A Delay in Adjuvant Therapy Is Associated With Worse Prognosis Only in Patients With Transitional Circulating Tumor Cells After Resection of Pancreatic Ductal Adenocarcinoma

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Objectives: The aim of the study was to assess the association of circulating tumor cells (CTCs) with survival as a biomarker in pancreatic ductal adenocarcinoma (PDAC) within the context of a delay in the initiation of adjuvant therapy.

Background: Outcomes in patients with PDAC remain poor and are driven by aggressive systemic disease. Although systemic therapies improve survival in resected patients, factors such as a delay in the initiation of adjuvant therapy are associated with worse outcomes. CTCs have previously been shown to be predictive of survival.

Methods: A retrospective study was performed on PDAC patients enrolled in the prospective CircuLating tUmor cellS in pancreaTic cancER trial (NCT02974764) on CTC-dynamics at the Johns Hopkins Hospital. CTCs were isolated based on size (isolation by size of epithelial tumor cells; Rarecells) and counted and characterized by subtype using immunofluorescence. The preoperative and postoperative blood samples were used to identify 2 CTC types: epithelial CTCs (eCTCs), expressing pancytokeratin, and transitional CTCs (trCTCs), expressing both pancytokeratin and vimentin. Patients who received adjuvant therapy were compared with those who did not. A delay in the receipt of adjuvant therapy was defined as the initiation of therapy ≥ 8 weeks after surgical resection. Clinicopathologic features, CTCs characteristics, and outcomes were analyzed.

Results: Of 101 patients included in the study, 43 (42.5%) experienced a delay in initiation and 20 (19.8%) did not receive adjuvant therapy. On multivariable analysis, the presence of trCTCs ($P = 0.002$) and the absence of adjuvant therapy ($P = 0.032$) were associated with worse recurrence-free survival (RFS). Postoperative trCTC were associated with poorer RFS, both in patients with a delay in initiation (12.4 vs 17.9 mo, $P = 0.004$) or no administration of adjuvant chemotherapy (3.4 vs NR, $P = 0.016$). However, it was not associated with RFS in patients with timely initiation of adjuvant chemotherapy ($P = 0.293$).

Conclusions: Postoperative trCTCs positivity is associated with poorer RFS only in patients who either experience a delay in initiation or no receipt of adjuvant therapy. This study suggests that a delay in the initiation of adjuvant therapy could potentially provide residual systemic disease (trCTCs) a window of opportunity to recover from the surgical insult. Future studies are required to validate these findings and explore the underlying mechanisms involved.

Keywords: adjuvant therapy, circulating tumor cells, pancreatic ductal adenocarcinoma, pancreatic neoplasms, survival, transitional CTCs

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Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease, with a 5-year survival of only 10%, and remains the third leading cause of all cancer-associated deaths in the United States.[1] These poor outcomes result from a predisposition for early systemic dissemination and an absence of adequate screening methods.[2] Surgical resection remains the only possibility for a cure; however, the rate of recurrence even in this group is nearly 80%, suggesting that minimal residual disease drives patient outcomes (survival).[3]

Currently, predictive biomarkers that are capable of accurately informing clinical decision-making have not been identified in PDAC. Although CA19-9 is useful, it is limited by its poor sensitivity and specificity. Circulating tumor cells (CTCs) have shown promise as prognostic markers in PDAC.[4–6] Previous studies have established phenotypic heterogeneity in CTCs and their association with patient outcomes. Specifically, our group and others have shown that the transitional type CTCs (trCTCs), expressing both epithelial and mesenchymal markers, are associated with poor patient outcomes and that locoregional and systemic therapy can alter CTC characteristics.[4–15]

Previous work has shown that delays in or missed adjuvant chemotherapy are associated with worse survival.[16] One explanation for this observation is that a delay in chemotherapy provides residual circulating disease a window to recover from the surgical insult or systemic therapy administered in the neoadjuvant setting. Given that CTCs are hypothesized to be a reflection of minimal residual disease, the aim of the current study was to assess the association of CTCs with survival in the context of a delay in the initiation of adjuvant therapy.

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METHODS

Patient Recruitment and Data Collection

Patients were selected from the cohort enrolled in the longitudinal prospective randomized CircuLating tUmor cellS in pancreaTic cancER (CLUSTER) trial (NCT02974764) conducted at Johns Hopkins Hospital from March 2016 to March 2018.[4] Of the 200 patients enrolled, those who underwent successful resection, had postoperative blood samples analyzed, and had data available on adjuvant therapy status were included in this study (Fig. 1).

Clinicopathologic data, including demographic information, details on presentation, surgical approach, histopathologic data and information regarding administration of systemic therapy, and survival were extracted from a prospectively maintained institutional registry on patients managed for pancreatic diseases at the Johns Hopkins Hospital. Missing data were collected retrospectively from patients' electronic medical records.

Sample Collection and CTC Characterization

Patients included in the study were sampled at multiple time points during their treatment course. CTC results from the postoperative blood sample, collected and analyzed 4 to 6 days postoperatively, before discharge, were used for analysis in this study. The 10 mL of blood drawn at this time point was processed within 4 hours for CTC enrichment using the Isolation by Size of Epithelial Tumor Cells assay (Rarecells). Details regarding the methods used for CTC isolation and characterization have previously been reported by our group.[4,5] In brief, CTCs were fixed using formaldehyde, filtered on to a 10-core membrane, and stored in -20°C until characterization. In addition, commercially available pancreatic cancer cell lines were spiked in healthy donor blood, run through this assay and stained using immunofluorescence to establish baseline and control parameters; donor blood was subjected to the same protocol and used as a negative control, verifying the absence of circulating epithelial cells in the healthy cohort (Gemenetzis et al[4], supplementary methods).

CTC characterization was achieved through immunofluorescent staining, performed as described in Gemenetzis et al.[4] Cells that stained positive with the epithelial marker anti-pancytokeratin (Thermo Fisher; FITC) only were characterized as epithelial-type CTCs (eCTCs); cells positive for pancytokeratin that also stained positive with the mesenchymal marker antivimentin (Thermo Fisher; AlexaFluor 594) were characterized as epithelial-mesenchymal, or transitional CTCs (trCTCs). White blood cells were excluded from analysis utilizing concurrent staining with anti-CD45, anti-CD11b, anti-CD14, and anti-CD34 (Thermo Fisher, AlexaFluor 647). DAPI ProLong Gold was used as a nuclear counterstain and mountant (Thermo Fisher).

CTCs were identified using the Nikon Ti-E inverted microscope system (Nikon, Japan) based on CD-marker negativity and pancytokeratin and/or vimentin positivity, as described in Gemenetzis et al[4] and Poruk et al.[5] Cellular morphology, such as the shape of nuclei, overall size, and nuclear/cytoplasmic ratio, was further used to confirm cells as CTCs on phase-contrast light microscopy. Reviewers were blinded to patient clinical data, and the results are reported as CTCs per milliliter of blood.

No cells with a purely mesenchymal phenotype (pancytokeratin negative, vimentin positive, and CD positive) were observed. Morphologic details and genetic information of observed CTCs and white blood cells were as previously described.[4,5] CTCs were not observed in any healthy volunteer samples.

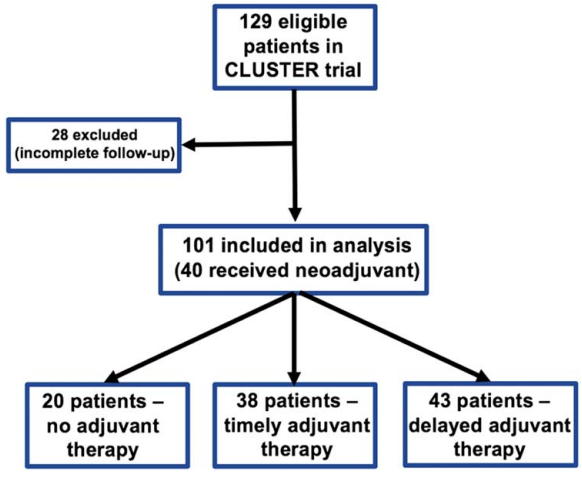

FIGURE 1. Patient selection and stratification by pattern of administration of adjuvant therapy.

Definitions and Patient Stratification

The primary outcome of the study was recurrence-free survival (RFS), which was defined as the time between surgical resection and recurrence of disease or date of death, whichever came first. For patients without recurrence of disease at their most recent follow-up censoring occurred at the time of last follow-up. Survival data for this study were censored at March 1, 2020. The pattern of receipt of adjuvant therapy was defined by stratifying the patient population into 3 groups: those with timely initiation of adjuvant therapy (< 8 week after surgical resection), those having a delay in initiation of adjuvant therapy (≥ 8 week after surgical resection), and those who did not receive any adjuvant therapy (Fig. 1). These cutoffs were based on prior literature on delay in initiation of adjuvant therapy in PDAC.[16,17] However, there remains a debate about the appropriate cutoff to define a delay in initiation of adjuvant therapy. The PRODIGE 24 trial on adjuvant therapy in resected pancreatic cancer included patients who underwent resection 3 to 12 weeks before randomization. As a result, it was decided to perform an additional analysis using the 12-week cutoff to define a delay in initiation of adjuvant therapy.[18]

Statistical Analysis

Frequencies and percentages were reported for categorical variables, and continuous variables were reported as means with SDs or medians with interquartile ranges as deemed appropriate. Categorical variables were assessed using a χ^2 test or Fisher exact test, whereas continuous variables were assessed using the Student t test. Survival analysis was conducted using Kaplan-Meier estimates, and a Cox regression model was used to assess differences. A P value of <0.05 was considered statistically significant. Analysis was performed using STATA v.19 (Texas).

The study was approved by the Institutional Review Board for Human Research at each of the 4 participating institutions and complied with all Health Insurance Portability and Accountability Act regulations.

RESULTS

Patient Characteristics

The current study included 101 (50.5%) of the 200 patients enrolled in the CLUSTER trial. The clinicopathologic

TABLE 1. Clinicopathologic Characteristics Stratified by Pattern of Adjuvant Initiation

Variable	Timely Adjuvant (N = 38), n (%)	Delayed Adjuvant (N = 43), n (%)	No Adjuvant (N = 20), n (%)	*P*
Age, ≥ 65 y	21 (55)	26 (60)	17 (85)	0.072
Sex, female	22 (58)	16 (37)	9 (45)	0.174
Neoadjuvant, received	16 (42)	13 (30)	11 (55)	0.160
Median duration of neoadjuvant, months	3.81 (2.43-5.06)	3.40 (2.90-4.00)	3.44 (2.30-6.05)	0.451
Size, > 2 cm	32 (86)	32 (76)	17 (85)	0.456
Nodal disease, present	24 (63)	27 (63)	10 (50)	0.246
Perineural invasion, present	31 (82)	32 (74)	15 (75)	0.456
Lymphovascular invasion, present	20 (53)	22 (51)	9 (45)	0.853
Margin, positive	4 (11)	6 (14)	2 (10)	0.856
Grade of tumor differentiation				
Well/moderate	25 (66)	29 (67)	7 (35)	**0.039**
Poor/undifferentiated	11 (29)	9 (21)	7 (35)	—
NA due to treatment response	2 (5)	5 (12)	6 (30)	—
Postoperative trCTCs, present	7 (18)	9 (21)	11 (55)	**0.006**
Postoperative eCTCs, present	27 (71)	29 (67)	19 (95)	0.056

characteristics of these patients are summarized in Table 1. The majority of patients were male (N = 54, 53.4%) and had an age 65 years or above (N = 64, 63.4%). Neoadjuvant therapy was administered to 40 patients (39.6%). Nodal disease was observed in 61 patients (60.4%), and the majority (N = 89, 88.1%) had margin-negative resections. Perineural invasion was observed in 78 patients (77.2%), and a majority (N = 61, 60.4%) had well-differentiated/moderately differentiated tumors.

Patterns of Administration of Adjuvant Therapy

Adjuvant therapy was administered in 81 patients (80.1%), of which 38 (37.6%) patients had timely and 43 (42.6%) patients had delayed initiation of adjuvant therapy (Fig. 1). In the majority of patients experiencing a delay in initiation of adjuvant therapy, there was no reported reason for the delay (N = 29, 67%). In addition, 14 patients (32.6%) had a delay in the initiation of adjuvant due to postoperative complications. Patient characteristics stratified by type of adjuvant therapy are summarized in Table 1. All the 3 groups were balanced in terms of their clinicopathologic characteristics except the grade of tumor differentiation and trCTCs positivity (Table 1). More patients who did not receive adjuvant therapy had postoperative trCTCs present (timely vs delayed vs no adjuvant therapy: 18% vs 21% vs 55%, $P = 0.006$).

CTC Characteristics and Survival

CTCs were identified in 76 patients (75.2%) at the postoperative time point; 49 (48.5%) had eCTCs alone, and 27 (26.7%) were trCTC positive. Clinicopathologic features stratified by trCTC positivity are summarized in Table 2. Patients found to have trCTCs has higher rates of eCTCs ($P = 0.002$) and lower rates of adjuvant therapy ($P = 0.006$). Patients who were trCTC negative postoperatively were found to have prolonged RFS compared with trCTCs-positive patients (median RFS: 16.6 vs 8.9 mo, $P < 0.001$) (Fig. 2).

Univariate and Multivariable Analysis for RFS

The median follow-up of the study population was 18.0 months [interquartile range (IQR): 12.4–24.5], at which 47 (47%) patients were alive and 27 (27%) were disease free. Median RFS was 15.0 months (IQR: 7.7–NR), and median overall survival was 24.6 months (IQR: 13.4–29.6). On univariate analysis, factors that were significantly associated with shorter RFS included presence of perineural invasion, poor/undifferentiated tumor grade, no receipt of adjuvant chemotherapy, and trCTCs positivity (all $P < 0.05$). On multivariable analysis, the factors found to be independently associated with RFS included poor tumor differentiation [well/moderately vs poorly differentiated, hazard ratio (HR): 4.19, 95% CI, 1.14–15.43, $P = 0.031$], absence of administration of adjuvant chemotherapy (timely initiation of adjuvant vs no administration of adjuvant, HR: 2.56, 95% CI, 1.09–6.05, $P = 0.032$), and trCTCs positivity (absence vs presence of trCTCs, HR: 2.53, 95% CI, 1.39–4.59, $P = 0.002$) (Table 3).

Survival analysis of the entire study population revealed no significant difference in RFS between patients receiving timely or delayed adjuvant therapy (median RFS: 16.5 vs 12.9 mo, $P = 0.497$). However, a significantly shorter RFS was observed in patients who did not receive any adjuvant therapy (12.88 vs 4.67 mo, $P = 0.037$) (Fig. 3).

When patients were further stratified by trCTC status, it was seen that in patients with timely initiation of adjuvant therapy, there was no significant difference in median RFS between trCTCs-positive and trCTCs-negative patients (trCTCs positive vs negative: 9.92 vs 14.92 mo, $P = 0.633$) (Fig. 4A). In contrast, there was a significant difference in median RFS between trCTCs-positive and trCTCs-negative patients in the cohort that experienced a delay in initiation (12.35 vs 17.97 mo, $P = 0.004$) or had no administration of adjuvant chemotherapy

TABLE 2. Clinicopathologic Characteristics Stratified by trCTC Status

Variable	trCTC Negative, n (%)	trCTC Positive, n (%)	*P*
Age, ≥ 65 y	46 (62.2)	18 (66.7)	0.678
Sex, female	36 (48.7)	11 (40.7)	0.481
Neoadjuvant, received	30 (40.5)	10 (37.0)	0.750
Size, > 2 cm	56 (77.8)	25 (92.6)	0.089
Nodal disease, present	41 (55.4)	20 (74.1)	0.090
Perineural invasion, present	55 (74.3)	23 (85.2)	0.249
Lymphovascular invasion, present	34 (45.9)	17 (62.9)	0.130
Margin, positive	6 (8.1)	6 (22.2)	0.079
Grade of tumor differentiation	—	—	0.660
Well/moderate	46 (62.2)	15 (55.6)	—
Poor/undifferentiated	18 (24.3)	9 (33.3)	—
NA because of treatment response	10 (13.5)	3 (11.1)	—
Pattern of adjuvant therapy	—	—	**0.006**
Timely adjuvant (< 8 wk)	31 (41.9)	7 (25.9)	—
Delayed adjuvant (> 8 wk)	34 (45.9)	9 (33.3)	—
No adjuvant	9 (12.2)	11 (40.7)	—
Postoperative eCTCs, present	49 (66.2)	26 (96.3)	**0.002**

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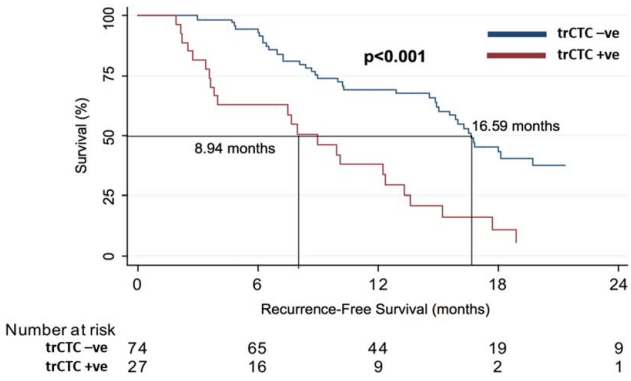

FIGURE 2. Recurrence-free survival stratified by postoperative trCTC status.

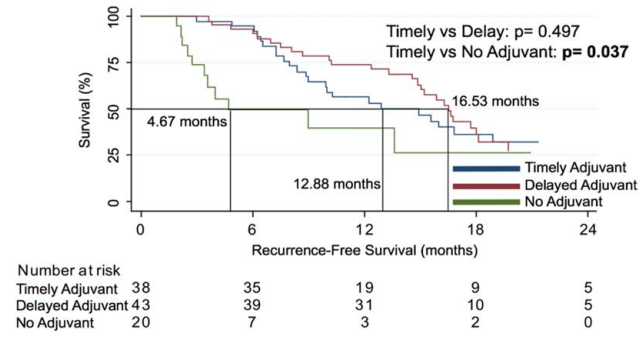

FIGURE 3. Recurrence-free survival stratified by patterns of administration of adjuvant therapy.

(3.38 vs NR [Not reached], $P = 0.016$) (Figs. 4B, C). In addition, the rate of recurrence was observed to be significantly higher in the patients with a delay in or no administration of adjuvant therapy ($P = 0.050$ and <0.001, respectively) (Figure S1, Supplemental Digital Content 1, http://links.lww.com/SLA/E260).

Finally, the majority of patients who were classified as having a delay in the initiation of chemotherapy started their therapy between 8 and 12 weeks postoperatively. Therefore, we wanted to assess whether these findings would remain consistent when a cutoff of ≥ 12 weeks was used. Upon survival analysis using this cutoff, no difference was observed in the cohort of patients with timely adjuvant (Figure S2a, Supplemental Digital Content 1, http://links.lww.com/SLA/E260). A significant difference was observed for the patients with a delay in the administration of adjuvant therapy; however, only 13 patients were included in this analysis (Figure S2b, Supplemental Digital Content 1, http://links.lww.com/SLA/E260).

DISCUSSION

Surgical resection of clinically localized disease provides the best chance for cure in PDAC.[2] The majority of treatment failures are systemic in nature resulting from progression from minimal residual disease to macroscopic disease that is appreciable on imaging.[3] CTCs can provide a window into the nature and extent of minimal residual disease and are predictive of response.[10] Adjuvant chemotherapy provides a survival benefit over resection alone as has been shown by multiple studies.[18–20] Furthermore, the PRODIGE 24 trial reported a median survival of 54.4 months in the group receiving modified FOLFIRINOX after resection.[18] A delay or no receipt of adjuvant therapy is associated with worse survival, but the underlying mechanisms are poorly understood.[16,21] One hypothesis is that delayed or insufficient systemic therapy facilitates the establishment and proliferation of minimal residual disease as reflected by CTC counts and phenotype.[22] In the current study, we demonstrate that a delay or lack of adjuvant therapy is associated with a significantly worse survival only among patients with persistent trCTCs after resection. This suggests that patients exhibiting residual trCTCs are most likely to derive benefit from timely administration of adjuvant therapy.

Recently, studies focusing on mechanisms of disease progression have identified a role of CTCs in the systemic biology of PDAC. These studies have identified phenotypic heterogeneity and demonstrated that cells expressing both mesenchymal and epithelial features (trCTCs) are associated with poorer outcomes.[4,6] A

TABLE 3. Univariate and Multivariable Analyses for Recurrence-Free Survival

		Univariate		Multivariable	
Variables	**N (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age, ≥65 y	64 (63.4)	1.00 (0.99-1.01)	0.603	—	—
Sex, female	47 (46.5)	0.78 (0.47-1.30)	0.346	—	—
Neoadjuvant, received	40 (39.6)	1.17 (0.69-1.96)	0.562	—	—
Size, >2 cm	81 (81.8)	1.89 (0.90-4.00)	0.092	—	—
Nodal disease, present	61 (60.4)	1.20 (0.72-2.02)	0.475	—	—
Perineural invasion, present	78 (77.2)	2.64 (1.29-5.40)	**0.008**	1.66 (0.69-3.97)	0.254
Lymphovascular invasion, present	51 (50.5)	1.33 (0.80-2.20)	0.267	—	—
Margin, positive	12 (11.9)	2.05 (0.96-4.36)	0.061	—	—
Grade of tumor differentiation					
Well/moderately	61 (60.4)	—	—	—	—
Poor/undifferentiated	27 (26.7)	2.40 (1.39-4.16)	0.002	4.19 (1.14-15.43)	**0.031**
Treatment response	13 (12.9)	0.54 (0.19-1.51)	0.237	1.88 (0.53-6.65)	0.328
Pattern of adjuvant therapy					
Timely adjuvant (<8 wk)	38 (37.6)	—	—	—	—
Delayed adjuvant (≥8 wk)	43 (42.6)	0.83 (0.47-1.44)	0.497	0.99 (0.56-1.74)	0.963
No adjuvant	20 (19.8)	2.15 (1.05-4.42)	**0.037**	2.56 (1.09-6.05)	**0.032**
Postoperative trCTCs, present	27 (26.7)	3.09 (1.82-5.23)	**<0.001**	2.53 (1.39-4.59)	**0.002**
Postoperative eCTCs, present	75 (74.3)	1.29 (0.72-2.31)	0.393	—	—

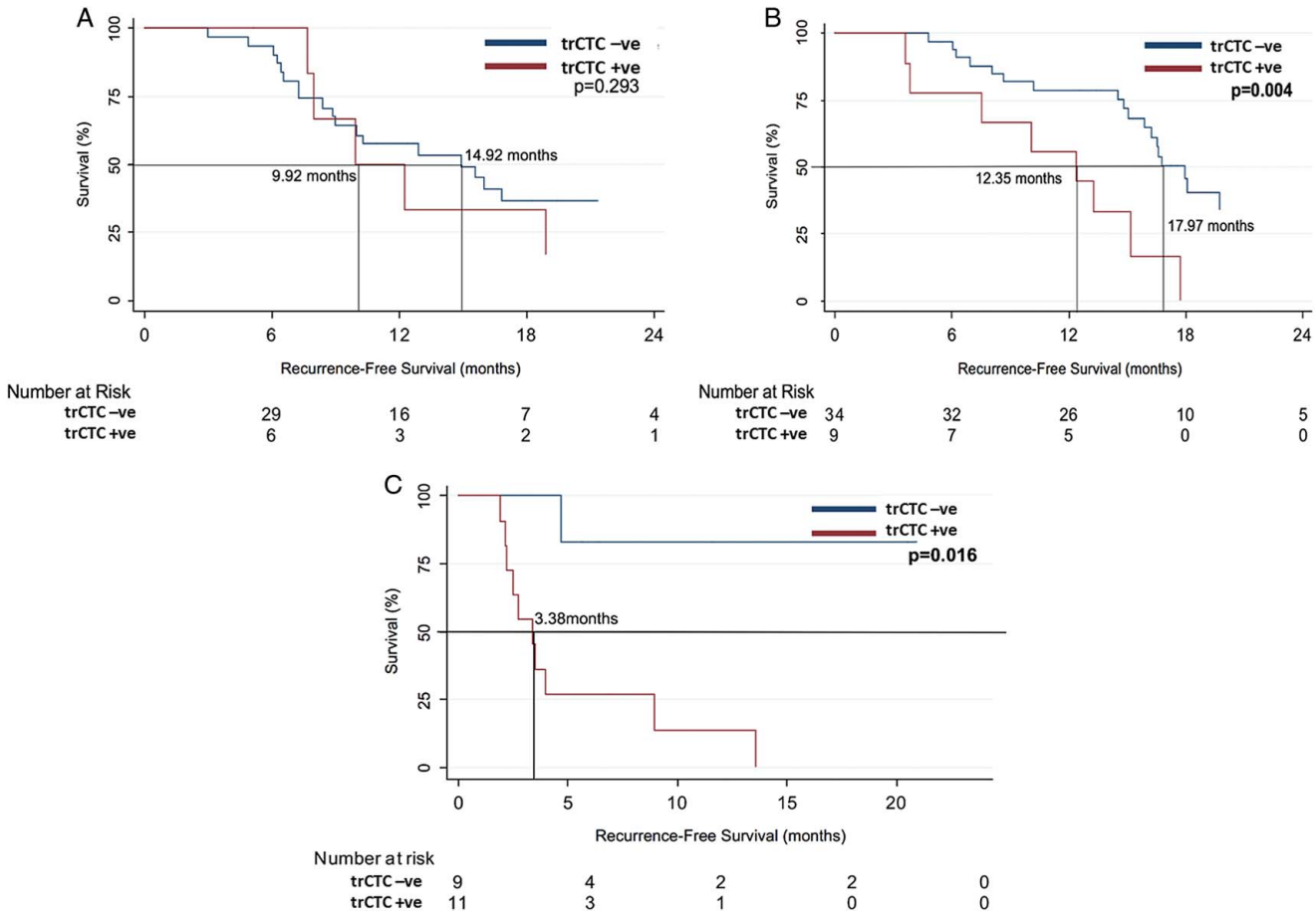

FIGURE 4. Recurrence-free stratified by trCTCs status in patients with timely initiation of adjuvant therapy (A), delayed initiation of adjuvant therapy (B), and no administration of adjuvant therapy (C).

majority of prior studies focus on the total number of CTCs observed, and a recent meta-analysis by Han et al[9] reported a significant association between the presence of CTCs and poorer patient outcomes. Similarly, an association between CTCs and RFS was observed in the current study. Furthermore, in prior work by our group, we have shown that it is the dynamic changes in CTC enumerations that can help prognosticate patients.[4] This was remonstrated by Ma et al[13] in a meta-analysis, where CTC characteristics at different time points during a patient's course of therapy were associated with patient outcomes. In addition, previous studies have demonstrated that CTC subtypes are associated with PDAC outcomes and that both surgical and systemic treatment can affect CTC characteristics and enumeration.[4,5,7,8,10,11,14]

The importance of an epithelial-mesenchymal transition and the reverse process (mesenchymal-epithelial transition) has become more evident, where cells lose or gain epithelial or mesenchymal characteristics.[23–26] This transition facilitates intravasation of tumor cells and seeding of distant locations. Our group has previously shown that of the CTC subtypes, trCTCs are the ones that are most strongly associated with worse outcomes.[4] In the current study, a similar trend was observed where presence of the transitional, and not the epithelial, subtype was associated with RFS.

Limited reports are available on the impact of a delay in the initiation of adjuvant therapy on patient outcomes, with mixed results. Although Wu et al[16] identified that a delay in initiation of therapy was associated with poorer outcomes in PDAC, Valle et al[21] in their follow-up study on the ESPAC-3 trial, reported that it was the completion of recommended adjuvant therapy, and not a delay in initiation of therapy, that was associated with survival. Similar to Wu and colleagues, 2 other studies reported an association between timely initiation of adjuvant therapy and improved survival.[27,28] Although time to adjuvant therapy is considered as one of the factors affecting outcomes in these patients, other less studied processes might be at play that can confound these findings. Tohme et al[22] suggested that surgical resection could paradoxically augment the development of metastasis via the initiation of an inflammatory response that can facilitate distant tumor growth. In addition, it has been suggested that postoperative immunosuppression can result in the progression of disease.[29] Lastly, it could be that slower recovery in patients who have a delay in the initiation of or do not receive adjuvant therapy may be a surrogate of poorer disease biology.

Given these contrasting findings, we wanted to explore whether there is a subset of patients, based on their CTC characteristics, who have poorer outcomes when there is a delay in the administration of adjuvant therapy. The initial investigation on the patterns of administration of adjuvant therapy yielded results similar to those reported by Valle and colleagues, where

patients with timely or delayed initiation of adjuvant therapy had similar outcomes. However, when these trends were studied in the context of trCTC positivity, it was observed that in the subgroup of patients who had a delay in initiation of adjuvant therapy, it was only those who were trCTC positive postoperatively who had a shorter RFS. This was not observed in patients receiving timely adjuvant therapy. Furthermore, although no administration of adjuvant therapy on its own was associated with poorer survival, patients who had trCTCs in their circulation postoperatively and did not receive any adjuvant therapy had the worst survival observed across all groups investigated in this study. We hypothesize that the delay in the administration of adjuvant therapy in patients with persistent trCTCs allows these cells to proliferate and recover from the surgical insult, subsequently resulting in higher rates and earlier recurrence of disease.

There are multiple clinical applications of these data. On the basis of these findings, it can be recommended that adjuvant therapy should be administered to all patients in a timely manner whenever possible. Although a majority of literature identified postoperative complications as the driver of a delay in or no administration of adjuvant therapy, the current study found it to be otherwise. In a majority of our patients, a reason for a delay in administration of therapy could not be identified. This is an easily modifiable factor, and in the future, clinicians should try and initiate adjuvant therapy shortly after surgical resection. In addition, the use of trCTCs as a biomarker to identify patients who would benefit most from timely initiation of therapy is another potential application. Postoperative evaluation of CTCs could allow clinicians to identify patients who have residual trCTCs and are at a higher risk of disease progression. With timely initiation of therapy, clinicians could potentially help improve outcomes in this cohort. Whether trCTC status could be used to identify patients who would not benefit from adjuvant therapy remains to be studied. Larger follow-up studies and incorporation of CTC detection in randomized controlled trials are required so these findings can be validated and integrated into clinical practice.

There are several limitations of this study. First, because of the small sample size of the study, various aspects of the adjuvant therapy, including the duration, type, and dose of chemotherapy regimens used, could not be studied. Similarly, various aspects of neoadjuvant therapy administration (duration, type, and dosage) were not studied in detail, which could impact the patterns of survival. However, in this patient population, receipt of neoadjuvant therapy was found not to be associated with RFS and even when controlling for neoadjuvant therapy the findings remained consistent. When examining the survival curves, the number of patients in each group is low, which limits the generalizability of these findings. Second, data on reasons for a delay in the receipt of adjuvant therapy were not collected as part of the CLUSTER trial and had to be collected retrospectively from the patients' electronic medical records. Despite the considerable efforts, the reason remained unknown for a majority of these patients. Finally, patients who received no adjuvant chemotherapy may possibly have had more aggressive disease or experienced more severe disease-related complications, which may have driven their outcomes rather than the patterns of receipt of adjuvant therapy. In the current study, patients who demonstrated early progression of disease, which could have led to a delay or no receipt of adjuvant therapy, were not included. In the future, larger prospective and randomized studies are required to validate these findings and study these aspects of systemic therapy in greater detail. Furthermore, to decrease the human error involved in CTC enumeration and characterization, improved techniques for CTC assessment are required.

In conclusion, postoperative trCTCs positivity is associated with poorer RFS only in patients who either experience a delay in initiation of or do not receive of adjuvant therapy. This study suggests that a delay in the initiation of adjuvant therapy could potentially provide residual systemic disease (trCTCs) a window of opportunity to recover from the surgical or chemotherapeutic (neoadjuvant) insult. Future studies are required to validate these findings and explore the underlying mechanisms involved.

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