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Transitional Subtype of Circulating Tumor Cells in Early Post-Operative Period of 5-Year Survivors Following Resection of Pancreatic Ductal Adenocarcinoma

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Abstract

Objectives

The objective was to assess the ability of circulating tumor cells (CTCs) to predict long-term survival (LTS, >5 years after resection) in pancreatic ductal adenocarcinoma (PDAC).

Summary of background data

Predictors of LTS remain poorly understood in PDAC.

Methods

Patients enrolled in the prospective CLUSTER Trial for serial assessment of CTCs, undergoing PDAC resection were included (2016-2018). Number of epithelial (eCTCs) and transitional (trCTCs) CTCs were serially assessed. Clinicopathological factors and CTC characteristics associated with LTS were identified and their ability to predict LTS was assessed.

Results

In 133 patients, 41% and 82% received neoadjuvant and/or adjuvant therapy, respectively. LTS was achieved by 17% patients. Nodal disease and perineural invasion (PNI) were present in 62%, and 80% of patients, respectively. Preoperatively eCTCs and trCTCs were observed in 97% and 68% of patients as compared to 77% and 27% postoperatively.

PNI (OR:0.19,95%CI:0.06–0.60), nodal disease (OR:0.28,95%CI:0.09–0.82), and postoperative trCTCs (OR:0.04,95%CI:0.01–0.38) were independently associated with LTS. A clinical score based on PNI and nodal disease demonstrated an AUC of 0.79 (95%CI:0.69–0.89) in predicting LTS. Addition of postoperative trCTC into a translational score demonstrated an AUC of 0.84 (95%CI:0.75–0.92). Upon internal validation the clinical and translational scores had AUCs of 0.78 (95%CI:0.67–0.89) and 0.84 (95%CI:0.73–0.92), respectively ($p<0.001$).

Conclusions

Patients with residual postoperative trCTCs are unlikely to achieve LTS and trCTCs emerge as one of the strongest predictors of LTS in resected PDAC. Inclusion of postoperative trCTC status to clinicopathological factors improves our ability to predict LTS.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) has a high propensity for early distant spread, and when diagnosed at a clinically localized, potentially curable stage, it is most often a systemic disease.[1-6] The only potential chance for cure and long-term survival is through an oncologic resection in combination with systemic therapy.[7-11] Despite improvements in multiagent systemic therapies for control of minimal residual disease (MRD), the majority of patients undergoing resection die from systemic progression.[2,3,12,13] Therefore, effective control of MRD remains the greatest challenge in improving long-term survival (LTS) in clinically localized PDAC.

Circulating tumor cells have been implicated in systemic progression of disease and are hypothesized to be the origin of MRD and metastases.[1,14-21] Cells originating in the primary tumor extravasate into circulation. Most of these cells are unable to establish distant disease and are rapidly eliminated.[22-24] Importantly, a subset of CTCs reprogrammed via the epithelial-to-mesenchymal transition can withstand the harsh environment in circulation and are capable of establishing micrometastic niches in distant organs.[15,17,18,20,25,26] Our group, along with others, has shown that circulating tumor cells can be isolated from the peripheral blood of patients with pancreatic cancer.[1,17,20,27-29] Phenotypic heterogeneity exists among CTCs with epithelial (eCTCs) or mesenchymal subtypes.[29] CTCs that demonstrate both epithelial and mesenchymal features are known as transitional CTCs (trCTCs).[1,29] To better understand the clinical importance of CTCs in PDAC, our group performed a prospective longitudinal study (CircuLating tUmor cellS in panceatTic cancer, CLUSTER) designed to characterize the impact of CTC on cancer-specific outcomes.[1] In that study it was shown that both neoadjuvant therapy and surgical resection were associated with a reduction in the burden of CTCs. Importantly, the

trCTC subtype was prognostic of survival and was able to predict recurrence of disease, prior to standard imaging-based determinants.

Although relatively rare, long-term survival (LTS) as defined by survival of >5 years after resection is consistently found in patients undergoing resection of clinically localized PDAC.[30-35] We have demonstrated that the impact of established prognostic factors including nodal disease, perineural and lymphovascular invasion, grade of tumor differentiation, and margin status on LTS is time-varying. Using the Aalen linear hazards model analysis, we have shown that multiple factors including the grade of tumor differentiation, tumor size, and lymphovascular invasion lose prognostic value after 2 to 4 years, depending on the specific feature. Moreover, this work suggests yet to be identified prognostic factors exist. As such, no robust predictors of LTS in PDAC have been identified to date.[30,31,36-39] The prognostic significance of CTCs on LTS remains unknown. Given the strong predictive capability of CTC in median and OS, we hypothesized that CTC are prognostic of true LTS in clinical localized and resected patients. The goal of the current study was to evaluate the ability of CTCs to predict LTS in resected patients with PDAC within the CLUSTER cohort. The CLUSTER study completed accrual in 2018, and the actual 5-year vital status is now known for the entire study population. This is a retrospective analysis of the prospective CLUSTER cohort.[1]

METHODS

Patient Recruitment & Data Collection

The prospective CLUSTER Trial (NCT02974764) was conducted from March 2016 to March 2018.[1] A total of 200 patients with a presumed diagnosis of PDAC were enrolled and longitudinal sampling of peripheral blood was performed to evaluate CTCs. Of the study

population, patients who had histopathological confirmed PDAC and underwent successful resection were included in the current study. Patients who had a pathological diagnosis other than PDAC on surgical resection, had an R2 resection, or experienced postoperative 90-day mortality were excluded.

Clinicopathological data were extracted from a prospectively maintained institutional database. This included demographic information, details on presentation and surgical approach, histopathological findings, information on neoadjuvant and adjuvant therapy, and survival outcomes. Missing data were collected retrospectively from patients' electronic medical records. Based on CA19-9 serum levels patients were categorized into non-secretors (<5 units/mL), normal (5-37 units/mL), and elevated (≥37 units/mL).

Most patients underwent initial evaluation by a pancreas protocol computed tomography (CT) scan, endoscopic ultrasound with biopsy, biliary drainage if needed, and serum carbohydrate antigen 19-9 (CA19-9) levels. The subsequent decision regarding management and resectability in most patients was determined in a multidisciplinary setting (including surgeons, oncologists, radiation oncologists, pathologists, and radiologists). Recommendations for treatment generally followed the National Comprehensive Cancer Network (NCCN) guidelines.[40]

The pathological analysis of the pancreatic specimen included the grade of tumor differentiation, tumor size, and extent of invasion. Following the College of American Pathologists (CAP) pancreatic protocol, counts of the number of harvested lymph nodes and involved nodes were also reported.[41] A microscopic clearance margin of less than 1mm was considered to be a positive margin (R1).

Sample Collection and CTC Characterization

Patients were sampled at multiple time points during their treatment course (during neoadjuvant therapy, prior to resection, after resection, prior to initiation of adjuvant therapy, during adjuvant therapy, and after adjuvant till recurrence of disease or death). A total of 10 mL of blood was drawn at each time point and was processed within 4 hours for CTC enrichment using the Isolation by Size of Epithelial Tumor Cells assay (ISET; Rarecells). Details regarding the methods used for CTC isolation and characterization have previously been reported.[1] In short, CTCs were fixed used formaldehyde, filtered on to a 10-core membrane and stored in -20° C until characterization steps. Additionally, 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.[1]

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

CTCs were identified using the Nikon Ti-E inverted microscope system (Nikon, Japan) based on CD-marker negativity and pan-cytokeratin and/or vimentin positivity, as described in Gemenetzis et al.[1] and Poruk et al.[29] 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 mL of blood.

No cells with a purely mesenchymal phenotype (pancytokeratin-, vimentin+, CD-) were observed. Morphological details and genetic information of observed CTCs and white blood cells (WBCs) were as reported in Gemenetzis et al.[1] No CTCs were observed in any healthy volunteer samples.

Definitions and patient stratification

The primary outcome of this follow up study was LTS, which was defined as a survival of ≥5 years after resection. Patients who were found to have a follow up of less than 5 years but were alive at their most recent follow-up were considered to be lost to follow-up and were censored in the survival analysis. Overall survival (OS) was defined as the time between the date of surgery and the date of death. For all patients found to be alive at the time of last follow-up, the survival was censored at the date of last contact. Survival data for this study were updated till April 1, 2024.

The cohort was divided into trCTC negative (no trCTCs), low-burden (1 trCTC) and high-burden (≥1 trCTCs) patients. The cut-off for the high burden of trCTCs was derived by analyzing the distribution of trCTC counts and it was found that 1 or more trCTC represented the

75th quartile of patients (**supplementary Fig 1**, Supplemental Digital Content 1, http://links.lww.com/SLA/F628).

Statistical Analysis

Categorical variables were reported as frequencies and percentages while continuous variables were reported as means with standard deviations or medians with interquartile ranges as deemed appropriate. Categorical variables were assessed using a Chi^2 test or Fisher's exact test, while continuous variables were assessed using the Student's T-test. Survival analysis was conducted using Kaplan-Meier estimates, and a backwards selection Cox-regression model was used to assess differences. Patients were categorized into LTS and non-LTS and the groups and factors associated with LTS were identified using a penalized logistic regression model. Firth's correction was used to reduce bias arising from a small number of events or separation. This is achieved by penalizing the likelihood, which results in the generation of finite and more reliable parameter estimates. The ability of various clinicopathological factors with or without CTC status to predict LTS was evaluated using the area under the receiver operating curve (AUC).

This study was carried out in compliance with the ethical principles for medical research involving human subjects outlined in the Declaration of Helsinki and was approved by the Institutional Review Board for Human Research and complied with all Health Insurance Portability and Accountability Act regulations. The study abided by the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines.

A p-value of <0.05 was considered statistically significant. Analysis was performed using R statistical software (Version 4.2).

RESULTS

Patient Characteristics

Of the 200 patients enrolled in the CLUSTER trial, 133 were included in the current study. A majority of excluded patients were found to have pathology other than PDAC at the time of surgical resection or did not undergo resection due to progression of disease. The general demographics are summarized in Table 1. In summary, the median age was 68.0 years (IQR 59.3-74.5) and 47% were female. Neoadjuvant therapy was administered in 54 (41%) of patients. Nodal disease and perineural and lymphovascular invasion were present in 83 (62%), 107 (80%), and 71 (53%) of patients, respectively. A negative margin was achieved in 112 (85%) patients. Adjuvant therapy was administered in 101 (82%) patients.

In terms of CTCs, 97% of patients were found to have eCTCs as compared to 68% of patients with trCTCs preoperatively. After surgical resection eCTCs and trCTCs were observed in 77% and 27% of patients, respectively. Lastly, after completion of adjuvant therapy 95% patients had eCTCs compared to 40% patients with trCTCs.

Factors Associated with Overall Survival

The median overall survival was 23.2 (IQR: 13.4 to 44.7) months and the median follow up of all surviving patients at last follow up was 75.2 months (IQR: 68.3 to 78.6) months. At the time of last follow up 110 (82.7%) patients had died within 5 years of resection, 22 (16.5%) patients were alive beyond five years, and one patient was lost to follow up at 24 months. On univariable analysis factors found to be significantly associated with worse OS included older age, current smoking status, higher grade of tumor differentiation, presence of perineural and

lymphovascular invasion, nodal disease, absence of complete pathological response to therapy, presence of presurgical trCTCs, and presence of postsurgical trCTCs. On multivariable Cox-regression analysis factors that were found to be independently associated with worse OS included increasing tumor size (HR:1.18, 95%CI: 1.03-1.36 per mm increase, p=0.021), nodal disease (HR:1.72, 95%CI: 1.05-2.83, p=0.032), and postoperative presence of trCTCs (HR:1.72, 95%CI: 1.06-2.78, p=0.028). Adjuvant therapy was associated with improved OS (HR:0.52, 95%CI: 0.30-0.90, p=0.019). Patients who were found to have postoperative trCTCs had a median survival of 16.0 months (95%CI: 12.6-22.2) as compared to 24.9 months (95%CI:21.0-36.5) in those who did not have trCTCs after resection (p<0.001) (Figure 1).

Factors Associated with Long-Term Survival

A total of 22 (16.7%) patients achieved LTS. Upon comparing patients who achieved LTS to those who did not, LTS population had significantly lower rates of elevated CA19-9 at diagnosis (46% vs. 62%, p=0.02) and smaller tumors (median: 2.45 vs. 3.20 cm, p=0.016). Higher rates of nodal disease (69% vs. 27%, p<0.001), and perineural (86% vs. 50%, p<0.001) and lymphovascular invasion (58% vs. 27%, p<0.001), were observed in those who did not achieve LTS. Of those achieving LTS, 18% demonstrated complete pathological response following neoadjuvant treatment as compared to 1.8% in the non-LTS cohort (p=0.007). Both groups had similar characteristics in terms of age, sex, smoking status, BMI, receipt of neoadjuvant therapy, surgery type, grade of tumor differentiation, margin positivity, and receipt of adjuvant therapy (Table 1).

In terms of CTCs, those who achieved LTS were more likely to have no trCTCs at the time of presurgical (p=0.013) and postsurgical (p=0.002) blood draw, however, no difference was observed in the postadjuvant trCTC status (p=0.808). Additionally, eCTC status was not significantly different in LTS compared to the non-LTS cohort at all three time points.

Logistic regression analysis demonstrated that factors that were independently associated with LTS included perineural invasion (OR:0.19, 95%CI: 0.06 – 0.60, p=0.004), presence of nodal disease (OR:0.28, 95%CI: 0.09 – 0.82, p=0.020), and postoperative presence of trCTCs (OR:0.04, 95%CI: <0.01 – 0.38, p=0.001). A clinical score based on perineural invasion and nodal disease demonstrated an AUC of 0.79 (95%CI:0.69 – 0.89) in predicting LTS. Addition of postoperative trCTC status in form of a translational score demonstrated an AUC of 0.84 (95%CI:0.75 – 0.92). Upon internal validation via bootstrapping the clinical and translational scores had AUCs of 0.78 (95%CI: 0.67 – 0.89) and 0.84 (95%CI: 0.73 – 0.92), respectively (p<0.001) (Figure 2).

Trends of Transitional CTCs

Patients were stratified into three categories (no trCTCs, low burden of trCTCs (1 cell per 10ml blood volume), and high burden of trCTCs (≥2cells per 10ml blood volume) based on the number of trCTCs observed at multiple time points (Figure 3). A total of 85 patients had available values prior to surgery, after surgery, and at follow up after completion of adjuvant treatment if applicable. At the time of resection, 29 patients were trCTC negative, of whom a majority (N=28) remained negative after resection, while one patient developed a low burden of trCTCs postoperatively. At longer term follow up, 16 (55%), 6 (21%), and 7 (24%) showed

negative, low-burden, and high-burden of disease, respectively. Of the 24 patients who had low burden of disease 21 (88%) became negative postoperatively and none progressed to high burden. Lastly, of the 32 with a high-burden of trCTCs, preoperatively, 15 (47%) became negative. However, a relevant proportion still had low (n=13, 41%) or high burden (n=4, 13%) of disease. Twenty (63%) patients did progress to a high-burden again during follow-up. The trends in trCTC burden stratified by LTS are presented in Figure 3. LTS was only achieved in patients with a negative or low burden of CTCs preoperatively and exclusively for patients with negative CTCs postoperatively. While 15 (47%) of patients with high trCTC burden preoperatively did normalize trCTCs after resection, none achieved LTS.

DISCUSSION

Poor outcomes in PDAC are driven by systemic disease as is evident by high rates of distant recurrence in patients undergoing an oncological resection.[2,3,13,42,43] While LTS still remains rare, with the increased utilization of multiagent systemic therapies it is becoming more common.[30-32,34-37,39,44,45] Factors associated with LTS remain poorly studied and to date no strong predictors of LTS in resected PDAC have been identified.[30-32,36,39,44,46,47] We previously reported that trCTCs are associated with poor overall and recurrence free survival.[1,29] However, to date the effect of CTCs at the time of diagnosis and after resection on LTS in PDAC has not been studied. In the current study we demonstrate that the presence of postoperative trCTCs is independently associated with LTS. Importantly, no patient with residual trCTCs after resection achieved long-term survival. Furthermore, it was seen that addition of trCTCs to a predictive model comprising of clinicopathological features improved our ability to predict LTS in PDAC.

The findings of the study indicate that the presence of postoperative trCTCs is a significant predictor of LTS. Specifically, patients with residual trCTCs after surgical resection were found to have a markedly reduced likelihood of achieving LTS. This underscores the importance of trCTCs as a potential biomarker for identifying patients at higher risk of poor outcomes despite undergoing a successful local tumor resection. The ability of trCTCs to predict LTS, even in the context of traditional clinicopathological factors such as perineural invasion and nodal disease, provides further insight into disease biology and could potentially have a role in patient education and management. As has been demonstrated in the past, integration of multiple clinicopathological features and biomarkers into multianalyte models can help improve its predictive ability.[48-50] This was demonstrated in the current study as well, where the inclusion of postoperative trCTC status into our predictive model improved the accuracy of LTS prediction. This suggests that trCTCs provide additional valuable information beyond conventional clinicopathological factors, highlighting their role as a potential adjunct to existing prognostic models. This study builds on prior work by demonstrating that trCTCs are not only predictive of overall survival but also of LTS. This distinction is critical, as overall survival is a more general measure that may not fully capture the unique aspects of LTS in the context of aggressive cancers like PDAC.

The ability to predict LTS with higher accuracy has implications for patient management and treatment planning. Identifying patients who are less likely to achieve LTS with standard therapy can facilitate trial design to study alternative treatment approaches, such as targeted investigational therapies. On the other hand, patients predicted to have a higher likelihood of LTS could be studied as a group potentially not requiring additional chemotherapy in the context of a clinical trial. The results of this study confirm earlier findings of the ability of trCTCs in

predicting survival outcomes and serving as an objective measurement of what we now assign the nebulous term "tumor biology".[1,27-29,50] Previous studies have shown that CTCs, particularly those with both epithelial and mesenchymal characteristics, are associated with poorer overall survival in various cancer types.[51-53] This study highlights the specific role of trCTCs in the context of PDAC and its association with long-term outcomes. The finding that trCTCs, and not eCTCs, are independently associated with LTS emphasizes the need for more nuanced assessments of CTC phenotypes in predicting patient outcomes. In the future, identification of CTC subtypes on a genomic or transcriptomic level could further improve our ability to identify unique populations of CTCs which have distinct association with tumor characteristics and outcomes. Overall, incorporating trCTC status into clinical practice may possibly lead to identification of targeted therapies, resulting in more personalized treatment strategies, and potentially improve outcomes.

In terms of the trends of trCTCs, while some patients with high trCTC burden preoperatively had a substantial reduction in their trCTC numbers after resection, they still did not achieve LTS. This finding suggests that such patients harbor residual sources of trCTC beyond the primary tumor consistent with the properties of MRD. As such trCTC are a direct window of MRD biology, and unlike ctDNA, represent the actual residual disease. Our findings suggest that if upon removal of the primary tumor the number of trCTCs is reduced to zero i.e. normalized, the source of all trCTCs in that patient was the primary tumor and the operation was curative. In case of reduction in the trCTC burden it is possible that while some trCTCs that are shed from the primary tumor are eradicated the second source i.e. disseminated tumor cells still exist. In these patients the residual trCTCs will result in progression to grossly metastatic disease and death. Additionally, it was seen that even when you have normalization of trCTCs after

resection, there may be a possibility that trCTCs are detected in your bloodstream during follow up which could be owing to the limit of detection of the device that was used.

Transitional CTCs are characterized by phenotypic and genetic changes that suggest a hybrid state between epithelial and mesenchymal traits which may facilitate the cell's ability to adapt to different microenvironments, survive in the bloodstream, and potentially seed metastases or contribute to disease recurrence, which is the hallmark of MRD. Poruk et al. investigated the tumor initiating cell (TIC) phenotype of CTCs in PDAC which are CTCs that exhibit stem cell like properties.[20] A higher rate of TIC positivity was observed in PDAC patients than that reported for other malignancies. This is congruent with clinical observations where the rate of recurrence in higher in PDAC as compared to other malignancies. The findings of this study suggest that the TIC phenotype of CTCs may exhibit stem cell-like properties such as potential for dormancy and reactivation and could serve as a source of metastases. Indeed TIC positivity was prognostic and was associated with higher rates of recurrence. On the contrary, this transitional state could be part of the tumor's natural evolutionary process rather than an indicator of its metastatic potential. Some trCTCs may not survive in circulation or have metastatic potential. It’s possible that trCTCs are involved in early-stage metastasis and may not represent the surviving, dormant cells that characterize MRD. This could mean that the presence of transitional CTCs is more of a transient event rather than a stable marker of MRD. Further work is required in the field to investigate the genetic and phenotypic heterogeneity in these cells which could provide critical insights into the evolution of MRD after therapy.

It is possible that other factors, such as the extent of disease dissemination, the biological characteristics of the remaining disease, or presence of premetastatic niches play a critical role in determining long-term outcomes. The clinical implications of this finding remain to be

elucidated. Additionally, while our current subtyping has been shown to correlate with clinical outcomes recent reports also suggest that genetic and transcriptomic subtypes may exists which may be associated with outcomes.[54,55] This needs to be further investigated and may potentially help identify CTC characteristics that are even more predictive of LTS in resected PDAC.

While this study provides novel insights into the biology of PDAC, it does have several limitations that need to be acknowledged. First, the time points for assessment of CTCs for each patient were not uniform. The blood collection for CTCs assessment was performed in conjunction with routine clinical treatment and not based on a study timeline. 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. Despite that, this is the largest study investigating longitudinal changes in CTCs in patients with PDAC. Future studies should aim to validate these findings in larger, multicenter cohorts and explore the biological mechanisms underlying trCTCs' prognostic significance. Third, assessment of CTCs is dependent on the technique used and therefore the generalizability of these results is limited. However, ISET is one of the most well-established devices for CTC isolation and assessment. The strength of the study was a near complete follow-up on all patients. Patients has either died prior to achieving LTS or were still alive beyond 5 years. A majority of prior studies are limited by having patients with insufficient follow up.

In conclusion, this study demonstrates that patients with residual trCTCs after resection are unlikely to achieve LTS and trCTCs emerge as one of the strongest predictors of LTS in patients with resected PDAC. Addition of postoperative trCTC status to clinicopathological factors improves our ability to predict LTS in resected PDAC. Transitional CTCs can serve as a

valuable biomarker in the management of PDAC, paving the way for more personalized and effective treatment strategies. Future research is required to validate these findings and explore the broader implications of CTC dynamics on LTS in PDAC.

References

1.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 268:408-420, 2018
2.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 25:2475-2483, 2018
3.Groot VP, Rezaee N, Wu W, et al: Patterns, Timing, and Predictors of Recurrence Following Pancreatectomy for Pancreatic Ductal Adenocarcinoma. Ann Surg 267:936-945, 2018
4.Habib JR, Kinny-Koster B, Bou-Samra P, et al: Surgical Decision-Making in Pancreatic Ductal Adenocarcinoma: Modeling Prognosis Following Pancreatectomy in the Era of Induction and Neoadjuvant Chemotherapy. Ann Surg 277:151-158, 2023
5.Mizrahi JD, Surana R, Valle JW, et al: Pancreatic cancer. Lancet 395:2008-2020, 2020
6.Wolfgang CL, Herman JM, Laheru DA, et al: Recent progress in pancreatic cancer. CA Cancer J Clin 63:318-48, 2013
7.Aliseda D, Marti-Cruchaga P, Zozaya G, et al: Neoadjuvant therapy versus upfront surgery in resectable pancreatic cancer: reconstructed patient-level meta-analysis of randomized clinical trials. BJS Open 8, 2024
8.Bradley A, Van Der Meer R: Upfront Surgery versus Neoadjuvant Therapy for Resectable Pancreatic Cancer: Systematic Review and Bayesian Network Meta-analysis. Sci Rep 9:4354, 2019
9.Chen X, Liu G, Wang K, et al: Neoadjuvant radiation followed by resection versus upfront resection for locally advanced pancreatic cancer patients: a propensity score matched analysis. Oncotarget 8:47831-47840, 2017
10.Gemenetzis G, Groot VP, Blair AB, et al: Survival in Locally Advanced Pancreatic Cancer After Neoadjuvant Therapy and Surgical Resection. Ann Surg 270:340-347, 2019
11.Javed AA, Wright MJ, Siddique A, et al: Outcome of Patients with Borderline Resectable Pancreatic Cancer in the Contemporary Era of Neoadjuvant Chemotherapy. J Gastrointest Surg 23:112-121, 2019
12.Groot VP, Blair AB, Gemenetzis G, et al: Recurrence after neoadjuvant therapy and resection of borderline resectable and locally advanced pancreatic cancer. Eur J Surg Oncol 45:1674-1683, 2019
13.Groot VP, Gemenetzis G, Blair AB, et al: Defining and Predicting Early Recurrence in 957 Patients With Resected Pancreatic Ductal Adenocarcinoma. Ann Surg 269:1154-1162, 2019
14.Baccelli I, Schneeweiss A, Riethdorf S, et al: Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol 31:539-44, 2013
15.Court CM, Ankeny JS, Sho S, et al: Circulating Tumor Cells Predict Occult Metastatic Disease and Prognosis in Pancreatic Cancer. Ann Surg Oncol 25:1000-1008, 2018
16.Han L, Chen W, Zhao Q: Prognostic value of circulating tumor cells in patients with pancreatic cancer: a meta-analysis. Tumour Biol 35:2473-80, 2014
17.Hasanain A, Blanco BA, Yu J, et al: The importance of circulating and disseminated tumor cells in pancreatic cancer. Surg Open Sci 1:49-55, 2019
18.Houg DS, Bijlsma MF: The hepatic pre-metastatic niche in pancreatic ductal adenocarcinoma. Mol Cancer 17:95, 2018

19.Ma XL, Li YY, Zhang J, et al: Prognostic role of circulating tumor cells in patients with pancreatic cancer: a meta-analysis. Asian Pac J Cancer Prev 15:6015-20, 2014
20.Poruk KE, Blackford AL, Weiss MJ, et al: Circulating Tumor Cells Expressing Markers of Tumor-Initiating Cells Predict Poor Survival and Cancer Recurrence in Patients with Pancreatic Ductal Adenocarcinoma. Clin Cancer Res 23:2681-2690, 2017
21.Z'Graggen K, Centeno BA, Fernandez-del Castillo C, et al: Biological implications of tumor cells in blood and bone marrow of pancreatic cancer patients. Surgery 129:537-46, 2001
22.Meng S, Tripathy D, Frenkel EP, et al: Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 10:8152-62, 2004
23.Micalizzi DS, Maheswaran S, Haber DA: A conduit to metastasis: circulating tumor cell biology. Genes Dev 31:1827-1840, 2017
24.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 2:25ra23, 2010
25.Koch M, Kienle P, Hinz U, et al: Detection of hematogenous tumor cell dissemination predicts tumor relapse in patients undergoing surgical resection of colorectal liver metastases. Ann Surg 241:199-205, 2005
26.Pommier A, Anaparthy N, Memos N, et al: Unresolved endoplasmic reticulum stress engenders immune-resistant, latent pancreatic cancer metastases. Science 360, 2018
27.Javed AA, Ding D, Hasanain A, et al: Persistent Circulating Tumor Cells at One Year after Oncologic Resection Predict Late Recurrence in Pancreatic Cancer. Ann Surg, 2022
28.Javed AA, Floortje van Oosten A, Habib JR, et al: A Delay in Adjuvant Therapy is Associated with Worse Prognosis only in Patients with Transitional Circulating Tumor Cells Following Resection of Pancreatic Ductal Adenocarcinoma. Ann Surg, 2022
29.Poruk KE, Valero V, 3rd, Saunders T, et al: Circulating Tumor Cell Phenotype Predicts Recurrence and Survival in Pancreatic Adenocarcinoma. Ann Surg 264:1073-1081, 2016
30.Belfiori G, Crippa S, Francesca A, et al: Long-Term Survivors after Upfront Resection for Pancreatic Ductal Adenocarcinoma: An Actual 5-Year Analysis of Disease-Specific and Post-Recurrence Survival. Ann Surg Oncol 28:8249-8260, 2021
31.Bengtsson A, Andersson R, Ansari D: The actual 5-year survivors of pancreatic ductal adenocarcinoma based on real-world data. Sci Rep 10:16425, 2020
32.Javed AA, Mahmud O, Fatimi AS, et al: Predictors for Long-Term Survival After Resection of Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis. Ann Surg Oncol, 2024
33.Oettle H, Neuhaus P, Hochhaus A, et al: Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. JAMA 310:1473-81, 2013
34.Picozzi VJ, Oh SY, Edwards A, et al: Five-Year Actual Overall Survival in Resected Pancreatic Cancer: A Contemporary Single-Institution Experience from a Multidisciplinary Perspective. Ann Surg Oncol 24:1722-1730, 2017
35.Yamamoto T, Yagi S, Kinoshita H, et al: Long-term survival after resection of pancreatic cancer: a single-center retrospective analysis. World J Gastroenterol 21:262-8, 2015
36.Conroy T, Castan F, Lopez A, et al: Five-Year Outcomes of FOLFIRINOX vs Gemcitabine as Adjuvant Therapy for Pancreatic Cancer: A Randomized Clinical Trial. JAMA Oncol 8:1571-1578, 2022
37.Kardosh A, Lichtensztajn DY, Gubens MA, et al: Long-Term Survivors of Pancreatic Cancer: A California Population-Based Study. Pancreas 47:958-966, 2018

38.Nakagawa K, Akahori T, Nishiwada S, et al: Prognostic factors for actual long-term survival in the era of multidisciplinary treatment for pancreatic ductal adenocarcinoma. Langenbecks Arch Surg 403:693-700, 2018
39.van Goor I, Schouten TJ, Verburg DN, et al: Predicting Long-term Disease-free Survival After Resection of Pancreatic Ductal Adenocarcinoma: A Nationwide Cohort Study. Ann Surg 279:132-137, 2024
40.Tempero MA, Malafa MP, Al-Hawary M, et al: Pancreatic Adenocarcinoma, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 19:439-457, 2021
41.Kakar S SC, Adsay NV, Fitzgibbons P, Frankel WL,, Klimstra DS KA, Mino-Kenudson M, Pawlik T, Vauthey J, Washington MK: Protocol for the Examination of Specimens From Patients With Carcinoma of the Pancreas With Guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees, 2017
42.WHO Classification of Tumours of the Digestive System. Anticancer Research 31:1089-1089, 2011
43.Rompen IF, Levine J, Habib JR, et al: Progression of Site-Specific Recurrence of Pancreatic Cancer and Implications for Treatment. Ann Surg, 2023
44.Han SH, Heo JS, Choi SH, et al: Actual long-term outcome of T1 and T2 pancreatic ductal adenocarcinoma after surgical resection. Int J Surg 40:68-72, 2017
45.Luu AM, Braumann C, Belyaev O, et al: Long-term survival after pancreaticoduodenectomy in patients with ductal adenocarcinoma of the pancreatic head. Hepatobiliary Pancreat Dis Int 20:271-278, 2021
46.Addeo P, Fattori A, Grunder C, et al: Prognostic significance of residual lymphovascular invasion after resection of locally advanced and borderline resectable pancreatic adenocarcinomas treated by neoadjuvant chemotherapy. HPB (Oxford) 23:1285-1295, 2021
47.Dusch N, Weiss C, Strobel P, et al: Factors predicting long-term survival following pancreatic resection for ductal adenocarcinoma of the pancreas: 40 years of experience. J Gastrointest Surg 18:674-81, 2014
48.Cohen JD, Javed AA, Thoburn C, et al: Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. Proc Natl Acad Sci U S A 114:10202-10207, 2017
49.Cohen JD, Li L, Wang Y, et al: Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 359:926-930, 2018
50.Kinny-Köster B, Habib JR, Wolfgang CL, et al: Favorable tumor biology in locally advanced pancreatic cancer-beyond CA19-9. Journal of gastrointestinal oncology 12:2484-2494, 2021
51.Heerboth S, Housman G, Leary M, et al: EMT and tumor metastasis. Clin Transl Med 4:6, 2015
52.Qi LN, Xiang BD, Wu FX, et al: Circulating Tumor Cells Undergoing EMT Provide a Metric for Diagnosis and Prognosis of Patients with Hepatocellular Carcinoma. Cancer Res 78:4731-4744, 2018
53.Satelli A, Batth I, Brownlee Z, et al: EMT circulating tumor cells detected by cell-surface vimentin are associated with prostate cancer progression. Oncotarget 8:49329-49337, 2017
54.Amantini C, Morelli MB, Nabissi M, et al: Expression Profiling of Circulating Tumor Cells in Pancreatic Ductal Adenocarcinoma Patients: Biomarkers Predicting Overall Survival. Front Oncol 9:874, 2019
55.Franses JW, Philipp J, Missios P, et al: Pancreatic circulating tumor cell profiling identifies LIN28B as a metastasis driver and drug target. Nat Commun 11:3303, 2020

Figure 1. Survival stratified by (a) epithelial CTCs and (B) transitional CTCs in patients with resected PDAC.

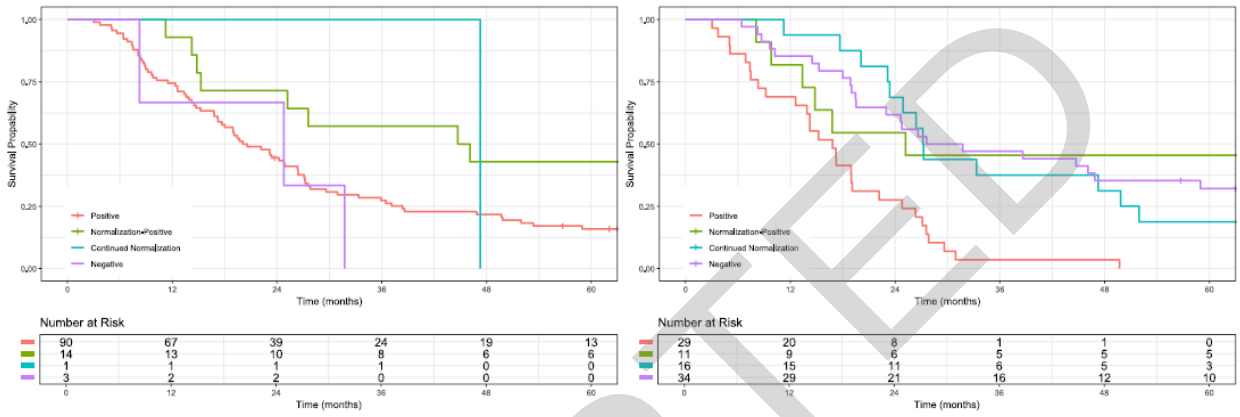

Figure 1. Survival stratified by (a) epithelial CTCs and (B) transitional CTCs in patients with resected PDAC

Figure 2. Comparison between a clinical score developed based on presence of nodal disease and perineural invasion and a translational score developed based on presence of nodal disease, perineural invasion, and postoperative trCTCs in their ability to predict LTS in patients with resected PDAC.

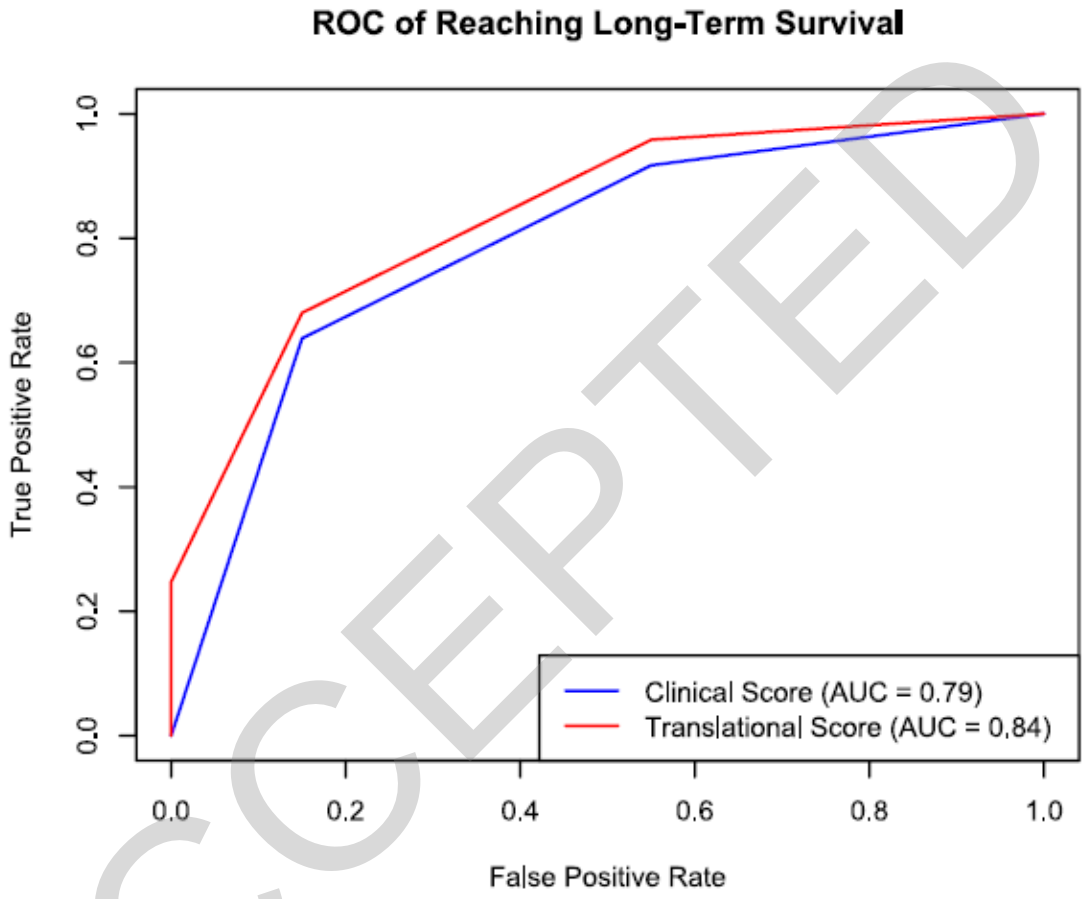

Figure 2. Comparison between a clinical score developed based on presence of nodal disease and perineural invasion and a translational score developed based on presence of nodal disease, perineural invasion, and postoperative trCTCs in their ability to predict LTS in patients with resected PDAC.

Figure 3. Trajectories of transitional CTCs and association with long-term survival.

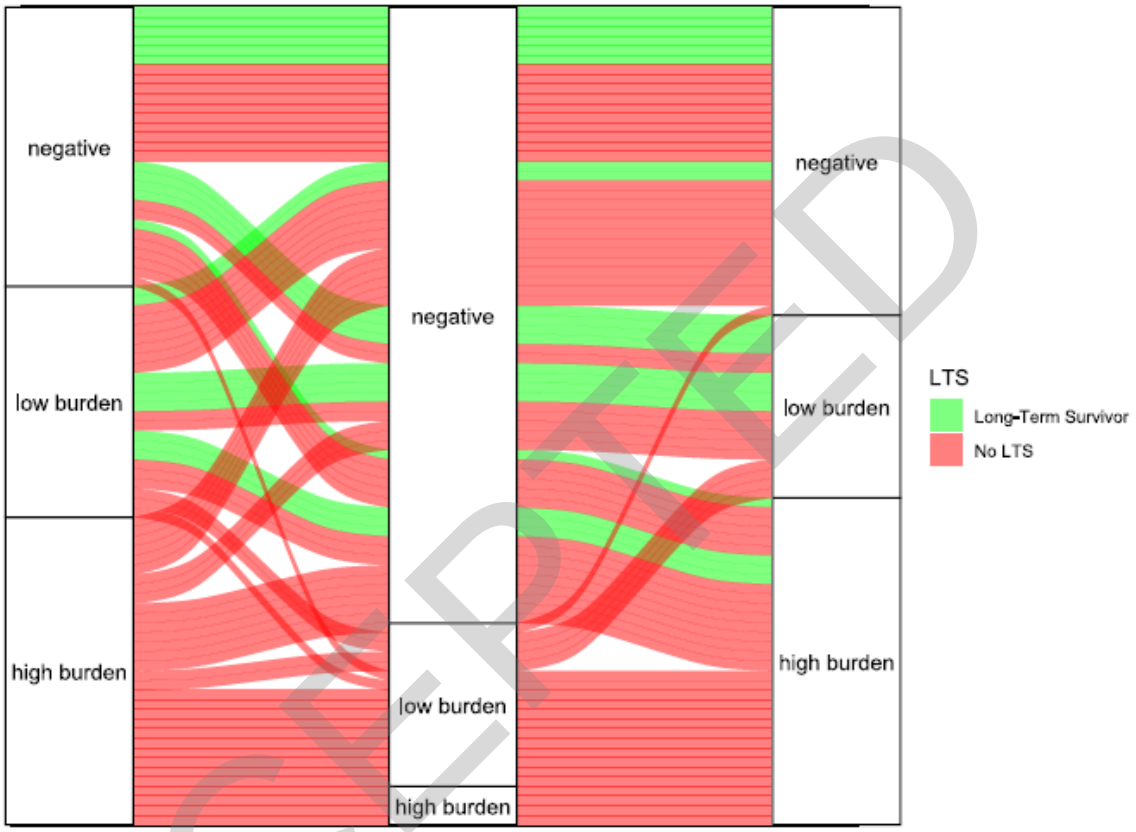

Figure 3. Trajectories of transitional CTCs and association with long-term survival

Table 1. Clinicopathological features and CTC characteristics stratified by LTS			
Variables	Non-LTS (N=110)	LTS (N=22)	P-value
Age, median (IQR)	69.2 (59.9 – 74.8)	64.4 (54.2 – 69.3)	0.082
Sex, female	50 (45.0)	11 (50)	0.696
Smoking status			0.119
Non-smoker	55 (50)	15 (68)	
Smoker	55 (50)	7 (32)	
BMI, kg/m^2			0.327
Normal	95 (86)	17 (77)	
Obese	15 (14)	5 (23)	
CA19-9 at diagnosis			**0.022**
<37	19 (17)	9 (41)	
≥37	68 (62)	10 (45)	
Non-producers/Not available	23 (21)	3 (14)	
Neoadjuvant, received	42 (38)	11 (50)	0.302
Surgery type			0.545
PD	80 (73)	17 (77)	
DP	22 (20)	5 (23)	
Total	8 (7.3)	-	
Tumor size, cm	3.2 (2.38 – 4.33)	2.45 (1.65 – 3.25)	**0.016**
Grade of tumor differentiation			0.857
Well/Moderate	24 (67)	54 (68)	
Poor	12 (33)	25 (32)	
Unknown	3	11	
Margin, R1 positive	18 (17)	2 (9.1)	0.525
Perineural invasion, present	95 (86)	11 (50)	**<0.001**
Lymphovascular invasion, present	64 (58)	6 (27)	**<0.001**
AJCC T-stage			**<0.001**
T0-T2	45 (41)	22 (100)	
T3/T4	65 (59)	-	
Nodal disease, present	76 (69)	6 (27)	**<0.001**
Complete pathological response	2 (1.8)	4 (18)	**0.007**
Adjuvant chemotherapy, received	84 (82)	17 (81)	0.999
Presurgery eCTCs, present	106 (97)	21 (95)	0.525
Presurgery trCTCs, present	79 (72)	10 (45)	**0.013**
Postsurgery eCTCs, present	76 (78)	15 (71)	0.568
Postsurgery trCTCs, present	32 (33)	-	**0.002**
Postadjuvant eCTCs, present	37 (95)	15 (94)	0.999
Postadjuvant trCTCs, present	16 (41)	6 (38)	0.808