases returning to the peripancreatic region. However, only 51.7% of patients had records of recurrence patterns and the follow-up time may not be enough to reach significant results. The same follow-up limitation affects the stratification for higher death risk using trCTCs at 1-year postsurgery. The Kaplan-Meier curve showed a notable trend for a better overall survival for trCTCs-negative patients but not statistically significant (Fig. S1, Supplemental Digital Content 1, http://links.lww.com/SLA/E259). Further studies may be able to address these questions in the future.

Limitations

First, the CTCs assay time points for each patient are not uniform. The blood sample for CTCs test was always done in conjunction with routine clinical treatment or follow-up. The last available CTC assay within a window of 12 months +/− 3 months after surgery was used for each patient, making the start time of calculating point to recurrence slightly different. However, this may be the most common scenario clinicians would meet when CTCs are introduced in clinical practice. Moreover, we tried to avoid lead-time bias by blinding the providers to the CTCs result so that positive CTCs results would not trigger earlier or additional clinical or imaging tests. Second, the variances were large with wide confidence intervals for hazard ratios and odds ratios in the Cox and logistic models. The main reason was the small sample size and controlled confounders in limited sample size, especially for the logistic models. The odds ratios and hazard ratios might change with larger sample size.

Furthermore, additional statistical significance may be observed in similar studies with increased sample sizes. Third, the CLUSTER study is a prospective trial that was not designed for this late recurrence study. This study was retrospective in nature to allow for this secondary analysis. Patients who were lost to follow-up or without available CTCs assay around 1 year were excluded from this study. Although the comparisons of demographic and clinicopathologic features between the present late recurrence study and the CLUSTER study showed no significant differences (Table S1, Supplemental Digital Content 1, http://links.lww.com/SLA/E259), there may still be selection bias introduced in the process. This study does not have follow-up CA19-9 data at the same trCTC time points, which was not collected when CLUSTER study was designed. We acknowledge that having data on CA19-9 could potentially impact the findings of the study. In the future, studies evaluating the impact of CA19-9 on the findings are required. Lastly, some patients' follow-up time is not long enough for late recurrence patterns to be adequately observed, especially among the trCTC-negative group. Future studies should be able to give a clearer picture of recurrence patterns with the use of dynamic CTC analyses.

REFERENCES

1. Gemenetzis G, Groot VP, Blair AB, et al. Survival in locally advanced pancreatic cancer after neoadjuvant therapy and surgical resection. *Ann Surg*. 2019;270:340–347.
2. Hishinuma S, Ogata Y, Tomikawa M, et al. Patterns of recurrence after curative resection of pancreatic cancer, based on autopsy findings. *J Gastrointest Surg*. 2006;10:511–518.
3. Sergeant G, Ectors N, Van Steenbergen W, et al. Patterns of recurrence after curative resection of pancreatic ductal adenocarcinoma. *Eur J Surg Oncol*. 2009;35:600–604.
4. Riall TS, Cameron JL, Lillemoe KD, et al. Resected periampullary adenocarcinoma: 5-year survivors and their 6-to 10-year follow-up. *Surgery*. 2006;140:764–772.

5. Cameron JL, He J. Two thousand consecutive pancreaticoduodenectomies. *J Am Coll Surg*. 2015;220:530–536.
6. Fortner JG, Klimstra DA, Senie RT, et al. Tumor size is the primary prognosticator for pancreatic cancer after regional pancreatectomy. *Ann Surg*. 1996;223:147.
7. Pawlik TM, Gleisner AL, Cameron JL, et al. Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. *Surgery*. 2007;141:610–618.
8. Butturini G, Stocken DD, Wente MN, et al. Influence of resection margins and treatment on survival in patients with pancreatic cancer: meta-analysis of randomized controlled trials. *Arch Surg*. 2008;143:75–83.
9. Groot VP, Gemenetzis G, Blair AB, et al. Implications of the pattern of disease recurrence on survival following pancreatectomy for pancreatic ductal adenocarcinoma. *Ann Surg Oncol*. 2018;25:2475–2483.
10. Poruk KE, Valero V, 3rd, Saunders T, et al. Circulating tumor cell phenotype predicts recurrence and survival in pancreatic adenocarcinoma. *Ann Surg*. 2016;264:1073–1081.
11. Chang MC, Chang YT, Chen JY, et al. Clinical significance of circulating tumor microemboli as a prognostic marker in patients with pancreatic ductal adenocarcinoma. *Clin Chem*. 2016;62:505–13.
12. Court CM, Ankeny JS, Sho S, et al. Circulating tumor cells predict occult metastatic disease and prognosis in pancreatic cancer. *Ann Surg Oncol*. 2018;25:1000–1008.
13. Gemenetzis G, Groot VP, Yu J, et al. Circulating tumor cells dynamics in pancreatic adenocarcinoma correlate with disease status: results of the prospective CLUSTER study. *Ann Surg*. 2018;268:408–420.
14. Groot VP, Mosier S, Javed AA, et al. Circulating Tumor DNA as a Clinical Test in Resected Pancreatic Cancer. *Clin Cancer Res*. 2019;25:4973–4984.
15. Groot VP, Rezaee N, Wu W, et al. Patterns, timing, and predictors of recurrence following pancreatectomy for pancreatic ductal adenocarcinoma. *Ann Surg*. 2018;267:936–945.
16. Groot VP, Gemenetzis G, Blair AB, et al. Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann Surg*. 2018;269:1154–1162.
17. Nieto MA, Huang RY-J, Jackson RA, et al. EMT: 2016. *Cell*. 2016;166:21–45.
18. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. *Cell*. 2017;168:670–691.
19. Rasheed ZA, Yang J, Wang Q, et al. Prognostic significance of tumorigenic cells with mesenchymal features in pancreatic adenocarcinoma. *J Natl Cancer Inst*. 2010;102:340–351.
20. Stott SL, Lee RJ, Nagrath S, et al. Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. *Sci Transl Med*. 2010;2:25ra23–25ra23.
21. Meng S, Tripathy D, Frenkel EP, et al. Circulating tumor cells in patients with breast cancer dormancy. *Clin Cancer Res*. 2004;10:8152–8162.
22. Laget S, Broncy L, Hormigos K, et al. Technical insights into highly sensitive isolation and molecular characterization of fixed and live circulating tumor cells for early detection of tumor invasion. *PloS One*. 2017;12:e0169427.
23. Sparano J, O'Neill A, Alpaugh K, et al. Association of circulating tumor cells with late reurrence of estrogen receptor-positive breast cancer: a secondary analysis of a randomized clinical trial. *JAMA Oncol*. 2018;4:1700–1706.
24. Moon G, Noh H, Cho IJ, et al. Prediction of late recurrence in patients with breast cancer: elevated neutrophil to lymphocyte ratio (NLR) at 5 years after diagnosis and late recurrence. *Breast Cancer*. 2020;27:54–61.
25. Ning Y, Zhang W, Hanna DL, et al. Clinical relevance of EMT and stem-like gene expression in circulating tumor cells of metastatic colorectal cancer patients. *Pharmacogenomics J*. 2018;18:29–34.
26. Satelli A, Mitra A, Brownlee Z, et al. Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression. *Clin Cancer Res*. 2015;21:899–906.
27. Uen Y-H, Lu C-Y, Tsai H-L, et al. Persistent presence of postoperative circulating tumor cells is a poor prognostic factor for patients with stage I–III colorectal cancer after curative resection. *Ann Surg Oncol*. 2008;15:2120.
28. Kwan TT, Bardia A, Spring LM, et al. A digital RNA signature of circulating tumor cells predicting early therapeutic response in localized and metastatic breast cancer. *Cancer Discov*. 2018;8:1286–1299.
29. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. *Cell*. 2012;148:349–361.
30. Rhim AD. Epithelial to mesenchymal transition and the generation of stem-like cells in pancreatic cancer. *Pancreatology*. 2013;13:114–117.
31. Alvarez-Cubero MJ, Vazquez-Alonso F, Puche-Sanz I, et al. Dormant circulating tumor cells in prostate cancer: therapeutic, clinical and biological implications. *Curr Drug Targets*. 2016;17:693–701.
32. Gomis RR, Gawrzak S. Tumor cell dormancy. *Mol Oncol*. 2017;11: 62–78.
33. Micalizzi DS, Maheswaran S, Haber DA. A conduit to metastasis: circulating tumor cell biology. *Genes Dev*. 2017;31:1827–1840.
34. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer*. 2007;7:834–846.
35. Lenk L, Pein M, Will O, et al. The hepatic microenvironment essentially determines tumor cell dormancy and metastatic outgrowth of pancreatic ductal adenocarcinoma. *Oncoimmunology*. 2017;7:e1368603.
36. Alix-Panabieres C, Riethdorf S, Pantel K. Circulating tumor cells and bone marrow micrometastasis. *Clin Cancer Res*. 2008;14:5013–21.
37. Effenberger KE, Schroeder C, Hanssen A, et al. Improved risk stratification by circulating tumor cell counts in pancreatic cancer. *Clin Cancer Res*. 2018;24:2844–2850.
38. Jolly MK, Boareto M, Huang B, et al. Implications of the hybrid epithelial/mesenchymal phenotype in metastasis. *Front Oncol*. 2015; 5:155.
39. Barriere G, Fici P, Gallerani G, et al. Circulating tumor cells and epithelial, mesenchymal and stemness markers: characterization of cell subpopulations. *Ann Transl Med*. 2014;2:109.
40. Cayrefourcq L, Mazard T, Joosse S, et al. Establishment and characterization of a cell line from human circulating colon cancer cells. *Cancer Res*. 2015;75:892–901.
41. Bulfoni M, Gerratana L, Del Ben F, et al. In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis. *Breast Cancer Res*. 2016;18:30.
42. Kang Y, Pantel K. Tumor cell dissemination: emerging biological insights from animal models and cancer patients. *Cancer cell*. 2013;23: 573–581.
43. Alix-Panabieres C, Mader S, Pantel K. Epithelial-mesenchymal plasticity in circulating tumor cells. *J Mol Med (Berl)*. 2017;95:133–142.
44. Mani SA, Guo W, Liao M-J, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008;133:704–715.
45. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer*. 2009;9:265–273.
46. Weinberg RA. Mechanisms of malignant progression. *Carcinogenesis*. 2008;29:1092–1095.
47. Francart ME, Lambert J, Vanwynsberghe AM, et al. Epithelial-mesenchymal plasticity and circulating tumor cells: travel companions to metastases. *Dev Dyn*. 2018;247:432–450.
48. Willipinski-Stapelfeldt B, Riethdorf S, Assmann V, et al. Changes in cytoskeletal protein composition indicative of an epithelial-mesenchymal transition in human micrometastatic and primary breast carcinoma cells. *Clin Cancer Res*. 2005;11:8006–8014.
49. Frick M. Persistence of circulating tumor cells as a biomarker for recurrent disease following stereotactic body Radiation Therapy for Stage I Non-Small Cell Lung Cancer. *Int J Radiat Oncol Biol Phys*. 2019;105: S115.