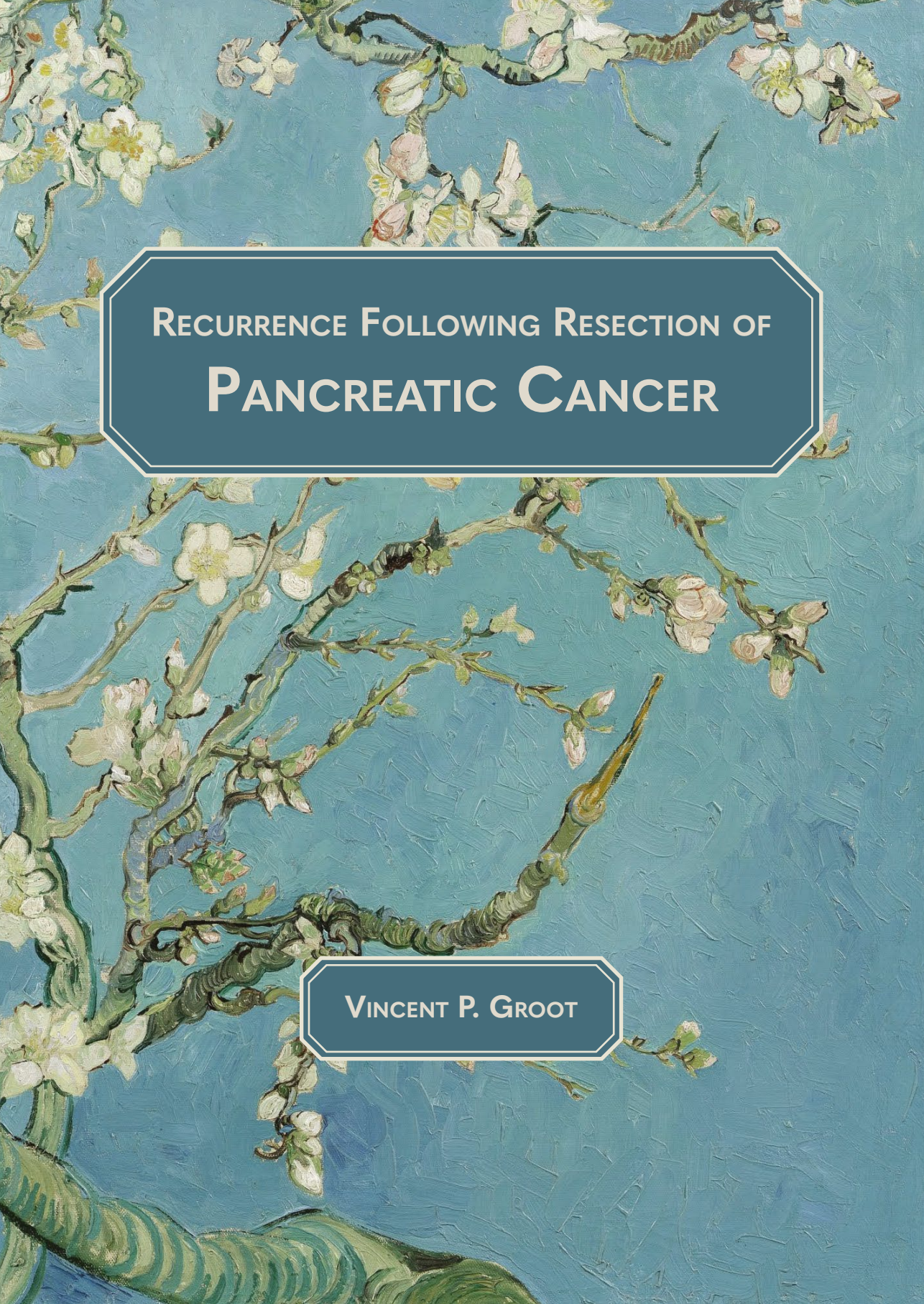

Recurrence Following Resection of Pancreatic Cancer

Vincent P. Groot

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Recurrence Following Resection of Pancreatic Cancer
PhD thesis, Utrecht University, The Netherlands

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Recurrence Following Resection of Pancreatic Cancer

Recidieven na Resectie van Pancreaskanker
(met een samenvatting in het Nederlands)

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ingevolge het besluit van het college voor promoties
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door

Vincent Paul Groot

geboren op 20 april 1990
te 's-Gravenhage

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	Dr. J. He

To my parents and brother,
for their love and invaluable support

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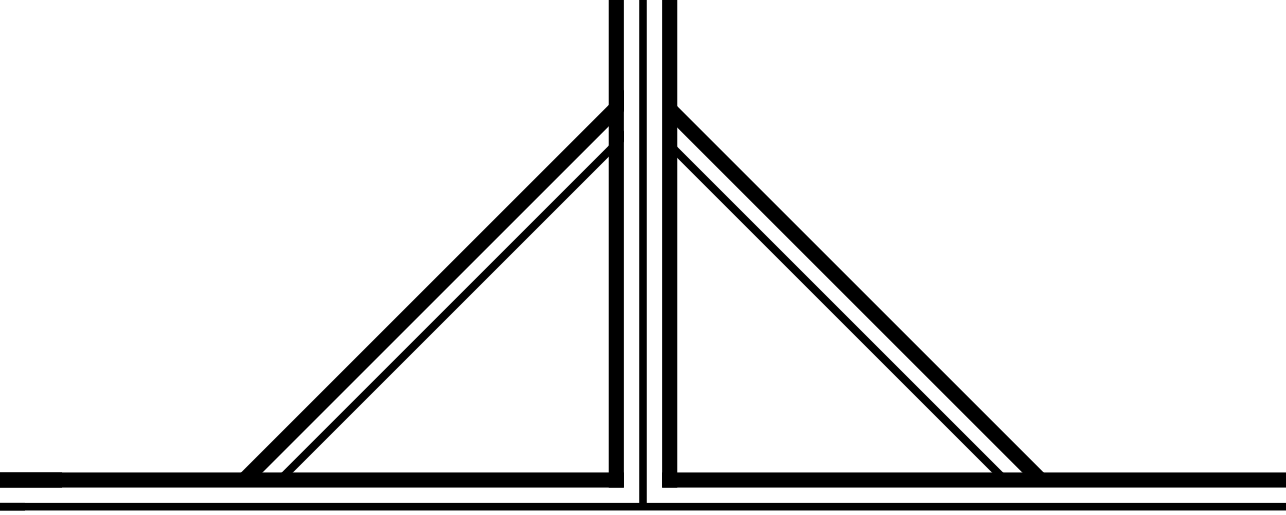

CHAPTER 1

General Introduction and Thesis Outline

Pancreatic Cancer

Epidemiology

Pancreatic cancer is the seventh most common cause of cancer-related death worldwide, while only being the fourteenth most common cancer, with 432,000 deaths and 459,000 new cases projected for 2018.[1] In the Netherlands, 2300 new patients were diagnosed with pancreatic cancer in 2018.[2] The most common type of pancreatic cancer is invasive ductal adenocarcinoma (PDAC), which accounts for ~95% of exocrine pancreatic cancers.[3] Throughout this thesis, when the term "pancreatic cancer" is mentioned, PDAC is implied. Despite decades of effort, the prognosis for patients with PDAC remains devastating. Only 8% of patients with this disease are cured, one of the lowest rate among solid tumors.[4] Furthermore, with progress being made in other cancer fields and an ageing population, PDAC is projected to become the second most common cause of cancer-related death in the Western world by 2030.[5] Due to lack of effective screening methods, coupled with late onset of clinical symptoms and a propensity for early metastasis, most PDAC patients present with either metastatic (40-50%) or advanced localized disease (30-40%) at initial presentation.[6,7] Consequently, only 10-20% of newly diagnosed patients present with a localized carcinoma amenable to resection.[8]

Multimodality Treatment

Complete surgical removal offers the best chance of long-term survival in patients with localized PDAC. However, surgery for PDAC has historically been associated with considerable morbidity and a high risk of peri-operative mortality. This was particularly true for the pancreatoduodenectomy (Whipple operation[9]), which carried a hospital mortality rate of around 25% up to the early 1980s.[10] Since that time, the mortality rate has decreased considerably, in great part due to extraordinary efforts by Dr. John L. Cameron, former Chair of Surgery of the Johns Hopkins Hospital (Figure 1).[11] Dr. Cameron set a new standard by publishing his personal series of, first 1000 pancreatoduodenectomies, and then 2000 pancreatoduodenectomies with a 1% peri-operative mortality rate.[10,12]

Resection of the primary carcinoma has been the foundation of treatment for patients with localized PDAC.[8] However, due to locally invasive and micrometastatic disease at time of resection, high rates of local and systemic recurrence following curative-intent pancreatectomy were observed.[6] As a result, survival after resection remained disappointing at 18-20 months.[13] Following several landmark randomized controlled clinical trials, adjuvant chemotherapy was acknowledged as standard of care to improve systemic control and survival outcomes. By the

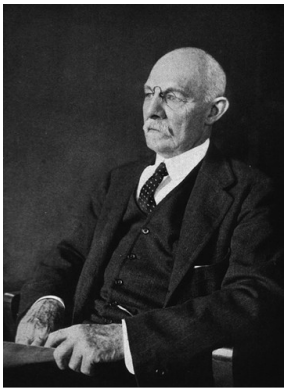

Figure 1. As 1st and 5th Chairs of Surgery at the Johns Hopkins Hospital (Baltimore, USA), Dr. William Stewart Halsted (left) performed the first successful resection of a periampullary tumor in 1898, while Dr. John Lemuel Cameron (right) minimized the peri-operative mortality rate and ushered in the modern era of pancreatic surgery.

late 2000s, the CONKO-001 and ESPAC-3 trials had established both 5-FU and Gemcitabine as potential options for adjuvant chemotherapy, albeit with modest effectiveness.[14,15] More recently, the ESPAC-4 trial showed that addition of Capecitabine to Gemcitabine improved post-pancreatectomy survival by 2.5 months (28.0 vs 25.5 months) when compared to resection followed by Gemcitabine alone.[16]

Why Does Resection Fail?

Even after combination of "curative-intent" resection and systemic therapy, less than 4% of resected PDAC patients will live 10 years or more.[17] Near the end of 2018, the PRODIGE-24 trial published remarkable results of adjuvant treatment with a modified FOLFIRINOX (fluorouracil, leucovorin, irinotecan, and oxaliplatin) regimen as compared with Gemcitabine in patients with resected PDAC.[18] In selected patients with a post-operative serum carbohydrate antigen (CA) 19-9 level of <180 U/mL, an unprecedented median survival of 54 months in the FOLFIRINOX group was observed, firmly establishing FOLFIRINOX as a standard of care treatment.[19] Despite the encouraging survival, more than 50% of patients in the FOLFIRINOX group, and more than 70% in the Gemcitabine group developed disease recurrence. As the data of this trial remain immature, with 61% of all patients being alive at the time of analysis, this number of patients with recurrence will undoubtedly continue to rise.

Even though disease recurrence remains the major barrier to curing PDAC, few have studied PDAC recurrence as a primary outcome of interest. A more accurate understanding of disease failure after seemingly successful surgery is deemed essential in the pursuit of improved

outcomes for PDAC patients. Therefore, the current thesis aims to provide an extensive comprehension of multiple aspects of recurrence following pancreatectomy for localized PDAC. **Part I** explores the incidence and predictors of specific PDAC recurrence patterns. Additionally, the impact on survival of the location and timing of recurrence are examined. In **Part II**, the new paradigm of neoadjuvant therapy is studied, with a focus on recurrence and survival outcomes. **Part III** underlines the difficulties and controversies surrounding post-pancreatectomy follow-up and detection of recurrence. In **Part IIIa** the performance and role of imaging during post-operative surveillance are discussed. **Part IIIb** considers the value of biomarkers for the detection of recurrence PDAC, including two prospective longitudinal studies that investigated the potential clinical utility of both circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in this setting. Lastly, **Part IV** portrays the potential of individualized treatment of recurrent disease, based on experiences with local and pulmonary recurrence.

Aspects of Pancreatic Cancer Recurrence

Patterns, Timing and Implications of Recurrence

Not all PDACs exhibit the same biological behavior. Patients undergoing resection represent one way to study the heterogeneity in biological behavior of PDAC, as they form a relatively homogeneous population rendered free of clinical disease at a defined time point. Thus, the natural pattern and rate of recurrence can be assessed based on this consistent frame of reference. This is of importance, as a more detailed understanding of the timing of disease failure and the factors predicting specific recurrence patterns after curative-intent surgery for PDAC can potentially help guide a more personalized treatment approach (**Chapter 2**). Additionally, knowledge on the prognostic impact of both location and timing of recurrence can further assist patient and physician alike when discussing the balance between quality of life and further treatment options (**Chapter 3** and **Chapter 4**).

Recurrence After Neoadjuvant Therapy and Resection

Approximately 30-50% of newly diagnosed patients are considered to have advanced localized PDAC, due to local involvement of adjacent veins and arteries.[8] The more aggressive utilization of potent chemotherapies in the neoadjuvant setting have increasingly allowed these patients to undergo curative-intent pancreatectomy. Recent retrospective series have suggested a survival benefit for these patients who were resected after neoadjuvant therapy.[20-22] However, these reports were limited by cohort heterogeneity and lack of comparison with patients who were treated with chemotherapy alone. Therefore, it is of interest to determine if surgical

resection of locally advanced PDAC is associated with improved survival compared with non-operative treatment (**Chapter 5**).

Following neoadjuvant therapy, a pathologic complete response is observed in around 3-11% of resected PDAC specimens.[23] In other cancers, a complete response is associated with greatly improved survival.[24] However, the independent prognostic value of a complete response in PDAC remains unclear (**Chapter 6**). As with primary resected PDAC, disease recurrence remains the principal cause of limited survival in patients undergoing post-neoadjuvant resection. A greater understanding of PDAC recurrence can provide valuable insight when contemplating appropriate strategies regarding adjuvant treatment, oncologic surveillance, and management of recurrence. However, the incidence, timing, and implications of disease recurrence in patients undergoing post-neoadjuvant resection of advanced localized disease are not well established (**Chapter 7**).

Post-Pancreatectomy Follow-Up

Diagnostic follow-up could facilitate earlier detection of PDAC recurrence when the tumor load is still low, arguably increasing the chance of effective systemic and local control. Potential disadvantages of routine, recurrence-focused diagnostic testing are introduction of stress and morbidity that can result in a decrease in quality of life, while the cost-effectiveness of treatment and impact on survival are yet unclear. Given the ongoing debate on this topic, it is of interest to provide an overview of the best available evidence for frequency, methodology and focus of follow-up after PDAC resection (**Chapter 8**). Additionally, assessment of the current practice of follow-up in the Netherlands could aid in the development of desirable novel studies and guidelines on post-pancreatectomy follow-up (**Chapter 9**).

Routine imaging is the foundation of recurrence-focused diagnostic follow-up. Radiologic assessment of the extent and location of recurrent PDAC can provide critical information that determines patient eligibility for any additional treatment or inclusion in clinical trials. As radiological assessment after pancreatectomy is challenging, knowledge on the diagnostic performance of different imaging modalities for detection of recurrent PDAC is essential (**Chapter 10**). Adhering to the European guidelines, follow-up in the Netherlands generally does not include imaging for the detection of PDAC recurrence.[25] However, it is unknown how often patients undergo imaging for PDAC recurrence in actual daily practice (**Chapter 11**). Biomarker testing can complement imaging surveillance when monitoring disease progression after surgery, as a sudden elevation of serum tumor markers can indicate clinically occult proliferation of minimal residual PDAC. First discovered in 1979, CA 19-9 has become the

most studied and well-known biomarker for PDAC.[26] However, there is limited data on the diagnostic value of CA 19-9 and other serum tumor markers for the early detection of recurrent pancreatic cancer during follow-up (**Chapter 12**).

Clinical Utility of Liquid Biopsies

Liquid biopsies are rapidly emerging as important tools in precision oncology, possibly facilitating early detection of cancer, tracking of evolutionary dynamics and heterogeneity of tumors, and recognition of emergence of therapy resistance.[27,28] The term liquid biopsy is commonly used to refer to the isolation and analysis of tumor derived material from peripheral blood, or other fairly easily accessible fluids such as urine, ascites, pleural effusions or cerebrospinal fluid.[29] For example, intact circulating tumor cells can intravasate into the bloodstream (Figure 2). Additionally, cell-free circulating DNA is thought to be released into the circulation through apoptosis or necrosis of tumor cells. Due to the low-abundance of circulating tumor cells and tumor DNA amidst a backdrop of normal germline cells and DNA, detection and analysis of these liquid biopsies represent a considerable challenge. Consequently, methods for enrichment and detection are constantly expanding and evolving, resulting in different assays with varying advantages and drawbacks regarding sensitivity, limit of detection and breadth of coverage.[29,30]

Particularly in pancreatic cancer, the potential of liquid biopsies is tantalizing, as it may allow for non-invasive testing of an otherwise hardly accessible organ.[31] For instance, if liquid biopsies are able to reliably identify residual or progressive disease,

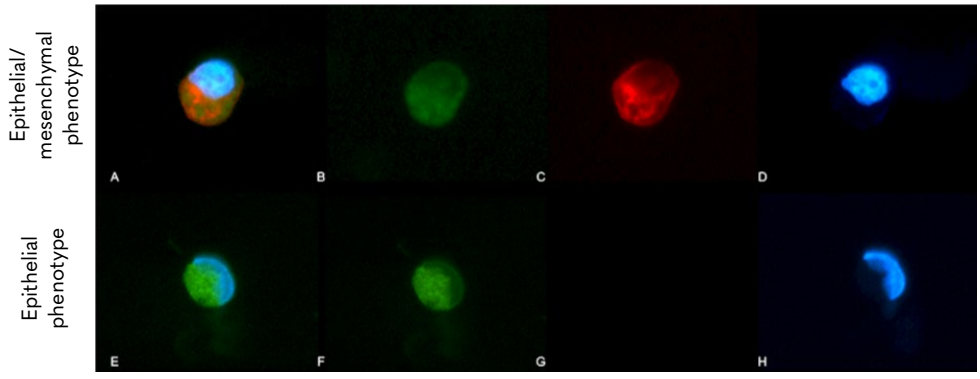

Figure 2. Two distinct phenotypes of circulating tumor cells: epithelial/mesenchymal phenotype (A), positive for pan-cytokeratin (B), vimentin (C) and DAPI (D), and epithelial phenotype (E), positive pan-cytokeratin (F), and DAPI (H), and negative for vimentin (G). All immunofluorescent images under 20X magnification (Nikon Eclipse Ti-E microscope).

refractory PDAC patients can be started or switched on to second- or third-line regimens in a timely manner. Despite the current high enthusiasm, the American Society of Clinical Oncology has advised that more robust research demonstrating clinical validity or clinical utility is needed before liquid biopsies can be deployed in routine clinical pratice.[32] To this effect, a prospective longitudinal cohort study (NCT02974764) was designed to investigate the clinical significance of circulating tumor cell dynamics in patients with resected PDAC (**Chapter 13**).[33] Additionally, analytical and clinical validation of a *KRAS* circulating tumor DNA assay using digital-droplet PCR was conducted, in preparation of offering this assay as an in-house clinical test in the Johns Hopkins Hospital (**Chapter 14**).

Treatment of Recurrence

Patients with PDAC recurrence constitute a substantial group that faces limited treatment possibilities and poor survival of only 3 to 9 months after recurrence detection.[34] Yet, more effective multi-drug regimens, local ablative therapies and even surgical re-resection represent expanded treatment options that could offer improved systemic and local control of PDAC recurrence.[35-37] Patients with isolated local and pulmonary recurrence in particular seem to exhibit a slower growing tendency and extended survival. Consequently, these patients could represent unique clinical subpopulations with potentially favorable tumor biology, in which a more intensive treatment strategy might be justified.[38,39] However, published data on treatment of local recurrence are limited, and little is known about which patients could benefit (**Chapter 15** and **Chapter 16**). Furthermore, it remains unclear to what degree patient factors, recurrence features (i.e. timing, extent, presentation), and distinct treatment strategies are related to survival in patients with pulmonary PDAC recurrence (**Chapter 17**).

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PART I

Recurrence Following Primary Resection

CHAPTER 2

Patterns, Timing, and Predictors of Recurrence Following Pancreatectomy for Pancreatic Ductal Adenocarcinoma

Vincent P. Groot, Neda Rezaee, Wenchuan Wu, John L. Cameron, Elliot K. Fishman, Ralph H. Hruban, Matthew J. Weiss, Lei Zheng, Christopher L. Wolfgang, Jin He

Annals of Surgery 2018;267:936-945

Abstract

Objective

After surgery for PDAC, most patients will develop disease recurrence. Understanding the patterns and timing of disease failure can help guide improvements in therapy. This study aims to describe accurately the pattern, timing, and predictors of disease recurrence after a potentially curative resection for pancreatic ductal adenocarcinoma (PDAC).

Methods

Patients who underwent pancreatectomy for PDAC at the Johns Hopkins Hospital between 2000 and 2010 were included. Exclusion criteria were incomplete follow-up records, follow-up <24 months, and neoadjuvant therapy. The first recurrence site was recorded and recurrence-free survival (RFS) was estimated using Kaplan-Meier curves. Predictive factors for specific recurrence patterns were assessed by univariable and multivariable analyses using Cox-proportional hazard regression models.

Results

From the identified cohort of 1103 patients, 692 patients had comprehensive and detailed follow-up data available. At a median follow-up of 25.3 months, 531 (76.7%) of the 692 had recurred after a median RFS of 11.7 months. Most patients recurred at isolated distant sites (n=307, 57.8%), while isolated local recurrence was seen in 126 patients (23.7%). Liver only recurrence (n=134, 25.2%) tended to occur early (median 6.9 months), while lung only recurrence (n=78, 14.7%) occurred later (median 18.6 months). A positive lymph node ratio >0.2 was a strong predictor for all distant disease recurrence. Patients receiving adjuvant chemotherapy or chemoradiotherapy had fewer recurrences and a longer RFS of 18.0 and 17.2 months, respectively.

Conclusion

Specific recurrence locations have different predictive factors and possess distinct RFS curves, supporting the hypothesis that unique biological differences exist among tumors leading to distinct patterns of recurrence.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related death in the United States by 2030.[1] Lack of screening and early detection, coupled with the propensity for early metastasis and minimally effective systemic therapy, remain major barriers to curing patients with PDAC. Only a minority of patients present with a tumor suitable for oncological resection, the best chance for long-term survival. However, even after curative-intent surgery, most patients will develop disease recurrence resulting in a 5-years survival of only 12% to 27%.[2-5]

Not all pancreatic tumors exhibit the same biological behavior. Among patients with PDAC, diverse patterns and timing of recurrence exist that cannot be explained by differences in disease stage alone. For example, while the majority of patients present with systemic disease or progress to develop systemic disease, a significant proportion of patients will develop isolated local recurrence. Even among the patients who develop systemic disease, metastatic spread will in some cases be limited to the liver, lung, or peritoneum. The current literature on patterns and timing of disease failure after curative-intent surgery for PDAC varies significantly, possibly due to smaller sample sizes and limited follow-up. Two important and often cited landmark studies on PDAC by Van den Broeck et al.[6] and Sperti et al.,[7] for instance, report variable local recurrence (17% vs. 33%), distant recurrence (60% vs. 28%), and distant + local recurrence (23% vs. 39%) rates. A more accurate and detailed understanding of the timing of disease failure and the factors predicting specific recurrence patterns after curative-intent surgery for PDAC can be of importance for prognosis stratification and possibly help guide a more personalized treatment approach.

Patients undergoing oncological resection of PDAC represent one way to study the heterogeneity in biological behavior of PDAC, as they form a relatively homogeneous population rendered free of clinical disease at a defined time point. Thus, the natural pattern and rate of recurrence can be assessed based on this consistent frame of reference. This report presents a large and detailed single institutional study that characterizes patterns and timing of disease recurrence following resection of PDAC. Furthermore, our prospectively maintained database allows for an accurate analysis of clinicopathological features and treatment characteristics associated with the site of first recurrence, to better understand these aspects of the biological behavior of PDAC.

Methods

Patient Selection

The study cohort consisted of patients who underwent standard pancreatoduodenectomy (PD), pylorus-sparing PD, or total pancreatectomy at the Johns Hopkins Hospital between 2000 and 2010. From a prospectively maintained database, only those patients with resectable localized and histologically confirmed PDAC in the head of the pancreas were included. To ensure accuracy and homogeneity of the follow-up data, patients with incomplete available records were excluded. This included patients with less than 24 months of follow-up, in which neither recurrence nor death had occurred. Exclusion criteria were use of neoadjuvant therapy, presence of distant metastasis at the time of resection, and grossly positive resection margin (R2). Patients with surgery-related mortality, defined as death during the index admission after pancreatectomy or death within 90 days of operation, were also excluded. The primary outcomes of interest were pattern of recurrence and timing of recurrence, defined by recurrence-free survival (RFS). Our institutional review board approved this retrospective study.

Demographics, Clinicopathological, and Treatment Characteristics

Resectability and staging were established using pancreatic protocol computed tomography (CT) and were typically discussed in a multidisciplinary setting. A team specializing in hepatobiliary and pancreatic surgery performed all pancreatic resections. The prospectively maintained mono-institutional database was complemented, where necessary, with information obtained from the electronic medical record. Surgical specimens were assessed by an experienced pancreatic pathologist who made the diagnosis of PDAC and described tumor characteristics including resection margin, tumor size, T-stage, lymph node status (N-stage), tumor differentiation grade, and presence of microscopic vascular or perineural invasion. Lymph node ratio (LNR) was defined as the number of lymph nodes harboring cancer divided by the number of total nodes harvested. Cancer stage was defined according to the 7th edition of the American Joint Committee on Cancer staging system.[8] As described for PDAC, resection margin (R) status was defined as R0 when distance of tumor cells to the closest resection margin was >1 mm or R1 when distance was ≤1 mm. Intraoperative frozen section and resection margin assessment was performed on the pancreatic neck, uncinate, and bile duct margin.

Evaluated treatment factors included type of operation, operative complications, and necessity of vascular resection and reconstruction. Vascular resections were done at the

surgeon's discretion. Complications were graded according to the Clavien-Dindo classification system.[9] Stratification of received adjuvant therapy occurred in 3 groups: chemotherapy, chemoradiotherapy, or none. Specific adjuvant therapy details were deemed to be beyond the scope of this study.

Follow-up and Pattern of Recurrence

Patient follow-up occurred at the outpatient clinic of our institution, performed by either a surgical, medical, or radiation oncologist. In general, following completion of all therapy, patients had at least 1 follow-up by computed tomography of the chest, abdomen, and pelvis every 6 months for the first 2 years and then yearly for 5 more years. When pre-operative elevated levels were observed, carbohydrate antigen 19-9 was evaluated every 6 months. Occasional additional imaging modalities performed to determine pattern of recurrence consisted of magnetic resonance imaging and fluorodeoxyglucose positron emission tomography.

When imaging findings were consistent with recurrence, biopsy was rarely performed. Either radiological or histological evidence was required for the diagnosis of recurrent disease. When considering patterns of recurrence, only the first site was documented in this study, using 6 mutually exclusive categories. Local recurrence was defined as recurrence in the remnant pancreas or in the surgical bed, such as soft tissue along the celiac or superior mesenteric artery, aorta or around the pancreatojejunostomy site. Distant recurrence was stratified into 3 different categories: "liver only" and "lung only" for isolated hepatic and pulmonic recurrence respectively, and "other" for recurrence occurring in other less common locations. When both isolated local recurrence and isolated distant recurrence were revealed at the same moment, recurrence was defined as "local + distant" recurrence. The category "multiple" contained patients in whom recurrence occurred in multiple distant sites and/or carcinomatosis was found.

Definitions and Statistical Analysis

Summary statistics were obtained using established methods. Length of follow-up was calculated from the date of operation to the date of death or the last follow-up. Date of recurrence was defined as the date of the first follow-up cross-sectional imaging study (CT, PET/CT, or magnetic resonance imaging) demonstrating recurrence. RFS was defined as the time interval between the date of the operation and the date of recurrence. Kaplan-Meier curves were used to estimate median RFS with corresponding 95% confidence intervals (95% CI) and the log-rank test was performed for pairwise comparison between recurrence locations.

To identify potential factors predicting patterns of recurrence, univariable and multivariable analyses of clinicopathological variables and treatment characteristics were performed using Cox-proportional hazard regression models. Employing backward elimination, all factors with a P-value of <0.10 in univariable analysis were included as a covariate in multivariable regression analysis to identify independent prognostic factors. A 2-tailed P-value of <0.05 was considered statistically significant. Values are expressed as median and range, unless specified otherwise. Statistical analysis was performed with SPSS statistical software v22.0 (IBM Corp., Armonk, NY).

Results

Cohort Characteristics

Between 2000 and 2010, a total of 1103 patients underwent upfront PD or total pancreatectomy for histologically confirmed PDAC of the head of the pancreas at the Johns Hopkins Hospital. Excluded from this cohort were 17 patients (1.5%) who died within 90 days after the operation and 188 patients (17.0%) with less than 24 months of follow-up, in which neither recurrence nor death had occurred. A further 206 patients (18.7%) were followed up at other institutions and did not have complete and accurate recurrence data available. After exclusion of these patients, a cohort of 692 patients with comprehensive and detailed follow-up data was available for further analysis. Demographics, clinicopathological, and treatment characteristics of these patients are summarized in Table 1.

Median follow-up for all patients was 25.3 months (95% CI 23.1-27.6). At last follow-up, recurrence was documented in 531 patients (76.7%) while 161 patients (23.3%) had no sign of recurrence after a minimum follow-up of 24 months. For patients with and without recurrence, median follow-up was 20.1 months (95% CI 18.1-22.1) and 53.5 months (95% CI 43.8-63.2) respectively.

Pattern of Recurrence

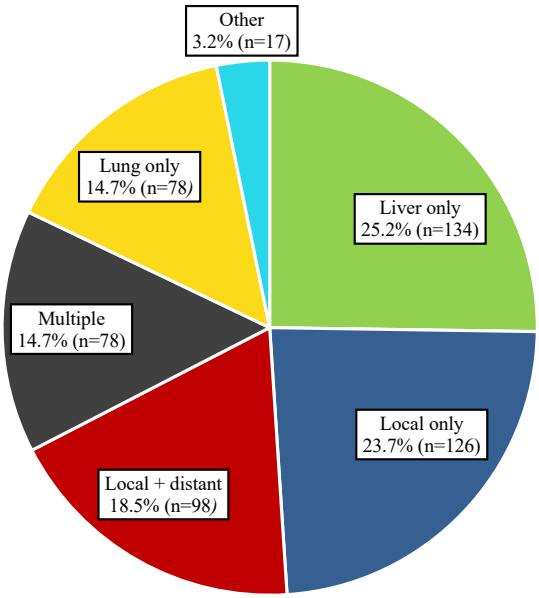
Overall, 531 patients (76.7%) had radiologic or histologic evidence of recurrent PDAC. Distribution of the 6 different patterns of recurrence can be seen in Figure 1A. Most patients first recurred at isolated distant sites (n=307, 57.8%), while isolated local recurrence was seen in 126 patients (23.7%). The remaining 98 patients (18.5%) had both a local and distant site as first recurrence location. In these 98 patients, liver (n=49) and lung (n=14) lesions were found most often in combination with local recurrence. Intra-abdominal recurrence occurred solitarily in 407 patients (76.6%) and together with extra-abdominal recurrence in 31 patients (5.8%). Solitary extra-abdominal recurrence was less common (n=93, 17.5%) and occurred

Table 1. Demographics, clinicopathologic and treatment characteristics of 692 included patients

Variable	All patients (n=692)
Male, n (%)	365 (52.7%)
Race, n (%)	
Caucasian	599 (86.6%)
Other	93 (13.4%)
Age, mean years (SD)	65.3 (10.6)
Operation procedure, n (%)	
Pylorus-preserving pancreatoduodenectomy	393 (56.8%)
Standard pancreatoduodenectomy	270 (39.0%)
Total pancreatectomy	29 (4.2%)
Vascular resection, n (%)	47 (6.8%)
Blood transfusion, n (%)	251 (36.3%)
Morbidity, n (%)	
≤Clavien-Dindo grade II	562 (81.2%)
≥Clavien-Dindo grade III	130 (18.8%)
Resection margin, n (%)	
R0 (>1.0 mm)	451 (65.2%)
R1 (≤1.0 mm)	241 (34.8%)
Tumor differentiation, n (%)	
Well/Moderate	445 (64.3%)
Poor	247 (35.7%)
Tumor size, mean cm (SD)	3.1 (1.5)
T-stage, n (%)	
1-2	155 (22.4%)
3-4	537 (77.6%)
Positive lymph nodes, n (%)	525 (75.9%)
Positive lymph node ratio, n (%)	
≤0.2	460 (66.5%)
>0.2	232 (33.5%)
Microscopic perineural invasion, n (%)	622 (89.9%)
Microscopic lymphovascular invasion, n (%)	404 (58.4%)
AJCC stage 7th edition, n (%)	
≤2A	156 (22.5%)
≥2B	536 (77.5%)
Adjuvant treatment, n (%)	
None	226 (32.7%)
Chemotherapy	142 (20.5%)
Chemoradiotherapy	324 (46.8%)

AJCC, American Joint Committee on Cancer; SD, standard deviation.

A



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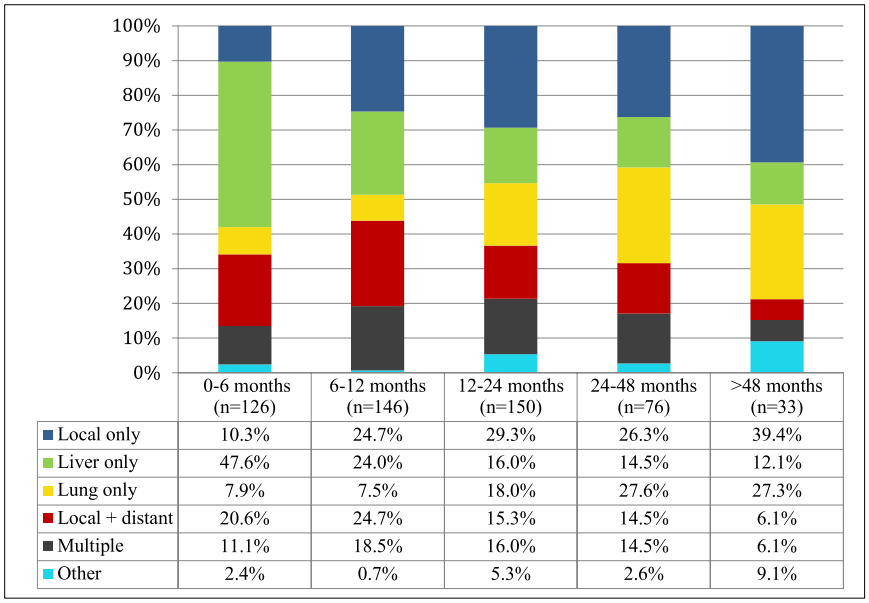


	0-6 months (n=126)	6-12 months (n=146)	12-24 months (n=150)	24-48 months (n=76)	>48 months (n=33)
Local only	10.3%	24.7%	29.3%	26.3%	39.4%
Liver only	47.6%	24.0%	16.0%	14.5%	12.1%
Lung only	7.9%	7.5%	18.0%	27.6%	27.3%
Local + distant	20.6%	24.7%	15.3%	14.5%	6.1%
Multiple	11.1%	18.5%	16.0%	14.5%	6.1%
Other	2.4%	0.7%	5.3%	2.6%	9.1%

Figure 1. (A) Distribution of recurrence patterns and (B) distribution of recurrence pattern at different time points.

predominantly in the lung (n=78). Other extra-abdominal recurrences were found in osseous structures, the brain, supraclavicular lymph nodes, the groin, thigh muscle, and the skin.

The proportion of the recurrence locations differed significantly at progressive time points (Figure 1B). At 6 months, liver only recurrence was responsible for almost half of all documented recurrences (47.6%). However, liver only recurrence accounted for just 12.1% of the recurrences occurring after 48 months (P<0.001). Whereas the prevalence of liver recurrence diminishes over time, both local and lung only recurrences combined for 57.8% of all recurrences occurring after 2 years after surgery.

Timing of Recurrence

Among the 531 patients who recurred, 272 (51.2%) had done so within 12 months and 422 (79.5%) within 24 months after operation (Figure 2). By 5 years after resection, almost 97% of all observed recurrences had occurred. Consistent with previous publications, 5 year RFS did not equal cure. Of the 90 patients who were free of recurrence after 5 years of follow-up, 17 patients (18.9%) went on to develop recurrence after a median follow-up of 83.7 months (62.5-154.7). In 10 of these 17 patients, late recurrence occurred locally. Local recurrence was described on imaging to be adjacent to the pancreatic resection bed in 8 patients. However, the other 2 patients presented with a new density in the pancreatic tail, possibly indicating a second primary tumor in the remnant pancreas. Locations of the remaining 7 late recurrences were liver (n=3), other (n=2), local + distant (n=1), and multiple (n=1). The cumulative recurrence rates per location category in different time periods after pancreatectomy are shown in Figure 2.

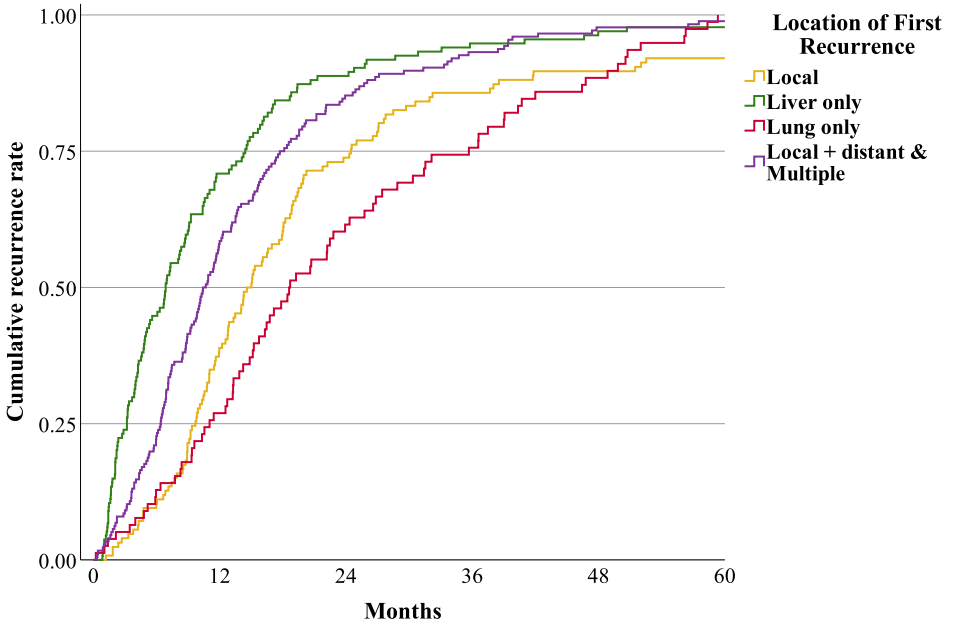
Median RFS was 11.7 months (95% CI 10.5-12.9) for the 531 patients who developed a recurrence and 15.9 months (95% CI 14.3-17.5) for the entire cohort. Median RFS for patients receiving adjuvant chemoradiotherapy (17.2 months; P=0.001) and chemotherapy (18.0 months; P=0.008) was significantly longer when compared with patients who did not receive any adjuvant treatment (10.5 months). A pairwise comparison of median RFS among the specific recurrent patterns is shown in Figure 3. Median RFS for patients with local only, lung only, and other recurrence exceeded 12 months and did not differ significantly from each other. Median RFS for patients with liver only recurrence (6.9 months, 95% CI 4.9-8.9) was significantly shorter when compared with all other recurrence locations, except for local + distant recurrence (10.0 months, 95% CI 8.5-11.6) (P=0.129). The limited RFS for patients with liver only recurrence is further reflected by the high cumulative early recurrence rate; after 6 months, almost 45% of liver only recurrences had occurred compared with only 10.3%

(P<0.001) and 12.8% (P=0.001) of the local and lung only recurrences, respectively (Figure 2). Figure 2 further illustrates how both lung (61.5%) and local (73.8%) recurrence start to "catch up" with liver only recurrence (88.8%) around the 2-year mark. At 48 months, this process is almost complete with almost 90% of local and lung only recurrence having occurred.

Factors Associated with Patterns of Recurrence

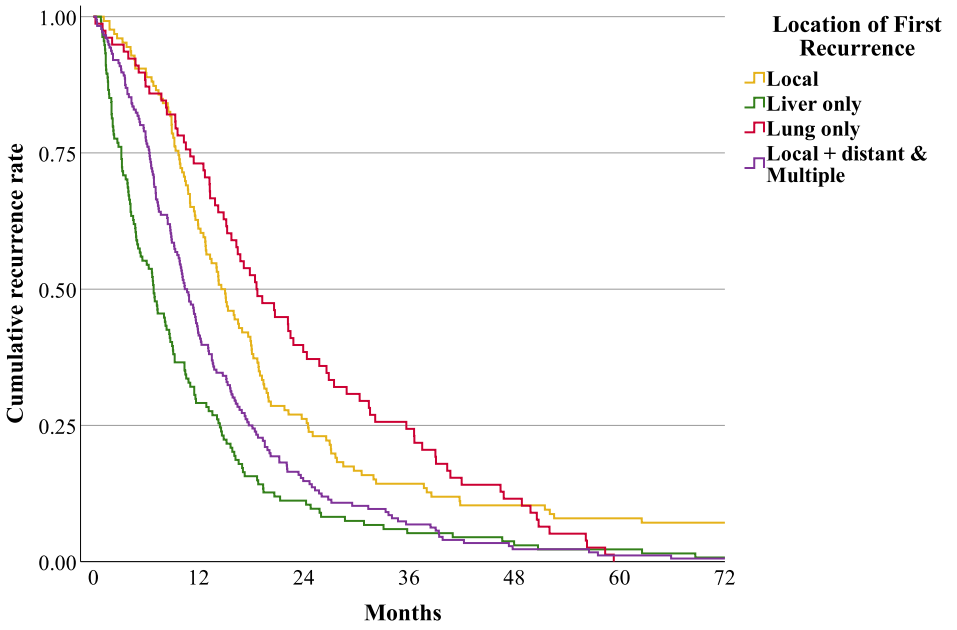
When adjusting for possible confounding variables using Cox-proportional hazards modeling, several prognostic clinicopathological factors were found to be independently associated with an increased likelihood of recurrence: poor differentiation grade (hazard ratio (HR) 1.41, 95% CI 1.18-1.69, P<0.001), lymphovascular invasion (HR 1.32, 95% CI 1.09-1.60, P=0.004), R1- resection margin (HR 1.37, 95% CI 1.14-1.64, P=0.001), and an LNR >0.2 (HR 1.93, 95% CI 1.60-2.34, P<0.001). Tumor size, analyzed as a continuous variable in centimeters, was an additional independent predictor for recurrence (HR 1.08, 95% CI 1.02-1.13, P=0.009). Patients receiving adjuvant chemotherapy (HR 0.75, 95% CI 0.57-0.97, P=0.027) and chemoradiotherapy (HR 0.73, 95% CI 0.61-0.89, P=0.001) had a decreased likelihood of recurrence when compared with patients who did not receive any adjuvant therapy.

Subsequent multivariable regression analysis further revealed independent predicting factors for the different specific recurrence locations; results with corresponding 95% CI are shown in Table 2. An LNR >0.2 was a strong prognostic factor for all recurrence locations, with the exception of local only recurrence (P=0.358). R1-resection margin was strongly predictive for local only recurrence (HR 2.46; P<0.001). Apart from the aforementioned LNR >0.2, poor tumor differentiation (HR 2.48; P<0.001) and increasing tumor size per centimeter (HR 1.13; P=0.006) were significantly associated with liver only recurrence. Also, poor tumor differentiation was an independent predictor for multiple recurrence and/or carcinomatosis (HR 1.72; P=0.019). In addition to the LNR, lymphovascular invasion was the only other independent predictor for lung only recurrence (HR 1.67; P=0.042). Multivariable analysis further revealed that patients who received adjuvant chemoradiotherapy had a significantly decreased likelihood of local only recurrence (HR 0.64; P=0.024) when compared with patients who did not receive adjuvant therapy. Furthermore, both chemotherapy and chemoradiotherapy were found to significantly reduce the likelihood of local + distant recurrence. The presence of perineural invasion did not correlate with recurrence or a specific recurrence pattern.



Recurrence patterns	*0-6 months*	*6-12 months*	*12-24 months*	*24-48 months*	*48-60 months*
Local only (n=126)	10.3% (n=13)	38.9% (n=49)	73.8% (n=93)	89.7% (n=113)	92.1% (n=116)
Liver only (n=134)	44.8% (n=60)	70.9% (n=95)	88.8% (n=119	97.0% (n=130)	97.8% (n=131)
Lung only (n=78)	12.8% (n=10)	26.9% (n=21)	61.5% (n=48)	88.5% (n=69)	100.0% (n=78)
Local + distant (n=98)	26.5% (n=26)	63.3% (n=62)	86.7% (n=85)	98.0% (n=96)	99.0% (n=97)
Multiple (n=78)	17.9% (n=14)	52.6% (n=41)	83.3% (n=65)	97.4% (n=76)	98.7% (n=77)
Other (n=17)	17.6% (n=3)	23.5% (n=4)	70.6% (n=12)	82.4% (n=14)	88.2% (n=15)
All recurrence (n=531)	23.7% (n=126)	51.2% (n=272)	79.5% (n=422)	93.8% (n=498)	96.8% (n=514)

Figure 2. Cumulative recurrence rate per specific recurrence pattern as a function of time. For clarity, the curve for "other" recurrence has been omitted and the curves for "local + distant" and "multiple" recurrence have been combined.



Recurrence patterns	*Median RFS months (95% CI)*	*Lung only*	*Other*	*Local only*	*Multiple*	*Local + distant*	*Liver only*
Lung only (n=78)	18.6 (14.3-22.9)		0.456	0.454	**0.002**	**<0.001**	**<0.001**
Other (n=17)	15.7 (10.8-20.7)	0.456		0.399	0.082	**0.015**	**0.003**
Local only (n=126)	14.6 (12.4-16.8)	0.454	0.399		**0.031**	**<0.001**	**<0.001**
Multiple (n=78)	11.7 (10.5-12.9)	**0.002**	0.082	**0.031**		0.299	**0.011**
Local + distant (n=98)	10.0 (8.5-11.6)	**<0.001**	**0.015**	**<0.001**	0.299		0.129
Liver only (n=134)	6.9 (4.9-8.9)	**<0.001**	**0.003**	**<0.001**	**0.011**	0.129	

Figure 3. Recurrence-free survival curves and pairwise comparison of median recurrence-free survival for different recurrence patterns. For clarity, the curve for "other" recurrence has been omitted and the curves for "local + distant" and "multiple" recurrence have been combined.

Discussion

This report presents a large and detailed single institutional study that characterizes patterns and timing of disease recurrence following resection of PDAC. Furthermore, our prospectively maintained database allowed for the first accurate analysis of clinicopathological features associated with the site of first recurrence. The current study found that 76% of recurrences occur at a distant site, demonstrating that most patients with PDAC have systemic disease at the time of resection. Additionally, almost 20% of the patients who were free of recurrence after 5 years went on to develop disease recurrence. Furthermore, specific recurrence patterns were shown to have unique RFS curves. Lastly, this study established that distinctive clinicopathological features correlate with different patterns of recurrence. All off these findings are highly suggestive of unique biological behaviors of tumors within a distinct recurrence pattern and open the door to identification of tumors for detailed molecular analysis.

Several prior studies have reported on the patterns of recurrence following pancreatectomy for PDAC. However, these studies showed significant disparity in recurrence rates and patterns, mainly due to small population sizes and limited follow-up. After an extensive literature search, 4 autopsy studies[10-13] and 15 clinical studies[6,7,11,14-25] were identified with (A) pattern of recurrence as primary or secondary outcome, (B) data available for stratification in “local”, “distant”, and “local + distant” recurrence groups, and (C) exclusion of patients with R2-resection margin, distal pancreatectomy and/or neo-adjuvant therapy (Table 3). Four autopsy studies were included, consisting of 81 examined autopsies of which 68 (84.0%) were found to have disease recurrence. Solitary local recurrence was only found in 6 patients (8.8%), indicative of the advanced disease stage at which the autopsies were performed.

For clinical studies, the sample size of patients with recurrence ranged from 15 to 209 patients. The recurrence rate differed significantly between the included studies, ranging from 54.7% to 91.1%. Similarly, reported local only recurrence rates (8.1%-38.2%), distant-only recurrence rates (13.3%-81.1%), and local + distant recurrence rates (0.0%-78.4%) showed great variations. However, when combining the outcomes of all clinical studies, pooled totals closely approach the recurrence rates of the current study. For instance, 1509 of 2101 patients (72%) developed disease recurrence in previously reported studies, similar to the 77% found in our cohort (Table 3). Likewise, Table 3 further shows that the local only recurrence rate (24%), distant-only recurrence rate (58%), and the local + distant recurrence rate (19%) found in this study differ from most of the individual other studies. However, when location rates from all previous clinical studies are combined, the pooled rates (26%, 53%, and 21%,

Table 2. Univariable and multivariable Cox-proportional regression analysis of independent prognostic variables for different recurrence patterns

	Local Only (n=126)				*Liver Only (n=134)*				*Lung only (n=78)*			
Clinical characteristics	***P¹***	***HR***	***95% CI***	***P²***	***P¹***	***HR***	***95% CI***	***P²***	***P¹***	***HR***	***95% CI***	***P²***
Age at surgery												
≥75 years vs. <75 years	0.089	0.69	0.40-1.19	0.179	0.647	-			0.913	-		
Gender												
Male vs. female	0.221	-			0.218	-			0.982	-		
Race												
Caucasian vs. all others	0.522	-			0.265	-			0.094	0.42	0.17-1.04	0.059
Blood transfusion												
Yes vs. no	0.133	-			0.185	-			0.763	-		
Vascular resection												
Yes vs. no	0.808	-			0.485	-			0.418	-		
Complications†												
Clavien ≥3 vs. Clavien ≤2	0.520	-			0.781	-			0.255	-		
Resection margin status												
R1 (≤1mm) vs. R0 (>1mm)	<0.001	**2.46**	**1.70-3.55**	**<0.001**	0.095	1.15	0.80-1.66	0.437	0.059	1.13	0.69-1.85	0.616
Tumor size (cm)*	0.658	-			0.001	**1.13**	**1.04-1.24**	**0.006**	0.189	-		
Tumor differentiation grade												
Poor vs. well/moderate	0.233	-			<0.001	**2.48**	**1.75-3.50**	**<0.001**	0.798	-		
Lymphovascular invasion												
Yes vs. no	0.111	-			0.046	1.09	0.75-1.58	0.662	0.001	**1.67**	**1.02-2.72**	**0.042**
Perineural invasion												
Yes vs. no	0.164	-			0.179	-			0.255	-		
Positive lymph node ratio												
>0.2 vs. ≤0.2	0.047	1.21	0.81-1.82	0.358	0.018	**1.49**	**1.02-2.17**	**0.040**	<0.001	**3.30**	**2.04-5.35**	**<0.001**
Adjuvant therapy												
Chemotherapy vs. none	0.146	-			0.135	-			0.123	-		
Chemoradiotherapy vs. none	0.020	**0.64**	**0.44-0.95**	**0.024**	0.846	-			0.506	-		

†According to Clavien-Dindo classification
*Continuous
CI, *Confidence Interval;* HR, *Hazard Ratio;* P¹, *univariable P-value;* P², *multivariable P-value.*

Table 2. Univariable and multivariable Cox-proportional regression analysis of independent prognostic variables for different recurrence patterns (*continued*)

	Local + distant (n=98)				*Multiple (n=78)*				*Other (n=17)*			
Clinical characteristics	***P¹***	***HR***	***95% CI***	***P²***	***P¹***	***HR***	***95% CI***	***P²***	***P¹***	***HR***	***95% CI***	***P²***
Age at surgery												
≥75 years vs. <75 years	0.700	-			0.556	-			0.934	-		
Gender												
Male vs. female	0.318	-			0.174	-			0.787	-		
Race												
Caucasian vs. all others	0.270	-			0.003	**2.16**	**1.26-3.68**	**0.005**	0.408	-		
Blood transfusion												
Yes vs. no	0.842	-			0.814	-			0.798	-		
Vascular resection												
Yes vs. no	0.073	1.52	0.78-2.94	0.218	0.223	-			0.516	-		
Complications†												
Clavien ≥3 vs. Clavien ≤2	0.660	-			0.424	-			0.110	-		
Resection margin status												
R1 (≤1mm) vs. R0 (>1mm)	0.279	-			0.031	1.33	0.83-2.12	0.239	0.002	**4.01**	**1.43-11.20**	**0.008**
Tumor size (cm)*	0.009	1.11	0.99-1.24	0.071	0.298	-			0.070	1.20	0.98-1.46	0.079
Tumor differentiation grade												
Poor vs. well/moderate	0.692	-			0.003	**1.72**	**1.09-2.71**	**0.019**	0.504	-		
Lymphovascular invasion												
Yes vs. no	0.001	**1.73**	**1.10-2.72**	**0.017**	<0.001	1.69	1.00-2.86	0.051	0.327	-		
Perineural invasion												
Yes vs. no	0.110	-			0.024	2.89	0.90-9.35	0.076	0.320	-		
Positive lymph node ratio												
>0.2 vs. ≤0.2	<0.001	**2.10**	**1.38-3.20**	**0.001**	<0.001	**2.65**	**1.64-4.29**	**<0.001**	0.005	**3.15**	**1.13-8.80**	**0.029**
Adjuvant therapy												
Chemotherapy vs. none	<0.001	**0.33**	**0.18-0.60**	**<0.001**	0.961	-			0.523	-		
Chemoradiotherapy vs. none	<0.001	**0.26**	**0.16-0.40**	**<0.001**	0.625	-			0.346	-		

†According to Clavien-Dindo classification
*Continuous
CI, *Confidence Interval*; HR, *Hazard Ratio*; P¹, *univariable P-value*; P², *multivariable P-value*.

Table 3. Studies on the recurrence pattern of pancreatic ductal adenocarcinoma after resection

Autopsy studies	***Total patients, n***	***Patients with recurrence, n (%)***	***Local only, n (%)***	***Distant only, n (%)***	***Local + distant, n (%)***	***Median RFS (months)***	***Median Follow-Up (months)***
Johnstone *et al.* (1993)	15	11 73.3%	1 9.1%	2 18.2%	8 72.7%	NR	Potential >9 years
Kayahara *et al.* (1993)	20	15 75.0%	1 6.7%	3 20.0%	11 73.3%	NR	15
Hishinuma *et al.* (2006)	24	22 91.7%	1 4.5%	4 18.2%	17 77.3%	NR	All patients until death
Iacobuzio *et al.* (2009)	22	20 90.9%	3 15.0%	4 20.0%	13 65.0%	14	NR
Totals from autopsy studies	81	68 84.0%	6 8.8%	13 19.1%	49 72.1%	-	-
Clinical studies	***Total patients, n***	***Patients with recurrence, n (%)***	***Local only, n (%)***	***Distant only, n (%)***	***Local + distant, n (%)***	***Median RFS (months)***	***Median Follow-Up (months)***
Griffin *et al.* (1989)	36	26 72.2%	7 26.9%	4 15.4%	15 57.5%	8	Until death
Westerdahl *et al.* (1993)	86	74 86.0%	6 8.1%	10 13.5%	58 78.4%	NR	NR
Kayahara *et al.* (1993)	25	15 60.0%	5 33.3%	2 13.3%	8 53.3%	NR	15
Nitecki *et al.* (1995)	174	112 64.4%	38 33.9%	74 66.1%	0 0%	12	22
Sperti *et al.* (1997)	88	78 88.6%	26 33.3%	22 28.2%	30 38.5%	NR	NR
Neoptolemos *et al.* (2004)*	289	158 54.7%	56 35.4%	53 33.5%	43 27.2%	NR	47 months for alive patients
Shibata *et al.* (2005)	69	52 75.4%	12 23.1%	38 73.1%	2 3.8%	NR	22
Schnelldorfer *et al.* (2008)	276	196 71.0%	25 12.8%	159 81.1%	12 6.1%	13	97 months for alive patients
Asinyanbola *et al.* (2009)	154	103 66.9%	22 21.4%	71 68.9%	10 9.7%	16	20
Tani *et al.* (2009)	68	55 80.9%	21 38.2%	34 61.8%	0 0%	NR	16
Van den Broeck *et al.* (2009)	139	110 79.1%	19 17.3%	66 60.0%	25 22.7%	10	19
Hernandez *et al.* (2010)	61	51 83.6%	19 37.3%	20 39.2%	12 23.5%	5	39
Zhang *et al.* (2012)	79	72 91.1%	22 30.6%	33 45.8%	17 23.6%	14.2	<24 months excluded
Gnerlich *et al.* (2012)	285	198 69.5%	47 23.7%	109 55.1%	42 21.2%	NR	20

Suenaga *et al.* (2014)	272	209 76.8%	60 28.7%	111 53.1%	38 18.2%	NR	16
Totals from clinical studies*	2101	1509 71.8%	385 25.5%	806 53.4%	312 20.7%	-	-
Current study (2018)	692	531 76.7%	126 23.7%	307 57.8%	98 18.5%	16	25

* Includes six patients with unknown location of recurrence
FU, *follow-up*; NR, *not reported*.

respectively) are remarkably similar to those found in the current study. This shows that the found recurrence pattern in the present study is an accurate representation of the true pattern of failure after resection for PDAC. Moreover, our detailed recurrence data allowed for further stratification of recurrence patterns in 6 separate groups: liver only (25%), local only (24%), local + distant (19%), multiple (15%), lung only (15%), and other (3%) recurrence.

The unique RFS curves for patients with different sites of first recurrence form another important finding of this study. In a relatively large study including 209 patients with recurrence after pancreatectomy, Suenaga et al.[25] reported a median RFS of 6.0 months for liver only recurrence, similar to the 6.9 months in the current cohort. Also, Suenaga et al. stated that RFS for hepatic recurrence was significantly shorter when compared with recurrences at other sites (median 6.0 months vs. 9.9 months, $P<0.001$). A study by Sperti et al.[7] reported that 48% of hepatic recurrence occurred within 6 months, almost equivalent to the 45% found in our cohort. Sperti et al., however, presented comparable RFS for local recurrence (median 9.5 months) and hepatic recurrence (9.0 months). In the current study, liver metastasis occurred significantly earlier than any other recurrence, with the exception of local + distant recurrence (median 6.9 months vs. 10.0 months, $P=0.13$). Unsurprisingly, half of the 98 patients with both local + distant recurrence in our cohort had hepatic metastatic disease. In contrast, patients with lung or local only recurrence had extended median RFS (18.6 months and 14.6 months, respectively). Van den Broeck et al.[6] similarly reported that retroperitoneal and lung metastases occurred later in the post-operative period than liver and peritoneal metastases. Following the apparent less aggressive tumor biology and slower growing tendency, patients might benefit from additional treatment of their pulmonary or local recurrence. Initial studies at our institution on metastasectomy for pulmonary metastases[26,27] and stereotactic body radiation therapy for isolated local recurrence,[28] for instance, have shown promising results.

The current study showed that an LNR >0.2 was a strong predictor for all distant disease recurrence, while a R1-resection margin was, expectedly, strongly associated with local only recurrence. Apart from the LNR, hepatic recurrence was associated with larger tumor size

and poor tumor differentiation. Furthermore, poor tumor differentiation was associated with development of multiple recurrence and/or carcinomatosis. Although poor tumor differentiation has been recognized as an indicator of poor prognosis, the exact relation remains unclear.[29-32] Shibata et al.,[18] however, offer an intriguing hypothesis, suggesting that several molecules that are expressed at high levels in undifferentiated tumors, including epidermal growth factor receptor, E-cadherin, and laminin γ-chain, may enhance the ability of PDAC to metastasize to the liver. Lastly, we found an LNR >0.2 and lymphovascular invasion to be associated with pulmonary recurrence. To the authors' knowledge, no other clinical predictors for lung only recurrence have previously been described in patients with PDAC.

Although effects of adjuvant therapy on overall survival are being studied extensively, few studies have evaluated the association of adjuvant therapy with patterns of recurrence. The current study showed that both adjuvant chemotherapy and chemoradiotherapy significantly reduce the likelihood of recurrence in general. Furthermore, RFS was significantly longer after both chemotherapy and chemoradiotherapy. A recent study by the Central Pancreatic Consortium showed similar results, reporting that chemotherapy and chemoradiotherapy led to fewer local and distant recurrences.[33] In our cohort however, only chemoradiotherapy resulted in significantly fewer local recurrences while both chemoradiotherapy and chemotherapy resulted in less local + distant recurrences. These results suggest that radiation therapy in the adjuvant setting continues to play an important role in preventing local recurrence, especially in patients with a positive resection margin. Although most patients will die from distant disease, localized disease can lead to death in up to 30% of PDAC patients.[13] Furthermore, local recurrence can be debilitating due to symptoms resulting from locally destructive growth, highlighting the importance of local tumor control even after resection.[34]

An important advantage of this study was the use of a database from a major tertiary referral center for patients with PDAC, providing a large and relatively homogenous patient population. Secondly, our mono-institutional study provided more uniform pathologic assessment, eliminating interinstitutional variability often limiting study accuracy and comparability. However, our study does have several limitations. First, our database lacked specific information with regard to adjuvant therapy after pancreatectomy. More accurate data on regimen, dose, and frequency of adjuvant treatment would potentially reveal associations not appreciated by the current study. Second, presented results only provide a cross-section of the natural history of resected PDAC since merely the first recurrence was recorded and further disease progression was not accounted for. Furthermore, although follow-up was long with 2 years, it can be argued that a higher percentage of patients would recur if followed long enough. Last, manifestation of recurrence was typically based on radiological evidence without pathological

confirmation. This could lead to a potential overestimation of, for instance, the incidence of lung only recurrence, as benign pulmonary nodules are common and often difficult to differentiate from true recurrence.[35] Despite these limitations, the current study accurately describes the patterns and timing of the different recurrence locations and identifies unique clinicopathological features independently associated with specific recurrence patterns.

The different clinicopathological features and RFS curves found in our study support the hypothesis that unique biological differences exist among primary tumors leading to distinct patterns of recurrence. Future research will have to clarify if unique genetic signatures exist that correspond to specific recurrence behavior. This possibility seems likely, as molecular profiling has been shown to predict cancer recurrence in prostate, stomach, and liver cancer.[36-39] Currently, *SMAD4* status is one example of how underlying genetic status can correlate with biological behavior in PDAC: PDAC not expressing *SMAD4* is associated with a high metastatic burden, while tumors with intact *SMAD4* tend to remain localized.[13] Evidence already exists for substantial molecular heterogeneity in PDAC. Jones et al.[40] identified over 1200 genetic alterations among 96 PDACs with the average tumor harboring 63 alterations. Similarly, in their genomic study of 99 PDAC samples Biankin et al.[41] reported that of 2627 detected high-confidence genetic mutations, 2016 were non-silent. There was an average of 26 mutations per patient, ranging from 1 to 116 mutations. Furthermore, mouse models studying metastasis biology in breast and renal carcinoma showed site-specific metastasis could be attributed to gene inactivation or suppression, protein modulators, and tumor- and stroma-supplied proteolytic networks.[42-47] Evidently, the promise of combining clinical factors with genetic signatures to predict recurrence patterns would have important implications for prognosis stratification and the quest toward personalized medicine for patients with PDAC.

Conclusion

This study provides an accurate and detailed understanding of the pattern and timing of disease recurrence after surgery for PDAC. Furthermore, unique clinicopathological features were identified that can help predict the specific location of first recurrence. These findings are highly suggestive of biologic heterogeneity in pancreatic cancer between individual patients and future studies might reveal genetic signatures associated with recurrence patterns, possibly opening the way for prognostic stratification, targets of treatment, and a more patient-tailored approach for patients with PDAC.

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CHAPTER 3

Implications of the Pattern of Disease Recurrence on Survival Following Pancreatectomy for Pancreatic Ductal Adenocarcinoma

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Abstract

Objective

After radical resection of pancreatic ductal adenocarcinoma (PDAC), approximately 80% of patients will develop disease recurrence. It remains unclear to what extent the location of recurrence carries prognostic significance. Additionally, stratifying the pattern of recurrence may lead to a deeper understanding of the heterogeneous biological behavior of PDAC. The aim of this study was to characterize the relationship of recurrence patterns with survival in patients with resected PDAC.

Methods

This single-center cohort study included patients undergoing pancreatectomy at the Johns Hopkins Hospital between 2000 and 2013. Exclusion criteria were neoadjuvant therapy and incomplete follow-up. Sites of first recurrence were stratified into five groups and survival outcomes were estimated using Kaplan-Meier curves. The association of specific recurrence locations with overall survival (OS) was analyzed using Cox proportional-hazards models with and without landmark analysis.

Results

Accurate follow-up data were available for 877 patients, 662 (75.5%) of whom had documented recurrence at last follow-up. Patients with multiple-site (n=227, 4.7 months) or liver only recurrence (n=166, 7.2 months) had significantly worse median survival after recurrence when compared with lung only (n=93) or local only (n=158) recurrence (15.4 and 9.7 months, respectively). On multivariable analysis, the unique recurrence patterns had variable predictive values for OS. Landmark analyses, with landmarks set at 12, 18, and 24 months, confirmed these findings.

Conclusion

This study demonstrates that specific patterns of PDAC recurrence result in different survival outcomes. Furthermore, distinct first recurrence locations have unique independent predictive values for OS, which could help with prognostic stratification and decisions regarding treatment after the diagnosis of recurrence.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second most common cause of cancer-related death in the US in the next 15 years and continues to be one of the most treatment-resistant malignancies.[1] Radical resection of localized PDAC provides the best chance for long-term survival, and many studies have investigated prognostic factors for predicting survival outcomes after pancreatectomy.[2-4]

Unfortunately, even after successful radical resection of PDAC, up to 80% of patients will develop disease recurrence.[5-9] A recent study from our institution found that the time to recurrence differed for distinct post-operative recurrence locations.[10] However, the relationship of these specific PDAC recurrence locations with overall survival (OS) remains poorly described. Knowledge on the prognostic impact of distinct recurrence sites might help clinicians with prognostic stratification and provide a better understanding of the heterogeneous biological behavior of PDAC.

The aim of this study was therefore twofold. First, to characterize the association of patterns of disease recurrence with survival after recurrence (SAR), and, second, to investigate the predictive value of specific recurrence locations on OS while adjusting for known prognostic clinicopathologic variables. To avoid bias in examining OS by recurrence location, careful statistical analysis was performed using proportional hazard models, with and without landmark analysis, in a large cohort of patients who underwent surgery for primary resectable PDAC in the head of the gland.

Methods

Patient Selection

This single-center cohort included patients who underwent a standard pancreatoduodenectomy, pylorus-preserving pancreatoduodenectomy, or total pancreatectomy for PDAC at the Johns Hopkins Hospital between 2000 and 2013. Only patients who underwent upfront resection of resectable or borderline resectable PDAC in the head of the pancreas were included. Resectability and staging were generally discussed in a multidisciplinary setting and based on pre-operative pancreatic protocol computed tomography (CT) imaging. Patients were staged with resectable disease (stage I) when there was no arterial and/or venous involvement, and with borderline resectable disease (stage II) when there was limited arterial and/or venous involvement allowing for safe and complete resection with or without vascular reconstruction. Apart from neoadjuvant therapy, other exclusion criteria were (1) synchronous

distant metastases at the time of surgery; (2) macroscopic margin-positive resection (R2); or (3) 90-day surgery-related mortality. Patients with incomplete follow-up data due to loss to follow-up or follow-up performed at other institutions were also excluded. Institutional Review Board approval was obtained for this retrospective study.

Demographic, Clinicopathological, and Treatment Characteristics

Demographic and clinical variables were extracted from a prospective institutional database. Perioperative complications were reported according to the Clavien-Dindo classification.[11] The resection margin (R) was defined as R0 when the distance of carcinoma cells to the closest resection margin was >1 mm, and R1 when the distance was ≤1 mm. If their performance status allowed, patients with recurrence were further treated with systemic therapy. In highly selected patients, localized treatments such as stereotactic body radiation therapy for isolated local recurrence, or metastasectomy for stable isolated lung recurrence, were sporadically performed.

Follow-Up and Recurrence Definitions

Follow-up methodology and definitions of disease recurrence after pancreatectomy at our institution have been described previously.[10] The first location of disease recurrence was documented based on either radiological or histological evidence; however, confirmation of recurrence with a biopsy was not routinely performed. Recurrence locations were stratified into five mutually exclusive categories: "local only", "liver only", "lung only", "multiple-site" and "other". Multiple-site recurrence included patients with local and distant recurrence, recurrence in multiple distant sites (i.e. lung and liver) and/or carcinomatosis.

Survival Outcomes

Recurrence-free survival was defined as the time interval between the date of surgery and the date of recurrence, while OS was defined as the time from the date of surgery to either death from any cause or last follow-up. Cause of death was retrieved from our institutional database, cross-referenced, and supplemented with data obtained from the National Death Index of the Centers for Disease Control and Prevention. Disease-specific survival was defined as the time from the date of operation to either death due to PDAC or last follow-up, with censoring occurring for patients who died of any other cause. Lastly, SAR was defined as the time from recurrence to either death or last follow-up.

Statistical Analysis

Kaplan-Meier curves and the log-rank test were used to estimate median survival outcomes with a corresponding 95% confidence interval (CI), and to compare between subgroups. To establish whether recurrence location as a time-dependent covariate has a prognostic effect on the OS of patients with resected PDAC, while adjusting for other clinicopathologic variables, two distinct multivariable Cox proportional-hazards regression models were used.

First, a conventional Cox model was created in which recurrence locations were considered as fixed covariates. However, since specific recurrence locations have different recurrence-free intervals,[10] this introduces a potential bias when assessing OS directly. More specifically, local or lung recurrence generally occur relatively late after surgery, which might result in a "lead time" or "guarantee time" bias towards improved OS when compared with multiple-site or liver only recurrence, which tend to arise after a shorter recurrence-free interval.

A statistical technique to account for this type of potential bias is a landmark analysis (see also Online Supplemental Methods).[12-15] A landmark time of 12 months was chosen,[16] and a multivariable Cox model was then conducted with survival from the landmark. To examine whether this choice of landmark affected either strength or direction of the found relationships between recurrence locations and survival, similar analyses were performed with landmarks set at 18 and 24 months.

A stepwise backward approach using Akaike information criterion (AIC) was used to select covariates for the multivariable models. Internal validation and optimism for the multivariable model with landmark analysis set at 12 months was performed with 200 times bootstrap. The proportional hazards assumption of the Cox models was checked using Schoenfeld residuals. A two-tailed P-value of <0.05 was considered to indicate statistical significance. Statistical analysis was performed using SPSS v25.0 (IBM Corporation, Armonk, NY, USA) and R v3.3.3 (R Foundation, Vienna, Austria).

Results

A total of 1325 patients who underwent upfront pancreatectomy for histologically confirmed PDAC were included in our study. Excluded were 427 patients (32.2%) with incomplete follow-up data due to loss to follow-up or follow-up performed at other institutions, as well as a further 21 patients (1.6%) who died within 90 days after surgery. Consequently, a final cohort of 877 patients with accurate recurrence and survival data was established.

Cohort Characteristics

Detailed demographic, clinicopathologic, and treatment characteristics of the included patients are summarized in Table 1. Median follow-up for all patients was 23.6 months (95% CI 21.8-25.3). At last follow-up, disease recurrence was recorded in 662 patients (75.5%). A total of 215 patients (24.5%) had no sign of recurrence after a median follow-up of 46.3 months (95% CI 36.4-54.3). The most common manifestation was multiple-site recurrence (n=227, 34.3%), followed by liver only (n=166, 25.1%), local only (n=158, 23.9%), and lung only (n=93, 14.0%) recurrence. Other recurrence sites (n=18, 2.7%) included osseous structures, the brain, supraclavicular lymph nodes, groin, thigh muscle, and the skin.

Cause of Death

At last follow-up, 186 patients (21.2%) were alive after a median follow-up of 56.4 months (95% CI 45.0-67.8). Of the 691 patients who died, 81.8% died of PDAC (n=565). Other causes included cardiovascular disease (n=28, 4.1%), other primary cancers (n=26, 3.8%), infectious disease (n=13, 1.9%), and lung disease causing respiratory failure (n=9, 1.3%). Several other less common causes, including but not limited to accidents, suicide, or drug overdose, amounted to 2.3% (n=16) of the reported deaths.

Thirty-four patients (4.9%) died of unknown cause but without documented recurrence on the most recent imaging. Of the 140 patients still alive 5 years after surgery, 50 (35.7%) went on to die during later follow-up, of whom 23 (46.0%) were due to causes other than PDAC. In patients who survived <5 years, the ratio of death due to other causes (103/641, 16.1%) was significantly lower (P<0.001). Furthermore, 45/147 patients (30.6%) who were >75 years of age and died, passed away due to other causes, compared with 81/544 patients (14.9%) who were ≤75 years of age at the time of surgery (P<0.001). Rates of death due to PDAC did not differ significantly among the different recurrence patterns.

Survival Outcomes

Median survival outcomes for all patients and per different recurrence location are presented in Table 2. Patients with multiple-site or liver only recurrence had a limited median SAR of 4.7 months (95% CI 3.8-5.1) and 7.2 months (95% CI 6.0-8.5), respectively, which were both significantly worse when compared with survival after local- or lung only recurrence (Figure 1). Patients with lung only recurrence had a median SAR of 15.4 months (95% CI 10.9-19.9), significantly longer than the second longest median SAR of patients with local only recurrence (9.7 months, 95% CI 7.5-12.0, P=0.001). Further analysis showed that patients who recurred early had relatively limited survival after their recurrence. Overall, 338 patients with

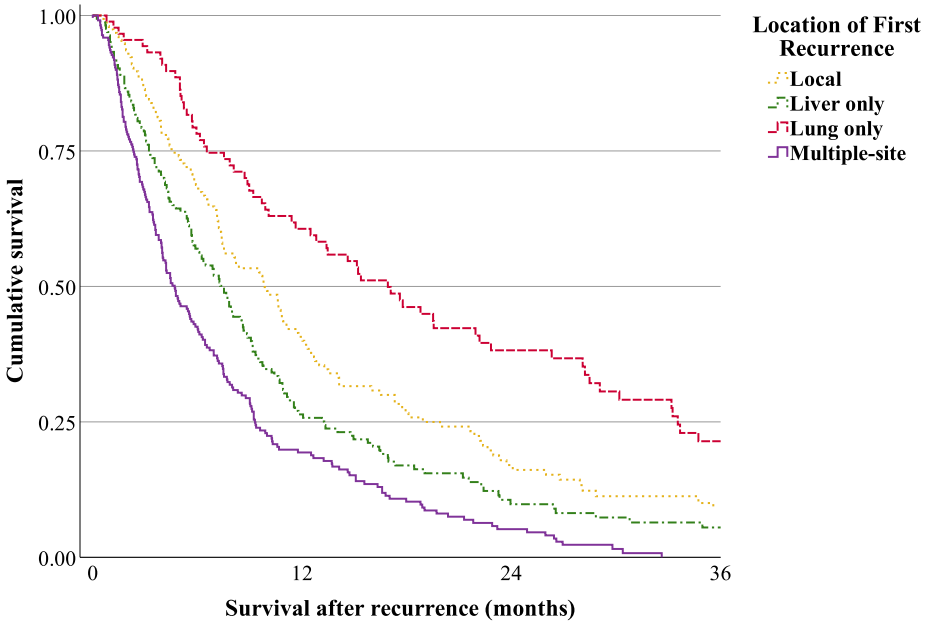
Table 1. Demographics, clinicopathologic and treatment characteristics of included patients

Variable	*All patients (n=877)*	*No recurrence (n=215)*	*Recurrence (n=662)*	*P*-value
Male, *n* (%)	463 (52.8%)	131 (60.9%)	332 (50.2%)	**0.006**
Race, *n* (%)				0.942
Caucasian	760 (86.7%)	186 (86.5%)	574 (86.7%)	
Other	117 (13.3%)	29 (13.5%)	88 (13.3%)	
Age (years)				**0.002**
Mean (SD)	65.7 (10.5)	67.7 (10.7)	65.1 (10.4)	
Operation procedure, *n* (%)				0.315
Pylorus-preserving pancreatoduodenectomy	421 (47.7%)	104 (48.4%)	323 (48.8%)	
Standard pancreatoduodenectomy	421 (48.0%)	98 (45.6%)	314 (47.4%)	
Total pancreatectomy	38 (4.3%)	13 (6.0%)	25 (3.8%)	
Blood transfusion, *n* (%)	267 (30.4%)	66 (30.7%)	201 (30.4%)	0.926
Morbidity, *n* (%)				**0.036**
≤Clavien-Dindo grade II	719 (82.0%)	166 (77.2%)	553 (83.5%)	
≥Clavien-Dindo grade III	158 (18.0%)	49 (22.8%)	109 (16.5%)	
Resection margin, *n* (%)				**<0.001**
R0 (>1.0 mm)	597 (68.1%)	180 (83.7%)	417 (63.0%)	
R1 (≤1.0 mm)	280 (31.9%)	35 (16.3%)	245 (37.0%)	
Tumor differentiation, *n* (%)				**0.001**
Well/Moderate	541 (61.7%)	154 (71.6%)	387 (58.5%)	
Poor	336 (38.3%)	61 (28.4%)	275 (41.5%)	
Tumor size (cm)				**0.007**
Mean (SD)	3.1 (1.4)	2.9 (1.7)	3.1 (1.3)	
T-stage, *n* (%)				**0.007**
1-2	232 (26.5%)	72 (36.7%)	160 (24.2%)	
3-4	645 (73.5%)	136 (63.3%)	502 (75.8%)	
Positive lymph nodes, *n* (%)	667 (76.1%)	121 (64.4%)	531 (80.2%)	**<0.001**
Positive lymph node ratio, *n* (%)				**<0.001**
≤0.2	593 (67.6%)	182 (84.7%)	411 (62.1%)	
>0.2	284 (32.4%)	33 (15.3%)	251 (37.9%)	
Microscopic perineural invasion, *n* (%)	788 (89.9%)	175 (81.4%)	613 (92.6%)	**<0.001**
Microscopic lymphovascular invasion, *n* (%)	518 (59.1%)	90 (41.9%)	428 (64.7%)	**<0.001**
AJCC stage 7th edition, *n* (%)				**<0.001**
≤2A	241 (27.5%)	80 (37.2%)	161 (24.3%)	
≥2B	636 (72.5%)	135 (62.8%)	501 (75.7%)	
Adjuvant therapy, *n* (%)				**<0.001**
No adjuvant	269 (30.7%)	51 (23.7%)	218 (32.9%)	
Chemotherapy	200 (22.8%)	74 (34.4%)	126 (19.0%)	
Chemoradiotherapy	408 (46.5%)	90 (41.9%)	318 (48.0%)	
Recurrence site, *n* (%)				NA
No recurrence	215 (24.5%)	215 (100%)	NA	
Multiple-site	227 (25.9%)	NA	227 (34.3%)	
Liver only	166 (18.9%)	NA	166 (25.1%)	
Local only	158 (18.0%)	NA	158 (23.9%)	
Lung only	93 (10.6%)	NA	93 (14.0%)	
Other	18 (2.1%)	NA	18 (2.7%)	

AJCC, *American Joint Committee on Cancer*; NA, *not applicable*; SD, *standard deviation*.

a recurrence-free survival of B 12 months had a median SAR of 6.0 months (95% CI 5.2-6.8), significantly shorter than the 10.0 months (95% CI 9.1-12.1) for the 324 patients who recurred after more than 12 months (P<0.001).

Median OS of 662 patients with recurrence (21.1 months, 95% CI 19.0-23.2) was considerably shorter when compared with the 83.9 months (95% CI 59.3-108.6) found for 215 patients without recurrence (P<0.001) (Table 2). Pairwise comparison per specific recurrence patterns revealed that median OS varied significantly based on recurrence location (Figure 2). Lung only, local only and other recurrence were all associated with considerably longer median OS when compared with multiple-site or liver only recurrence.



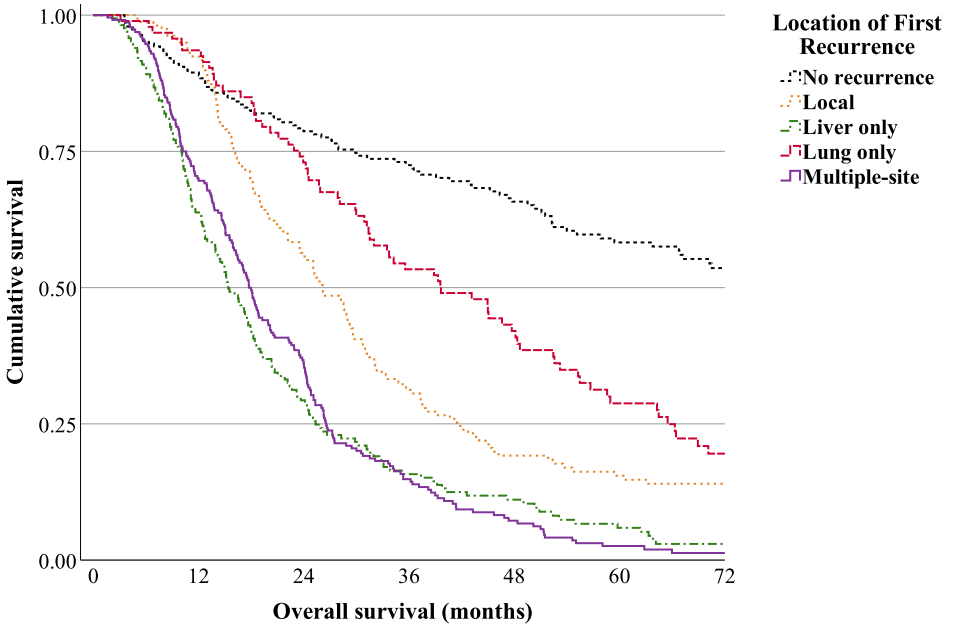
Recurrence pattern	*Lung only*	*Local only*	*Other*	*Multiple-site*	*Liver only*	*Median SAR months (95% CI)*
Lung only (n=93)		**0.001**	**0.034**	**<0.001**	**<0.001**	15.4 (10.9-19.9)
Local only (n=158)	**0.001**		0.559	**<0.001**	**0.008**	9.7 (7.5-12.0)
Other (n=18)	**0.034**	0.559		**0.041**	0.389	8.0 (1.8-14.3)
Multiple-site (n=227)	**<0.001**	**<0.001**	**0.041**		**0.001**	4.7 (3.8-5.1)
Liver only (n=166)	**<0.001**	**0.008**	0.389	**0.001**		7.2 (6.0-8.5)

CI, *confidence interval*; SAR, *survival after recurrence*.

Figure 1. Kaplan-Meier curves showing survival after recurrence (SAR) for different recurrence patterns with pairwise comparison of SAR using the log-rank test. For clarity, the curve for "other" recurrence has been omitted.

Multivariable Analysis

In the multivariable Cox regression model with recurrence locations as fixed covariates, all recurrence patterns were significantly associated with worse OS when compared with non-recurrent patients (Table 3). Multiple-site and liver only recurrence both had hazard ratios (HRs) of more than 4, indicative of their strong independent association with a limited OS. While local only, lung only, and other recurrence were also significantly correlated with decreased OS, their HRs of <2.5 showed a weaker relationship. Furthermore, adjuvant therapy (HR 0.61, 95% CI 0.52-0.72, P<0.001) was independently associated with prolonged OS.



Recurrence pattern	*No recurrence*	*Lung only*	*Local only*	*Other*	*Multiple-site*	*Liver only*	*Median OS months (95% CI)*
No recurrence (n=215)		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	83.9 (59.3-108.6)
Lung only (n=93)	**<0.001**		**0.003**	0.341	**<0.001**	**<0.001**	39.6 (29.3-50.0)
Local only (n=158)	**<0.001**	**0.003**		0.626	**<0.001**	**<0.001**	26.2 (22.8-29.5)
Other (n=18)	**<0.001**	0.341	0.626		**0.003**	**0.005**	29.4 (23.5-35.3)
Multiple-site (n=227)	**<0.001**	**<0.001**	**<0.001**	**0.003**		0.855	17.7 (16.2-19.3)
Liver only (n=166)	**<0.001**	**<0.001**	**<0.001**	**0.005**	0.855		15.4 (13.1-17.6)

CI, *confidence interval*; OS, *overall survival*.

Figure 2. Kaplan-Meier curves showing overall survival (OS) for different recurrence patterns with pairwise comparison of OS using the log-rank test. For clarity, the curve for "other" recurrence has been omitted.

Table 2. Median survival outcomes for included patients

Outcome (months)	*Median RFS (95% CI)*	*Median SAR (95% CI)*	*Median OS (95% CI)*	*Median DSS (95% CI)*
All patients (*n*=877)	15.5 (14.1-16.8)	7.3† (6.6-7.95)	24.8 (23.2-26.4)	27.5 (25.0-30.0)
No recurrence (*n*=188)	NA*	NA	83.9 (59.3-108.6)	NA*
Lung only (*n*=93)	18.4 (14.8-22.1)	15.4 (10.9-19.9)	39.6 (29.3-50.0)	44.9 (36.5-53.4)
Local only (*n*=158)	14.2 (12.6-15.9)	9.7 (7.5-12.0)	26.2 (22.8-29.5)	28.5 (25.2-31.9)
Other (*n*=18)	15.5 (14.9-16.1)	8.0 (1.8-14.3)	29.4 (23.5-35.3)	29.4 (12.5-46.3)
Multiple-site (*n*=227)	11.1 (9.8-12.4)	4.7 (3.8-5.1)	17.7 (16.2-19.3)	18.1 (16.3-20.0)
Liver only (*n*=166)	7.3 (5.9-8.7)	7.2 (6.0-8.5)	15.4 (13.1-17.6)	15.5 (13.3-17.7)

* Not computable separately as all cases are censored.
† For all patients with recurrence (*n*=662).
DSS, *disease-specific survival*; NA, *not applicable*; RFS, *recurrence-free survival*; SAR, *survival after recurrence*; OS, *overall survival*.

Table 3. Multivariable Cox regression analysis for overall survival with recurrence locations as fixed covariates

	Multivariable analysis		
Clinical characteristics	***HR***	***95% CI***	***P-value***
Age at surgery, years†	**1.01**	**1.01-1.02**	**<0.001**
Complications			
Clavien-Dindo grade ≥III vs. Clavien-Dindo grade ≤II	**1.22**	**1.00-1.50**	**0.047**
Tumor size, cm†	**1.11**	**1.05-1.17**	**<0.001**
Tumor differentiation grade			
Poor vs. well/moderate	**1.24**	**1.06-1.45**	**0.006**
Microscopic perineural invasion			
Yes vs. no	1.24	0.93-1.66	0.147
Positive lymph node ratio			
>0.2 vs. ≤0.2	**1.49**	**1.26-1.76**	**<0.001**
Resection margin status			
R1 (≤1.0 mm) vs. R0 (>1.0 mm)	**1.37**	**1.16-1.61**	**<0.001**
Adjuvant therapy			
Adjuvant vs. no adjuvant	**0.61**	**0.52-0.72**	**<0.001**
Recurrence site			
Multiple-site vs. no recurrence	**4.78**	**3.65-6.25**	**<0.001**
Liver only vs. no recurrence	**5.75**	**4.39-7.55**	**<0.001**
Local only vs. no recurrence	**2.46**	**1.86-3.24**	**<0.001**
Lung only vs. no recurrence	**1.90**	**1.39-2.60**	**<0.001**
Other vs. no recurrence	**2.37**	**1.38-4.08**	**0.002**

Significant values on multivariable analysis ($P<0.05$) are shown in bold
† Continuous.
CI, *confidence interval*; HR, *hazard ratio*.

Table 4. Multivariable Cox regression analysis for overall survival using a landmark set at 12 months post-surgery

	Multivariable analysis		
Clinical characteristics	***HR***	***95% CI***	***P-value***
Age at surgery, years†	**1.02**	**1.01-1.02**	**0.001**
Microscopic perineural invasion			
Yes vs. no	1.38	0.99-1.90	0.055
Positive lymph node ratio			
>0.2 vs. ≤0.2	**1.49**	**1.23-1.80**	**<0.001**
Resection margin status			
R1 (≤1.0 mm) vs. R0 (>1.0 mm)	**1.28**	**1.06-1.55**	**0.012**
Recurrence site			
Multiple-site vs. no recurrence	**5.83**	**4.29-7.92**	**<0.001**
Liver only vs. no recurrence	**5.77**	**4.21-7.92**	**<0.001**
Local only vs. no recurrence	**3.61**	**2.67-4.88**	**<0.001**
Lung only vs. no recurrence	**2.29**	**1.63-3.21**	**<0.001**
Other vs. no recurrence	**2.57**	**1.41-4.67**	**0.002**

Significant values on multivariable analysis ($P<0.05$) are shown in bold
† Continuous.
CI, *confidence interval*; HR, *hazard ratio*.

To adjust for potential bias in the first model, multivariable Cox models for OS using landmark analyses were additionally performed. At the chosen landmark time of 12 months, 200 patients (22.8%) had died or were lost to follow-up. As a result, 677 patients were available for analysis, 180 (26.6%) of whom had recurrence at that time. Similar to the first Cox model with fixed covariates, the landmark analysis established that all recurrence locations were associated with worse OS, with the strongest relationship found for multiple-site and liver only recurrence (Table 4). The model with landmark at 12 months had a C-statistic of 0.696. Since internal validation with 200 times bootstrap showed an optimism of 0.009, the optimism-corrected performance of the model was 0.687. Further multivariable analyses using landmarks set at 18 and 24 months found comparable relationships between the different recurrence patterns and OS (Online Supplemental eTables S1 and S2).

Discussion

This study demonstrates that OS and survival after PDAC recurrence differ based on the site of first recurrence. Specifically, the current study found that patients with multiple-site recurrence had a limited median SAR of <5 months, while patients who developed pulmonary metastases had a median survival of >15 months after first diagnosis of recurrence. Furthermore, patients with isolated lung or local recurrence had a considerably prolonged median OS. Lastly, using two different techniques of multivariable Cox regression analysis, specific recurrence patterns were shown to have distinct independent predictive values for the prognosis of OS.

A prior study focusing on the timing and predictors of PDAC recurrence, consisting of a subset of the present cohort, showed that specific recurrence locations occur after distinct recurrence-free intervals.[10] Furthermore, different recurrence locations were shown to have unique predictive factors. The current findings that show that specific recurrence locations have different post-pancreatectomy and post recurrence survival further supplement and expand on these previous findings, and continue to support the hypothesis that unique biological behaviors exist among PDACs.

Relatively few studies have focused on the impact of the initial relapse pattern on post-pancreatectomy survival outcomes.[9] Moreover, associations observed in earlier studies vary considerably, possibly due to limited sample size and length of follow-up. For instance, van den Broeck et al.[6] found no association between recurrence location and survival, while Tani et al.[17] found similar median OS for patients with and without initial liver metastases. However, other studies did find significant correlations between patterns of recurrence and survival outcomes. When considering SAR, Sperti et al.[5] showed that median survival time from the detection of recurrence until death was 7 months for patients with isolated local recurrence versus 3 months for patients with multiple-site or liver only recurrence ($P<0.05$). Similar to our results, Zhang and colleagues[18] found that patients with locoregional recurrence (10.4 months) had significantly improved SAR when compared with those with isolated distant (5.5 months, $P=0.002$) or multiple-site (5.8 months, $P=0.044$) recurrence.

The current study demonstrates that recurrence at multiple sites or in the liver leads to a relatively limited OS of 18 and 15 months, respectively. In addition, both of these manifestations of recurrent PDAC were strong independent predictors for decreased OS in all multivariable models. Similarly, in a study of 209 patients, Suenaga et al.[8] reported that patients with hepatic relapse had significantly shorter OS than those without hepatic relapse (12.9 months vs. 18.4 months, $P<0.001$), and that local recurrence was associated with a more favorable prognosis than recurrences at other sites (18.4 months vs. 14.7 months, $P=0.010$).

This study aimed to accurately identify the correlation between five unique PDAC recurrence patterns and subsequent SAR. Recurrence of PDAC following resection imposes a difficult choice when considering the pursuit of additional treatment. The presented results provide prognostic data to further assist patient and physician alike when discussing challenging clinical choices and the balance of quality of life with further treatment options. Patients with isolated local or pulmonary recurrence were found to have relatively favorable survival outcomes. The favorable prognosis for patients with isolated local recurrence might stem from the fact that local recurrence can originate from microscopic residual disease in the remnant pancreas that

has not gone through the process of hematogenous dissemination. This seemingly less-hostile tumor biology and slower-growing propensity might thus warrant additional local treatment. Initial experiences at our institution with stereotactic body radiation therapy for isolated local recurrence have shown encouraging results, especially in patients who had a relatively long recurrence-free interval.[19,20]

Patients with pulmonary metastases had both the longest median SAR (15 months) and OS (40 months), possibly representing another unique clinical and biological subtype of PDAC in which more aggressive and/or localized additional therapy might be justified. A study from our institution, for example, showed improved median OS of 51 months after pulmonary metastasectomy in highly selected patients.[21] Although the present literature is limited, several other studies have reported on the benefit of either surgical resection or stereotactic radiosurgery for lung metastases, highlighting increased interest in additional treatment options.[22-26]

The records of specific causes of death revealed that up to 18% of patients died from causes other than PDAC after surgical resection. Interestingly, more than 35% of patients who died after being recurrence-free for 5 years did so due to other causes. In addition, approximately 30% of deaths among patients older than 75 years at the time of operation were attributed to other diseases. However, these results should be interpreted with caution as several of the causes of death that were recorded, such as cholangitis, liver abscesses, or even suicide, might have been indirectly related to PDAC or the treatment thereof. Similarly, the taxing aftermath of pancreatic surgery and subsequent adjuvant therapy might have exacerbated existing comorbidities or weakened the immune system of older patients, resulting in more deaths credited to other causes such as cardiovascular events and infectious diseases.

Our study has several other important limitations worthy of mentioning. First, our database merely captured the recurrence location, without commenting on the extent of recurrent disease. Second, recurrence location was generally based on radiographic findings without additional tissue confirmation. This might have influenced the identified rates of recurrence, as, for instance, benign pulmonary nodules are seen commonly and are often difficult to differentiate from true pulmonary metastases.[27] Third, many patients choose to receive further therapy from local institutions, limiting the accuracy of data on type, dosage, and frequency of therapy for recurrence that would have potentially exposed correlations not currently established. Lastly, the number of patients who were lost to follow-up was noteworthy, potentially limiting the generalizability of our findings. On the other hand, a significant strength of this study was the use of a prospective database from a large tertiary

referral center for patients with PDAC. Furthermore, additional multivariable models using landmark analyses were performed to adjust for potential bias caused by the distinct timing of different recurrence patterns.

Conclusion

This study demonstrates that specific patterns of PDAC recurrence are associated with different survival outcomes. Furthermore, distinct locations of the first recurrence were shown to have unique independent predictive values for OS, which could help with prognostic stratification and decisions regarding treatment after the diagnosis of recurrence. In support of earlier findings, these results continue to suggest that ductal adenocarcinoma of the pancreas is a highly heterogenic disease and future studies should focus on developing patient-tailored multimodality treatment approaches that take into account these diverse biological behaviors.

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Supplemental Material to Chapter 3

- **Supplemental Methods.**
- **Supplemental eTable S1.** Multivariable Cox regression analysis for overall survival using a landmark set at 18 months post-surgery.
- **Supplemental eTable S2.** Multivariable Cox regression analysis for overall survival using a landmark set at 24 months post-surgery.

Supplemental material can be consulted online per the website of the journal and/or publisher.

CHAPTER 4

Defining and Predicting Early Recurrence in 957 Patients with Resected Pancreatic Ductal Adenocarcinoma

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Abstract

Objective

A clear definition of "early recurrence" after pancreatic ductal adenocarcinoma resection is currently lacking. This study aims to establish an evidence-based cut-off to differentiate between early and late recurrence and to compare clinicopathologic risk factors between the two groups.

Methods

Patients undergoing pancreatectomy for pancreatic ductal adenocarcinoma between 2000 and 2013 were included. Exclusion criteria were neoadjuvant therapy and incomplete follow-up. A minimum P-value approach was used to evaluate the optimal cut-off value of recurrence-free survival to divide the patients into early and late recurrence cohorts based on subsequent prognosis. Potential risk factors for early recurrence were assessed with logistic regression models.

Results

Of 957 included patients, 204 (21.3%) were recurrence-free at last follow-up. The optimal length of recurrence-free survival to distinguish between early (n=388, 51.5%) and late recurrence (n=365, 48.5%) was 12 months (P<0.001). Patients with early recurrence had 1-, and 2-year post-recurrence survival rates of 20 and 6% compared with 45 and 22% for the late recurrence group (both P<0.001). Pre-operative risk factors for early recurrence included a Charlson age-comorbidity index ≥4 (OR 1.65), tumor size >3.0 cm on computed tomography (OR 1.53) and CA 19-9 >210 U/mL (OR 2.30). Post-operative risk factors consisted of poor tumor differentiation grade (OR 1.66), microscopic lymphovascular invasion (OR 1.70), a lymph node ratio >0.2 (OR 2.49), and CA 19-9 >37 U/mL (OR 3.38). Adjuvant chemotherapy (OR 0.28) and chemoradiotherapy (OR 0.29) were associated with a reduced likelihood of early recurrence.

Conclusion

A recurrence-free interval of 12 months is the optimal threshold for differentiating between early and late recurrence, based on subsequent prognosis.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease that is projected to become the second most common cause of cancer-related death in the United States by 2030.[1] Due to lack of early clinical symptoms and effective screening methods, most patients suffer from locally advanced or metastatic cancer at the time of initial presentation. Consequently, it is estimated that only 20% of newly diagnosed patients are initial candidates for resection without undergoing neoadjuvant treatement.[2] Yet, even in this most favorable cohort with resectable PDAC, up to 80% of patients recur after a short recurrence-free interval.[3-5] This high rate of recurrence has been attributed to the presence of occult micrometastatic disease at the time of resection and lack of effective systemic therapies.[6,7]

Although the term "early recurrence" is often utilized in both the academic and clinical setting, a clear definition is currently lacking, with arbitrary cut-off values varying between 6 and 12 months found in the literature.[8-11] Primary surgical resection is the standard of care for localized PDAC.[2] However, resectable patients susceptible to early recurrence constitute a key cohort worthy of further study, as these selected patients may benefit from a neoadjuvant-first approach.[12-15] The goal of this study, therefore, was two-fold: first, to establish an evidence-based cut-off value to differentiate between early and late recurrence based on the difference in prognosis after recurrence, and second, to identify perioperative risk factors for early PDAC recurrence after resection. An evidence-based cut-off value for early recurrence has the potential to aid clinicians with prognostic stratification of post-pancreatectomy patients, while identified risk factors might help guide neoadjuvant and adjuvant treatment decisions.

Methods

Study Population

Our institutional review board approved of this retrospective study. Patients who underwent pancreatectomy for primary resectable PDAC between 2000 and 2013 were included from an institutional database. Exclusion criteria were grossly positive resection margin (R2), synchronous distant disease at the time of resection, use of neoadjuvant therapy, and 90-day post-operative mortality. Patients with incomplete records due to follow-up done at other institutions, or with less than 24 months of follow-up in which neither recurrence nor death occurred, were also excluded. The primary outcomes of interest were recurrence-free survival (RFS), post-recurrence survival (PRS), and overall survival (OS).

Data Collection and Follow-up

Both pre- and post-operative demographics, clinicopathologic, and treatment variables were extracted from a prospectively maintained institutional database. The pre-operative Charlson age-comorbidity index (CACI) was calculated from available data as a measure of frailty and patients were dichotomized using a threshold of 4 points based on recent literature.[16-18] Pre- and post-operative carbohydrate antigen (CA) 19-9 values were obtained, when available. CA 19-9 values acquired at time of jaundice (total bilirubin >5 mg/dL) or later than 2 months post-operatively were excluded from analysis. Furthermore, patients who had 3 or more consecutive undetectable CA 19-9 values (<1.0 U/mL) were deemed Lewis antigen negative and were also excluded from analysis. The resection margin (R) was defined as R0 when the distance of carcinoma cells to the closest resection margin was >1 mm, and R1 when the distance was ≤1 mm. After resection, patients were routinely referred to a medical or radiation oncologist for adjuvant treatment recommendations. Adjuvant therapy was stratified into three groups: chemotherapy, chemoradiotherapy (including patients who underwent chemotherapy followed by radiotherapy with or without radio-sensitizing chemotherapy), and no adjuvant therapy.

Our institutional follow-up strategy and definitions for diagnosis of PDAC recurrence have been described previously. When imaging findings were consistent with recurrence, biopsy was seldom performed. Magnetic resonance imaging and/or fluorodeoxyglucose positron emission tomography were performed if necessary to clarify ambiguous computed tomography (CT) findings. Recurrence locations were stratified into five mutually exclusive categories: "local only", "liver only", "lung only", "multiple-site", and "other". Patients with recurrence and good performance status were generally further treated with systemic therapy or enrolled in experimental clinical trials.

Outcomes and Statistical Analysis

RFS was calculated from the date of pancreatectomy to the date of recurrence or last follow-up if recurrence did not occur. OS was defined as the time from surgery to either death or last follow-up. PRS was defined as the time from first recurrence to either death or last follow-up. Median survival outcomes were estimated with a Kaplan-Meier curve. The log-rank test was performed to compare between subgroups. A minimum P-value approach was used to evaluate the optimal threshold of RFS to divide the patients in an early and late recurrence cohort based on the length of PRS. In this approach, the log-rank test is performed for different lengths of RFS to determine the optimal cut-off point with the lowest P-value.

Table 1. Demographics, clinicopathologic and treatment characteristics of included patients

Variable	*All patients (n=957)*	*No recurrence (n=204)*	*Recurrence (n=753)*	*P*-value
Female, *n* (%)	456 (47.6%)	88 (43.1%)	368 (48.9%)	0.146
Race, *n* (%)				0.367
Caucasian	821 (85.8%)	179 (87.7%)	642 (85.3%)	
Other	136 (14.2%)	25 (12.3%)	111 (14.7%)	
Age, mean years (SD)	65.8 (10.5)	68.2 (10.4)	65.2 (10.5)	**<0.001**
Charlson age-comorbidity index, *n* (%)				**0.042**
<4 points	657 (68.7%)	152 (74.5%)	505 (67.1%)	
≥4 points	300 (31.3%)	52 (25.5%)	248 (32.9%)	
Pre-operative CA 19-9 (U/ml)[a]				
Median (IQR)	130 (50-398)	72 (28-269)	148 (56-455)	**0.006**
Post-operative CA 19-9 (U/ml)[b]				
Median (IQR)	38 (19-113)	28 (16-45)	43 (22-138)	**<0.001**
Operation procedure, *n* (%)				0.100
Pylorus-preserving pancreatoduodenectomy	415 (43.4%)	92 (45.1%)	323 (42.9%)	
Classic pancreatoduodenectomy	383 (40.0%)	83 (40.7%)	300 (39.8%)	
Total pancreatectomy	34 (3.6%)	11 (5.4%)	23 (3.1%)	
Distal pancreatectomy	125 (13.1%)	18 (8.8%)	107 (14.2%)	
Morbidity, *n* (%)				0.123
≤Clavien-Dindo grade II	803 (83.9%)	164 (80.4%)	639 (84.9%)	
≥Clavien-Dindo grade III	154 (16.1%)	40 (19.6%)	114 (15.1%)	
Resection margin, *n* (%)				**<0.001**
R0 (>1.0 mm)	658 (68.8%)	167 (81.9%)	491 (65.2%)	
R1 (≤1.0 mm)	299 (31.2%)	37 (18.1%)	262 (34.8%)	
Tumor differentiation, *n* (%)				**<0.001**
Well/Moderate	591 (61.8%)	148 (72.5%)	443 (58.8%)	
Poor	366 (38.2%)	56 (27.5%)	310 (41.2%)	
Tumor size, mean cm (SD)	3.2 (1.5)	3.0 (1.7)	3.2 (1.4)	**0.010**
T-stage, *n* (%)				**0.028**
1-2	283 (29.6%)	73 (35.8%)	210 (27.9%)	
3-4	674 (70.4%)	131 (64.2%)	543 (72.1%)	
Positive lymph nodes, *n* (%)	719 (75.1%)	130 (63.7%)	589 (78.2%)	**<0.001**
Positive lymph node ratio, *n* (%)				**<0.001**
≤0.2	646 (67.5%)	174 (85.3%)	472 (62.7%)	
>0.2	311 (32.5%)	30 (14.7%)	281 (37.3%)	
Microscopic perineural invasion, *n* (%)	859 (89.8%)	163 (79.9%)	696 (92.4%)	**<0.001**
Microscopic lymphovascular invasion, *n* (%)	544 (56.8%)	81 (39.7%)	463 (61.5%)	**<0.001**
AJCC stage 7th edition, *n* (%)				**0.001**
≤2A	671 (28.3%)	76 (37.3%)	195 (25.9%)	
≥2B	686 (71.7%)	128 (62.7%)	558 (74.1%)	
Adjuvant therapy, *n* (%)				**0.002**
No adjuvant	307 (32.1%)	55 (27.0%)	252 (33.5%)	
Chemotherapy	207 (21.6%)	62 (30.4%)	145 (19.3%)	
Chemoradiotherapy	443 (46.3%)	87 (42.6%)	356 (47.3%)	
Recurrence site, *n* (%)				NA
Local only	190 (19.9%)	0 (0%)	190 (25.2%)	
Metastatic	563 (58.8%)	0 (0%)	563 (74.8%)	

Survival (median months, 95% CI)				
Recurrence-free survival	15.2 (14.0-16.4)	NA	11.7 (10.8-12.6)	NA
Post-recurrence survival	NA	NA	7.5 (6.8-8.2)	NA
Overall survival	24.8 (23.3-26.3)	93.0 (61.0-125.1)	21.1 (19.2-22.9)	**<0.001**

[a] 398 patients had pre-operative CA 19-9 levels available for analysis. Excluded from analysis were 65 Lewis-antigen negative patients and 494 patients with missing pre-operative values.
[b] 532 patients had post-operative CA 19-9 levels available for analysis. Excluded from analysis were 65 Lewis-antigen negative patients and 360 patients with missing post-operative values.
AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; CI, *confidence interval*; IQR, *interquartile range*; NA, *not applicable*; SD, *standard deviation*.

Receiver operating characteristics (ROC) curves were constructed to estimate the optimal threshold for both pre- and post-operative CA 19-9 as a risk factor for early recurrence. The optimal cutoff value was determined to be the point of the ROC curve closest to the upper-left corner of the graph. Associations between potential risk factors and early PDAC recurrence were assessed by univariable logistic regression. Variables with a P-value of <0.10 were included as covariate in two separate multivariable logistic regression models: one for pre-operative and one for post-operative risk factors. Results were presented as odds ratio (OR) with corresponding 95% confidence interval (CI). A 2-tailed P-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS statistical software v25.0 (IBM Corp., Armonk, NY).

Results

Patient Cohort

In the study period of 2000 to 2013, 1520 patients underwent upfront pancreatectomy for newly diagnosed PDAC. Excluded from this cohort were 24 patients (1.6%) who died within 90 days post-surgery and 220 patients (14.5%) with less than 24 months of follow-up, in which neither death, nor recurrence occurred. An additional 319 patients (21.0%) who were followed post-operatively at other institutions were also excluded. Therefore, a total of 957 patients were included in the final analysis. Demographics, clinicopathologic, and treatment characteristics of the entire study population, and dichotomized for patients with and without recurrence, are summarized in Table 1.

Median follow-up for the entire cohort was 24.2 months (95% CI 22.7-25.8). At the time of last follow-up, 753 (78.7%) of 957 patients had recurred after a median RFS of 11.7 months (95% CI 10.8-12.6). Patients most often experienced multiple-site recurrence (n=253, 33.6%), followed by isolated local (n=190, 25.2%), liver only (n=184, 24.4%), or lung only (n=106, 14.1%) recurrence. The remaining 20 patients (2.7%) experienced first recurrence at more

unusual locations such as the brain, osseous structures, or the ovaries. Median OS for all patients with recurrence was 21.1 months (95% CI 19.2-22.9). Median PRS was 7.5 months (95% CI 6.8-8.2). Median OS for the entire cohort was 24.8 months (95% CI 23.3-26.3) with 175 patients (18.3%) currently alive after a median follow-up of 71.0 months (95% CI 63.3-78.7).

Defining Early and Late Recurrence

The evaluated early recurrence cut-off values and associated survival outcomes are shown in Table 2. In the current study cohort of 753 patients with recurrence, the optimal length of RFS to distinguish between early and late recurrence, based on subsequent PRS, was 12 months ($P=3.31x10^{-13}$) (Figure 1). Median RFS in the early (<12 months) recurrence cohort (n=388, 51.5%) was 6.5 months (95% CI 5.9-6.9), followed by a relatively limited PRS of 6.1 months (95% CI 5.5-6.8). Patients with recurrence after 12 months (n=365, 48.5%) had a median RFS of 20.9 months (95% CI 19.4-22.4) with a median PRS of 10.8 months (95% CI 9.4-12.2). Patients with early recurrence had 1- and 2-year PRS rates of 20 and 6% compared with 45 and 22% for the late recurrence group (both P<0.001). Median OS was significantly longer for patients with late recurrence (34.6 months, 95% CI 31.5-37.6) when compared with patients with early recurrence (13.0 months, 95% CI 12.2-13.8; P<0.001).

Patients with early recurrence more often had a larger tumor, a poorly differentiated tumor, positive lymph nodes, and microscopic lymphovascular invasion (Table 3). Additionally, both pre- and post-operative CA 19-9 values were significantly higher in patients with early recurrence. On the other hand, patients with late recurrence had superior pre-operative performance status and less severe post-operative complications according to the CACI and Clavien-Dindo classification, respectively. Furthermore, patients with late recurrence had more often received adjuvant chemotherapy or chemoradiotherapy. Patients with a pre-operative CACI score of ≥4 (60.0 vs. 71.5%; P<0.001) or a post-operative complication Clavien-Dindo classification ≥III (60.4 vs. 69.4%; P=0.029) were less likely to receive any adjuvant therapy.

Observed recurrence patterns were also notably different. Patients with late recurrence presented more often with local only (31.2 vs. 19.6%; P<0.001) or lung only recurrence (20.0 vs. 8.5%; P<0.001). On the contrary, liver only (33.8 vs. 14.5%; P < 0.001) and multiple-site recurrence (36.9 vs. 30.1%; P= 0.050) were more prevalent among the patients recurring early.

Pre- and Post-operative CA 19-9 Analysis

Of the entire cohort of 957 patients, 398 patients had pre-operative CA 19-9 values available (median 130 U/mL, IQR 50-398). Within 2 months post-operatively, 532 patients had CA 19-9 values available (median 38 U/mL, IQR 19-113). Sixty-five patients (6.8%) were deemed Lewis antigen negative and were excluded from the analysis. For pre-operative CA 19-9, the area under the curve (AUC) was 0.640 and the optimal threshold for predicting early recurrence was 210 U/mL with a sensitivity of 70.9% and specificity of 51.1% (Figure 2A). The best cut-off value for post-operative CA 19-9 (AUC=0.732) was 37 U/mL with a sensitivity of 71.6% and specificity of 64.9% (Figure 2B). Eighty-nine of 157 patients (56.7%) with pre-operative CA 19-9 values exceeding 210 U/mL recurred early, versus 86 of 241 patients (35.7%) with pre-operative CA 19-9 less than 210 U/mL (P<0.001). Similarly, 175 of 272 patients (64.3%) with >37 U/mL post-operative CA 19-9 experienced early recurrence compared with 75 of 260 patients (28.8%) with <37 U/mL (P<0.001). Elevated pre- and post-operative CA 19-9 levels were both significantly associated with decreased RFS (Figure 3).

Table 2. Evaluated cut-off values for defining early and late recurrence based on the prognosis after recurrence

		Potential early recurrence cohort				*Potential late recurrence cohort*			
Evaluated cut-off	*P-value*	*n*	*RFS (months)*	*PRS (months)*	*OS (months)*	*n*	*RFS (months)*	*PRS (months)*	*OS (months)*
3 months	$3.43x10^{-1}$	85	1.6	7.9	9.1	604	13.7	7.5	23.7
4 months	$1.10x10^{-2}$	123	2.1	6.9	9.8	630	14.6	7.7	24.4
5 months	$6.79x10^{-4}$	157	2.6	6.5	9.2	596	15.1	7.9	25.1
6 months	$8.70x10^{-5}$	182	3.3	6.5	9.9	571	15.6	8.0	25.9
7 months	$1.70x10^{-5}$	222	3.8	6.5	10.1	531	16.6	8.0	26.6
8 months	$3.73x10^{-7}$	244	4.0	6.4	10.2	509	17.1	8.3	28.1
9 months	$1.29x10^{-9}$	277	4.4	6.1	10.7	476	18.1	8.9	29.6
10 months	$1.68x10^{-11}$	319	5.3	6.0	11.2	434	19.4	9.7	31.5
11 months	$2.56x10^{-11}$	353	5.9	6.2	12.3	400	20.2	10.3	32.5
12 months	**$3.31x10^{-13}$**	**388**	**6.5**	**6.1**	**13.0**	**365**	**20.9**	**10.8**	**34.6**
13 months	$4.21x10^{-13}$	410	6.8	6.2	13.6	343	23.0	10.8	35.6
14 months	$6.98x10^{-13}$	437	7.1	6.2	13.9	316	24.5	10.9	38.5
15 months	$3.47x10^{-12}$	462	7.5	6.4	14.4	291	25.7	10.6	40.2
16 months	$1.49x10^{-12}$	489	8.4	6.6	14.9	264	26.9	10.9	43.3
17 months	$4.75x10^{-11}$	508	8.6	6.7	15.2	204	27.6	11.7	44.9
18 months	$3.58x10^{-12}$	526	8.9	6.7	15.7	227	29.0	11.8	47.8
19 months	$1.21x10^{-11}$	543	9.1	6.9	16.0	210	30.6	12.1	48.4
20 months	$4.21x10^{-11}$	564	9.3	6.9	16.6	189	31.9	13.4	51.3

Shown in bold is the optimal cut-off threshold with the lowest *P*-value.
OS, *overall survival*; PRS, *post-recurrence survival*; RFS, *recurrence-free survival*.

Factors Associated with Early Recurrence

Results of univariable analysis and two separate multivariable logistic regression models with pre- and post-operative risk factors are presented in Table 4. Three pre-operative variables proved to be independently associated with recurrence within 12 months: CACI score of ≥4 (OR 1.65, 95% CI 1.06-2.55, P=0.025), tumor size on the last pre-operative CT scan >3.0 cm (OR 1.53, 95% CI 1.11-1.95, P=0.029) and pre-operative CA 19-9 of >210 U/mL (OR 2.30, 95% CI 1.51-3.50, P<0.001). Four post-operative risk factors were independently correlated with early recurrence, including poor tumor differentiation grade (OR 1.66, 95% CI 1.10-2.51, P=0.016), microscopic lymphovascular invasion (OR 1.70, 95% CI 1.10-2.63, P=0.018), positive lymph node ratio >0.2 (OR 2.49, 95% CI 1.62-3.84, P<0.001), and post-operative CA 19-9 of >37 U/mL (OR 3.38, 95% CI 2.25-5.08, P<0.001). Furthermore, both adjuvant chemotherapy (OR 0.28, 95% CI 0.16-0.51, P<0.001) and chemoradiotherapy (OR 0.29, 95% CI 0.18-0.47, P<0.001) were independently associated with a reduced likelihood of early recurrence.

Discussion

Although the prognostic relevance of initial recurrence is of significant clinical impact, there is presently no established and evidence-based definition for early recurrence of PDAC after pancreatectomy. Our study implied that the optimal cut-off value to differentiate between early and late recurrence, based on subsequent prognosis, is a recurrence-free interval of at least 12 months. Additionally, independent risk factors for the development of early PDAC recurrence after resection were found, including a pre-operative CACI score of ≥4, tumor size on the last pre-operative CT scan >3.0 cm and a CA 19-9 level of >210 U/mL. Additionally, pathologic findings of poor tumor differentiation grade, microscopic lymphovascular invasion, a positive lymph node ratio >0.2, and post-operative CA 19-9 level of >37 U/mL were independently associated with early recurrence. Lastly, adjuvant chemotherapy and chemoradiotherapy were associated with a reduced likelihood of early recurrence.

Throughout the present literature, varying cut-off values are being used to divide patients based on timing of recurrence, for instance: 6 months by Sugiura et al.[19] and Matsumoto et al.[8], 8 months by Niedergethmann et al.[9] and 12 months by Zhai et al.[10] and Nishio et al.[11] To the best of the authors' knowledge, just one previous study has been performed with the primary goal of classifying patients into early and late recurrence groups based on the statistical assessment of the best cut-off value to differentiate in prognosis.[20] In their study of 55 patients with recurrence, Yamamoto et al. also established an optimal cutoff of 12 months for differentiating early and late recurrence based on OS.

Figure 1. Different cut-off values of recurrence-free survival with corresponding P-values (log-rank test) show that the optimal threshold for defining early and late recurrence based on the difference of post-recurrence survival is 12 months.

Table 3. Demographics, clinicopathologic and treatment characteristics of all patients with recurrence

Variable	***Early recurrence <12 months (n=388)***	***Late recurrence >12 months (n=365)***	***P*-value**
Female, *n* (%)	191 (49.2%)	177 (48.5%)	0.840
Race, *n* (%)			0.232
Caucasian	325 (83.8%)	317 (86.8%)	
Other	63 (16.2%)	48 (. 13.2%)	
Age, mean years (SD)	65.4 (11.1)	64.9 (9.7)	0.457
Charlson age-comorbidity index, *n* (%)			**0.008**
<4 points	243 (62.6%)	262 (71.8%)	
≥4 points	145 (37.4%)	103 (28.2%)	
Pre-operative CA 19-9 (U/ml)[a]			**<0.001**
Median (IQR)	221 (87-685)	91 (30-294)	
Post-operative CA 19-9 (U/ml)[b]			**<0.001**
Median (IQR)	91 (30-294)	29 (16-60)	
Operation procedure, *n* (%)			0.311
Pylorus-preserving pancreatoduodenectomy	149 (38.4%)	151 (41.4%)	
Classic pancreatoduodenectomy	163 (42.0%)	160 (43.8%)	
Total pancreatectomy	15 (3.9%)	8 (2.2%)	
Distal pancreatectomy	61 (15.7%)	46 (12.6%)	
Morbidity, *n* (%)			**0.013**
≤Clavien-Dindo grade II	317 (81.7%)	322 (88.2%)	
≥Clavien-Dindo grade III	71 (18.3%)	43 (11.8%)	
Resection margin, *n* (%)			0.126
R0 (>1.0 mm)	243 (62.6%)	248 (67.9%)	
R1 (≤1.0 mm)	145 (37.4%)	117 (32.1%)	
Tumor differentiation, *n* (%)			**<0.001**
Well/Moderate	202 (52.1%)	241 (66.0%)	
Poor	186 (47.9%)	124 (34.0%)	
Tumor size, mean cm (SD)	3.5 (1.5)	3.0 (1.1)	**<0.001**
T-stage, *n* (%)			**0.005**
1-2	91 (23.5%)	119 (32.6%)	
3-4	297 (76.5%)	246 (67.4%)	
Positive lymph nodes, *n* (%)	322 (83.0%)	267 (73.2%)	**0.001**
Positive lymph node ratio, *n* (%)			**<0.001**
≤0.2	213 (54.9%)	259 (71.0%)	
>0.2	175 (45.1%)	106 (29.0%)	
Microscopic perineural invasion, *n* (%)	361 (93.5%)	335 (92.0%)	0.430
Microscopic lymphovascular invasion, *n* (%)	257 (68.5%)	206 (57.4%)	**0.001**
AJCC stage 7th edition, *n* (%)			**0.001**
≤2A	81 (20.9%)	114 (31.2%)	
≥2B	307 (79.1%)	251 (68.8%)	
Adjuvant therapy, *n* (%)			**<0.001**
No adjuvant	175 (45.1%)	77 (21.1%)	
Chemotherapy	65 (16.8%)	80 (21.9%)	
Chemoradiotherapy	148 (38.1%)	208 (57.0%)	

Recurrence site, *n* (%)			
Liver only	131 (33.8%)	53 (14.5%)	**<0.001**
Multiple-site	143 (36.9%)	110 (30.1%)	**0.050**
Lung only	33 (8.5%)	73 (20.0%)	**<0.001**
Local only	76 (19.6%)	114 (31.2%)	**<0.001**
Other	5 (1.3%)	15 (4.1%)	**0.016**

[a] 315 patients had pre-operative CA 19-9 levels available for analysis. Excluded from analysis were 50 Lewis-antigen negative patients and 388 patients with missing pre-operative values.
[b] 455 patients had post-operative CA 19-9 levels available for analysis. Excluded from analysis were 50 Lewis-antigen negative patients and 248 patients with missing post-operative values.
AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; IQR, *interquartile range*; SD, *standard deviation*.

The 37 patients with early recurrence had a limited 5-year survival rate of 9% compared with the 42% rate found for the 18 patients with late recurrence (P<0.001). However, no mention was made on PRS outcomes and potential differences between the two patient populations. Using OS as primary outcome when defining early and late recurrence potentially introduces bias, since OS will inevitably be better in the late recurrence cohort group as these patients already have a long recurrence-free interval. To avoid this bias, we made the conscious decision to use the difference in survival after recurrence (PRS) in our analyses to define early and late recurrence.

The current study shows that patients who recurred within 12 months had a PRS of 6.1 months compared with a PRS of 10.8 months for patients with late recurrence (P<0.001). The fact that patients with a prolonged RFS after surgery also tended to live longer after they recurred, may suggest favorable tumor biology. Conversely, more aggressive tumor biology may lead to shorter RFS followed by a more rapid progression to death. In this way, RFS could be a clinically useful surrogate for appreciating PDAC behavior. The impact of the timing of recurrence presented in the current study could potentially aid physicians with prognostic stratification and help aid decision-making regarding the treatment of recurrence. Multiple studies focusing on the treatment of recurrence have suggested that RFS is an important factor when selecting patients for further treatment of recurrence.[21] For instance, a study done at this institution on stereotactic body radiation therapy for isolated local recurrence showed that patients with a RFS > 9 months had superior survival after salvage treatment.[22] In another study, Boone et al.[23] performed re-resections in selected patients with isolated local, liver, or pulmonary recurrence and found that survival after treatment of recurrence was significantly longer for patients with >15 months of RFS (40.6 vs. 8.2 months; P<0.05). Lastly, after controlling for location and treatment of recurrence, a recent Dutch study found that an RFS of >10 months was independently associated with prolonged survival after recurrence.[24]

Table 4. Univariable and multivariable logistic regression for associations between pre- and post-operative risk factors and early recurrence of pancreatic ductal adenocarcinoma after resection (<12 months)

	Univariable		*Multivariable*	
Pre-operative risk factors	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***
Age: > 70 years vs ≤ 70 years	1.00 (0.77 - 1.30)	0.984	-	
Gender: Female vs. male	1.15 (0.89 - 1.49)	0.288	-	
Race: Caucasian vs. all others	0.82 (0.57 - 1.17)	0.272	-	
Abdominal pain: Yes vs. no	1.27 (0.96 - 1.67)	0.101	-	
Jaundice: Yes vs. no	0.82 (0.63 - 1.06)	0.130	-	
Diabetes: Yes vs. no	1.28 (0.94 - 1.75)	0.116	-	
Weight loss: Yes vs. no	1.22 (0.94 - 1.60)	0.138	-	
Smoking: Past/current vs. never	0.80 (0.59 - 1.09)	0.149	-	
Charlson age-comorbidity index: ≥4 vs. <4	1.48 (1.12 - 1.95)	**0.005**	**1.65 (1.06 - 2.55)**	**0.025**
Tumor size⁋: >3.0 cm vs. ≤3.0 cm	2.25 (1.69 - 2.99)	**<0.001**	**1.53 (1.11 - 1.95)**	**0.029**
Tumor location⁋: Body/tail vs. head/uncinate	1.40 (0.99 - 1.97)	**0.053**	1.45 (0.82 - 2.56)	0.198
Pre-op CA 19-9: >210 U/ml vs. ≤210U/ml	2.36 (1.56 - 3.56)	**<0.001**	**2.30 (1.51 - 3.50)**	**<0.001**
	Univariable		***Multivariable***	
Post-operative risk factors	***Odds Ratio (95% CI)***	***P-value***	***Odds Ratio (95% CI)***	***P-value***
Age: >70 years vs ≤70 years	1.00 (0.77 - 1.30)	0.984	-	
Gender: Female vs. male	1.15 (0.89 - 1.49)	0.288	-	
Race: Caucasian vs. all others	0.82 (0.57 - 1.17)	0.272	-	
Charlson age-comorbidity index: ≥4 vs. <4	1.48 (1.12 - 1.95)	**0.005**	1.45 (0.95 - 2.22)	0.087
Complications*: Clavien ≥III vs. Clavien ≤II	1.42 (1.00 - 2.00)	**0.047**	1.14 (0.65 - 2.00)	0.645
Tumor size: > 3.0 cm vs. ≤ 3.0 cm	1.61 (1.24 - 2.10)	**<0.001**	1.11 (0.74 - 1.68)	0.609
Tumor grade: Poor vs. well/moderate	1.97 (1.51 - 2.57)	**<0.001**	**1.66 (1.10 - 2.51)**	**0.016**
Lymphovascular invasion: Yes vs. no	1.99 (1.52 - 2.60)	**<0.001**	**1.70 (1.10 - 2.63)**	**0.018**
Perineural invasion: Yes vs. no	2.00 (1.25 - 3.18)	**0.004**	1.31 (0.57 - 3.02)	0.521
Positive lymph node ratio: >0.2 vs. ≤0.2	2.71 (2.05 - 3.58)	**<0.001**	**2.49 (1.62 - 3.84)**	**<0.001**
Resection margin status: R1 vs. R0	1.61 (1.23 - 2.13)	**0.001**	1.24 (0.81 - 1.91)	0.340
Post-op CA 19-9: >37 U/ml vs. ≤37 U/ml	4.45 (3.09 - 6.41)	**<0.001**	**3.38 (2.25 - 5.08)**	**<0.001**
Adjuvant therapy:				
- Chemotherapy vs. no adjuvant	0.34 (0.23 - 0.49)	**<0.001**	**0.28 (0.16 - 0.51)**	**<0.001**
- Chemoradiotherapy vs. no adjuvant	0.32 (0.24 - 0.43)	**<0.001**	**0.29 (0.18 - 0.47)**	**<0.001**

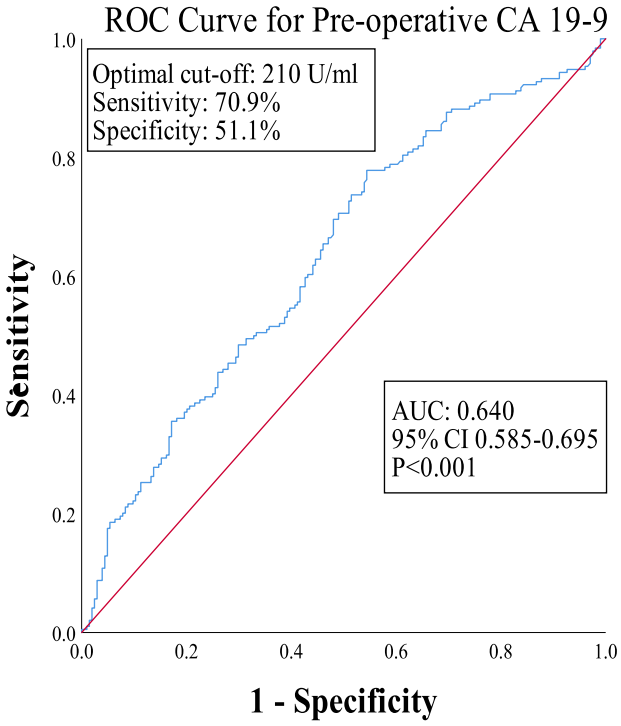
Shown in bold are univariable associations ($P<0.10$) that were selected for multivariable analysis and significant risk factors ($P<0.05$) on multivariable analysis.

⁋ Based on the last pre-operative computed tomography scan.

*According to Clavien-Dindo classification.

CA 19-9, *carbohydrate antigen 19-9*; CI, *confidence interval*.

A



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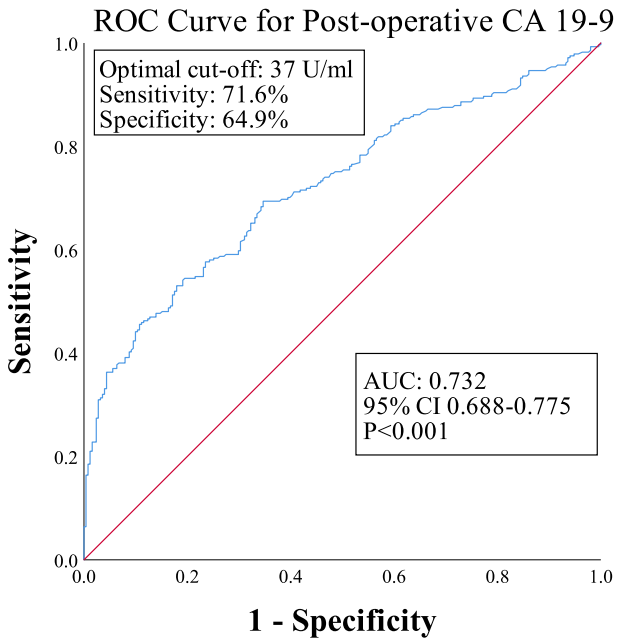

Figure 2. The ROC curve for (A) pre-operative CA 19-9 and (B) post-operative CA 19-9 for predicting early recurrence (<12 months).

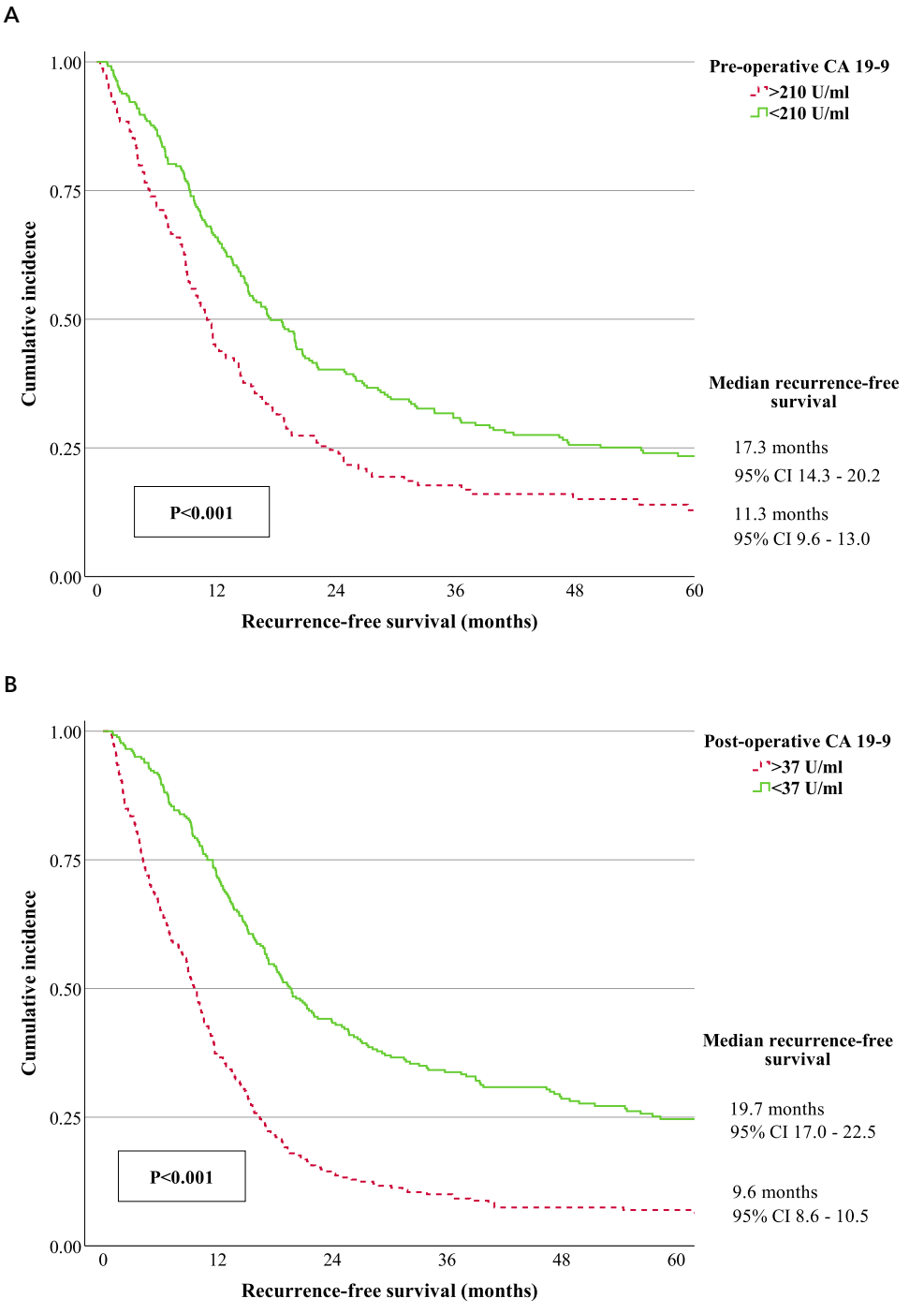

Figure 3. Kaplan-Meier curves showing worse recurrence-free survival outcomes for patients with elevated (A) pre-operative and (B) post-operative CA 19-9 values.

Several independent pre- and post-operative variables were identified that were associated with an increased likelihood of early recurrence after surgery for PDAC, including elevated pre- and post-operative CA 19-9. First discovered in 1979, CA 19-9 has become the most studied and well-known biomarker for PDAC.[25] Multiple reports have established the association between elevated pre- and post-operative CA 19-9 levels and decreased post-pancreatectomy survival, with varying thresholds between 37 and 400 U/mL being advocated.[26-29] However, far fewer studies have focused on the correlation between CA 19-9 and recurrence, and there is currently no consensus regarding the CA 19-9 threshold for prediction of early recurrence.[2,30] For instance, in a recent study by Nishio et al. (n=90), a pre-operative CA 19-9 of >529 U/mL was recommended as the optimal cutoff for predicting recurrence within 12 months.[11] Studies by Kim et al. (n=86) and Sugiura et al. (n=154) both found a pre-operative threshold of >100 U/mL to have the best correlation with recurrence within 6 months.[19,31] In this study, analyses of ROC curves and associated AUCs revealed optimal pre- and post-operative CA 19-9 thresholds for the prediction of early recurrence of >210 and >37 U/mL, respectively. However, with an AUC of 0.640, sensitivity of 71% and specificity of 51%, the predictive strength of finding elevated pre-operative CA 19-9 was fairly limited, highlighting the necessity of finding more accurate biomarkers in patients with PDAC.

A low performance status according to the CACI was shown to be an independent pre-operative risk factor for early recurrence. High CACI scores have previously been found to correlate with worse outcomes in patients with PDAC. For instance, Dias-Santos et al.[32] reported that a CACI score of >4 was predictive of death within 1 year of pancreatectomy (P<0.001). Similarly, another recent study showed that a CACI score of ≥4 was a predictor of poor survival on multivariable analysis (P= 0.024).[18] Interestingly, both a pre-operative CACI score ≥4 and a post-operative complication Clavien-Dindo classification ≥III were significantly associated with early recurrence on univariable analysis. However, both lost significance in a multivariable model that included post-operative risk factors. This could in part be explained by the strong confounding effect of adjuvant therapy as a variable in the post-operative multivariable model. For instance, a study from our group reported that post-operative complications delay the time to adjuvant therapy and reduce the likelihood of adjuvant therapy.[33] Likewise, Asano et al.[18] found that the rate of patients who received chemotherapy was significantly lower in those with a CACI score of ≥4 (69 vs. 87%, P<0.0001). Similar correlations were observed in the current study cohort, as patients with low pre-operative performance status (P<0.001) or severe post-operative complications (P=0.029) had a decreased likelihood of receiving any adjuvant therapy.

It was somewhat surprising to find that R1 resection, although associated with recurrence in

general, was ultimately not a predictor of early recurrence on multivariable analysis. There might be several explanations for this finding. Firstly, patients at our institution with a close resection margin are commonly recommended to undergo adjuvant radiation therapy with either conventional or stereotactic radiotherapy for margin attenuation, possibly suppressing the impact of R1 status. Secondly, R1 margin has previously been shown to be particularly associated with local recurrence, which was more commonly seen in the late recurrence cohort.[5] Lastly, recurrence following R1 margin might primarily be caused by microscopic residual disease in the remnant pancreas that has not undergone the process of hematogenous metastasis, possibly indicating a less aggressive and more favorable tumor biology resulting in a later recurrence.[22] Overall, the prognostic impact of the distance of PDAC cells to the final resection margin has not been fully clarified yet, and is an intensely debated topic in recent pancreatic surgery literature.[34-39]

From a total of 957 patients with primary resectable patients, more than 40% (n=388, 41.5%) recurred within 12 months. Of these patients with early recurrence, 80% had distant metastases, supporting the hypothesis that occult micrometastatic disease was present at the time of surgery. In recent years, it has been argued that a chemotherapy-first approach for resectable PDAC might help select for better tumor biology, while on the other hand sparing those patients who might have recurred early a major abdominal operation.[12-14] In the near future, prospective studies (such as the PREOPANC-trial) may be able to clarify the role of neoadjuvant chemotherapy in resectable PDAC.40 Although a CACI score of ≥4, a tumor size >3 cm on pre-operative CT and pre-operative CA 19-9 of >210 U/mL were shown to be independently associated with early recurrence, differences in tumor biology seem to exist that cannot be accounted for by currently identified risk factors alone. Accurate pre-operative identification of those patients with a high likelihood of early recurrence would greatly help clinicians and patients alike in selecting the appropriate sequence of therapies in PDAC. Recent advances in the field of "liquid biopsies" may result in a usable biomarker that reflects the presence of micrometastatic disease in patients with PDAC.[41,42] A current ongoing prospective trial at our institution (NCT02974764) aims to further elucidate the usefulness of both circulating tumor cells and circulating tumor DNA as a prognostic biomarker in PDAC patients.[43]

Previous work from our institution, using a subset of the current cohort consisting of patients who underwent resection for a tumor in the head of the pancreas between 2000 and 2010 (n=692), showed that specific recurrence locations have different predictive factors and possess distinct RFS curves.[5] The reported findings in the present study further complement and expand on those prior results by showing that an RFS of 12 months is the best cut-off

to separate early from late recurrence and by identifying risk factors that can help predict early recurrence. However, this study has several limitations worthy of consideration. First, a significant number of patients were excluded due to incomplete follow-up records, possibly limiting the generalizability of our findings to the population of PDAC patients as a whole. Second, although a prospective database from a large tertiary referral center was used for data extraction, this was a retrospective study with all the associated bias risks. Lastly, our database lacked specific information with regard to additional treatment for recurrence after pancreatectomy. Additional data on further treatments might have revealed associations not appreciated by the current study.

Conclusion

There is presently no established and evidence-based definition of early recurrence following surgery for primary resectable PDAC. This study found a recurrence-free interval of 12 months to be the optimal threshold for differentiating between early and late recurrence based on subsequent prognosis. Furthermore, pre-operatively (>210 U/mL) and post-operatively (>37 U/mL) elevated CA 19-9 were shown to be independently associated with early recurrence, albeit with relatively low sensitivity, specificity, and predictive value. Since currently acknowledged pre-operative risk factors are inadequate to accurately identify patients susceptible to early PDAC recurrence, further studies are needed to identify new biomarkers for the detection of clinically occult micrometastatic disease at the time of operation.

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PART II

Recurrence Following Post-Neoadjuvant Resection

CHAPTER 5

Survival in Locally Advanced Pancreatic Cancer after Neoadjuvant Therapy and Surgical Resection

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Abstract

Objective

An increasing number of LAPC patients who respond favorably to neoadjuvant therapy undergo surgical resection. The impact of surgery on patient survival is largely unknown. This study aims to identify the survival of patients with locally advanced pancreatic cancer (LAPC) and assess the effect of surgical resection after neoadjuvant therapy on patient outcomes.

Methods

All LAPC patients who presented to the institutional pancreatic multidisciplinary clinic (PMDC) from January 2013 to September 2017 were included in the study. Demographics, clinical data on neoadjuvant treatment, and surgical resection were documented. Primary tumor resection rates after neoadjuvant therapy and overall survival (OS) were the primary study endpoints.

Results

415 LAPC patients were included in the study. Stratification of neoadjuvant therapy in FOLFIRINOX-based, Gemcitabine-based, and combination of the two, and subsequent outcome comparison did not demonstrate significant differences in OS of 331 non-resected LAPC patients ($P=0.134$). Eighty-four patients underwent resection of the primary tumor (20%), after a median duration of five months of neoadjuvant therapy. FOLFIRINOX-based therapy and stereotactic body radiation therapy correlated with increased probability of resection ($P=0.006$). Resected patients had better performance status, smaller median tumor size ($P=0.029$), and lower median CA19-9 values ($P<0.001$) at PMDC. Patients who underwent surgical resection had significant higher median OS compared to those who did not (35.3 vs. 16.3 months, $P<0.001$). The difference remained significant when non-resected patients were matched for time of neoadjuvant therapy (19.9 months, $P<0.001$).

Conclusion

Surgical resection of LAPC after neoadjuvant therapy is feasible in a highly selected cohort of patients (20%) and is associated with significantly longer median overall survival.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is historically characterized by poor prognosis and high rates of disease-specific mortality. For 2017, the American Cancer Society estimated 53,670 new cases and 43,090 PDAC-related deaths in the United States.[1] Mortality continues to increase, and it is projected that by 2030, PDAC will be the second most common cancer-related cause of death.[2] The advanced stage of disease at the time of diagnosis is the main driver for decreased survival.[3] Systemic chemotherapy is the treatment of choice for patients presenting with advanced disease with a potential role for consolidative radiation, yet overall survival remains low compared to those who are eligible for upfront surgical resection.

Locally advanced pancreatic cancer (LAPC) accounts for 30% of all newly-diagnosed pancreatic cancer cases and is considered surgically unresectable, due to local involvement of adjacent vessels. Current guidelines for LAPC recommend non-operative treatment for patients with good performance status, through a multidisciplinary approach.[4] Randomized prospective trials have not yet provided definitive data to favor a specific chemotherapy regimen. Previous studies have shown that for patients with metastatic PDAC, the combination of leucovorin, fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX) is associated with improved survival.[5] A recent meta-analysis also suggested a potential survival benefit of FOLFIRINOX in patients with LAPC.[6] Similar results have also been reported with Gemcitabine-based multi-agent therapy.[7]

Recent retrospective series have suggested the possibility of potentially curative resection after induction chemotherapy in patients with LAPC.[8-11] Data from these studies indicated a survival benefit after tumor resection in patients with imaging response or no progression of disease after neoadjuvant therapy. Additionally, contemporary radiation modalities, such as stereotactic body radiation therapy (SBRT) can complement induction chemotherapy and improve tumor resectability in patients with LAPC.[10] However, these reports were limited by cohort heterogeneity, since they included patients with borderline resectable and/or metastatic PDAC, and by the lack of survival comparison with patients who were treated with non-operative therapy. In this large retrospective series, we sought to overcome these limitations by studying exclusively LAPC patients, who were reviewed in a single high-volume pancreatic multidisciplinary clinic (PMDC) within the past five years. The primary goal of this study is to determine if surgical resection is associated with improved survival compared to aggressive non-operative management in patients with LAPC. We secondarily sought to identify potential predictive factors for surgical resection.

Methods

Patient Cohort

The initial patient pool included all patients seen at the Johns Hopkins PMDC from January 1st, 2013 to September 30th, 2017. The primary eligibility criterion was the diagnosis of LAPC at the time of PMDC evaluation, based on the National Comprehensive Cancer Network guidelines.[12] More specifically, all patients who presented with encasement (>180° contact) of the celiac artery (CA) or superior mesenteric artery (SMA), unreconstructible portal or superior mesenteric vein, or tumor involvement of the first jejunal SMA branch were staged as LAPC and were included in the study. Most of these patients were seen more than once at PMDC along the course of their disease, to re-assess tumor resectability and re-evaluate treatment response. Primarily, all non-LAPC patients were excluded from the study. The rest of the exclusion criteria are shown in Figure 1; to ensure cohort homogeneity for resection eligibility, all LAPC patients who did not undergo standardized pancreatic protocol multi-detector computed tomography (MDCT) in our institution were excluded from the study. Follow-up data were documented until patient death, or date of last follow-up. Major eligibility criteria for surgical exploration included absence of local tumor extension and major vessel involvement, no deteriorating patient performance status, and/or response to neoadjuvant treatment per RECIST criteria (Response Evaluation Criteria in Solid Tumors)[13], assessed at PMDC. Subsequently, two cohorts were generated: LAPC patients who underwent surgical resection of the primary tumor, and LAPC patients who did not reach eligibility for resection and were treated with non-operative therapy. The same team of surgeons, specialized in pancreatic and hepatobiliary surgery, performed all surgical resections of LAPC patients. The institutional review board has approved this retrospective study.

Data Collection

All patient data were collected from the PMDC disposition notes and the prospectively maintained institutional database. Patient demographics included age, gender, race, and performance status based on the Eastern Cooperative Oncology Group (ECOG) scale. Data on tumor site, size, major vessel involvement, presence of regional lymphadenopathy, tumor involvement of the gastrointestinal tract, and presence or suspicion of distant metastases, as assessed by MDCT, were documented. Information about the type and timing of induction therapy were collected, including chemotherapy regimens, radiation therapy modalities, and treatment changes or complications, where applicable.

For patients who underwent surgical resection of the primary tumor, the type of surgery and

post-operative outcomes were evaluated. Tumor pathology characteristics, such as grade of differentiation, nodal disease, and resection margin status, were extracted from final pathology reports. Resections were characterized as R1, when malignant cells were identified within 1mm (≤1mm) from the surgical margin. Patient appointments at the institutional outpatient clinic and medical notes from local oncologists for out-of-state patients were utilized for tracking of adjuvant therapy plans and disease recurrence patterns. The date of death was obtained from institutional medical records, the Social Security Death Index, or online obituaries.

Definitions and Statistical Analysis

Summary statistics were obtained using established methods. Categorical variables were compared by the chi-square test, while continuous variables were compared using the Mann-Whitney U-test. Overall survival (OS) and post-resection survival were defined as the time from date of diagnosis (OS) or resection (post-resection survival) to either death or last follow-up. Recurrence-free survival (RFS) was defined as the time interval between date of operation and either date of recurrence or last follow-up, if recurrence was not observed. Kaplan-Meier curves were used to estimate median survival with corresponding 95% confidence intervals (CIs). The log-rank test was utilized for subgroup comparison. A two-sided P-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS statistical software v25.0 (IBM Corp., Armonk, NY).

Results

Patient Cohort

The flowchart for the study selection cohort is available in Figure 1. Within the studied period, 932 patients were evaluated at the institutional PMDC. In total, 109 patients (12%) met the exclusion criteria; most of them had undergone resection of the primary tumor prior to initial PMDC presentation (n=85). The remaining 823 patients were stratified based on their clinical disease stage and resectability status. According to the NCCN guidelines, 461 patients were characterized as LAPC (56%). Induction chemotherapy and/or chemoradiation, and re-evaluation were recommended in all LAPC cases, on an individual basis. After initial presentation, 46 patients (10%) were lost to follow-up and were excluded.

Resected LAPC Patients

One hundred and sixteen LAPC patients (28%) were deemed eligible for surgical exploration within the studied period. The decision for exploration was made based on tumor response to neoadjuvant therapy (n=13 with complete or partial response according to RECIST 1.1 criteria[13],

Table 1), or offered to patients without signs of local disease progression or metastases after completion of at least four months of neoadjuvant therapy (n=103). Resection of the primary tumor was achieved in 84 patients (20% of all LAPC patients, 72% of those eligible for exploration). For the remainder, resection was aborted intraoperatively, due to occult abdominal metastatic disease (n=12, 10%), or local extension of the tumor (n=20, 17%).

All resected LAPC patients had an ECOG performance status of 0 or 1 (Table 2). Most common tumor site was at the right side of the pancreas (head, uncinate process, and pancreatic neck, n=57, 68%), and mean tumor size was 35mm (±11mm, SD). The median value of carbohydrate antigen 19-9 (CA19-9) at presentation was 72 U/mL (IQR 35-268). A pancreatoduodenectomy was the most common type of performed operation (n=44, 53%), followed by distal pancreatectomy with en bloc celiac axis resection (n=22, 27%). Post-operative outcomes are

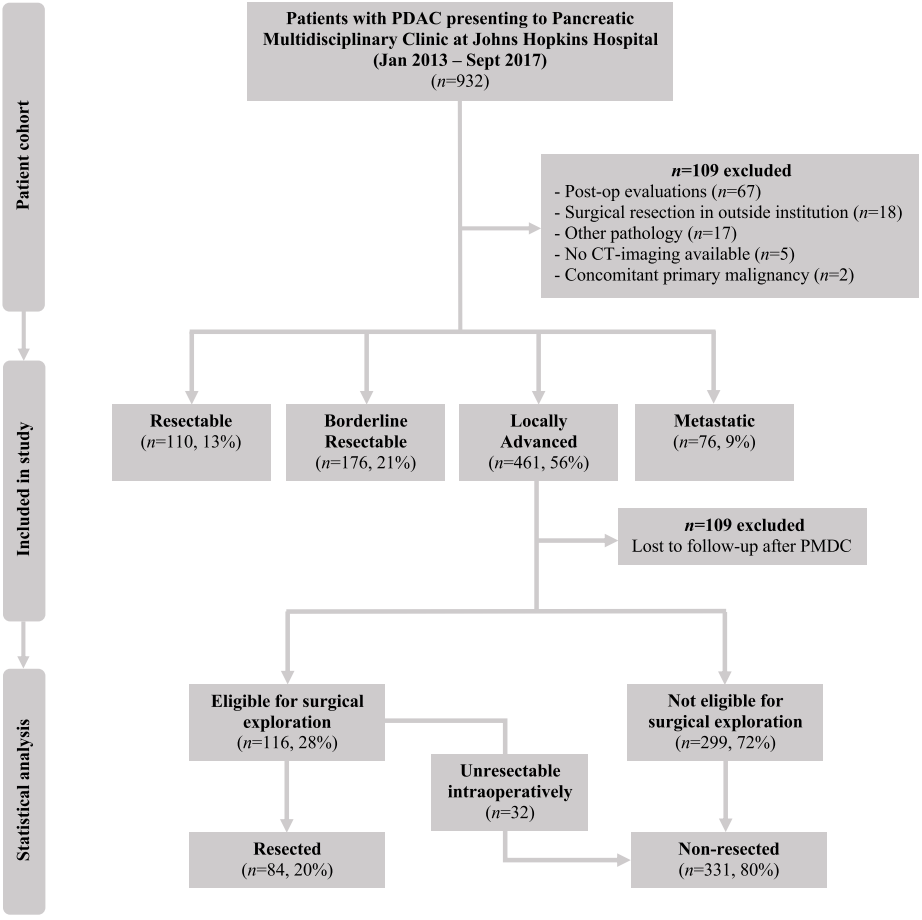

Figure 1. Patient study selection flowchart.

Table 1. Tumor response post neoadjuvant therapy in all explored LAPC patients, according to RECIST 1.1 criteria

Variable	***All explored LAPC patients (n=116)***	***Resected LAPC (n=84)***	***Non-resected LAPC (n=32)***	***P*-value**
RECIST 1.1, grade				0.463
Complete response	2 (2%)	2 (2%)	0	
Partial response (<30%)	11 (9%)	8 (10%)	3 (9%)	
Stable disease	96 (83%)	70 (83%)	26 (81%)	
Progressive disease	7 (6%)	4 (5%)	3 (9%)	

LAPC, *locally advanced pancreatic cancer*.

detailed in Table 3. 30-day morbidity per Clavien-Dindo classification[14] was 59% (n=49), and 90-day mortality 4% (n=3): one patient died from sepsis and subsequent multi-organ failure, one from a cardiovascular event, and another from failure to thrive post-operatively. Margin-negative resection (R0) was achieved in 89% of cases (n=75).

Significant variations were noted in the neoadjuvant chemotherapy regimens (Online Supplemental eTable S1): most of the patients received FOLFIRINOX5 (n=44, 52%), or "modified" FOLFIRINOX (omitted bolus doses of 5-FU or leucovorin, n=7, 8%), and regimens were adapted individually, depending on drug toxicity and tolerance. In 16 patients (19%) Gemcitabine/Nab-Paclitaxel was preferred.[15] Fourteen patients (17%) received both FOLFIRINOX and Gemcitabine/Nab-Paclitaxel at different time points, due to either drug toxicity (n=8), or increase in CA19-9 and disease progression on imaging under one regimen (n=6). Neoadjuvant therapy scheme was decided by a medical oncologist on an individual basis, taking multiple factors under consideration, including patient performance status and personal medical history. The median time of chemotherapy received for resected LAPC patients was five months (IQR 4-6). Radiation therapy was added as an adjunct after induction chemotherapy in almost all patients who were offered surgical exploration (n=111, 96%), to maximize the probability of margin-negative resection. SBRT was the modality of choice for the resected cohort (n=67, 80%). The median total time from diagnosis to surgery was 10 months (IQR 9-11).

Twenty-five patients received additional chemotherapy post-operatively (30%) for a median time of three months (IQR 2-4). The decision for adjuvant therapy was also made by the medical oncologist based on final pathology and patient status. The administered adjuvant regimens are summarized in Online Supplementary eTable S1. Disease recurrence after resection was recorded in 40 patients (48%), with local recurrence (n=13, 33%) being the most common, followed by distant liver metastases (n=9, 23%).

Table 2. Demographics, tumor characteristics, and neoadjuvant treatment data of resected and non-resected locally advanced pancreatic cancer patients

Variable	***All patients (n=415)***	***Resected (n=84)***	***Non-resected (n=331)***	**P-value**
Male, *n* (%)	195 (47%)	36 (43%)	159 (48%)	0.396
Age, mean years (SD)	65.2 (10.1)	63.3 (9.1)	65.6 (10.3)	**0.050**
ECOG performance status, *n* (%)				**<0.001**
ECOG 0	131 (32%)	42 (50%)	89 (27%)	
ECOG 1	260 (63%)	42 (50%)	218 (66%)	
ECOG 2	21 (5%)	0 (0%)	21 (6%)	
ECOG 3	3 (1%)	0 (0%)	3 (1%)	
CT-scan characteristics, *n* (%)				
Tumor size (mm), mean (SD)	38 (14)	35 (11)	39 (15)	**0.029**
Regional lymphadenopathy, *n* (%)	111 (27%)	24 (29%)	87 (26%)	0.672
Gastro-intestinal involvement, *n* (%)	91 (22%)	10 (12%)	81 (25%)	**0.013**
Duodenum	68 (75%)	6 (60%)	62 (77%)	
Stomach	22 (24%)	3 (30%)	19 (23%)	
Small bowel	1 (1%)	1 (10%)	0 (0%)	
Tumor site, *n* (%)				0.987
Head	174 (42%)	33 (39%)	141 (43%)	
Uncinate	62 (15%)	13 (16%)	49 (15%)	
Neck	51 (12%)	11 (13%)	40 (12%)	
Body	115 (28%)	24 (29%)	91 (28%)	
Tail	13 (3%)	3 (4%)	10 (3%)	
Atrophy of the pancreas, *n* (%)	258 (62%)	40 (48%)	218 (66%)	**0.002**
CA 19-9 at PMDC[a]				**<0.001**
Median (IQR)	181 (56-628)	72 (35-268)	206 (72-693)	
Final neoadjuvant chemotherapy, *n* (%)	378 (91%)	84 (100%)	294 (89%)	**0.001**
Stratified, *n* (%)				**0.018***
FOLFIRINOX-based	189 (50%)	53 (63%)	136 (46%)	
FOLFIRINOX-Gemcitabine combination	72 (19%)	14 (17%)	58 (20%)	
Gemcitabine-based	117 (31%)	17 (20%)	100 (34%)	
Time of therapy administered (months)				**<0.001**
Median (IQR)	4 (3-6)	5 (4-6)	4 (3-6)	
Final neoadjuvant radiotherapy, *n* (%)	222 (54%)	81 (96%)	141 (43%)	**<0.001**
Modality, *n* (%)				
SBRT	160 (72%)	67 (80%)	93 (66%)	
IMRT/EBRT	62 (28%)	14 (17%)	48 (34%)	

[a]30 patients had undetectable CA 19-9 (possibly not expressing the Lewis antigen).

* Post-hoc analysis (resected vs non-resected): FFX-based and Gemcitabine-based: ***P*=0.006**, FFX-Gemcitabine combination and Gemcitabine-based: *P*=0.376, FFX-based and FFX-Gemcitabine combination: *P*=0.155.

CA 19-9, *carbohydrate antigen 19-9*; CT, *computed* tomography; EBRT, *external beam radiation therapy*; ECOG, *Eastern Cooperative Oncology Group*; IMRT, *intensity-modulated radiation therapy*; IQR, *interquartile range*; PMDC, *pancreatic multidisciplinary clinic*; SBRT, *stereotactic body radiation therapy,* SD, *standard deviation*.

Table 3. Perioperative data and outcomes of resected locally advanced pancreatic cancer patients

Variable	*Resected patients (n=84)*
Type of operation, *n* (%)	
Whipple	44 (53%)
DP-CAR	23 (27%)
Distal pancreatectomy	16 (19%)
Total pancreatectomy	1 (1%)
Length of hospital stay (days)	
Median (IQR)	8 (6-11)
Morbidity, *n* (%)	
≤Clavien-Dindo grade IIIA	45 (54%)
≥Clavien-Dindo grade IIIB	4 (5%)
Thirty-day readmission, *n* (%)	24 (29%)
Thirty-day mortality, *n* (%)	1 (1%)
Ninety-day mortality, *n* (%)	3 (4%)
Adjuvant chemotherapy, *n* (%)	25 (30%)
Months of adjuvant administered	
Median (IQR)	3 (2-4)
Disease recurrence, *n* (%)	40 (48%)
Recurrence site, *n* (%)	
Local	13 (33%)
Liver only	9 (23%)
Carcinomatosis	7 (18%)
Multiple sites	7 (18%)
Lung	4 (10%)
Tumor size in pathology report (mm)	
Mean (SD)	20 (16)
Tumor differentiation, *n* (%)	
Well	2 (2%)
Moderate	45 (54%)
Poor	16 (19%)
Unknown	19 (23%)
Resection margin status, *n* (%)	
R0 (>1mm)	75 (89%)
R1 (≤1mm)	9 (11%)
Positive lymph nodes, *n* (%)	21 (25%)
Microscopic perineural invasion, *n* (%)	48 (57%)
Microscopic lymphovascular invasion, *n* (%)	18 (21%)
Treatment response, *n* (%)	
Complete (grade 0)	5 (6%)
Extensive (grade 1)	27 (32%)
Moderate (grade 2)	33 (39%)
Poor (grade 3)	13 (16%)
Unknown	3 (4%)

DP-CAR, *distal pancreatectomy with en bloc celiac artery resection (modified Appleby procedure);* IQR, *interquartile range;* SD, *standard deviation*.

Non-Resected LAPC Patients

Overall, 331 LAPC patients were treated only with chemotherapy and radiation therapy: 299 deemed not eligible for exploration and 32 who were declared unresectable intraoperatively. These patients developed distant metastases or significant local disease progression during neoadjuvant treatment. At the time of initial evaluation at the PMDC, non-resected patients were marginally older (P=0.050) and were more often characterized as ECOG 1 instead of ECOG 0 (P<0.001). Additionally, mean tumor size in baseline MDCT was larger compared to resected LAPC patients (39mm vs. 35mm, P=0.029), and gastrointestinal tract involvement was more frequent (P=0.013). Median CA 19-9 values were also significantly higher in the non-resected group (206 U/mL, IQR 72-693, P<0.001).

Variations in chemotherapy regimen combinations were also common in the unresected cohort. Thirty-seven patients (11%) in total did not receive chemotherapy after diagnosis. ECOG status of 2 or 3 was associated with no (P=0.004) or less chemotherapy (2.5 vs. 4 months for ECOG 0 or 1, P=0.020). When compared to the resected group, non-resected patients most often underwent Gemcitabine-based chemotherapy (P=0.006). Moreover, the median duration of administered chemotherapy was shorter (4 vs. 5 months, P<0.001). Less than half of the non-resected patients received complementary radiation therapy (n=141, 43%). However, the distribution of SBRT versus traditional external beam and intensity-modulated radiation therapy was similar in both cohorts.

Survival Rates in LAPC Patients

In this highly selected patient cohort, survival from the time of diagnosis was 35.3 months (95% CI 24.5-46.0) for the resected, and 16.2 months (95% CI 15.2-17.3) for the non-resected group (P<0.001, Figure 2A). In the non-resected cohort, OS was not associated with the type of chemotherapy regimen (Gemcitabine-based 16 months vs. FOLFIRINOX-based 17.4 months, P=0.134, Figure 3). Patients who switched chemotherapy during the course of their treatment also had comparable OS (17.2 months, 95% CI 14.5-20; P=0.099 vs. Gemcitabine-based, and P=0.675 vs. FOLFIRINOX-based only). Patients who received complementary neoadjuvant radiation therapy after chemotherapy had increased OS (20 vs. 14 months, P<0.001). As expected, patients who did not receive any treatment after diagnosis, due to poor performance status or personal preference (n=37) had worse survival than any treatment approach (5.4 months, IQR 3.5-7.3, P<0.001).

The plateau identified in the resected group survival curve (Figure 2A) depicts the median time interval between diagnosis and surgery (10 months, IQR 9-11). In an attempt to limit

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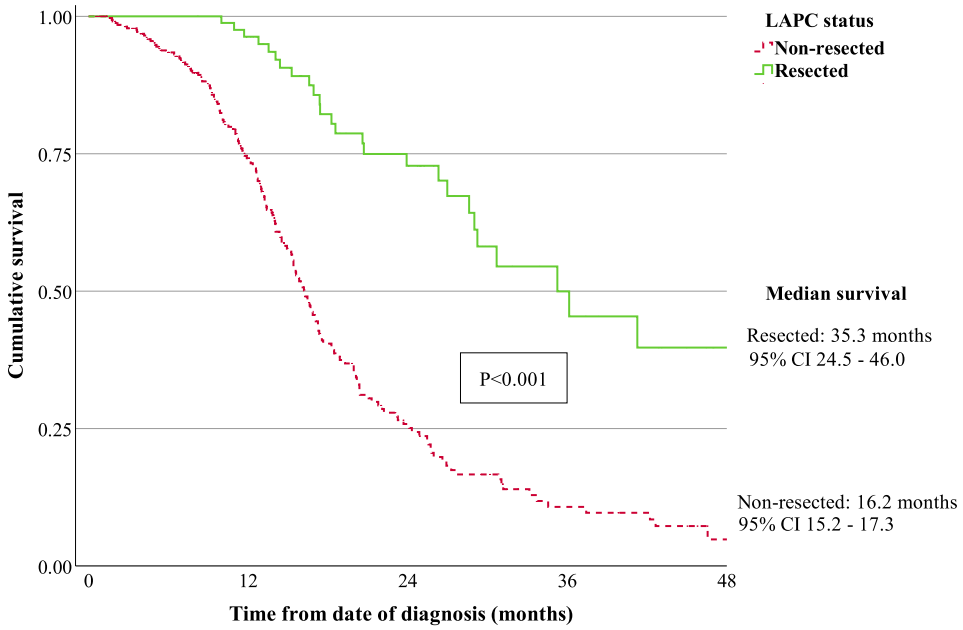

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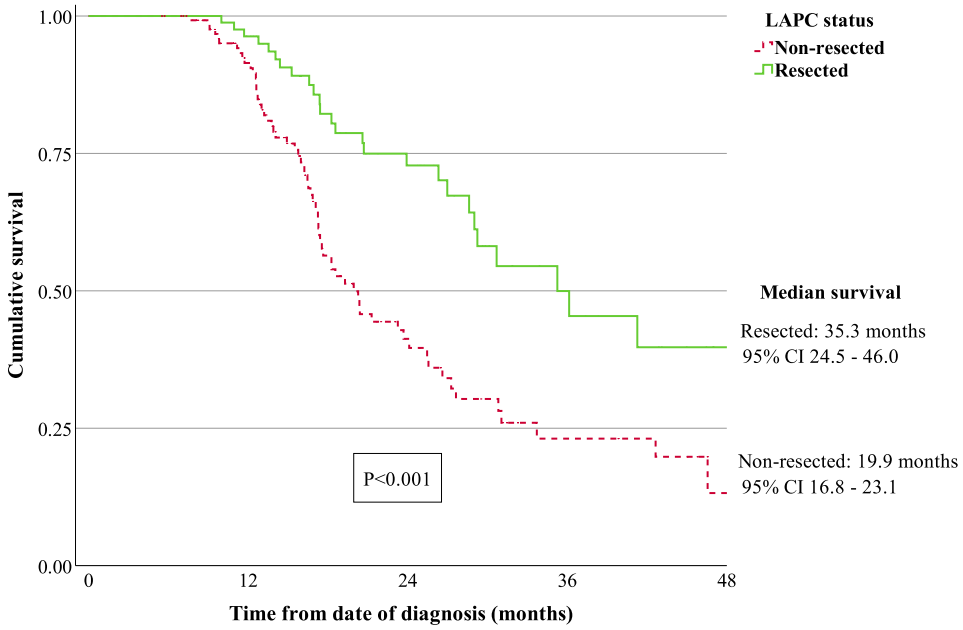

Figure 2. (A) Comparison of overall survival between resected and non-resected LAPC patients. (B) Comparison of overall survival between resected LAPC patients and non-resected who underwent at least five months of chemotherapy (excluded: non-resected patients who died, or progressed within five (5) months, or received less than 5 months of neoadjuvant chemotherapy).

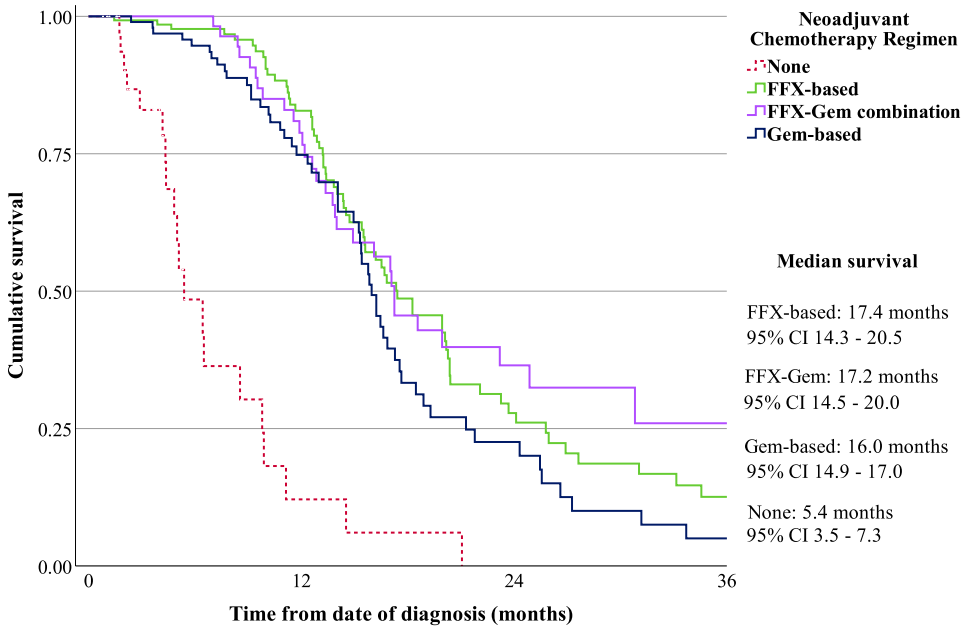

Figure 3. Comparison of overall survival between different chemotherapy regimens in non-resected locally advanced pancreatic cancer patients.

selection bias, all non-resected patients who progressed or died within this same time interval were excluded, and thus a matched group of non-resected patients who received at least five months of chemotherapy (n=132) was compared to the resected cohort: OS remained significantly different, favoring surgical resection (35.3 months vs. 19.9 months, P<0.001, Figure 2B). Statistical significance was preserved when we further limited the non-resected cohort to patients who received five months of chemotherapy and SBRT (n=74, 25.5 months, P=0.019). The impact of surgical resection on OS is also demonstrated in the percentage of alive patients at the 1-year and 3-year time points: in the resected group, 96% (n=81) and 50% (n=42) were alive at one year and three years, while in the non-resected group 74% (n=246, P<0.001) and 11% (n=37, P<0.001), respectively.

In the resected group, median post-operative RFS was 11.3 months (95% CI 9.3-13.3). Patients who received adjuvant chemotherapy did not have significantly longer post-operative RFS (15.2 vs. 9.9 months, P=0.135) or OS (36.1 vs. 28.6 months, P=0.577). However, patients with positive nodal disease in final pathology had significantly lower post-resection survival compared to node-negative patients (17.3 vs. 29.3 months, P=0.026, Figure 4A). Similarly, post-resection survival was worse with margin-positive resection (8.1 vs. 29.3 months, P=0.032, Figure 4B).

Discussion

This study provides detailed insight on the management and outcomes of patients with LAPC and clearly identifies a survival advantage in a selected cohort who underwent resection of the primary tumor, compared to those who did not. Moreover, we have overcome the confounding factor of previous reports that combined borderline resectable and LAPC patients. In previous retrospective studies, surgical resection of the primary tumor was the main driver for improved OS. Ferrone et al. showed increased resection rates after FOLFIRINOX therapy in 25 LAPC patients.[8] Hackert et al. also demonstrated that FOLFIRINOX resulted in primary tumor resectability of more than 60% in a large mixed cohort of borderline resectable, locally advanced, and metastatic PDAC patients.[9] In our cohort, FOLFIRINOX-based therapy correlated with increased resection rates (P=0.006). SBRT after FOLFIRINOX further contributed to that increase (P<0.001). SBRT as adjunct modality in LAPC has been previously associated with improvement of local disease control, and increased likelihood of margin-negative resection.[10,16-18]

The decision to offer surgical exploration was driven by three main factors: vessel involvement by the tumor, at least four months of chemotherapy, and/or absence of disease progression. After completion of scheduled treatment, patients with good performance status and no disease progression were re-reviewed and offered surgical exploration, with a median time to the operating room of six weeks. As Ferrone et al. previously suggested, imaging response alone may be inadequate to declare resectability after neoadjuvant treatment[8], and this also proves to be the case in our series: only 12% of resected patients had partial or complete response according to RECIST criteria.[13]

Surgical resection of the primary tumor correlated with significant improvement in OS in LAPC patients, with a median time of approximately three years from diagnosis. Moreover, half of the resected patients were alive at the 3-year time point. The decision to give adjuvant chemotherapy (n=25, 30%) was mainly based on patient preference, performance status, and final pathology findings. Interestingly, administration of adjuvant chemotherapy did not appear to further improve the post-resection survival of these patients (P=0.577). Additionally, we did not identify a correlation between administration of adjuvant chemotherapy and the time of disease recurrence. This conclusion coincides with previous reports, however data on adjuvant chemotherapy after resection in LAPC patients are scarce.[9,19] Increased resection rates with current neoadjuvant therapies make this an interesting field for future studies.

Distal pancreatectomy with en bloc celiac axis resection (DP-CAR) was the second most

common operation performed in our study. Until recently, involvement of the celiac artery (CA) in LAPC resulted to unresectability. The development of DP-CAR allowed surgical resection of body or tail tumors that involved the CA. The rationale behind this surgical approach is that the liver arterial supply will be preserved via retrograde blood flow from the gastroduodenal to the proper hepatic artery, after resection of the CA and ligation of the common hepatic artery. Despite an increased risk of liver ischemia, advancements in assessment of hepatic supply and optimized surgical technique allow improved post-operative morbidity and mortality rates in high-volume centers. Currently, DP-CAR, also known as the "modified Appleby" procedure, is the most common operation that involves arterial resection in PDAC.[20]

Positive nodal status (N1) and margin-positive resection (R1) had a direct impact on post-resection survival. In the resected cohort, 25% of the patients had N1 disease, a lower percentage than historically reported in patients with resectable PDAC.[21] Regional lymph node metastases correlated with lower median RFS and post-resection survival (P=0.047 and 0.026, respectively), in concordance with studies on stage I/II disease.[22-23] Furthermore, resected patients who underwent R0 resection (88%) had significantly higher median post-resection survival (P=0.032). Increased R0 resection rates have been previously identified after neoadjuvant therapy[9], compared to upfront resection in resectable PDAC[24], due to extensive necrosis of the tumor. Margin-positive resection is associated with increased risk of local recurrence and consequently shorter post-resection survival.[25-26]

The survival benefit of FOLFIRINOX[5] and Gemcitabine/Nab-Paclitaxel[15] against Gemcitabine monotherapy in metastatic PDAC patients paved the road for anecdotal treatment of LAPC in recent years. Multiple studies have associated both regimens with increased OS in patients with unresectable pancreatic cancer (borderline resectable and locally advanced).[9,16,17,27-30] In a recent meta-analysis, Suker et al. reported a median overall survival of 24.2 months after FOLFIRINOX therapy in LAPC patients.[6] Data on the role of radiation and chemoradiation therapy after induction chemotherapy, however, are more controversial. The LAP07 trial failed to show a survival benefit with the addition of chemoradiotherapy (54 Gy plus Capecitabine) after four months of chemotherapy[31], but even in this setting the chemoradiation arm experienced a local control benefit. Moreover, recent retrospective studies have demonstrated a potentially beneficial role of SBRT in LAPC, both for local control, as well as to help achieve a margin-negative resection.[10,18,32] In this study, we focused exclusively on LAPC patients and came across significant treatment variations, characterized by extensive micromanagement, due to drug toxicity or disease progression. Treatment stratification based on the main chemotherapy agent provided a valid comparison basis. Except for patients who did not

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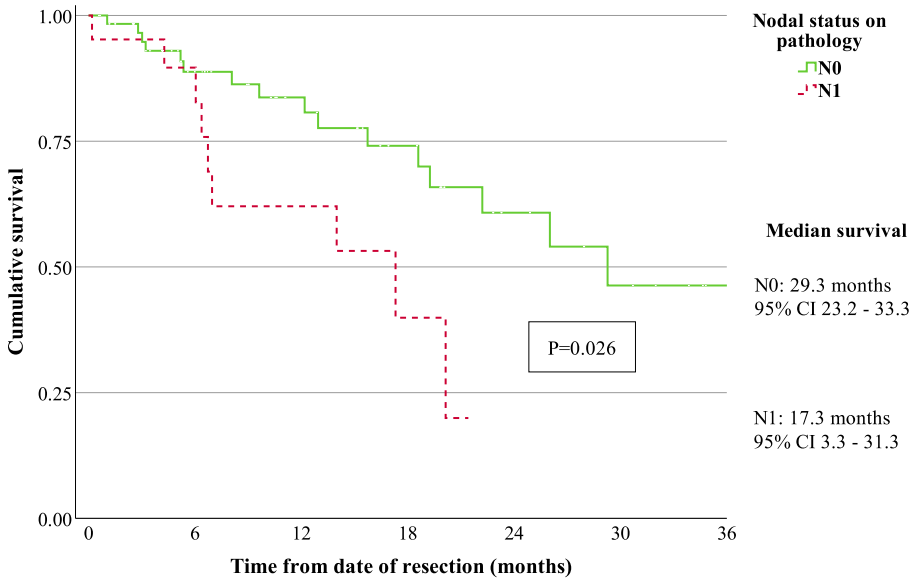

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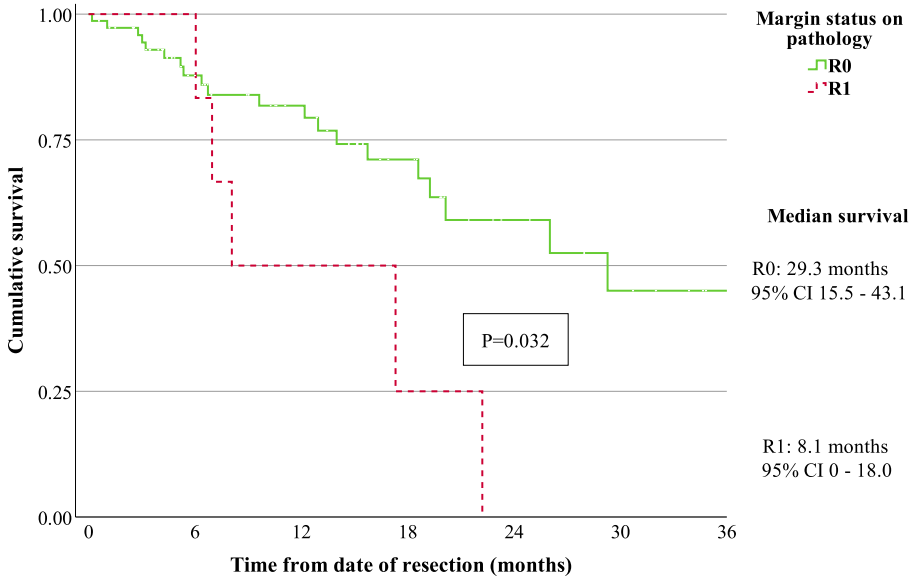

Figure 4. (A) Comparison of overall survival in patients with negative (N0) vs. positive (N1) nodal disease in final pathology report. (B) Comparison of overall survival in patients with negative (R0) vs. positive (R1) resection margin.

receive any treatment, OS was similar across different chemotherapy regimens in the non-resected cohort. A further positive effect of radiation therapy on OS was noted, most likely due to selection bias: patients whose disease did not progress during induction chemotherapy, had an option for additional radiation therapy, under the prism of future potential surgical resection.

In this large LAPC cohort, one can identify tumor biology as the common denominator for patient outcomes. In all stage III PDAC patients, a major goal at the time of diagnosis is potential surgical resection of the primary tumor. Our retrospective data indicate that regardless of neoadjuvant chemotherapy regimen, approximately 25% of patients had the chance to undergo surgical exploration with curative intent, among which 75% were resected, leading to a final patient ratio of 1:5 for surgical resection versus no resection in LAPC. This resection rate appears to be low compared to previous retrospective studies[27-28,33-34] and we can acknowledge two distinct factors for this difference: (1) borderline resectable PDAC patients were not part of the studied cohort (historically the resection rate for these patients in our institution is 65%), and (2) all LAPC patients who presented at PMDC were included in the initial pool, including those who did not receive neoadjuvant chemotherapy, or progressed early in the course of their disease. Most importantly however, surgical resection in LAPC patients was associated with an additional survival advantage, that was comparable to their resected stage I/II counterparts.[35] At the moment, we cannot identify with scientific certainty demographic and clinical parameters that will suggest which patients will become eligible for surgery. Genetic identification of PDAC subtypes may provide additional information about treatment response patterns[36], whereas optimal neoadjuvant treatment schemes will need to be determined through randomized prospective trials.[37]

This study has several limitations. Firstly, it is a retrospective study and eligible LAPC patients were not prospectively randomized to surgical resection or observation after neoadjuvant therapy. Therefore, it is subject to selection bias on multiple levels and its conclusions are observational. At PMDC, decision making for neoadjuvant therapy was based on patient characteristics and expert consensus. Additionally, micromanagement of neoadjuvant therapy and lack of adherence to standardized treatment protocols introduced further selection bias on which patients may respond, and how many will proceed to radiation therapy and surgical exploration. Specific data on regimen toxicity were also limited, since many out-of-state patients were not treated in our institution. Moreover, lead-time bias is possible, since a minority of patients were followed-up for a shorter period than the median time required from diagnosis to surgery, and they may be eligible for resection later in time. However, our data emanate from a realistic multidisciplinary approach in a high-volume institution for PDAC and

reflect the current landscape of LAPC treatment. Randomized prospective trials are needed to identify the optimal neoadjuvant chemotherapy regimen for improved resection rates and further assess the impact on overall survival in LAPC patients.

Conclusion

This large retrospective study demonstrates the current trends in LAPC treatment. Improved overall survival does not appear to correlate with a specific neoadjuvant regimen. Patients who receive at least four months of neoadjuvant treatment without signs of disease progression may be eligible for surgical exploration and resection. In our study, this resected cohort is 20% of all LAPC patients. Patients who underwent surgical resection of the primary tumor had significantly better overall survival, reaching a median of 35 months. A multidisciplinary approach in high-volume centers is necessary to identify these patients. Future prospective trials are required in order to define the optimal neoadjuvant treatment for LAPC.

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Supplemental Material to Chapter 5

– **Supplemental eTable S1.** Distribution of chemotherapy regimens utilized in resected and non-resected LAPC patients.

Supplemental material can be consulted online per the website of the journal and/or publisher.

CHAPTER 6

Is a Pathological Complete Response Following Neoadjuvant Chemoradiation Associated with Prolonged Survival in Patients with Pancreatic Cancer?

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Abstract

Objective

Patients with BR/LA-PDAC are often treated with neoadjuvant chemoradiation in an attempt to downstage the tumor. Uncommonly, a pCR may result. This study aims to describe the survival outcome of patients with borderline resectable or locally advanced pancreatic ductal adenocarcinoma (BR/LA-PDAC) who have a pathologic complete response (pCR) following neoadjuvant chemoradiation.

Methods

A retrospective review of a prospectively maintained database was performed at a single institution. pCR was defined as no viable tumor identified in the pancreas or lymph nodes by pathology. A near complete response (nCR) was defined as a primary tumor less than 1cm, without nodal metastasis. Overall survival (OS) and disease-free survival (DFS) were reported.

Results

186 patients with BR/LA-PDAC underwent neoadjuvant chemoradiation and subsequent pancreatectomy. 19 patients (10%) had a pCR, 29 (16%) had an nCR and the remaining 138 (74%) had a limited response. Median DFS was 26 months in patients with pCR, which was superior to nCR (12 months, P=0.019) and limited response (12 months, P<0.001). The median OS of nCR (27 months, P=0.003) or limited response (26 months, P=0.001) was less than that of pCR (more than 60 months). In multivariable analyses pCR was an independent prognostic factor for DFS (HR=0.45; 0.22-0.93, P=0.030) and OS (HR=0.41; 0.17-0.97, P=0.044). Neoadjuvant FOLFIRINOX (HR=0.47; 0.26-0.87, P=0.015) and negative lymph node status (HR=0.57; 0.36-0.90, P=0.018) were also associated with improved survival.

Conclusion

Patients with BR/LA-PDAC who had a pCR after neoadjuvant chemoradiation had a significantly prolonged survival compared to those who had nCR or a limited response.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a poor prognosis. Despite advancements in surgical, systemic and radiation therapies, mortality of PDAC is projected to surpass colorectal cancer to become the second leading cause of cancer-related death in the United States by 2030.[1] Complete surgical resection remains the only potentially curative option. Even the most encouraging results in the era of "contemporary chemotherapy" reported a median overall survival of 28 months after resection and adjuvant chemotherapy.[2,3] In addition, patients with borderline resectable or locally advanced PDAC (BR/LA-PDAC) may have margin-negative surgery after a period of neoadjuvant chemotherapy or chemoradiotherapy.[4] Patients with successfully resected BR/LA-PDAC following neoadjuvant therapy have similar overall survival when compared to those with initially resectable disease.[5,6] Furthermore, neoadjuvant chemoradiation has increasingly been utilized even in patients with resectable PDAC due to its potential ability to reduce micrometastatic disease, increase likelihood of a complete resection and offer a potential survival advantage.[7]

A pathological complete response (pCR) in PDAC is uncommon and observed in only 3-11% of resected specimen treated with neoadjuvant therapy.[6,8-13] In esophageal and locally advanced rectal adenocarcinoma, a pCR is associated with greatly improved outcomes with lower rates of local recurrence and improved survival.[14-16] Initial reports have suggested a similarly improved survival for PDAC patients with pCR.[9-12] However, due to the rarity of a complete response in PDAC, pCR has been grouped with other significant responders in multivariable analysis. Thus, the independent prognostic value of pCR remains unclear. Small tumor size and negative nodal metastases are well-established prognostic indicators for the improved long-term overall survival for patients with resectable PDAC following curative resection.[17,18] Therefore, we defined tumors less than 1cm without nodal metastasis as "near complete response" (nCR) and used it as an established comparison cohort in this study.

In this study, we present the survival outcomes of a cohort of patients with BR/LA-PDAC who had a pCR or a nCR after neoadjuvant treatment. We evaluated the prognostic impact of pCR on survival.

Methods

Patients with BR/LA-PDAC who presented to Johns Hopkins Hospital between 2008 and 2016 were identified from a prospectively maintained database and included in this study. Patients were identified with an initial diagnosis of BR/LA-PDAC as determined in our Pancreas Multidisciplinary Clinic or tumor board according to published definitions.[19] Patients

that failed to complete neoadjuvant chemoradiation therapy or did not proceed to surgery secondary to local disease progression, distant metastasis, or death were excluded from the study. Patients with death attributed to perioperative morbidity within 90 days of the operation were also excluded. The primary outcomes of interest were overall survival (OS) and disease-free survival (DFS).

Demographics, Clinicopathological and Treatment Characteristics

The following features were extracted from our single-institutional prospective database: age, sex, location of tumor, type of neoadjuvant chemotherapy, type of radiation therapy, lymph node status, lymphovascular invasion, perineural invasion, margin status, type of surgery, requirement of vascular resection, post-operative complications and treatment response. pCR was defined as an absence of any viable tumor in the pancreas or lymph nodes on final pathology. A near complete response (nCR) was defined as a primary tumor less than 1cm of total measured area, without nodal metastasis. The remaining neoadjuvant patients were deemed to be limited responders (LR). Identified cases of pCR had all histopathology, including the initial biopsies, re-reviewed by a pathologist with expertise in pancreatic malignancies.

All patients included in this study received neoadjuvant chemotherapy and radiation as either conventional external beam radiotherapy (CRT) or stereotactic body radiation therapy (SBRT). Chemotherapy regimens were selected at the discretion of the treating medical oncologist and were divided into single agent therapy, Gemcitabine based combination regimens (predominately Gemcitabine/Nab-Paclitaxel or Gemcitabine/Capecitabine) and FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin and irinotecan). Modified-FOLFIRINOX was commonly utilized at our institution and was not separated from full dose FOLFIRINOX therapy.

Patients that proceeded to the operating suite underwent pancreatoduodenectomy, distal pancreatectomy or total pancreatectomy as determined by the location and extent of the tumor. An R0 resection was defined as ≥1mm of margin free of malignant cells. Vascular resections were performed if involvement of the superior mesenteric vein, portal vein, celiac axis or hepatic artery was appreciated. Vascular reconstruction was preferentially performed by primary repair. Patch venorrhaphy or interposition grafting was used only when primary repair was not feasible. Perioperative complications within 90 days from resection were scored by the Clavien grading system.[20] Clavien grade III or greater were considered severe complications.

Table 1. Clinicopathologic characteristics of pathologic complete response (pCR) and non pCR patients

Variable	*pCR (n=19)*	*nCR + LR (n=167)*	*P-value*
Age, median years (IQR)	62 (56-68)	63 (57-69)	0.84
Male, *n* (%)	11 (58%)	94 (56%)	0.89
Radiation modality, *n* (%)			0.09
Stereotactic body radiation therapy	11 (58%)	63 (38%)	
Conventional radiotherapy	8 (42%)	104 (62%)	
Chemotherapy regimen, *n* (%)			0.41
FOLFIRINOX	11 (58%)	72 (43%)	
Multi-agent Gemcitabine based	5 (26%)	69 (41%)	
Single agent	3 (16%)	26 (16%)	
Disease stage, *n* (%)			0.16
Borderline	6 (32%)	81 (49%)	
Locally advanced	13 (68%)	86 (51%)	
Operation procedure, *n* (%)			0.97
Pancreatoduodenectomy	14 (74%)	120 (72%)	
Distal pancreatectomy	4 (21%)	39 (23%)	
Total pancreatectomy	1 (5%)	8 (5%)	
Vascular resection, *n* (%)	5 (26%)	74 (44`%)	0.13
Resection margin, *n* (%)			**0.04**
R0 (>1mm)	19 (100%)	134 (80%)	
R1 (≤1mm)	0 (0%)	33 (20%)	
Morbidity, *n* (%)			0.25
≤Clavien-Dindo grade II	16 (84%)	120 (72%)	
≥Clavien-Dindo grade III	3 (16%)	47 (28%)	
Microscopic lymphovascular invasion, *n* (%)	0 (0%)	44 (26%)	**0.002**
Microscopic perineural invasion, *n* (%)	0 (0%)	91 (55%)	**<0.001**

IQR, *interquartile range*.

Follow-up and Survival

Follow-up data through August 2017 was retrieved from our database. The date of death was obtained from medical records, local obituary or the Social Security Death Index. OS was calculated from the date of neoadjuvant therapy initiation to the date of death from any cause, or censored until the date of last follow-up. DFS was calculated from the time of surgery to the date of recurrence defined radiographically or confirmed patient death. Tissue diagnosis of recurrence was not routinely performed. Post recurrence survival (PRS) was defined as the interval from documented recurrence to time of death or last follow-up.

Statistical Analysis

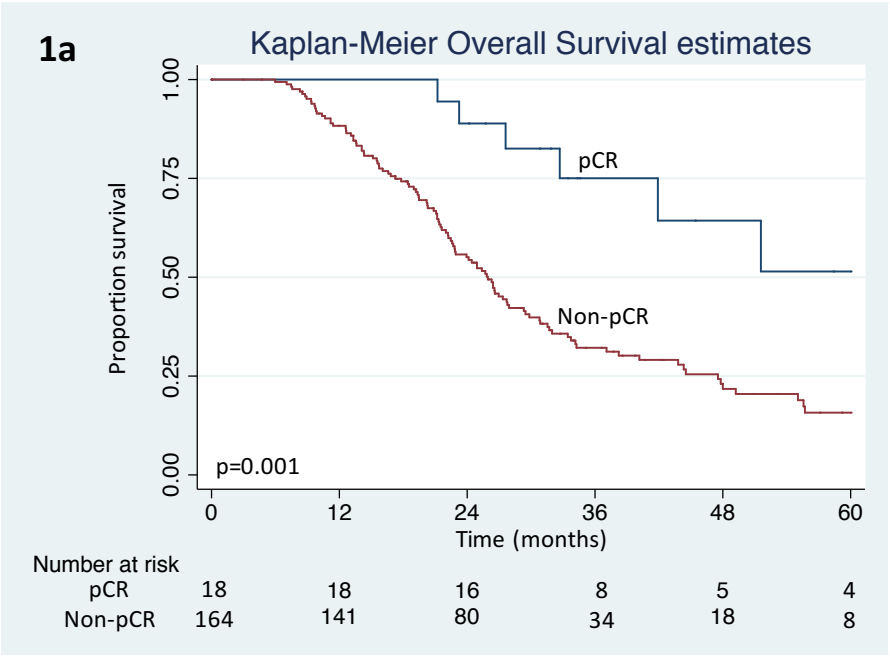
Statistical analysis was performed using Stata/MP 12.1 (Stata Corp, College station, TX, USA). Categorical variables were expressed as percentages and were compared using a chi-squared or a Fisher's exact test. Continuous variables were presented as median and interquartile range (IQR), and were compared using a Wilcoxon-Mann-Whitney test or a Kruskal-Wallis test. Survival analysis was performed using Kaplan-Meier survival estimates and a log-rank test. A P-value less than 0.05 was considered statistically significant. Multivariable analysis included all variables significant on univariable analysis and was performed using a Cox proportional-hazards model.

Results

Between 2008 and 2016, 186 patients with BR/LA- PDAC who completed neoadjuvant chemotherapy and radiation followed by pancreatectomy were identified. 105 patients were males (56.5%) and median age at time of operation was 63 (IQR 69-57). 87 cancers (47%) were defined as borderline resectable, and 99 (53%) locally advanced.[19] SBRT was utilized in 74 patients (40%), and CRT in the remaining 112 (60%). FOLFIRINOX was the most commonly utilized neoadjuvant regimen in 83 patients (45%) followed by combination Gemcitabine therapy in 74 patients (39.8%) and single agent chemotherapy in 29 (16%). Histopathologic specimens of the evaluable cohort were reviewed by pancreatic pathologists for treatment effect. A pCR was observed in 19 patients (10%). Of the patients without a pCR, 29 (16%) had a nCR with the remaining 138 (74%) a limited response. Median follow-up for all patients was 27 months (range 3-102 months). A median follow-up in the pCR group was 34 months (range 11-101 months), with 58% of pCR patients remaining alive with a median follow-up of 41 months (range 24-101 months). Additional clinicopathologic features of the pCR and remaining groups were similar and summarized in Table 1.

Predictors of Survival

Four patients (2%), including one with pCR, died within 90 days after the operation due to perioperative morbidity and were excluded from the survival analysis. A median OS of 27 months was appreciated in the 182 evaluable patients. In the pCR group, median OS was not yet met at 60 months, which was longer than patients without a pCR (26 months P=0.001) (Figure 1). With the additional sub-classification of the nCR cohort within patients without a pCR, median OS remained superior in pCR when compared to both nCR (27 months, P=0.003) and LR (26 months, P=0.001). No difference was appreciated between the patients with nCR or LR (P=0.773) (Figure 1).



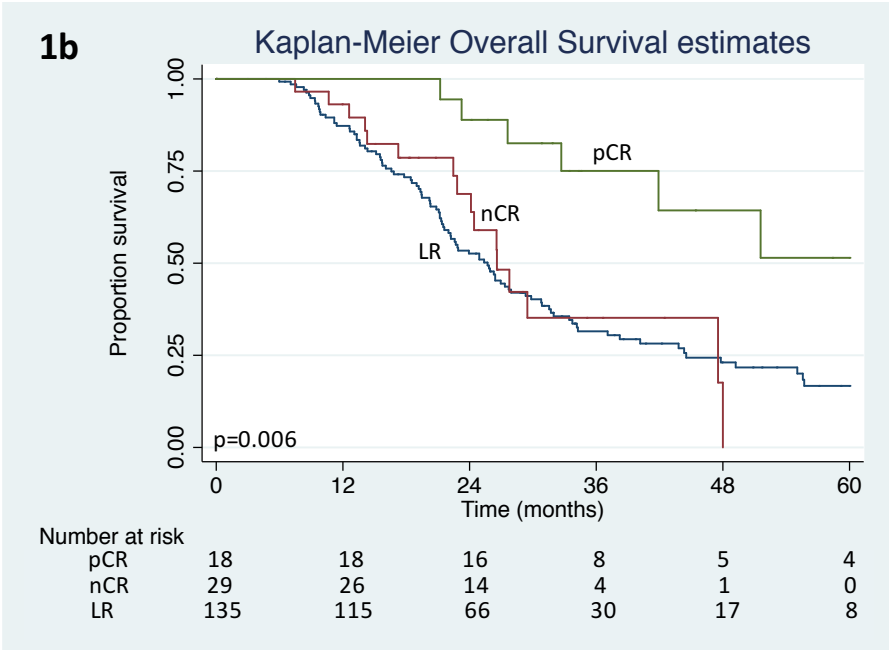


Figure 1. (A) Kaplan Meier estimates of overall survival (OS) in patients with BR/LA-PDAC treated with neoadjuvant chemoradiation followed by pancreatic resection based on histological response. pCR= pathologic complete response. (B) Kaplan Meier estimates of overall survival (OS) in patients with BR/LA-PDAC treated with neoadjuvant chemoradiation followed by pancreatic resection based on histological response.

Table 2. Univariable and multivariable Cox regression analysis of overall survival in patients with BR/LA-PDAC that underwent neoadjuvant chemoradiation therapy

Clinical characteristics	*Cohort (n=182)*	*Univariable P-value*	*HR*	*95% CI*	*Multivariable P-value*
Response, *n* (%)					
Limited response	135 (74%)	*ref.*			
Near complete response	29 (16%)	0.794	1.27	0.69-2.34	0.445
Pathologic complete response	18 (10%)	**0.002**	**0.41**	0.17-0.97	**0.044**
Age, *n* (%)					
<60 years	72 (40%)	*ref.*			
≥60 years	110 (60%)	0.125			
Gender, *n* (%)					
Male	103 (57%)	*ref.*			
Female	79 (43%)	0.197			
Radiation modality, *n* (%)					
Conventional radiotherapy	110 (60%)	*ref.*			
Stereotactic body radiation therapy	72 (40%)	**0.019**	0.88	0.55-1.41	0.605
Chemotherapy regimen, *n* (%)					
Single-agent	27 (15%)	*ref.*			
Multi-agent Gemcitabine based	74 (40%)	0.284	0.75	0.45-1.24	0.255
FOLFIRINOX	81 (45%)	**0.001**	**0.47**	0.26-0.87	**0.015**
Disease stage, *n* (%)					
Borderline	85 (47%)	*ref.*			
Locally advanced	97 (53%)	0.990			
Operation procedure, *n* (%)					
Pancreatoduodenectomy	131 (72%)	*ref.*			
Distal pancreatectomy	42 (23%)	0.267			
Total pancreatectomy	9 (5%)	0.838			
Vascular resection, *n* (%)					
No	105 (58%)	*ref.*			
Yes	77 (42%)	0.498			
Resection margin, *n* (%)					
R0 (>1mm)	23 (13%)	*ref.*			
R1 (≤1mm)	159 (87%)	**0.041**	0.63	0.38-1.04	0.069
Morbidity, *n* (%)					
≥Clavien-Dindo grade III	46 (25%)	*ref.*			
≤Clavien-Dindo grade II	136 (75%)	0.140			
Microscopic lymphovascular invasion, *n* (%)					
Yes	43 (24%)	*ref.*			
No	112 (62%)	0.269			
Microscopic perineural invasion, *n* (%)					
Yes	90 (49%)	*ref.*			
No	86 (47%)	**0.005**	0.85	0.55-1.29	0.442
Positive lymph node status, *n* (%)					
Yes	52 (29%)	*ref.*			
No	130 (71%)	**0.002**	0.67	0.43-1.03	0.071

CI, *confidence* interval; HR, *hazard ratio*.

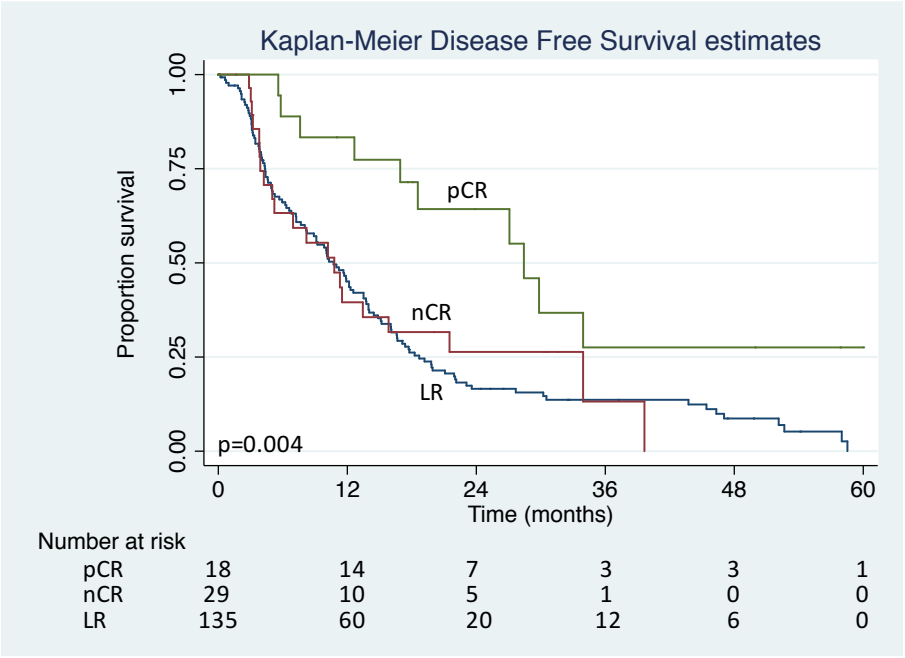


Figure 2. Kaplan Meier estimates of disease-free survival in patients with BR/LA-PDAC treated with neoadjuvant chemoradiation followed by pancreatic resection based on histological response.

Results of univariable and multivariable analyses of predictors of OS are presented in Table 2. Unadjusted factors significantly associated with improved survival were pCR, neoadjuvant SBRT, neoadjuvant FOLFIRINOX, lack of perineural invasion, negative node metastases and an R0 resection. On multivariable analysis, a pCR (HR 0.41, 95% CI 0.17-0.97, P=0.044), and neoadjuvant treatment with FOLFIRINOX (HR 0.47, 95% CI 0.26-0.87, P=0.015) remained independent predictors of OS. A trend was noted associating improved survival with a negative margin (HR 0.63, 95% CI 0.38-1.04, P=0.069) and negative nodal status (HR 0.67, 95% CI 0.43-1.03, P=0.071).

DFS was superior with Kaplan-Meier survival estimates with median DFS of 26 months in pCR cohort compared to 12 in nCR (P=0.019) and 12 in LR groups (P<0.001) (Figure 2). No difference was appreciated between nCR and LR (P=0.768). In 44% (n=8) of pCR patients, no recurrence or death was observed. In the nCR (n=8) and LR (n=19) cohort, only 27 patients (16%) did not have recurrence or death after surgery. Of the pCR patients with recurrence, 2 had loco-regional recurrence (20%) and 7 had distant recurrence including 3 liver metastases, 1 lung metastasis, and 3 carcinomatosis or multiple locations. The patterns of recurrence were similar in the non-pCR patients: with loco-regional recurrence documented in 30 (22%) patients and lung only metastases in 6 (4%). The remaining sites of distant recurrence included

Table 3. Univariable and multivariable Cox regression analysis of disease-free survival in patients with BR/LA-PDAC that underwent neoadjuvant chemoradiation therapy

Clinical characteristics	*Cohort (n=182)*	*Univariable P-value*	*HR*	*95% CI*	*Multivariable P-value*
Response, *n* (%)					
Limited response	135 (74%)	*ref.*			
Near complete response	29 (16%)	0.713	1.21	0.71-2.06	0.489
Pathologic complete response	18 (10%)	**0.002**	0.45	0.22-0.93	**0.030**
Age, *n* (%)					
<60 years	72 (40%)	*ref.*			
≥60 years	110 (60%)	0.313			
Gender, *n* (%)					
Male	103 (57%)	*ref.*			
Female	79 (43%)	0.588			
Radiation modality, *n* (%)					
Conventional radiotherapy	110 (60%)	*ref.*			
Stereotactic body radiation therapy	72 (40%)	0.547			
Chemotherapy regimen, *n* (%)					
Single-agent	27 (15%)	*ref.*			
Multi-agent Gemcitabine based	74 (40%)	0.308			
FOLFIRINOX	81 (45%)	0.185			
Disease stage, *n* (%)					
Borderline	85 (47%)	*ref.*			
Locally advanced	97 (53%)	0.445			
Operation procedure, *n* (%)					
Pancreatoduodenectomy	131 (72%)	*ref.*			
Distal pancreatectomy	42 (23%)	0.622			
Total pancreatectomy	9 (5%)	0.536			
Vascular resection, *n* (%)					
No	105 (58%)	*ref.*			
Yes	77 (42%)	0.908			
Resection margin, *n* (%)					
R0 (>1mm)	23 (13%)	*ref.*			
R1 (≤1mm)	159 (87%)	**0.025**	0.73	0.47-1.14	0.164
Morbidity, *n* (%)					
≥Clavien-Dindo grade III	46 (25%)	*ref.*			
≤Clavien-Dindo grade II	136 (75%)	0.133			
Microscopic lymphovascular invasion, *n* (%)					
Yes	43 (24%)	*ref.*			
No	112 (62%)	**0.072**	1.11	0.71-1.73	0.647
Microscopic perineural invasion, *n* (%)					
Yes	90 (49%)	*ref.*			
No	86 (47%)	**0.010**	0.91	0.60-1.39	0.669
Positive lymph node status, *n* (%)					
Yes	52 (29%)	*ref.*			
No	130 (71%)	**<0.001**	0.57	0.36-0.90	**0.018**

CI, *confidence* interval; HR, *hazard* ratio.

liver in 43 (31%) patients, carcinomatosis or multiple locations in 33 (24%) patients and other locations such as abdominal wall or ovary in 3 (2%) patients. Unfortunately, 1 (10%) patient with a pCR and 22 (16%) patients in the non-pCR cohort were identified as deceased without clear documentation of recurrence pattern. Despite similarities in recurrence patterns, a notable difference was appreciated in post recurrence survival (PRS), or the interval from recurrence to death. A PRS of only 6.4 months was observed in patients without a pCR compared to 11.2 months in those with a pCR (P=0.029). Of patients with documented recurrence, 33% of those with a pCR compared to 16% of those without pCR remain alive with disease.

On univariable analysis pCR, negative margins (R0), lack of lymphovascular invasion, lack of perineural invasion and negative nodal metastases correlated with prolonged time to recurrence or death. On multivariable analyses nodal metastases (HR 0.57, 95% CI 0.36-0.90, P=0.018) and pCR (HR 0.45, 95% CI 0.22-0.93, P=0.030) were independent predictors of improved disease-free survival (Table 3).

Discussion

This study presents our single institution cohort of pCR following neoadjuvant chemoradiation treatment in BR/LA-PDAC. Furthermore, we examine the association of pCR with survival. The magnitude of post-therapy response, and specifically a pCR, has been shown to be an important prognostic factor for survival in several malignancies.[14-16] In pancreatic cancer, small tumor size, negative margins and negative nodal metastases are the best established prognostic indicators for improved long-term overall survival following curative resection.[17,18] Due to these established factors, the nCR group, as defined by <1cm tumor with negative nodal metastases and negative margins, was used as an additional comparison group in our series. In this study, we found that BR/LA-PDAC patients with pCR had better survival, with the median not yet reached at 60 months, as compared to those with nCR or limited response. Median OS of the entire cohort was 27 months which is encouraging when compared to our institution's historical cohort of over 2000 upfront pancreatic resections yielding a median overall survival of 19 months.[17,21] Therefore, the prognostic impact of a pCR, which was significant for improved OS even within a pre-selected cohort, is notable. Moreover, in our study pCR was an independent prognostic factor for improved OS and DFS. These findings lend support to the use of neoadjuvant chemoradiation treatment in patients with BR/LA-PDAC and the use of pCR as an endpoint for future study.

Several prior studies have reported on patients with a pCR following neoadjuvant treatment for PDAC. After an extensive literature search, 21 studies and case series were identified with

Table 4. Literature review of prior reports of pathologic complete response in pancreatic cancer following neoadjuvant therapy

Reference	*Inclusion period*	*Resectability status of tumor*	*Neoadjuvant regimen*	*% pCR*	*Recurrence and survival status of patients with pCR*
Snady *et al.* (2000)	1989-1997	68 LAPC	CHT: 5-FU/Cisplatin RT: 54 Gy	3/20 (15%)	NR
Calvo *et al.* (2004)	1998-2001	15 resectable	CHT: Tegafur RT: 45-50.4 Gy	3/9 (33%)	3 pt alive with NED at 9, 25 and 36 mos.
Moutardier *et al.* (2004)	1996-2003	61 resectable	CHT: 5-FU/Cisplatin RT: 30-45 Gy	3/40 (8%)	1 pt died with liver mets at 27 mos. 2 pt alive with NED at 12 and 66 mos.
Turrini *et al.* (2009)	1996-2007	102 resectable	CHT: 5-FU/Cisplatin RT: 45 Gy	8/62 (13%)	NR
White *et al.* (2005)	1994-2005	51 BR + 16 LAPC	CHT: 5-FU RT: 30-45 Gy	4/54¶ (7%)	2 pt died of progression at 22 and 29 mos. 2 pt died with NED at 11 and 16 mos.
Katz *et al.* (2008)	1999-2006	125 BR	CHT: multiple RT: 30-50.4 Gy	4/66 (6%)	1 pt developed mets. 1 pt died of lung cancer. 2 pt alive with NED
Chatterjee *et al.* (2012)	NR	NR	CHT: multiple RT: 30-50.4 Gy	6/223 (3%)	6 pt alive with NED.
Zhao *et al.* (2012)	1995-2010	NR	CHT: multiple RT: 30-50.4 Gy	11/442 (3%)	6 pt alive with NED at 10-194 mos (median 63). 3 pt died of other causes. 1 pt with 2nd primary PDAC at 84 mo
Lee *et al.* (2016)	2008-2012	NR	CHT: multiple RT: 30-50.4 Gy	3/167 (2%)	3 pt alive with NED.
Cloyd *et al.* (2017)	1990-2015	NR	CHT: multiple RT: 30-50.4 Gy	23/583 (4%)	81 mos median survival for all pt. 4 pt developed distant recurrence
Tinkl *et al.* (2009)	1996-2006	120 LAPC	CHT: 5-FU or Gem/Cis RT: 50.4-55.8 Gy	3/38 (8%)	NR
Chun *et al.* (2011)	1987-2009	NR	CHT: multiple RT: 50.4 Gy	8/107 (7%)	NR
Rajagpalan *et al.* (2013)	2008-2011	7 BR + 5 LAPC	CHT: multiple SBRT: 24-46 Gy	3/12 (25%)	NR
Rose *et al.* (2014)	2008-2012	64 BR	CHT: Gem/Docetaxel RT: 50.4 Gy	3/31 (10%)	NR
Pietrasz *et al.* (2015)	2010-2013	47 BR + 33 LAPC	CHT: FFX RT: 54 Gy	12/80 (15%)	NR
Hirata *et al.* (2015)	2006-2011	104 resectable + 53 BR	CHT: Gem alone RT: 50 Gy	6/157 (4%)	NR
Lee *et al.* (2015)	2000-2012	35 resectable + 28 BR + 14 LAPC	CHT: Gem-based RT: 50-60 Gy	9/86 (10%)	4 pt with recurrence (2 liver, 2 peritoneum) and 5 pt alive with NED at 10-63 mos (median 21 mos)
Chuong *et al.* (2016)	2009-2012	36 BR	CHT: GTX RT: 32.5-40 Gy	4/36 (11%)	4 pt alive with NED
Rashid *et al.* (2016)	2006-2013	94 BR	CHT: GTX SBRT: 30-40 Gy	10/55 (18%)	NR

Hashemi-Sadraei *et al.* (2017)	2008-2014	53 BR/LAPC	CHT: multiple RT: 45-54 Gy	6/53 (11.3%)	2 pt died (median 11 mos), 4 pt alive median survival 28.7 mos. 3 of which have recurred at median 20.4 mos
Mellon *et al.* (2017)	2010-2015	8 LAPC + 73 BR	CHT: multiple SBRT: 30-50 Gy	6/81 (7%)	6 pt alive with NED at median 33 mos, including 1 pt with liver met at 7 mos, treated with RFA and FFX, alive with NED at 27 mos.

¶ 54/70 specimens available for examination.

* Follow-up data available for 10/11 patients with pathologic complete response.

5-FU, *5-flourouracil*; BR, *borderline resectable*; CHT, *chemotherapy*; Gem, *Gemcitabine*; GTX, *Gemcitabine, Docetaxel, and Capecitabine*; Gy, *Gray*; FFX, *FOLFIRINOX*; LAPC, *locally advanced unresectable pancreatic cancer*; mos, *months*; NR, *not reported*; RT, *radiotherapy*; pCR, *pathologic complete response*; pt, *patient*.

(A) outcomes for patients receiving neoadjuvant chemoradiation for PDAC and (B) at least 3 or more patients with a pCR (Table 4). The sample size of resected patients after neoadjuvant treatment ranged from 9 to 583 patients for a total of 2402 patients. Percentage of pCR ranged from 2-33% and pCR was observed in a total of 138 patients (6%), highlighting the rarity of a pCR in pancreatic cancer patients. Available recurrence and survival outcomes were encouraging and are shown in Table 4.

Statistically assessing impact of pCR is difficult due to the small number of patients. Consequently, patients with pCR are often combined with other major response groups in order to capture the prognostic value of pCR on survival in multivariable models. A study from Fox Chase Center by Chun and colleagues observed a major pathologic response (>95% fibrosis) in 21 patients of 107 patients (19%), including eight patients (7%) with pCR.[12] Median OS for major responders was 66 months and on subsequent multivariable analysis only major response was an independent predictor of OS (HR 2.26, 95% CI 1.11-4.61).12 Pietrasz et al. defined no evidence of tumor and ypT0-1N0 as complete and major pathologic response, respectively.[22] In their study, 21 of 80 patients (26%) had a major response, including 12 (15%) with pCR. No venous resection and a major response (HR 0.38, 95% CI 0.16-0.93) were independently associated with DFS on multivariable analysis.[22] However, a retrospective study of 6 patients (11%) with pCR reported less robust survival, with early death in 2 patients, and recurrence at a median of 20.4 months in 3 of the 4 remaining living patients.[23]

Several studies from MD Anderson with overlapping inclusion periods have reported on patients with pCR. In a cohort described by Chatterjee et al., 6 patients (3%) with pCR and 36 patients (16%) with minimal residual tumor (<5% viable residual tumor) where grouped together as "response group 1" while all other 181 patients were assigned to "response group 2" (5% or more viable residual tumor).[9] Subsequently, they showed in two separate multivariable analysis models that the pathologic response in group 1 was an independent

prognostic factor for OS in both models, but not DFS. Also from MD Anderson, Lee et al. reported 3 patients (2%) with a pCR and 18 patients (11%) with <5% residual carcinoma in the surgical specimen after neoadjuvant therapy.[24] Conversely from the results presented by Chatterjee et al., multivariable analysis showed an independent association with better DFS (P=0.03), but not OS (P=0.12).[9] The authors concluded that the lack of statistical significance for OS may have been due to the relatively small number of patients. Of note, these trials were not exclusively limited to BR/LA-PDAC patients.

In our study, neoadjuvant FOLFIRINOX was also an independent prognostic factor for better OS in patients with BR/LA-PDAC who underwent neoadjuvant chemoradiation and pancreatic resection. We have seen increasing use of FOLFIRINOX regimen for the treatment of PDAC on the basis of the ACCORD trial demonstrating improved survival when compared with Gemcitabine alone for patients with metastatic PDAC.[25] Similar success has been reported on the utilization of FOLFIRINOX to convert advanced PDAC to resectability, resulting in encouraging OS rates.[26,27] The combination of Gemcitabine + Nab-Paclitaxel has similarly offered benefit over single agent Gemcitabine alone in advanced PDAC.[28] Two ongoing prospective clinical trials will better assess a potential difference between these two agents as neoadjuvant therapy for resectable (NCT02243007) and BR-PDAC (NCT02717091). As the number of patients receiving Gemcitabine + Nab-Paclitaxel was limited in our cohort, any Gemcitabine-based combination was grouped together and compared to FOLFIRINOX or single agent therapy.

The use of neoadjuvant SBRT was associated with improved OS on univariable analysis. SBRT is a newer treatment modality and thus was more frequently paired with aggressive multi-agent chemotherapeutic regimens such as FOLFIRINOX. In our study, the use of SBRT did not maintain statistical significance when analyzed in a multivariable model, perhaps indicating confounding impact by the independent factors of chemotherapy and pCR. A prospective study comparing SBRT to CRT may help further identify if survival advantages do exist based on radiation modality. Concomitantly, the Alliance A021501 trial will evaluate the role of radiation in combination with chemotherapy comparing FOLFIRINOX versus FOLFIRINOX and SBRT followed by surgical resection in patients with BR-PDAC. An advantage of SBRT is that it can be delivered in only 5 days as opposed to 5-6 weeks with CRT, resulting in a shorter interval between neoadjuvant radiation therapy and surgical exploration and consequently allowing less time off from systemic chemotherapy. This may decrease the potential of systemic progression and improve quality of life.[29] The expanded timing of CRT however, allows better opportunity to develop and test new radiosensitizing therapeutics that may improve treatment effects.[30] At this time, both SBRT and CRT are valid modalities with

different benefits and do not appear in our cohort to independently impact survival.

DFS was similarly improved in the pCR cohort and identified as an independent prognostic factor on multivariable analysis. While a benefit was noted, a number of patients still had recurrence following a pCR contrary to some existing data that have reported a 0% recurrence in pCR cohorts.[9] Patterns of recurrence in pCR patients were similar to both the non-pCR patients in this cohort and previously reported patterns in up-front resected PDAC.[31] However, despite recurrence, a nearly 5 month longer PRS was observed in pCR patients compared to the remaining cohort (11.2 vs 6.4; P=0.029). This further suggests the important prognostic impact of a pCR on survival and as a surrogate marker for tumor biology. Moreover, these patients had a significant initial response to chemotherapy, and appear to continue to have beneficial treatment effect from adjuvant treatment following recurrence prolonging PRS. Our study is limited by our method of determining recurrence by radiographic change with a paucity of pathologic confirmation with biopsy. Furthermore, all patients in our study underwent radiation and a large number of vascular resections were performed, both of which can increase the appearance of local inflammation often misinterpreted as recurrence on imaging. Despite this, 7 patients with pCR had distant recurrence including 2 with peritoneal carcinomatosis. Sampling error or the failure to identify viable cancer cells on pathologic analysis may be a potential contributing factor towards the recurrence noted in our pCR patients. Retrospective molecular analysis of the specimen of pCR patients who recurred may help further illuminate this question.

Conclusion

A pCR in patients with BR/LA-PDAC following neoadjuvant chemoradiation therapy is associated with better survival than those with nCR or limited response. Additionally, pCR, the use of FOLFIRINOX and a negative lymph node status were identified as independent prognostic factors for improved OS. These findings suggest that pCR is an important prognostic factor and viable endpoint for future study of neoadjuvant therapy in patients with PDAC.

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CHAPTER 7

Recurrence After Neoadjuvant Therapy and Resection of Borderline Resectable and Locally Advanced Pancreatic Cancer

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Abstract

Objective

This study aims to establish the incidence, timing, and implications of recurrence in patients who underwent neoadjuvant treatment and surgical resection of borderline resectable (BRPC) or locally advanced pancreatic cancer (LAPC).

Methods

Patients with BRPC/LAPC who underwent post-neoadjuvant resection between 2007 and 2015 were included. Associations between clinicopathologic characteristics and specific recurrence locations, recurrence-free survival (RFS), and overall survival from resection (OS) were assessed using Cox regression analyses.

Results

For 231 included patients, median survival from diagnosis and resection were 28.0 and 19.8 months, respectively. After a median RFS of 7.9 months, 189 (81.8%) patients had recurred. Multiple-site (n=87, 46.0%) and liver only recurrence (n=28, 14.8%) generally occurred earlier and resulted in significantly worse OS when compared to local only (n=52, 27.5%) or lung only recurrence (n=18, 9.5%). Microscopic perineural invasion, yN1-yN2 status and elevated pre-surgery CA 19-9 >100 U/mL were associated with both local only and multiple-site/liver only recurrence, while R1-margin was associated with local only recurrence (HR 2.03). yN1-yN2 status and microscopic perineural invasion were independent predictors for both poor RFS and OS, while yT3-yT4 tumor stage (HR 1.39) and poor tumor differentiation (HR 1.60) were only predictive of poor OS. Adjuvant therapy was independently associated with both prolonged RFS (HR 0.73; median 7.0 vs. 10.9 months) and OS (HR 0.69; median 15.4 vs. 22.7 months).

Conclusion

Despite neoadjuvant therapy leading to resection and relatively favorable pathologic tumor characteristics in BRPC/LAPC patients, more than 80% of patients experienced disease recurrence, 72.5% of which occurred at distant sites.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is associated with poor survival. In recent years, more aggressive utilization of potent neoadjuvant therapy regimens has increasingly allowed patients with borderline resectable (BRPC) or locally advanced (LAPC) pancreatic cancer to undergo curative-intent pancreatectomy.[1-5] In these patients, meta-analyses indicate that rates of R0-resection and survival can be achieved similar to those of patients with initially resectable disease.[6-11] However, as with primary resected PDAC, disease recurrence remains the principal cause of limited survival in resected BRPC/LAPC patients.

Treatment of BRPC/LAPC requires a patient-tailored and multidisciplinary approach.[12-14] A comprehensive understanding of PDAC recurrence can provide valuable insight when contemplating appropriate strategies regarding adjuvant treatment, oncologic surveillance, and management following recurrence. However, the incidence, timing, and implications of disease recurrence in patients undergoing post-neoadjuvant resection of BRPC/LAPC are not well established. Furthermore, it is unknown if specific clinicopathological features and treatment factors are associated with distinct patterns of recurrence in these patients.

Therefore, the aim of this single-institution cohort study was to accurately report on different aspects of PDAC recurrence in BRPC/LAPC patients who underwent neoadjuvant treatment followed by surgical resection. These aspects include the patterns and timing of recurrence, clinical factors associated with distinct recurrence locations and implications of recurrence on survival outcomes. Additionally, we sought to identify predictors of recurrence-free survival (RFS) and overall survival (OS) in this unique cohort of post-neoadjuvant resected PDAC patients.

Methods

Patient Selection

This study included patients with BRPC or LAPC who underwent post-neoadjuvant resection at the Johns Hopkins Hospital between 2007 and 2015. Resectability and staging were based on computed tomography (CT) imaging and assessed conform the National Comprehensive Cancer Network (NCCN) guidelines.[15] Further imaging studies to exclude distant metastases, such as magnetic resonance imaging (MRI) and/or positron-emission tomography/computed tomography (PET/CT) scans were performed on individual patient basis, based on recommendations of the pancreatic multidisciplinary clinic. Similarly, utilization of diagnostic laparoscopy was decided based on individual assessment. Excluded were patients with

metastatic disease at the time of diagnosis or surgery, or those who experienced 90-day post-resection mortality. Also excluded were patients with <12 months of follow-up (in which neither recurrence nor death had occurred), or patients with incomplete follow-up records.

Data Collection

Demographics, clinicopathologic and treatment characteristics were extracted from a prospective single-institution pancreatectomy database. Neoadjuvant chemotherapy regimens were chosen at the discretion of the treating medical oncologist, and predominantly included Gemcitabine-based regimens, 5-fluorouracil-based regimens such as FOLFIRINOX (5-fluorouracil, leucovorin, oxaliplatin, and irinotecan), or a combination of the two. After completion of neoadjuvant treatment, patients were re-evaluated with CT and those without evidence of local disease progression or distant metastasis were offered operative exploration.

Thirty-day morbidity was graded using the Clavien-Dindo classification system.[16] Tumor staging was reported according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system for pancreatic cancer.[17] Treatment effect was measured using the College of American Pathologists (CAP) Cancer Protocol.[18] When available, pre-neoadjuvant and pre-surgery carbohydrate antigen (CA) 19-9 values were documented. CA 19-9 values acquired more than 1 month before start of neoadjuvant treatment or date of surgery were not included for analysis. Based on receiver operating characteristics curves, cut-offs of 150 and 100 U/ml were utilized for pre-neoadjuvant and pre-surgery CA 19-9 values, respectively.

Follow-up and Recurrence

Follow-up methodology at our institution has been described previously and is performed according to the NCCN guidelines.[15,19] Follow-up data were retrieved through November 2018. The first location of disease recurrence was documented using five mutually exclusive groups: "local only", "liver only", "lung only", "multiple-site" and "other". Multiple-site recurrence included patients with recurrence at multiple distant sites (i.e. lung and liver), patients with carcinomatosis, and/or patients with simultaneous first recurrence at local and distant sites.

Survival Outcomes and Statistical Analysis

RFS was defined as the time interval between the date of surgery and the date of recurrence. OS and post-recurrence survival were defined as the time from the date of resection (OS) or the date of recurrence (post-recurrence survival) to either death or last follow-up. Kaplan-Meier curves were used to estimate median survival outcomes with a corresponding 95%

Table 1. Demographic, clinicopathologic, and treatment characteristics of included patients

Variable	*All patients (n=231)*	*No recurrence (n=42)*	*Recurrence (n=189)*	*P*-value
Male, *n* (%)	128 (55.4%)	23 (54.8%)	105 (55.6%)	0.925
Caucasian, *n* (%)	204 (88.3%)	36 (85.7%)	168 (88.9%)	0.562
Age, mean years (SD)	62.7 (9.4)	62.6 (9.6)	62.8 (9.4)	0.905
Disease stage, *n* (%)				0.975
Borderline resectable	138 (59.7%)	25 (59.5%)	113 (59.8%)	
Locally advanced	93 (40.3%)	17 (40.5%)	76 (40.2%)	
Neoadjuvant therapy *n* (%)				0.477
Chemotherapy	51 (22.1%)	11 (26.2%)	40 (21.2%)	
Chemotherapy & radiotherapy	180 (77.9%)	31 (73.8%)	149 (78.8%)	
Chemotherapy regimen, *n* (%)				0.155
FOLFIRINOX-based	98 (42.4%)	20 (47.6%)	78 (41.3%)	
FOLFIRINOX-Gemcitabine combination	44 (19.0%)	11 (26.2%)	33 (17.5%)	
Gemcitabine-based	89 (38.5%)	11 (26.2%)	78 (41.3%)	
Time of CHT administered, median months (IQR)	4 (2-5)	4 (2-5)	3 (2-5)	0.380
Neoadjuvant radiotherapy modality, *n* (%)				0.706
Conventional radiotherapy	81 (45.0%)	13 (41.9%)	68 (45.6 %)	
IMRT/SBRT	99 (55.0%)	18 (58.1%)	81 (54.4 %)	
Time from diagnosis to resection, median months (IQR)	8 (6-10)	8 (6-10)	7 (5-9)	0.214
Pre-neoadjuvant CA 19-9, median U/ml (IQR)[a]	170 (68-554)	103 (39-294)	184 (79-587)	**0.018**
Pre-surgery CA 19-9, median U/ml (IQR)[b]	39 (21-85)	27 (20-38)	47 (24-97)	**0.010**
Type of operation, *n* (%)				0.765
Pancreatoduodenectomy	164 (71.0%)	30 (71.4%)	134 (70.9%)	
Distal pancreatectomy	42 (18.2%)	9 (21.4%)	33 (17.5%)	
Total pancreatectomy	12 (5.2%)	1 (2.4%)	11 (5.8%)	
DP-CAR	13 (5.6%)	2 (4.8%)	11 (5.8%)	
Vascular resection, *n* (%)	91 (39.4%)	15 (35.7%)	76 (40.2%)	0.590
Morbidity, *n* (%)				0.516
≤Clavien-Dindo grade II	190 (82.3%)	36 (85.7%)	154 (81.5%)	
≥Clavien-Dindo grade III	41 (17.7%)	6 (14.3%)	35 (18.5%)	
R0 margin (>1.0mm), *n* (%)	180 (77.9%)	36 (85.7%)	144 (76.2%)	0.178
Tumor size, mean cm (SD)	2.5 (1.5)	2.4 (1.5)	2.5 (1.5)	0.629
T-stage (AJCC 8th edition), *n* (%)				0.092
yT0-yT2	127 (55.0%)	28 (67.7%)	99 (52.4%)	
yT3-yT4	104 (45.0%)	14 (33.3%)	90 (47.6%)	
N-stage (AJCC 8th edition), *n* (%)				**0.011**
yN0-status	140 (60.6%)	34 (81.0%)	106 (56.1%)	
yN1-status	72 (31.2%)	6 (14.3%)	66 (34.9%)	
yN2-status	19 (8.2%)	2 (4.8%)	17 (9.0%)	
Tumor differentiation, *n* (%)				0.353
Well/Moderate	162 (70.1%)	32 (76.2%)	130 (68.8%)	
Poor	69 (29.9%)	10 (23.8%)	59 (31.2%)	
Microscopic perineural invasion, *n* (%)	133 (57.6%)	16 (38.1%)	117 (61.9%)	**0.005**
Microscopic lymphovascular invasion, *n* (%)	91 (39.4%)	12 (28.6%)	79 (41.8%)	0.113

CAP score treatment response, *n* (%)				0.190
Complete (grade 0)	15 (6.5%)	4 (9.5%)	11 (5.8%)	
Extensive (grade 1)	43 (18.6%)	8 (19.0%)	35 (18.5%)	
Moderate (grade 2)	75 (32.5%)	18 (42.9%)	57 (30.2%)	
Poor (grade 3)	98 (42.4%)	12 (28.6%)	86 (45.5%)	
Adjuvant chemotherapy, *n* (%)				0.425
None	93 (40.3%)	20 (47.6%)	73 (38.6%)	
Chemotherapy	109 (47.2%)	16 (38.1%)	93 (49.2%)	
Chemotherapy & radiotherapy	29 (12.6%)	6 (14.3%)	23 (12.2%)	
Survival, median months (95% CI)				
Recurrence-free survival	9.8 (8.3-11.3)	NA	7.9 (7.1-8.8)	NA
Post-recurrence survival	NA	NA	8.4 (7.3-9.6)	NA
Overall survival	19.8 (17.3-22.4)	Not reached	18.0 (16.4-19.5)	**<0.001**
Survival from diagnosis	28.0 (25.5-30.5)	Not reached	26.1 (24.3-27.9)	**<0.001**

[a] 149 patients had documented pre-neoadjuvant CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

[b] 149 patients had documented pre-operative CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; CAP, *College of American Pathologists*; CHT, *chemotherapy*; CI, *confidence interval*; DP-CAR, *distal pancreatectomy with celiac axis resection*; IMRT, *intensity-modulated radiation therapy*; IQR, *interquartile range*; NA, *not applicable*; SBRT, *stereotactic body radiation therapy*; SD; *standard deviation*.

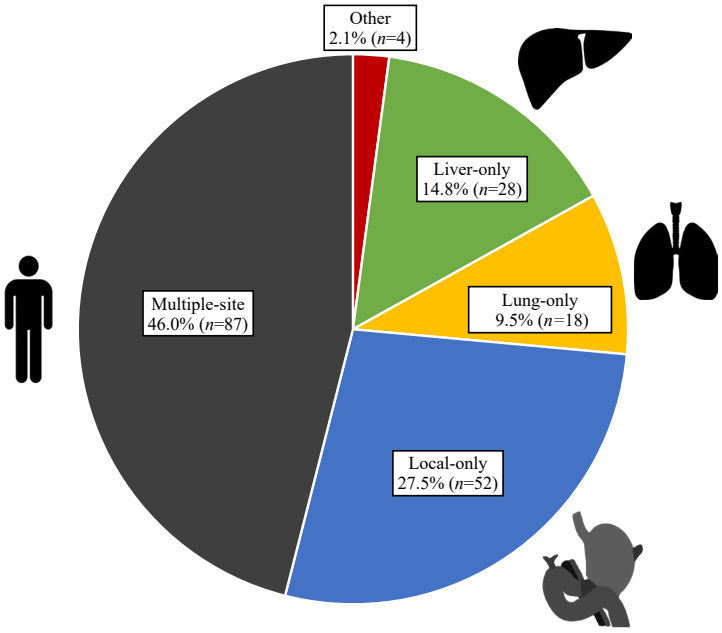
confidence interval (CI), and the log-rank test was used for subgroup comparison. Univariable Cox-proportional hazard regression analyses were performed to describe associations between clinicopathologic characteristics and specific patterns of recurrence, RFS, and OS. A stepwise, backward approach using the Akaike information criterion (AIC) was used to select covariates for inclusion in multivariable Cox models predicting RFS and OS. A two-tailed P-value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS v25.0 (IBM Corporation, Armonk, NY, USA) and R v3.3.3 (R Foundation, Vienna, Austria).

Results

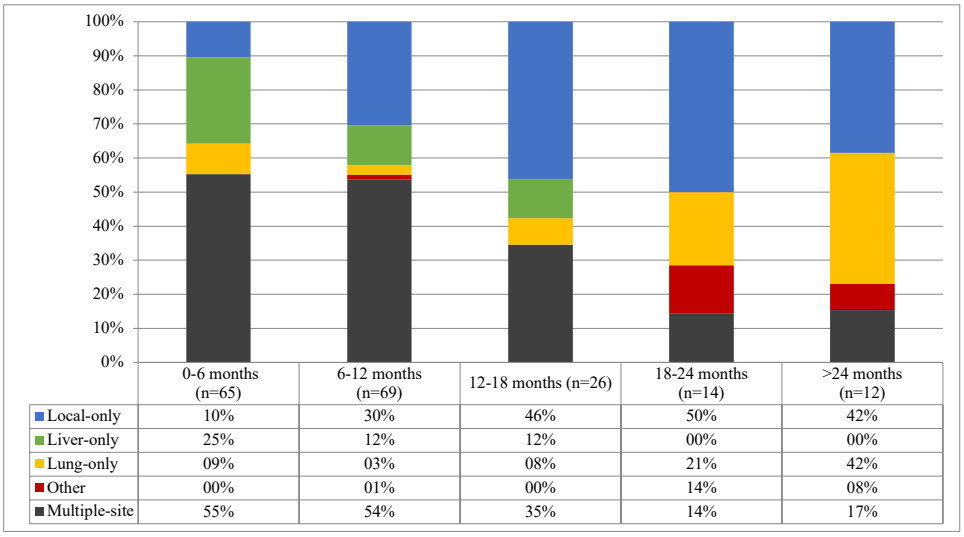
Patient Cohort

A total of 272 BRPC/LAPC patients underwent neoadjuvant treatment followed by a pancreatectomy between 2007 and 2015. From this cohort, 7 patients (2.6%) with 90-day mortality were excluded. Also excluded were 34 patients (12.5%) with <12 months of follow-up or with incomplete follow-up reports. Consequently, 231 patients (84.9%) were included in the final study cohort. At last follow-up, 57 patients (24.7%) were alive after a median follow-up of 44.9 months (95% CI 39.8-50.0) from the time of diagnosis and 37.3 months (95% CI 31.2-44.4) from the date of surgery. Disease recurrence was documented in 189 patients (81.8%) after a median RFS of 7.9 months (Table 1).

A



B



	0-6 months (n=65)	6-12 months (n=69)	12-18 months (n=26)	18-24 months (n=14)	>24 months (n=12)
Local-only	10%	30%	46%	50%	42%
Liver-only	25%	12%	12%	00%	00%
Lung-only	09%	03%	08%	21%	42%
Other	00%	01%	00%	14%	08%
Multiple-site	55%	54%	35%	14%	17%

Figure 1. (A) Distribution of recurrence patterns and (B) distribution of recurrence pattern at different time points.

Cohort and Treatment Characteristics

Complete demographic, clinicopathologic, and treatment characteristics are shown in Table 1. All patients underwent neoadjuvant chemotherapy with significant variations noted in their corresponding regimens (Online Supplemental eTable S1). The majority of patients (n=180, 77.9%) also underwent neoadjuvant radiotherapy, either with conventional external beam radiotherapy (n=81), intensity-modulated radiotherapy (n=17) or stereotactic body radiotherapy (n=82). After resection, 138 patients (59.7%) received additional adjuvant treatment, consisting of either chemotherapy alone (n=109, 47.2%) or chemotherapy and radiotherapy (n=29, 12.6%) (Online Supplemental eTable S1).

Pattern of Recurrence

Of the 189 patients (81.8%) with recurrent PDAC, 62 patients (32.8%) had pathologic confirmation of recurrence with a tissue biopsy while the other patients were diagnosed based on imaging evidence alone. Most patients first recurred at multiple sites (n=87, 46.0%) (Figure 1A). Of these 87 patients, 57 had simultaneous first recurrence at local and distant sites (Online Supplemental eTable S2). Isolated local recurrence occurred in 52 patients (27.5%). Isolated distant recurrence was most often seen in the liver (n=28, 14.8%) or in the lungs (n=18, 9.5%). Four patients (2.1%) recurred at less common distant sites, specifically: the abdominal wall, the left ovary, the uterus and the right calf muscle. The proportion of different recurrence locations at successive time intervals is displayed in Figure 1B. The rate of recurrence for patients with pre-neoadjuvant BRPC (81.9%) and LAPC (81.7%) was nearly identical (P=0.975). Additionally, recurrence patterns did not differ between patients with initial BRPC or LAPC (P=0.622). Specifically, the fraction of local recurrence for BRPC (35/113; 31.0%) and LAPC (17/76; 22.4%) were not significantly different (P=0.194).

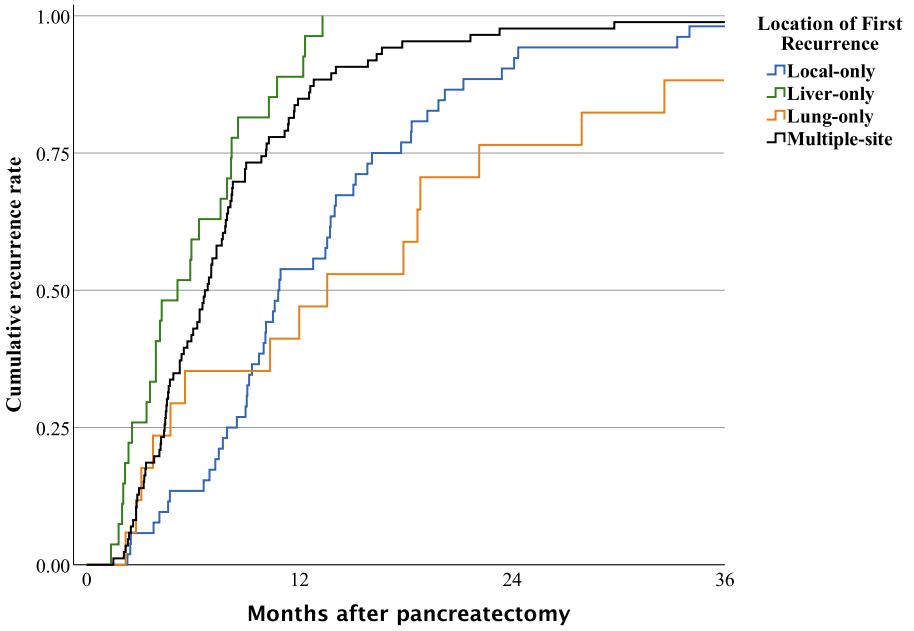
An historical cohort of primary resectable patients undergoing upfront resection at our institution between 2000-2013, which has been extensively described previously, was utilized to compare recurrence patterns and outcomes (Table 2).[20,21] The current cohort of post-neoadjuvant BRPC/LAPC patients had significantly lower pre-surgery CA 19-9 and more favorable pathologic tumor characteristics than patients with primary resectable PDAC. However, overall recurrence rates (81.8% vs 78.6%; P=0.291) and median survival from diagnosis (28.0 vs. 26.1 months; P=0.371) for post-neoadjuvant BRPC/LAPC and primary resectable patients were similar. While survival from diagnosis per recurrence location did not differ for the two patient cohorts, liver only recurrence occurred less frequent in the post-neoadjuvant patients (Table 2).

Table 2. Comparison of clinicopathologic characteristics, recurrence patterns and survival outcomes between the study cohort of resected post-neoadjuvant patients and a historical cohort of patients who underwent upfront resection

Variable	*Post-neoadjuvant (n=231)*	*Upfront surgery (n=957)*	*P*-value
Male, *n* (%)	128 (55.4%)	501 (52.4%)	0.403
Caucasian, *n* (%)	204 (88.3%)	821 (85.8%)	0.317
Age, mean years (SD)	62.7 (9.4)	65.8 (10.5)	**<0.001**
Pre-surgery CA 19-9, median U/ml (IQR)	39 (21-85)[a]	130 (50-398)[b]	**<0.001**
Type of operation, *n* (%)			**<0.001**
Pancreatoduodenectomy	164 (71.0%)	798 (83.4%)	
Distal pancreatectomy	42 (18.2%)	125 (13.1%)	
Total pancreatectomy	12 (5.2%)	34 (3.6%)	
DP-CAR	13 (5.6%)	0 (0%)	
R0 margin (>1.0mm), *n* (%)	180 (77.9%)	658 (68.8%)	**0.006**
Tumor size, mean cm (SD)	2.5 (1.5)	3.2 (1.5)	**<0.001**
T-stage (AJCC 8th edition), *n* (%)			**<0.001**
T0-T2	127 (55.0%)	99 (52.4%)	
T3-T4	104 (45.0%)	90 (47.6%)	
N-stage (AJCC 8th edition), *n* (%)			**<0.001**
N0-status	140 (60.6%)	238 (24.9%)	
N1-status	72 (31.2%)	400 (41.8%)	
N2-status	19 (8.2%)	319 (33.3%)	
Positive lymph node ratio, median (IQR)	0.00 (0.00-0.08)	0.11 (0.02-0.25)	**<0.001**
Tumor differentiation, *n* (%)			**<0.001**
Well/Moderate	162 (70.1%)	591 (61.8%)	
Poor	69 (29.9%)	366 (38.2%)	
Microscopic perineural invasion, *n* (%)	133 (57.6%)	859 (89.8%)	**<0.001**
Microscopic lymphovascular invasion, *n* (%)	91 (39.4%)	544 (56.8%)	**<0.001**
Adjuvant chemotherapy, *n* (%)			**<0.001**
None	93 (40.3%)	307 (32.1%)	
Chemotherapy	109 (47.2%)	207 (21.6%)	
Chemotherapy & radiotherapy	29 (12.6%)	443 (46.3%)	
Recurrence, *n* (%)	189 (81.8%)	753 (78.6%)	0.291
Recurrence pattern, *n* (%)			
Lung only	18 (9.5%)	106 (14.1%)	0.098
Local only	52 (27.5%)	190 (25.2%)	0.521
Multiple-site	87 (46.0%)	253 (33.6%)	**0.001**
Liver only	28 (14.8%)	184 (24.4%)	**0.005**
Other	4 (2.1%)	20 (2.7%)	0.674
Overall survival (from diagnosis), median months (95% CI)			
Lung only	31.5 (12.6-50.5)	43.5 (32.3-54.8)	0.992
Local only	34.6 (30.3-39.0)	27.8 (24.5-31.2)	0.297
Multiple-site	23.8 (22.6-25.0)	19.1 (17.4-20.8)	0.193
Liver only	17.8 (9.5-26.2)	16.2 (14.3-18.1)	0.766
Other	64.2 (0-not reached)	34.9 (19.6-50.1)	0.114

Survival outcomes, median months (95% CI)			
Recurrence-free survival (from resection)	9.8 (8.3-11.3)	15.2 (14.0-16.4)	**<0.001**
Post-recurrence survival	8.4 (7.3-9.6)	7.5 (6.8-8.2)	0.403
Survival from diagnosis	28.0 (25.5-30.5)	26.1 (24.5-27.7)	0.371
Overall survival (from resection)	19.8 (17.3-22.4)	24.8 (23.3-26.3)	**0.015**

[a] 149 patients had documented pre-operative CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.
[a] 398 patients had documented pre-operative CA 19-9 values, of whom 65 patients were Lewis-antigen negative and excluded.
AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; CI, *confidence interval*; DP-CAR, *distal pancreatectomy with celiac axis resection*; IQR, *interquartile range*; SD, *standard deviation*.



Recurrence patterns	*0-6 months*	*6-12 months*	*12-18 months*	*18-24 months*	*24-48 months*	*48-60 months*
Local only (n=52)	13.5% (*n*=7)	53.8% (*n*=28)	76.9% (*n*=40)	90.4% (*n*=47)	98.1% (*n*=51)	100% (*n*=52)
Liver only (n=28)	60.7% (*n*=17)	89.3% (*n*=25)	100% (*n*=28)	100% (*n*=28)	100% (*n*=28)	100% (*n*=28)
Lung only (n=18)	33.3% (*n*=6)	44.4% (*n*=8)	55.6% (*n*=10)	72.2% (*n*=13)	94.4% (*n*=17)	100% (*n*=18)
Multiple-site (n=87)	42.5% (*n*=37)	85.1% (*n*=74)	95.34% (*n*=83)	97.7% (*n*=85)	98.9 % (*n*=86)	100% (*n*=87)
Other (n=4)	0.0% (*n*=0)	25.0% (*n*=1)	25.0% (*n*=1)	75.0% (*n*=3)	75.0% (*n*=3)	100% (*n*=4)
All recurrence (n=189)	35.4% (*n*=67)	72.0% (*n*=136)	85.7% (*n*=162)	93.1% (*n*=176)	97.9% (*n*=185)	100% (*n*=189)

Figure 2. Cumulative recurrence rate per specific recurrence pattern as a function of time.

Timing of Recurrence

At 6 months after resection, 67 of 189 patients with recurrence (35.4%) had recurred, and 136 (72.0%) had done so within 12 months (Figure 2). No recurrences were documented more than 5 years post-surgery. Forty-two patients (18.2%) had no sign of recurrence after a median follow-up of 42.5 months (95% CI 35.2-49.8) after diagnosis and 32.2 months (95% CI 24.9-39.5) after resection. Figure 2 further demonstrates that nearly 90% of liver only recurrences occurred in the first 12 months. Similarly, 95.3% of multiple-site recurrences transpired in the first 18 months. Median RFS of the specific recurrence patterns with pairwise comparison are presented in Online Supplemental eFigure S1A. The high cumulative early recurrence rates of liver and multiple-site recurrence are further illustrated by their relatively short median RFS of 4.2 months and 6.7 months, respectively. Local only, lung only, and other recurrence generally occurred later, reflected by their median RFS exceeding 10 months.

Implications of Recurrence Pattern and Timing on Survival

Median survival outcomes for all patients and per different recurrence location are presented in Online Supplemental eTable S3. Median OS was not yet reached at 108 months in 42 patients without recurrence, compared to a median OS of 18.0 months for patients with recurrence ($P<0.001$). Pairwise comparison of specific recurrence patterns revealed that median post-recurrence survival and OS varied significantly based on recurrence location (Online Supplemental eFigure S1B-C). Patients with liver only or multiple-site recurrence had a median post-recurrence survival of 6.6 and 8.0 months, resulting in relatively limited median OS of 11.9 and 15.7 months, respectively. Similar to the observed differences in RFS, lung only, local only and other recurrence were all associated with considerably longer median post-recurrence survival and OS.

The timing of recurrence was strongly correlated with subsequent survival after recurrence. Using the log-rank test and a minimum P-approach[20], the optimal length of RFS to distinguish between early and late recurrence, based on subsequent post-recurrence survival, was 9 months ($P=6.30x10^{-5}$). Median post-recurrence survival in the early (RFS <9 months) recurrence cohort (n=108, 57.1%) was 7.9 months (95% CI 6.9-8.8). Patients with recurrence after 9 months (n=81, 42.9%) had a median post-recurrence survival of 13.8 months (95% CI 10.2-17.4). Patients with early recurrence had 1- and 2-yr post-recurrence survival rates of 21.9% and 7.0% compared with 54.3% and 25.4% for the late recurrence group (both $P<0.001$).

Table 3. Univariable Cox regression analysis for the identification of clinicopathologic and treatment characteristics associated with different recurrence patterns

	Local only (n=52)			*Multiple-site/Liver only (n=115)*			*Lung only/Other (n=22)*		
Clinical characteristics	***HR***	***95% CI***	***P***	***HR***	***95% CI***	***P***	***HR***	***95% CI***	***P***
Gender									
Male vs. female	1.42	0.81-2.48	0.223	1.24	0.86-1.80	0.249	**0.34**	**0.13-0.87**	**0.025**
Race									
Caucasian vs. all others	1.30	0.51-3.26	0.583	1.00	0.57-1.75	0.992	1.26	0.29-5.45	0.754
Age at surgery (years)*	1.02	0.99-1.05	0.204	1.00	0.98-1.02	0.850	0.97	0.92-1.01	0.140
Disease stage at diagnosis									
LAPC vs. BRPC	0.72	0.40-1.29	0.268	1.29	0.90-1.86	0.170	0.60	0.24-1.49	0.274
Tumor location									
Body/tail vs. head/uncinate	0.76	0.38-1.51	0.431	1.17	0.79-1.75	0.437	0.98	0.38-2.57	0.976
Neoadjuvant therapy									
CHRT vs. CHT	0.80	0.44-1.46	0.473	1.36	0.86-2.17	0.192	2.18	0.64-7.39	0.213
Neoadjuvant regimen									
Gem-based vs. FFX-based	0.74	0.40-1.38	0.342	**1.52**	**1.02-2.26**	**0.042**	1.21	0.45-3.22	0.705
Gem-FFX vs. FFX-based	0.72	0.34-1.53	0.388	0.97	0.57-1.67	0.917	1.14	0.37-3.52	0.815
CA 19-9 pre-neoadjuvant[a]									
>150 U/mL vs. <150 U/mL	1.58	0.78-3.21	0.207	**1.84**	**1.12-3.01**	**0.016**	1.43	0.51-4.02	0.499
CA 19-9 pre-surgery[b]									
>100 U/mL vs. <100 U/mL	**2.61**	**1.11-6.13**	**0.028**	**2.61**	**1.56-4.37**	**<0.001**	1.33	0.53-3.36	0.268
Vascular resection									
Yes vs. no	0.72	0.40-1.31	0.283	1.25	0.86-1.80	0.240	1.10	0.47-2.59	0.820
Resection margin status									
R1 (≤1.0mm) vs. R0 (>1.0mm)	**2.03**	**1.12-3.66**	**0.019**	1.15	0.75-1.79	0.524	0.73	0.22-2.48	0.614
T-stage (AJCC 8th edition)									
yT3-yT4 vs. yT0-yT2	1.27	0.74-2.20	0.391	**1.46**	**1.01-2.12**	**0.042**	1.83	0.77-4.33	0.169
N-stage (AJCC 8th edition)									
yN1-yN2 vs. yN0	**2.18**	**1.25-3.81**	**0.006**	**1.88**	**1.30-2.71**	**0.001**	2.13	0.85-5.35	0.106
Tumor differentiation grade									
Poor vs. well/moderate	1.20	0.63-2.31	0.580	**1.85**	**1.26-2.70**	**0.002**	1.35	0.48-3.74	0.571
Micr. lymphovascular invasion									
Yes vs. no	1.26	0.72-2.21	0.413	1.36	0.94-1.97	0.098	1.60	0.68-3.92	0.271
Micr. perineural invasion									
Yes vs. no	**1.98**	**1.12-3.44**	**0.019**	**1.75**	**1.20-2.56**	**0.004**	1.57	0.67-3.68	0.297
CAP score treatment response									
0 vs. 2/3	0.90	0.35-2.29	0.818	**0.35**	**0.13-0.94**	**0.038**	0.64	0.15-2.77	0.546
1 vs. 2/3	1.36	0.70-2.62	0.364	0.86	0.53-1.38	0.523	0.46	0.11-1.97	0.292
Adjuvant therapy									
Any vs. none	1.64	0.86-3.14	0.133	**0.61**	**0.42-0.88**	**0.008**	0.63	0.27-1.46	0.277

* Continuous variable

[a] 149 patients had documented pre-neoadjuvant CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

[b] 149 patients had documented pre-operative CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

AJCC, *American Joint Committee on Cancer*; BRPC, *borderline resectable pancreatic cancer*; CA, *carbohydrate antigen*; CAP, *College of American Pathologists*; CHRT, *chemoradiotherapy*; CHT, *chemotherapy*; CI, *confidence interval*; FFX, *FOLFIRINOX*; GEM, *Gemcitabine*; HR, *hazard ratio*; LAPC, *locally advanced pancreatic cancer*; Micr, *microscopic*.

Factors Associated with Patterns of Recurrence

On univariable Cox regression analysis, several clinicopathologic and treatment variables were associated with distinct recurrence locations (Table 3). Based on observed survival outcomes, recurrence patterns were grouped in local only (n=52), multiple-site/liver only (n=115) and lung only/other (n=22). Microscopic perineural invasion and yN1-yN2 status were correlated with both local and multiple-site/liver recurrence. An R1-margin was only associated with local recurrence (hazard ratio [HR] 2.03, P=0.019), while poor tumor differentiation (HR 1.85, P=0.002) and yT3-yT4 tumor stage (HR 1.46, P=0.042) correlated with multiple-site/liver recurrence. Whereas lung/other recurrence occurred less in males (HR 0.34, P=0.025), no other associations were found.

There was no significant difference observed in the rate of local recurrence between patients who received radiotherapy in either the neoadjuvant or adjuvant setting (43/201; 21.4%), and those who did not receive any radiation treatment (9/30; 30.0%) (P=0.292). Gemcitabine-based neoadjuvant therapy was associated with a higher likelihood of multiple-site/liver recurrence when compared to FOLFIRINOX-based regimens (HR 1.52, P=0.042). Alternatively, adjuvant therapy was associated with a decreased likelihood of multiple-site/liver recurrence (HR 0.61, P=0.008). A pathologic complete response (CAP score 0) was significantly associated with a decreased chance of multiple-site/liver recurrence (HR 0.35, P=0.038) when compared to moderate/poor treatment effect.

Association of CA 19-9 with Recurrence and Survival

Patients with pre-neoadjuvant elevated CA 19-9 >150 U/mL had significantly decreased survival from PDAC diagnosis (24.0 vs. 34.7 months; P=0.015), RFS (7.9 vs. 13.7 months; P=0.005) and OS (15.9 vs. 25.0 months; P=0.020) (Online Supplemental eFigure S2). Similarly, patients with pre-operatively elevated CA 19-9 >100 U/mL had worse RFS (4.6 vs. 10.9 months; P<0.001) and OS (11.2 vs. 20.9 months; P=0.001) (Online Supplemental eFigure S3).

Factors Associated with Recurrence-Free and Overall Survival

Median survival outcomes for patients with and without recurrence, and for different recurrence patterns are shown in Table 1 and Online Supplemental eTable S3. For all 231 patients, 1-yr, 2-yr and 5-yr rates of RFS and OS were 40%, 21%, 9% and 76%, 43%, 15%, respectively.

Table 4 shows the results of univariable and multivariable Cox regression analyses for RFS and OS. On univariable analysis, several pathologic features were associated with both decreased

RFS and OS. On multivariable analysis, yN1-yN2 status (HR 1.68, P=0.003) and microscopic perineural invasion (HR 1.43, P=0.041) were independent predictors for reduced RFS. Independent prognostic factors for poor OS were yT3-yT4 tumor stage (HR 1.39, P=0.046), yN1-yN2 status (HR 1.91, P=0.0024), poor tumor differentiation grade (HR 1.60, P=0.006) and microscopic perineural invasion (HR 1.48, P=0.041). Furthermore, adjuvant therapy was independently associated with both prolonged RFS (HR 0.73, P=0.043) and OS (HR 0.69, P=0.025). Estimated median RFS and OS for patients who received adjuvant therapy were 10.9 and 22.7 months respectively, while median RFS and OS for patients who did not receive adjuvant therapy were 7.0 and 15.4 months.

Patients with a pathologic complete response did not have a significantly lower recurrence rate (11/15; 73.3% vs. 178/216; 82.4% (P=0.378)), although a near significant difference in median RFS was observed (21.3 months (95% CI 12.5-30.0) vs. 9.1 months (95% CI 7.8-10.4); P=0.051). The 11 patients who recurred after a complete response did have significantly extended post-recurrence survival (24.8 months (95% CI 8.7-40.9) vs. 8.3 months (95% CI 7.5-9.1); P=0.003). Notably, patients with a complete response had significantly improved OS (46.8 months (95% CI 0-not reached) vs. 19.2 months (95% CI 17.1-21.2); P=0.002).

Discussion

Patients initially diagnosed with BRPC/LAPC who underwent post-neoadjuvant resection represent a growing cohort of importance. While low rates of R1-margin and lymph node positivity were observed in this study, PDAC recurrence nevertheless occurred in more than 80% of patients. Furthermore, 72.5% of recurrences occurred at distant sites, suggesting that despite a radiographic progression-free period leading to resection, viable micrometastatic PDAC can persist after systemic neoadjuvant treatment. Additionally, similar to primary resectable PDAC, different recurrence patterns were found to be associated with unique survival curves, with liver and multiple-site recurrence resulting in particularly poor outcomes. Lastly, several clinicopathologic and treatment features were found to be associated with distinct recurrence locations and patient survival. This detailed knowledge on the timing, pattern and factors associated with disease failure may help clinicians with important decisions regarding the challenging multimodality care of these patients with advanced localized disease.

Few studies on BRPC/LAPC have studied disease recurrence following resection as a primary outcome of interest. Therefore, currently published recurrence rates differ considerably. For instance, seven large contemporary studies that included data on recurrence after resection of BRCP/LAPC reported overall recurrence rates ranging from 38% to 65%.[12,22-27] However,

Table 4. Univariable and multivariable Cox regression analysis for recurrence-free and overall survival

	Recurrence-free survival						*Overall Survival*					
	Univariable Analysis			*Multivariable Analysis*			*Univariable Analysis*			*Multivariable Analysis*		
Clinical Characteristics	***HR***	***95% CI***	***P***	***HR***	***95% CI***	***P***	***HR***	***95% CI***	***P***	***HR***	***95% CI***	***P***
Gender												
Male vs. female	1.12	0.84-1.50	0.209	-			1.23	0.91-1.66	0.186	1.27	0.93-1.73	0.136
Race												
Caucasian vs. all others	1.10	0.70-1.73	0.691	-			1.19	0.73-1.94	0.489	-		
Age at surgery (years)*	1.00	0.99-1.02	0.951	-			1.01	0.99-1.03	0.094	-		
Disease stage at diagnosis												
LAPC vs. BRPC	0.02	0.76-1.37	0.902	-			1.03	0.76-1.39	0.873	-		
Tumor location												
Body/tail vs. head/uncinate	1.02	0.73-1.41	0.918	-			0.98	0.70-1.37	0.897	-		
Neoadjuvant therapy												
CHRT vs. CHT	1.22	0.86-1.72	0.276	1.40	0.97-2.03	0.071	1.16	0.81-1.66	0.429	1.42	0.96-2.08	0.076
Neoadjuvant regimen												
Gem-based vs. FFX-based	1.19	0.86-1.63	0.290	-			1.31	0.94-1.82	0.114	1.29	0.92-1.81	0.140
Gem-FFX vs FFX-based	0.89	0.59-1.35	0.590	-			0.94	0.61-1.44	0.769	0.86	0.55-1.33	0.496
CA 19-9 pre-neoadjuvant[a]												
>150 U/mL vs. <150 U/mL	1.70	1.17-2.48	**0.006**	NA[ϕ]			1.60	1.07-2.39	**0.021**	NA[ϕ]		
CA 19-9 pre-surgery[b]												
>100 U/mL vs. <100 U/mL	2.30	1.49-3.55	**<0.001**	NA[ϕ]			2.12	1.34-3.35	**0.001**	NA[ϕ]		
Vascular resection												
Yes vs. no	1.08	0.80-1.44	0.630	-			1.02	0.75-1.38	0.912	0.78	0.55-1.09	0.142
Resection margin status												
R1 vs. R0	1.31	0.94-1.83	0.126	-			1.36	0.96-1.93	0.087	-		
T-stage (8[th])												
yT3-yT4 vs. yT0-yT2	1.45	1.09-1.94	**0.012**	-			1.53	1.13-2.06	**0.006**	**1.39**	**1.01-1.92**	**0.046**
N-stage (8[th])												
yN1-yN2 vs. yN0	1.93	1.44-2.59	**<0.001**	**1.68**	**1.19-2.38**	**0.003**	1.91	1.41-2.59	**<0.001**	**1.50**	**1.06-2.13**	**0.024**
Tumor differentiation grade												
Poor vs. well/moderate	1.69	1.23-2.31	**0.001**	1.37	0.98-1.89	0.062	1.97	1.43-2.71	**<0.001**	**1.60**	**1.14-2.23**	**0.006**
Micr. lymphovascular invasion												
Yes vs. no	1.34	1.00-1.79	**0.050**	-			1.52	1.13-2.06	**0.006**	-		
Micr. perineural invasion												
Yes vs. no	1.78	1.32-2.40	**<0.001**	**1.43**	**1.02-2.01**	**0.041**	1.87	1.36-2.55	**<0.001**	**1.48**	**1.02-2.15**	**0.041**
CAP score												
0 vs. 2/3	0.54	0.29-1.00	0.051	NA[j]			0.28	0.12-0.64	**0.002**	NA[j]		
1 vs. 2/3	0.93	0.65-1.35	0.717	NA[j]			0.89	0.60-1.31	0.548	NA[j]		

Adjuvant therapy												
Any vs. none	0.76	0.56-1.02	0.067	**0.73**	**0.54-0.99**	**0.043**	0.76	0.56-1.03	0.072	**0.69**	**0.50-0.95**	**0.025**

* Continuous variable

[ϕ] Pre-neoadjuvant and pre-surgery CA 19-9 were not considered for inclusion in the multivariable models due to significant amount of missing values.

[ʃ] Treatment response (CAP score) was not considered for inclusion in the multivariable models due to significant correlation with other pathologic tumor characteristics.

[a] 149 patients had documented pre-neoadjuvant CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

[b] 149 patients had documented pre-operative CA 19-9 values, of whom 11 patients were Lewis-antigen negative and excluded.

AJCC, *American Joint Committee on Cancer;* BRPC, *borderline resectable pancreatic cancer;* CA, *carbohydrate antigen;* CAP, *College of American Pathologists;* CHRT, *chemoradiotherapy;* CHT, *chemotherapy;* CI, *confidence interval;* FFX, *FOLFIRINOX;* GEM, *Gemcitabine;* HR, *hazard ratio;* LAPC, *locally advanced pancreatic cancer;* Micr, *microscopic;* NA, *not applicable*.

one of the first prospective randomized trials reported a recurrence rate of 88% in 17 BRPC patients undergoing post-neoadjuvant resection; more similar to the 82% found in the current study.[28] The discrepancies in the published recurrence rates can likely be attributed to a combination of multiple factors, including variations in neoadjuvant treatment regimen and differences in both follow-up strategies and length. To reduce these potential biases in this retrospective study, we utilized a large, contemporary single-institution cohort and excluded patients with incomplete follow-up records. In the near future, further prospective clinical trials on BRPC and LAPC, such as the ALLIANCE and SCALOP-2 trials, might provide more accurate recurrence and survival data for resected BRPC/LAPC patients.[29]

Several studies have attempted to compare recurrence outcomes between neoadjuvant- and surgery-first strategies. A recent meta-analysis by Schorn et al. has suggested that the overall recurrence rate is lower in patients who received neoadjuvant therapy (Risk Ratio 0.82; P<0.001).[30] Additionally, fewer hepatic metastases were observed in the neoadjuvant patients (20% vs. 29% (P=0.02)). The current study also found a decreased rate of liver only recurrence in patients who underwent post-neoadjuvant resection when compared to upfront resection. Prospective trials that randomize patients to neoadjuvant and surgery-first arms, such as the PREOPANC-trial, ought to provide a better understanding of differences in patterns and rates of recurrence between these two treatment-sequence strategies.[31]

While the clinical advantage of a neoadjuvant-first approach is still being intensely debated, multiple studies have shown that neoadjuvant treatment can lead to reduced tumor size, lower N-stage and R1-rates, and decreased frequency of microscopic perineural and lymphovascular invasion.[6,32] However, published predictive values of these historic pathologic risk factors vary significantly, with only post-neoadjuvant N-stage being fairly consistently associated with poor survival.[1,4,33-38] In this study, yN1-yN2 and yT3-yT4 status, poor tumor differentiation and microscopic perineural invasion were independent predictors for decreased OS. However,

these associations were relatively weak, suggesting that conventional pathologic characteristics might have less prognostic value in post-neoadjuvant patients. Recent studies indicate that liquid biopsies in the form of circulating tumor cells or circulating tumor DNA show promise as potential novel biomarkers in cancer patients.[39,40] For instance, in patients undergoing resection of PDAC, a recent study from our institution found that the pre-operative presence of circulating tumor cells was the only predictors of early recurrence within 12 months from surgery in both chemo-naïve and post-neoadjuvant patients.[41] Novel approaches to accurate prognostic stratification in PDAC patients undergoing neoadjuvant treatment and resection are warranted, as these could help the selection of patients for surgery and the guidance of further treatment in the adjuvant setting.

Additional adjuvant treatment was associated with less multiple-site/liver recurrence and was an independent predictor for both improved RFS and OS. However, these results should be interpreted with caution as there was significant heterogeneity in the length and regimen of received treatments based on patient and clinician preference, potentially introducing substantial selection bias. In the current literature, data on adjuvant therapy after neoadjuvant therapy and resection in BRPC/LAPC patients are limited and often contradictory. In a recent study of 1375 patients, no difference in median OS was found based on the receipt of additional adjuvant therapy (28 vs. 27 months, P=0.541).[42] On the other hand, a different study showed survival benefit with added adjuvant treatment, albeit only in patients with a positive lymph node ratio <0.15.[43] Due to the ostensible limitations of retrospective studies, many questions on the addition of adjuvant treatment currently remain unanswered. Hopefully, future prospective trials will provide more insights concerning the right indications and strategies for adjuvant therapy in these patients.

This retrospective study has several important limitations. Most importantly, both neoadjuvant and adjuvant treatment regimens and length varied considerably. Therefore, the associations between treatment factors and recurrence and survival outcomes should be interpreted cautiously, as selection bias could play a significant role. Additionally, a substantial number of patients did not have pre-neoadjuvant and/or pre-surgery CA 19-9 available, prohibiting the inclusion of this potentially important risk factor in the multivariable models. Lastly, diagnosing disease recurrence with radiographic imaging can be challenging, especially since neoadjuvant or adjuvant radiotherapy can lead to localized fibrosis at the pancreatectomy site, possibly resulting in over-estimation of the incidence of local recurrence.[44]

Conclusion

In summary, this study offers a comprehensive understanding of the different aspects of disease recurrence in BRPC/LAPC patients, including the identification of specific clinicopathologic factors associated with distinct recurrence locations. Despite neoadjuvant therapy leading to resection and relatively favorable pathologic tumor characteristics, more than 80% of patients experienced disease recurrence, 72.5% of which occurred at distant sites. These findings suggest that systemic disease continues to exist in BRPC/LAPC patients after neoadjuvant treatment, highlighting the need to improve systemic treatment options and identify occult disease prior to surgery. Future prospective studies should focus on finding the optimal treatment sequence and regimens to reduce recurrence rates and consequently improve survival in these patients.

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Supplemental Material to Chapter 7

- **Supplemental eTable S1.** Distribution of the receipt of various neoadjuvant and adjuvant chemotherapy in the study cohort.
- **Supplemental eTable S2.** Breakdown off specific recurrence location in patients with multiple-site recurrence.
- **Supplemental eTable S3.** Median survival outcomes for included patients.
- **Supplemental eFigure S1**. Kaplan-Meier curves for different recurrence patterns for (A) recurrence-free, (B) post-recurrence and (C) overall survival with pairwise comparisons using the log-rank test for different recurrence patterns.
- **Supplemental eFigure S2.** Kaplan-Meier curves showing worse (A) survival from diagnosis, (B) recurrence-free survival and (C) overall survival for patients with elevated pre-neoadjuvant CA 19-9 values >150 U/mL.
- **Supplemental eFigure S3.** Kaplan-Meier curves showing worse (A) survival from diagnosis, (B) recurrence-free survival and (C) overall survival for patients with elevated pre-surgery CA 19-9 values >100 U/mL.

Supplemental material can be consulted online per the website of the journal and/or publisher.

PART III

Detection of Recurrence

CHAPTER 8

Post-Operative Surveillance of Pancreatic Cancer Patients

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Abstract

Objective

Recommendations for surveillance in patients who underwent resection of pancreatic ductal adenocarcinoma (PDAC) vary widely throughout the world, and the importance of standardized post-operative surveillance is intensely debated. The aim of this study is to collect the best available evidence for modalities, frequency, and duration of surveillance after PDAC resection.

Methods

The latest guidelines of different medical societies were collected. Furthermore, an extensive search of the literature on surveillance after resection of PDAC was performed in PubMed and Embase from 2000 to 2019. Articles comparing different modalities and frequencies of postoperative surveillance in PDAC patients with regard to survival, quality of life, morbidity and cost-effectiveness were selected.

Results

The literature search resulted in 570 articles, which were screened for eligibility. A total of seven guidelines and twelve original clinical studies were eventually evaluated. PDAC guidelines increasingly recommend a combination of tumor marker testing and computed tomography (CT) imaging every three to six months during the first two years after surgery. These guidelines are, however, based on expert opinion and other low-level evidence, as prospective studies comparing different surveillance strategies are lacking. According to the current literature, postoperative surveillance with tumor marker testing and imaging at regular intervals results in the detection of PDAC recurrence prior to the onset of symptoms and more frequent administration of further therapy. Theoretically, early recurrence detection could result in improvements in overall survival and quality of life in selected groups of patients.

Conclusion

Current evidence for recurrence-focused surveillance after PDAC resection is limited and contradictory. Consequently, recommendations on surveillance are conflicting. To define the clinical merit of recurrence-focused surveillance, patients who are most likely to benefit from early detection and treatment of PDAC recurrence need to be identified. To this purpose, well-designed prospective studies are needed, accounting for both economical and psychosocial implications of surveillance.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related mortality in Europe and is expected to rise to second place by 2030.[1,2] For patients with non-metastasized, resectable disease, radical resection combined with (neo)adjuvant chemotherapy offers the best chances for long-term survival.[3] However, even after radical resection, at least 80% of these patients develop local and/or distant disease recurrence, often within two years.[4-7]

Patients with PDAC recurrence constitute a significant group that continues to face limited treatment possibilities and a poor survival of only 3-9 months after recurrence detection.[8-11] Therefore, even after optimal treatment of the primary tumor, PDAC continues to be associated with a 5-year survival rate of only 12-27%.[12-15] However, more effective multi-drug regimens, e.g. FOLFIRINOX and Gemcitabine/Nab-Paclitaxel, and local ablative therapies such as stereotactic body radiation therapy (SBRT), irreversible electroporation (IRE (Nanoknife®)) and radiofrequency ablation (RFA), represent expanded treatment options that could offer improved systemic and/or local control of PDAC recurrence. Several small and non-randomized studies have suggested that patients with recurrent PDAC might benefit from these therapies, both in terms of survival and quality of life.[9,16-19] This has prompted questions on the importance of early detection of recurrent PDAC.

Without recurrence-focused surveillance, disease recurrence is commonly identified after the manifestation of clinical symptoms, which is associated with a more advanced disease stage and worse performance status. Surveillance with serial tumor marker testing and imaging could facilitate earlier detection of PDAC recurrence when the tumor load is still low, arguably increasing the chance of effective systemic and/or local treatment. Furthermore, deterioration of the patients' performance status limits the ability to receive treatment for PDAC recurrence. As a consequence, recurrence-focused surveillance is increasingly recommended. Evidence for this strategy is, however, lacking. Nevertheless, most studies on the potential benefits of postoperative surveillance have been performed prior to the introduction of recent treatment options.

Potential disadvantages of recurrence-focused surveillance are introduction of stress and morbidity that can result in an overall decrease in quality of life, while the impact of treatment on survival is yet unclear. Therefore, most European countries, including the Netherlands, have raised (ethical) concerns regarding recurrence-focused surveillance given the lack of high-quality evidence. Consequently, most PDAC guidelines provide minimal guidance

on modalities and frequency of postoperative surveillance. This has led to widely varying surveillance strategies throughout the world, potentially depriving certain PDAC patients of meaningful surveillance, while wrongfully harming others. Therefore, the aim of this study is to collect the best available evidence for modalities, frequency, and duration of surveillance after PDAC resection.

Methods

First, the latest guidelines of different medical societies were collected. Second, to evaluate the current evidence on modalities (e.g. serum tumor marker testing and imaging procedures), frequency (e.g. every 3 or 6 months) and duration (e.g. 2 years) of surveillance after resection of PDAC, an extensive search of the literature was performed in PubMed and Embase from 2000 to 2019. Synonyms, medical subject headings (MeSH) terms and keywords for "pancreatic cancer" were combined with different terms for "postoperative surveillance". The search was restricted to title and abstract, and English or Dutch language. Titles, abstracts, and subsequently full-text articles were screened for relevance. Conference abstracts and "Letter to the Editor"-papers were excluded.

Articles were found to be eligible when reporting on survival, quality of life, morbidity, and cost-effectiveness of postoperative surveillance. A study-population of patients with PDAC who underwent pancreatic resection with or without (neo)adjuvant chemo- and/or radiotherapy was deemed representative. Articles reporting on postoperative surveillance in patients with different pancreatic tumors, such as benign neoplasms, neuroendocrine tumors, or pancreatic metastases were excluded. No restriction in study design was made. Of original clinical studies, type of study, study period, methods, results and conclusions were extracted and summarized.

Results

Search Results

Five guidelines from different continents were evaluated, as well as the recently revised French, British and Dutch guidelines (Table 1). The literature search resulted in 570 titles and abstracts, which were screened for eligibility. After full-text assessment, twelve original studies were included (Table 2).

Guidelines on Follow-Up After Resection of PDAC

Recommendations on surveillance after radical resection of PDAC are conflicting, and

based on expert opinion and other low-level evidence (Table 1).[20-27] The European Society for Medical Oncology (ESMO) explicitly recommends no surveillance with regular additional testing, based on the poor prognosis upon diagnosis of disease recurrence and limited effective treatment options.[21] The International Association of Pancreatology/European Pancreatic Club (IAP/EPC) has, according to the 2016 guidelines, no recommendations for surveillance.[22] Recently, both the French and British guidelines were revised.[23,24] In contrast to the ESMO guidelines, the French guidelines now state that it might be beneficial to detect recurrence at an early stage, using serum CA 19-9 measurement when elevated at diagnosis, and a CT-scan of the chest, abdomen and pelvis every 3 months for 2-3 years, then every 6-12 months up to 5 years.[23] Within the guidelines of the UK National Institute for Health and Care Excellence on diagnosis and management of pancreatic cancer, it was stated that the committee was not able to make any recommendations about the method, frequency or duration of postoperative surveillance. Herewith, the guideline committee acknowledged the lack of evidence on whether detecting recurrence improves overall survival.[24] Similar to the British guidelines, recurrence-focused surveillance was not recommended within the revised 2019 National Dutch Guideline for pancreatic cancer (unpublished), considering the current lack of high-quality evidence on postoperative surveillance and recurrence treatment. Contrastingly, the National Comprehensive Cancer Network (NCCN; United States) and Japan Pancreas Society both recommend routine testing of serum carbohydrate antigen (CA) 19-9 levels and computed tomography (CT) imaging of the abdomen and pelvis, every 3-6 months for the first 2 years after surgery.[25,26] The American Society of Clinical Oncology (ASCO), however, states that the benefit of imaging is unclear. They recommend a surveillance existing of history and physical evaluation every 3-6 months, with addition serum CA 19-9 testing if this tumor marker was elevated pre-operatively.[27]

Serum Tumor Marker Testing

In current clinical practice, the most widely assessed serum biomarker after resection of PDAC is CA 19-9.[28,29] For the detection of PDAC recurrence, postoperative serum CA 19-9 has a moderate value with a sensitivity of 68-89% and a specificity of 77-89%.[30] It was found that CA 19-9 elevation could precede radiographic evidence of recurrence by three to six months.[31,32] In this context, it was suggested that CA 19-9 patterns during postoperative surveillance could be used in patient counselling, and benefits of tumor marker-guided treatment were shown. Patient interviews, however, have demonstrated that patients perceive detection of recurrence through CA-19-9 as the focal point of post-pancreatectomy surveillance, hampering discussions about other potential troublesome problems.[33] As such, they are considering not using this relatively unprecise tumor marker for the detection of recurrence.

Table 1. Current guidelines and follow-up recommendations

Continent	*Organization*	*Recommendation*	*Level of evidence*
Europe	ESMO (2015)	No recurrence-focused surveillance.	No evidence (4D)
	IAP/EPC (2016)	No recommendation.	Not applicable
	French clinical practice guidelines (2018)	(i) clinical examination, (ii) serum CA 19-9 testing when elevated at diagnosis, (iii) thoraco-abdomino-pelvic CT, every 3 months for 2-3 years, then every 6-12 months up to 5 years	Non-uniform expert opinion (2B)
	UK National Institute for Health and Care Excellence guidelines (2018)	No recurrence-focused surveillance.	Low (C)
	Dutch national guidelines (2019)	No recurrence-focused surveillance.	
North America	NCCN (2018)	History and physical examination for symptom assessment every 3-6 months for 2 years, then every 6-12 months as clinically indicated.	Uniform expert opinion (2A)
		CA 19-9 and follow-up CT scans (chest, abdomen, and pelvis) with contrast every 3-6 months for 2 years.	Non-uniform expert opinion (2B)
	ASCO (2017)	History and physical examination every 3-6 months after completion of therapy. Additional serum CA 19-9 if elevated pre-operatively.	Low (C)
		No recommendations regarding the use of imaging procedures, surveillance intervals and duration.	
Asia	JPS (2016)	Measurement of tumor markers and a dynamic CT scan every 3 to 6 months for 2 years post-operatively and every 6 to 12 months subsequently, at least for 5 years.	Low (C)
Australia	AGIGT (2016)	No recommendation.	Not applicable

ASCO, *American Society of Clinical Oncology*; AGIGT, *Australasian Gastro-Intestinal Trials Group*; ESMO, *European Society for Medical Oncology*; IAP/EPC, *International Association of Pancreatology/European Pancreatic Club*; JPS, *Japan Pancreas Society*; NCCN, *National Comprehensive Cancer Network*.

More recently, there has been increasing interest in the use of liquid biopsies in the form of circulating tumor DNA as potential novel biomarkers that could aid in the detection of PDAC recurrence.[34,35] Despite excitement about the potential uses of liquid biopsies and the rapid improvement in technology, a joint review by the ASCO and College of American Pathologists state that there currently insufficient evidence of clinical validity and utility for the majority of liquid biopsy assays in oncology care outside of study purposes.[36]

Imaging Procedures

to be the preferred imaging modality.[25,37,38] A meta-analysis on the diagnostic performance of CT and PET-CT imaging for the detection of PDAC recurrence showed that CT alone has a moderate diagnostic value with a sensitivity of 70% and a specificity of 80%; PET-CT alone showed a sensitivity and specificity of 88% and 89%, respectively, and for a combination of PET-CT and CT, a sensitivity of 95% and specificity of 81% was found.[39] Only a few small retrospective cohort studies reported on imaging as part of a standardized surveillance strategy; most studies used imaging procedures only when recurrent disease was clinically suspected.[40-42] In the context of standardized surveillance with regular imaging, diagnostic accuracy values of CT and PET-CT were found to be similar compared to values of imaging procedures performed when recurrence was clinically suspected.[39] In a study by Balaj et al., early local recurrence of PDAC was detected in the first 12 months following surgery in 69% of patients (n=33), with an average of 1.5 scans (range 1-3). The authors suggested that routine CT monitoring during the first year after surgery, using an interval of three months, may be appropriate to detect early local PDAC recurrence and provide treatment for local recurrence.[37]

Survival

Few studies have been published evaluating the consequences of radiologic surveillance on survival after resection of PDAC (Table 2). Furthermore, existing studies were retrospective, small and often reported contradictory results. Witkowski et al. performed a retrospective study to determine the potential survival benefit of abdominal imaging after pancreatic cancer resection.[43] In 222 patients with a superior OS of more than 51 months, survival was not improved in patients who received annual abdominal CT scans (n=72) compared to those who did not (n=150). For this subgroup analysis no survival data or hazard ratios were reported by the authors.[43] Furthermore, Tzeng et al. evaluated the cost-effectiveness of five surveillance strategies using a Markov model, populated with data from patients who had received intensive surveillance. They showed that an increased frequency and intensity of postoperative surveillance beyond 6-monthly clinical evaluation and CA 19-9 testing increased the costs, without survival benefits.[44] Samawi et al. found that multimodality surveillance at specialized cancer centers resulted in earlier detection of PDAC recurrence, although a survival benefit could not be demonstrated.[45] However, it was also shown that patients with more advanced tumors were more likely to be seen at cancer centers, potentially having a worse a priori prognosis compared to patients who received follow-up at primary care physicians. Nevertheless, the authors stated that primary care physicians may play a larger role in postoperative surveillance for selected low risk patients.[45]

Conversely, other studies reported data that could support a surveillance strategy with regular CT imaging. Two retrospective observational studies by Nordby et al. and Tzeng et al. showed that patients with asymptomatic PDAC recurrence, detected during systematic CT-based surveillance, had an improved survival and received oncological treatment more frequently.[16,17] Nordby et al. found a median OS of 24.5 months in patients with asymptomatic PDAC recurrence, compared to 11.0 months in patients with symptomatic recurrence ($P<0.0001$), with respectively 72% and 37% of patients undergoing further treatment.[16] Moreover, Tzeng et al. showed a median OS of 29.6 and 18.0 months in asymptomatic and symptomatic patients ($P=0.003$). Further therapy was administered in 91.2% of patients with asymptomatic recurrence and in 61.4% of patients with symptomatic recurrence.[17] They both stated that the detection of asymptomatic recurrence on CT surveillance might identify patients with good performance status and tumor biology that are most likely to benefit from subsequent treatment. Furthermore, it was mentioned that detection of recurrent disease by CT may facilitate patient inclusion in clinical trials.[16,17]

Moreover, an observational study among 85 patients in whom PDAC was resected showed that even when a symptomatic surveillance strategy without routine imaging was utilized, the majority of patients underwent additional imaging procedures for the detection of recurrence at least once. Although patients received an average of three imaging procedures, most recurrences were detected at a late stage and after the manifestation of clinical symptoms. In this observational cohort study, only 17 of 68 patients (25%) underwent treatment for recurrence, which was found to be an independent predictor for prolonged survival after recurrence.[46] It was argued that the imaging procedures could be used more effectively in the context of a recurrence-focused surveillance, with a 3- or 6-monthly interval, possibly increasing the number of patients eligible for treatment of PDAC recurrence.[46]

Table 2. Results of articles reporting on the consequences of routine imaging surveillance

Author	*Design*	*n*	*Methods*	*Results*	*Conclusion*
Witkowski *et al*. (2011)	Retrospective cohort study (1991-2005)	2217	Mean number of imaging procedures in 1991 vs. 2005.	3.0 vs. 6.0 procedures ($P<0.0001$)	Substantial increase in application of complex imaging.
			Mean annual rate of CT scans (1991 vs. 2005), adjusted for number of months of survival.	2.9 vs. 5.1 procedures ($P<0.0001$)	
			Survival analysis was performed in patients with superior OS (>51 months; $n=222$); patients who received annual abdominal CT scans ($n=72$) were compared to those who did not ($n=150$).	NA, $P=0.008$	No survival benefit of routine imaging.
Tzeng *et al*. (2012)	Retrospective cohort study (1998-2008)	327	All patients had imaging surveillance. OS and further therapy were compared in patients with asymptomatic ($n=118$) vs. symptomatic recurrence ($n=98$).	Median OS: 29.6 vs. 18.0 months ($P=0.003$)	OS was longer in asymptomatic patients.
				Further therapy was administered in 91.2% vs. 61.4% ($P<0.001$)	Treatment was administered more frequently to asymptomatic patients.
			PROS was compared in treated ($n=157$) vs. untreated patients ($n=59$).	Median PROS: 11.8 vs. 2.6 months ($P<0.001$)	Administration of further therapy was associated with longer PROS.
Tzeng *et al*. (2013)	Retrospective cohort study (1998-2008)	254	The cost-effectiveness of 5 surveillance strategies was compared using a Markov model, populated with data from patients who had received intensive surveillance.	A limited 6-month strategy* has a significantly lower ICER than other standardized strategies	Increased frequency and intensity of surveillance beyond 6-monthly clinical evaluation and CA 19-9 tests increases costs, without survival benefits.
Nordby *et al*. (2015)	Retrospective cohort study (2000-2010)	164	All patients underwent imaging every 6 months or in case of suspicious symptoms. OS and further therapy were compared in patients with asymptomatic ($n=29$) vs. symptomatic ($n=115$) recurrence.	Median OS was 24.5 vs. 11.0 months ($P<0.0001$)	Patients with asymptomatic recurrence have an improved survival.
				Treatment was administered in 72% vs. 37%	Detection of asymptomatic recurrence may facilitate patient eligibility for treatment.
Deobald *et al*. (2015)	Qualitative longitudinal descriptive study with semi-structured interviews.	15	Individual interviews were undertaken to assess patient and clinician attitudes towards surveillance, as well as perceived benefits and challenges.	FU visits help patients to fulfil their need for reassurance and desire to know whether recurrence occurs, without experiencing difficulties with the FU process. Clinicians recognize their desires.	
				A considerable contrast exists between patients' understanding and expectations, and clinicians' perspectives regarding disease and prognosis.	

Table 2. Results of articles reporting on the consequences of routine imaging surveillance (continued)

Author	*Design*	*n*	*Methods*	*Results*	*Conclusion*
Tjaden *et al*. (2016)	Prospective cohort study (2012)	184	All patients underwent imaging every 3 months for 2 years and every 6 months thereafter. FU data was analysed with a focus on symptoms, radiological findings and therapeutic consequences in terms of supportive and cancer-directed therapies.	In 49% of FU visits, oncological or diabetes management was changed, or symptom-directed therapy was induced or modified to improve QoL. PDAC recurrence was asymptomatic in 55/75 patients (74%). All patients with recurrence underwent cancer-directed treatment.	Structured surveillance is important to optimize (symptom-directed) treatment and outcome. FU imaging is important because recurrence is often asymptomatic, although its detection allows for treatment and potentially improved prognosis.
Elmi et al. (2017)	Retrospective cohort study (2005-2011)	229	Survival was compared in patients who underwent regular imaging surveillance (IS) (n=163) vs. those with clinical FU only (C)** (n=66) in a multivariable model.	Median OS: 30.4 ± 3.85 vs. 17.1 ± 2.42 months (P=0.002)	Routine imaging surveillance is associated with prolonged OS in a multivariable model.
Groot et al. (2017)	Prospective cohort study (2011-2015)	85	The number of imaging procedures to detect PDAC recurrence was determined in a setting where imaging was not performed routinely (symptomatic FU). The pattern, timing, and treatment of recurrent PDAC during symptomatic FU were assessed.	74 patients (87%) underwent at least one imaging procedure, with a mean amount of 3.1 ± 1.9 imaging procedures. 85% of patients with recurrence had symptoms. 25% of patients underwent treatment for recurrence, which was associated with a longer PROS (HR 0.32, 95% CI 0.16-0.65, P=0.002).	Although symptomatic FU does not include routine imaging, most patients underwent imaging for the detection of recurrence at least once. Most recurrences were detected at a late stage and after the manifestation of clinical symptoms, potentially limiting patients to undergo additional treatment for recurrence.
Rieser et al. (2018)	Prospective cohort study (2010-2016)	525	CA 19-9 was measured at 6-monthly intervals. Recurrence was defined by radiographic evidence. CA 19-9 levels were correlated with RFS and OS.	CA 19-9 had a poor PPV (35%) but a high NPV (92%) for radiographic recurrence. CA 19-9 elevation predicted subsequent RFS at each time point.	High CA 19-9 frequently may precede recurrence on imaging by more than 6 months. CA 19-9 patterns predict RFS and OS, which could be used in patient counselling and to direct protocols of salvage chemotherapy.

Table 2. Results of articles reporting on the consequences of routine imaging surveillance (continued)

Author	*Design*	*n*	*Methods*	*Results*	*Conclusion*
Dengsø et al. (2018)	Qualitative longitudinal descriptive study with	184	Interviews were undertaken to explore patients' experiences of and perspectives on surveillance within the first year after curative treatment of cancer in the pancreas, duodenum or bile-duct.	Patients experienced monitoring of CA 19-9 for the detection of recurrence as the main point of FU. Consultations were influenced considerably by the test results and implications of these, leaving discussions of other potential patient-important symptoms insufficiently addressed. The authors consider not using this relatively unprecise tumor marker for relapse in the future.	
Samawi et al. (2018)	Retrospective cohort study (2001-2015)	147	Surveillance strategies were classified: (1) Surveillance at cancer centers (CC, 66%) with regular lab tests, and/or imaging. (2) Surveillance at primary care (PCP, 44%). Patient and disease characteristics, percentage of patients receiving chemotherapy for recurrence, OS and RFS were compared in the CC vs. the PCP group.	Percentage of patients with T3/4 tumors was 78% vs. 62% (P=0.03). Percentage of patients receiving chemotherapy for recurrence was 58% vs. 34% (P=0.03). No difference in OS (HR 1.23; 95% CI 0.74-2.04, P=0.40); RFS favoured the PCP group (HR 1.62; 95% CI 1.01-2.56, P=0.04 for the CC group).	Patients with more advanced tumors were more likely to be seen at CC. Surveillance tests and imaging performed by CC detected recurrences earlier, but did not result in OS differences. PCP may play a larger role in the FU care of selected low risk patients
Li et al. (2019)	Prospective cohort study (2014-2017)	80	All patients underwent CA 19-9 testing and imaging every 3 months. RFS and OS were compared in patients who started treatment based on rising CA 19-9 (without radiographic confirmation) vs. patients who did not start until radiographic confirmation of recurrence.	60 patients (75%) had CA 19-9 elevation that preceded radiographic recurrence by ± 3 months. Median RFS: 23.6 vs. 12.1 months (P<0.001) Median OS: 28.1 vs. 20.7 months (P=0.049)	CA 19-9 could precede recurrence confirmed by radiographic examinations. Tumor marker-guided treatment can significantly prolong RFS and OS in patients under surveillance after pancreatic resection.

*A limited 6-month strategy existed of clinical evaluation and CA 19-9 testing with a 6-monthly interval, and imaging in case of symptoms, clinical findings or elevation of CA 19-9.

FU, *follow-up*; ICER, *incremental cost-effectiveness ratio*; NPV, *negative predictive value*; OS, *overall survival*; PDAC, *pancreatic ductal adenocarcinoma*; PPV, *positive predictive value*; PROS, *post-recurrence overall survival*; QoL, *Quality of Life*; RFS, *recurrence-free survival*.

Recently, a comparative retrospective study by Elmi and colleagues found that routine imaging surveillance after pancreatectomy, compared to symptom-oriented imaging surveillance, was associated with a prolonged overall post-operative survival in a multivariable model (30.4 months vs. 17.1 months; P=0.002).[18] The mean interval for diagnosis of recurrent disease in the routine imaging surveillance group was significantly shorter and more patients received chemotherapy for recurrent disease. The authors argue that the possibility for more aggressive treatment at an early stage could therefore have contributed to improved survival rates, and consequently suggested that CT-imaging at 3-4 months interval can be beneficial.[18]

Quality of Life

Previous studies showed that patients undergoing postoperative surveillance with regular diagnostic testing exhibit significant anxiety and fear of cancer recurrence, with a substantial impact on quality of life (QoL).[47] In general, however, patients seem to desire active surveillance after resection of PDAC, and clinicians support an active surveillance as well.[47] Unfortunately, no studies were found to evaluate the direct impact of recurrence-focused surveillance on QoL in PDAC patients. Only one study by Tjaden et al. reported on a potential QoL benefit of standardized surveillance. They found that in 49% of all surveillance-related visits, symptom-directed, oncological or diabetes treatment was induced or modified to improve QoL.[48]

Discussion

Currently improved systemic and local treatment options for PDAC might warrant early diagnosis of cancer recurrence. Since it can be difficult to obtain histological evidence of PDAC recurrence, a combination of a rise in serum tumor markers and suspicious, progressive findings on imaging could aid in the early clinical diagnosis. However, current evidence for potential survival benefits of early detection and treatment of PDAC recurrence is limited and contradictory Consequently, high-quality evidence is lacking to provide recommendations for surveillance exists.

The hypothesis that recurrence-focused surveillance has survival benefits follows the assumption that detection of disease recurrence at an early stage improves the eligibility to undergo treatment and allows for a timely start of further treatment while the tumor burden is still low. However, as the efficacy of recent treatments is poorly evaluated in patients with PDAC recurrence, survival benefits of treatment for recurrence are yet unclear. Given this lack of evidence showing beneficial effect, ethical concerns remain that the early detection of recurrent PDAC may compromise quality of life and patient reported outcomes. A survey undertaken by the Dutch Pancreatic Cancer Group among Dutch pancreatic surgeons

assessed their current practice of surveillance and motivation to participate in new studies concerning the detection and treatment of PDAC recurrence.[49] It was shown that current surveillance strategies of Dutch pancreatic surgeons mainly consist of regular outpatient visits with physical examination, in accordance with the European guidelines. The omission of standard tumor marker testing and imaging resulted from the belief that there exists a lack of effective therapies for local and distant recurrence. Because therapeutic consequences of recurrence diagnosis were stated to be minimal, mentally burdensome surveillance was not deemed justifiable.[49] On the other hand, it has been shown that a standardized surveillance can contribute to the induction or modification of symptom-directed clinical management to improve quality of life for symptomatic patients.[47,50,51] Furthermore, early detection of (asymptomatic) PDAC recurrence could facilitate patient recruitment in intervention studies to evaluate the potential benefits of (early) treatment. This might contribute to the expansion of treatment possibilities for patients with recurrent PDAC. To diminish unrealistic expectations of treatment benefits, however, it is highly important that patients are aware of the current limitations of the detection and treatment of PDAC recurrence.[16]

Potential benefits of recurrence-focused surveillance have been investigated in patients who underwent treatment with curative intent for different types of cancer, such as epithelial ovarian cancer and colorectal cancer.[52-54] In these cancer types, prospective studies showed no clear disease-specific survival benefit of multimodality surveillance with a certain intensity, and the cost-effectiveness of recurrence-focused surveillance remains unclear. Therefore, the importance and consequences of active surveillance in these patients is increasingly being questioned. An individualized approach to identify patients who potentially benefit most from early detection and treatment of colorectal or ovarian cancer recurrence was recommended. Furthermore, it was concluded that surveillance should be terminated if the likely benefits no longer outweigh the potential risks and disadvantages, such as morbidity from diagnostic testing, emotional distress and monetary costs.[53] For PDAC patients, unfortunately, the risk of disease recurrence after primary resection is relatively high when compared to these other cancers. Therefore, if treatment of PDAC recurrence actually improves survival, recurrence-focused postoperative surveillance might be more advantageous for these patients. As prospective studies on surveillance after PDAC resection are currently lacking, however, definite conclusions about the utility of a recurrence-focused surveillance in these patients cannot be made. This absence of evidence reflects an urgent need of high-quality studies to help guide surveillance recommendations.

Current research focuses on the development of new tests to improve prognostication in patients undergoing PDAC resection, such as circulating tumor DNA (ctDNA) and new

biomarkers like ADAM12.[36,55] These promising detection techniques might prove to be helpful during postoperative surveillance as well, as they can potentially contribute to the identification of patients with a high risk of recurrence, who might benefit most from an intensified surveillance. For clinical applicability, further development of these detection methods is needed.

Conclusion

Expert and high-volume pancreatic centers worldwide increasingly implement standardized postoperative surveillance with serum tumor marker testing and routine imaging in PDAC patients. However, the risk-benefit and cost-effectiveness of recurrence-focused surveillance is still unclear. Therefore, potential side-effects have to be considered, including psychosocial harm, morbidity related to diagnostic testing as well as the economic burden that comes with frequent additional testing and treatment. In this context, patients who are most likely to benefit from early detection of recurrent disease, i.e. patients who are able and willing to undergo treatment, need to be identified. Future well-designed prospective studies are exceedingly needed to define the clinical merit as well as economical and psychosocial implications of recurrence-focused surveillance after resection of PDAC.

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CHAPTER 9

Current Strategies for Detection and Treatment of Recurrence of Pancreatic Ductal Adenocarcinoma After Resection: Nationwide Survey

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Abstract

Objective

Consensus regarding follow-up strategies after resection of pancreatic ductal adenocarcinoma (PDAC) is lacking. The aim of this study was to evaluate current clinical practice and opinion on follow-up for PDAC and treatment of isolated local recurrence was among Dutch pancreatic surgeons.

Methods

An online survey was sent to all surgeons of the Dutch Pancreatic Cancer Group. Questions related to actual follow-up protocol, opinion on imaging and tumor marker testing and treatment of isolated local recurrence.

Results

Thirty-nine pancreatic surgeons from all 20 hospitals in the Netherlands that perform surgery for PDAC responded, yielding a response rate of 100%. Most surgeons (91%) used a follow-up scheme consisting of outpatient clinic visits with physical examination on indication. Routine imaging (11%) or tumor marker testing (9%) were seldom performed. The most used follow-up strategy consisted of appointments at 3, 6, 12, 18, 24, 30, 36, 48 and 60 months. Nearly all surgeons (n=37; 95%) were willing to participate in a new national multi-institutional study concerning the detection and treatment of (asymptomatic) recurrence.

Conclusion

Imaging and tumor markers are rarely used in the follow up after resection of PDAC in the Netherlands. Further prospective studies should demonstrate whether improved (adherence to) follow-up strategies and standardized treatment of asymptomatic recurrence could improve overall survival.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related mortality in Europe, with 43.400 deaths predicted for 2017.[1,2] It is often seen as a systemic disease with over 50% of all patients having evidence of metastatic spread at the time of diagnosis.[3] Even after radical resection, up to 80% of patients will experience disease recurrence.[4-6] Detection and treatment of disease recurrence therefore offers a potential strategy to improve overall survival in PDAC. Current national and international PDAC guidelines regarding surveillance and follow-up after resection of PDAC are based on expert opinion and other low-level evidence, as no randomized controlled trials or other high-quality evidence exist to guide recommendations.[7-13]

American guidelines seem to favor a more intensive approach to follow-up, with the National Comprehensive Cancer Network recommending routine testing of serum carbohydrate antigen (CA) 19-9 levels and CT imaging of the abdomen and pelvis every 3-6 months for the first 2 years after surgery.[8] Contrarily, the European Society for Medical Oncology explicitly does not recommend follow-up with regular additional testing, based on the poor prognosis upon diagnosis of disease recurrence and the lack of impact of regular follow-up on the survival outcome.[9] Instead of focusing on disease recurrence (oncologic follow-up), they argue that surveillance should concentrate on symptoms, nutrition and psychosocial support (supportive follow-up).

Management of recurrent PDAC is less well established as it is for other stages of PDAC, despite that the majority of patients will experience disease recurrence after pancreatic resection. The lack of effective therapeutic options for this significant group of patients seems to have led to a certain nihilistic stance towards the detection of disease recurrence during follow-up after resection.[14] However, the emergence of more potent treatments for PDAC, such as FOLFIRINOX, have led to a rising interest in follow-up strategies and the early diagnosis and treatment of recurrent pancreatic cancer.[15] Especially in patients who develop isolated local recurrence after a prolonged disease-free interval, additional therapy is sometimes considered.[16]

The most recent Dutch guidelines for pancreatic cancer care currently do not contain recommendations for follow-up frequency, methodology or focus.[12] Furthermore, they do not contain recommendations for the treatment of recurrent PDAC. Therefore, a survey was undertaken among Dutch pancreatic surgeons to assess their current practice of follow-up and opinion on diagnostic methods used during follow-up. The motivation of the respondents

to participate in new studies concerning the treatment of isolated local recurrence after radical pancreatic resection of PDAC was also evaluated.

Methods

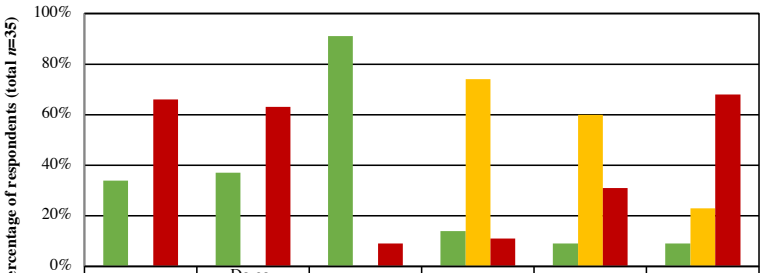
All surgeon members of the Dutch Pancreatic Cancer Group (DPCG) were requested to complete an online web-based survey using SurveyMethods® (www.surveymethods.com). The survey was conducted between February and October 2016. Non-responders received up to three reminders and were contacted by telephone once.

The survey included 17 questions, with a total of four questions with open answers that were categorized afterwards. The first six questions related to indications and contraindications for follow-up of patients after pancreatic resection of PDAC. The following three questions related to the use of serum CA 19-9 testing, serum carcinoembryonic antigen (CEA) testing and imaging monitoring during follow-up. A further three questions with open answers inquired into the current practice of follow-up frequency and opinion on methodology as well as perceived primary and secondary goals of follow-up. The second part, consisting of the last five questions of the questionnaire, focused on the opinion on and attitude towards possible treatment options for isolated local recurrence of PDAC after pancreatic resection. Lastly, the opinion and feasibility for a new trial for patients with recurrence was sought. Variables were processed using SPSS Statistics v22.0 (IBM Corporation, Armonk, NY, USA). Data were reported as number with percentage.

Results

Thirty-nine surgeons from all 20 hospitals in the Netherlands that perform surgery for PDAC responded, resulting in a response rate of 100%. Four surgeons (10%) declared that they did not directly participate in the follow-up care of resected patients and only completed the second part of the survey relating to the treatment of isolated local recurrence.

When questioned about indications and contraindications for follow-up after pancreatic resection, 12 surgeons (34%) regarded the age of the patient as a contributory factor when deciding on follow-up frequency and methodology. A more intensive follow-up strategy was advocated to relatively young patients and follow-up in reduced form was deemed justifiable for patients aged 80 years and older. Thirteen surgeons (37%) advised patients with significant co-morbidities to undergo less intensive follow-up. An often-used argument for this position was that patients with low performance status would not be able to undergo any further palliative treatment if recurrence was detected. A positive resection margin was not seen as



	Does patient age influence FU strategy?	Do co-morbidities influence FU strategy?	FU at standardized time points?	Routine use of imaging during FU?	Routine use of CA 19-9 during FU?	Routine use of CEA during FU?
Yes	34%	37%	91%	14%	9%	9%
Only when recurrence is suspected				74%	60%	23%
No	66%	63%	9%	11%	31%	68%

Figure 1. Use of imaging monitoring and serum tumor markers during follow-up after resection of pancreatic ductal adenocarcinoma in the Netherlands.

an indication or contraindication for follow-up, as only 5 surgeons (14%) let margin-status influence their decision-making.

In Figure 1, the current practice of imaging monitoring and serum tumor marker testing during follow-up is shown. Most pancreatic surgeons (91%) used a follow-up scheme that consisted of clinic visits at more or less standardized time points. Standard imaging (11%), CA 19-9 testing (9%) or CEA testing (9%) were seldom performed during these clinic visits. When recurrence was suspected during follow-up, 74% and 60% of surgeons advised imaging and CA 19-9 testing, respectively. CT imaging was the modality of choice for all surgeons, with magnetic resonance imaging (MRI) and fluorodeoxyglucose positron emission tomography (FDG-PET) named as possible additional options.

Table 1 shows the percentage of surgeons who adhered to a certain follow-up test at a specific moment. In general, clinic visits including a physical examination were scheduled frequently, especially during the first three years of follow-up. Three surgeons (9%) indicated that they discharged patients from follow-up after three years, while only four (11%) continued regular clinic visits after five years. The most used follow-up strategy consisted of appointments at 3, 6, 12, 18, 24, 30, 36, 48 and 60 months, with discharge from further follow-up after this period. No follow-up scheme consisted of more than four clinic visits every 12 months.

Different primary goals for follow-up were identified. The majority of surgeons (60%) regarded the primary goal of follow-up to be supportive patient care (supportive follow-up). More specifically, this included identifying and treating post-operative complications, monitoring pancreatic endocrine/exocrine insufficiency and nutritional status, patient guidance and

Table 1. Scheme of the current practice of follow-up after resection of pancreatic ductal adenocarcinoma in the Netherlands

Year	*1*				*2*				*3*		*4*		*5*		*>5*	
Month	*3*	*6*	*9*	*12*	*3*	*6*	*9*	*12*	*6*	*12*	*6*	*12*	*6*	*12*	*6*	*12*
Physical examination	**77**	**86**	63	**91**	37	**80**	31	**91**	57	**86**	31	**80**	26	**80**	3	11
Computed tomography imaging	6	6	3	11	3	3	3	11	3	9	0	9	0	9	0	0
Carbohydrate antigen 19-9 + carcinoembryonic antigen	6	9	6	9	6	9	6	9	9	9	0	9	0	9	0	0

In each box, the percentage of surgeons that carries out the examination at that time is given. All percentages >75% are in bold.

reassurance, and guiding in end of life decision-making. Seven other surgeons (20%) viewed the primary goal of follow-up to be based more on further therapeutical care (oncologic follow-up), such as early detection and treatment of local and distant recurrence and planning of palliative treatment options. The remaining 20% of surgeons named a combination of supportive and therapeutical goals. The last part of the survey focused on the opinion and attitude towards treatment of isolated local recurrence after resection. The respondents estimated that a median number of 5 (range, 1-30) patients with isolated local recurrence were seen every year per institution. Current treatment options for isolated local recurrence used by the surgeons consisted of chemotherapy (86%), stereotactic body radiation therapy (26%) and conventional radiotherapy (18%). Mentioned requirements for localized treatment were a prolonged disease-free interval before diagnosis of isolated local recurrence, stability of local disease and lack of emergence of distant metastases after a period of chemotherapy and a minimally invasive approach. Nearly all surgeons (n=37; 95%) were willing to participate in a new national multi-institutional study concerning the detection and treatment of (asymptomatic) recurrence.

Discussion

This survey reflects the lack of a standardized approach to follow-up after resection of PDAC. Furthermore, it reveals the doubts and uncertainty on the role of imaging and tumor marker testing during follow-up. Several goals for follow-up after resection of PDAC can be discerned.[17,18] First, timely re-evaluation after pancreatic resection is essential to identify and treat long term post-operative complications, monitor nutritional status of the patient and regulate endocrine- and exocrine insufficiency. Second, initial follow-up allows for audit and quality control of the performed surgical procedure. Third, follow-up plays an important role in the reassurance and psychosocial support of patients. Last, follow-up with tumor marker testing and imaging might detect asymptomatic tumor recurrence, which may lead to

additional therapy or facilitate patient recruitment into investigational studies. Also, accurate recurrence data can benefit new research concerning, for instance, novel biomarkers.[19] However, these possible beneficial results should always be considered in the context of both the economic and emotional burden of extensive follow-up for the patient.[7]

The current literature and available guidelines seem to agree that a standardized approach to follow-up is warranted for the post-surgical care, nutritional and psychosocial support of pancreatic cancer patients after pancreatic resection.[7,20] However, opinions, evidence and current practice differ when it comes to adding further diagnostic testing for the detection of PDAC recurrence. This study shows that the current follow-up strategies of Dutch pancreatic surgeons, in accordance with European guidelines, mainly consist of regular outpatient visits with physical examination. Although additional serum tumor marker testing was often performed when a recurrence was suspected, standard CA 19-9 and CEA monitoring was only performed by a minority of surgeons. The omittance of standard biomarker testing might be caused by the low confidence in the value of CA 19-9 and CEA as accurate tumor markers for disease recurrence. For recurrence diagnosis during follow-up after surgery for PDAC, CA 19-9 seems to be the most suitable tumor marker. When serum CA 19-9 levels normalize after resection, an increase can indicate tumor recurrence.[21-23] Failure of normalization of CA 19-9 after resection often indicates the presence of radiological occult metastatic disease.[24] As a biomarker, however, CA 19-9 has several limitations. For instance, approximately 6% of the white population and 22% of black population in the United States do not produce CA 19-9.[25]

Additional imaging (74%) during follow-up was often performed when recurrence was suspected based on symptoms. However, similarly to tumor marker testing, standard radiologic surveillance was seldom performed during follow-up clinic visits. Detailed and prospective studies on the role of imaging during follow-up after resection of PDAC are limited. Tzeng et al. retrospectively reviewed 327 patients who underwent pancreatoduodenectomy for PDAC.[26] Following completion of all therapy, follow-up consisted of re-evaluation every 3-4 months with a physical examination, serum CA 19-9 testing, chest radiograph and abdominal CT scan for the first two years. Two hundred sixteen patients (66%) with recurrence were detected, of whom 118 (55%) were asymptomatic. These asymptomatic patients were able to receive treatment more often than symptomatic patients (91% vs. 61%; $P<0.001$) and had a longer survival after recurrence (13 months vs. 5 months; $P<0.001$). In a different study, in which a chest and abdominal CT scan was performed every 6 months after pancreatic resection of PDAC, 144 of 164 patients (88%) were diagnosed with recurrent disease.[27] In this cohort, only 29 patients (20%) had asymptomatic recurrence. Nevertheless, asymptomatic recurrence was similarly associated with longer survival after recurrence (10 months vs.

4 months; P<0.0001). Also, 72% of asymptomatic patients went on to receive further oncological treatment, compared to only 37% of symptomatic patients.

When asked about their opinion on primary and secondary goals of follow-up, the views of Dutch pancreatic surgeons in general reflect the philosophy of the European guidelines. An often-reiterated opinion was that follow-up should primarily focus on management of post-surgical complications, exocrine- and endocrine insufficiency, nutritional status and psychosocial support of the patient. Consequently, it was their widespread conviction that follow-up schemes should be established based on the patient's wishes through the process of shared decision-making. The omission of standard tumor marker testing and imaging during follow-up was the result of the belief that there exists a lack of effective therapies for local and distant recurrence. As therapeutical consequences of recurrence diagnosis are currently minimal, mentally and financially burdensome follow-up was not deemed justifiable. However, several surgeons did argue that with the emergence of more effective systemic and local therapies, a renewed look into the effectiveness of follow-up and impact on survival and quality of life might be warranted.

For the diagnosis of asymptomatic tumor recurrence to be meaningful, (1) there should be an accurate way of diagnosing asymptomatic recurrence, (2) there should be an effective intervention available to treat recurrent disease, either proven or in the form of a clinical trial, with the intent to improve overall survival or quality of life, and (3) patients should be able to undergo this intervention when the recurrence is diagnosed.[17] Current controversy around standard tumor marker testing and imaging in follow-up especially revolves around the apparent lack of therapeutic consequences of recurrence diagnosis. Tzeng et al.[26] showed in their study that over 50% of patients can be diagnosed with recurrence before onset of clinical symptoms with a surveillance regimen combining CA 19-9 testing with abdominal CT-scans every 3-4 months. However, in a more recent cost-effectiveness analysis by the same authors, Tzeng et al.[28] found that additional routine imaging every 6 months increased total cost of follow-up by $3465 without increasing overall survival. This study further argued that post-operative surveillance consisting of clinical evaluation and CA 19-9 testing every six months is the most cost-effective strategy. This lack of impact on overall survival was also recognized by a retrospective analysis of the Surveillance, Epidemiology, and End Results (SEER)-Medicare database by Witkowski et al.[29] This analysis showed that, although the median number of imaging studies doubled between 1991-2005 (from three to six), there was no associated survival benefit in patients who underwent routine annual imaging within 5 years of resection.[7] Proponents of systematic follow-up, with tumor marker testing and imaging, argue that current guidelines may be outdated due to treatment advances (e.g. FOLFIRINOX) possibly improving

survival outcomes.[18] Although the current evidence remains slim, several studies have shown improved survival when treating recurrent disease. In the studies by Tzeng et al.[26] and Nordby et al.[27], patients with asymptomatic recurrence received more additional treatment and had a longer survival after recurrence when compared to symptomatic patients. Meyers et al. retrospectively grouped patients into three categories: group 1 received treatment after recurrence (n=45), group 2 was not offered treatment (n=9), and group 3 received no treatment due to poor performance status (n=16).[30] The median survival after recurrence was 10 months, 6 months, and 1 month, respectively, for the three groups ($P<0.0001$). Xue et al. compared patients with recurrent PDAC after radical resection (n=83) with 186 patients with an initial diagnosis of unresectable disease, receiving palliative chemotherapy mainly consisting of Gemcitabine.[31] The overall survival was longer for the patients with recurrent PDAC (383 days vs. 308 days; $P<0.01$), showing that treatment for recurrent PDAC is warranted and might lead to extended survival.

For a recent and detailed study regarding structured follow-up after pancreatic surgery, Tjaden et al. prospectively collected data of all post-operative visits during a one-year period at the University of Heidelberg (Germany).[14] Surveillance consisted of clinic visits every 3 months post-operatively, including CEA testing, CA 19-9 testing and cross-sectional imaging with CT or MRI. Analysis of symptoms, diagnostic procedures and therapeutic consequences showed that 55 of 74 (74%) of detected recurrence occurred in asymptomatic patients. For the 74 patients with recurrence, de novo chemotherapy was recommended for 37 patients (50%), a change of the present chemotherapy regimen in 15 patients (21%) and combined radio-chemotherapy in 13 patients (18%). Eleven patients (15%) with localized cancer recurrence underwent a reoperation. Concluding, the authors argued that imaging during follow-up is advisable, especially in PDAC patients who may develop asymptomatic recurrence, as diagnosis can result in major therapeutical consequences.

Conclusion

The lack of guidelines regarding follow-up results in different strategies among pancreatic surgeons in the Netherlands. Current evidence on the benefit of additional imaging and tumor marker testing for the detection and treatment of recurrence remains limited. However, detection of recurrent PDAC during follow-up will facilitate patient recruitment into investigational studies and recurrence data can benefit novel research. The high motivation among Dutch pancreatic surgeons to participate in a new study for the detection and treatment of recurrence appears to support this, making a national trial achievable.

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PART IIIa

Imaging Surveillance

CHAPTER 10

The Diagnostic Performance of CT Versus FDG PET-CT for the Detection of Recurrent Pancreatic Cancer: A Systematic Review and Meta-Analysis

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Abstract

Objective

Radiologic surveillance after resection of pancreatic ductal adenocarcinoma (PDAC) can provide information on the extent and location of disease recurrence. This systematic review and meta-analysis aim to give an overview of the literature on the diagnostic performance of different imaging modalities for the detection of recurrent disease after surgery for PDAC.

Methods

A systematic search was performed in PubMed, EMBASE and Cochrane Library up to 20 December 2017. All studies reporting on the diagnostic value of imaging modalities for the detection of local and/or distant disease recurrence during follow-up after resection of PDAC were eligible. Both histologic confirmation of recurrent PDAC and clinical confirmation by disease progression on follow-up imaging were considered as suitable reference standard. The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool was used for critical appraisal of methodological quality. Diagnostic accuracy data were extracted or calculated and presented in forest plots. A bivariate random-effects model was used to calculate pooled estimates of sensitivity and specificity.

Results

A total of seven retrospective studies with 333 relevant patients were ultimately eligible for data extraction. Overall, the methodological quality of the included studies was acceptable. All seven articles described test results of contrast-enhanced CT, whilst five and three articles reported outcomes on diagnostic accuracy of FDG PET-CT and FDG PET-CT combined with contrast-enhanced CT, respectively. For CT, pooled estimates for sensitivity were 0.70 (95% CI 0.61-0.78) and for specificity 0.80 (95% CI 0.69-0.88). For FDG PET-CT, pooled estimates for sensitivity and specificity were 0.88 (95% CI 0.81-0.93) and 0.89 (95% CI 0.80-0.94), respectively. For FDG PET-CT in combination with contrast-enhanced CT, pooled estimates for sensitivity were 0.95 (95% CI 0.88-0.98) and for specificity 0.81 (95% CI 0.63-0.92).

Conclusion

According to the current literature, post-operative CT has a moderate diagnostic accuracy in the detection of recurrent disease. FDG PET-CT imaging could be of additional value when disease recurrence is suspected despite negative or equivocal CT findings. Nevertheless, evidence supporting radiologic surveillance after resection of PDAC is limited. Future prospective studies are needed to optimize surveillance strategies after resection of pancreatic cancer.

Introduction

Despite many efforts towards improvement, the prognosis of pancreatic ductal adenocarcinoma (PDAC) remains dismal. To date, radical resection followed by adjuvant chemotherapy provides the best chance for long-term survival.[1] However, even after pancreatic resection, only 12-27% of patients manage to achieve 5-year survival.[2-5] Main reasons for this limited survival are the aggressive tumor biology of PDAC and the lack of effective systemic therapies. This combination results in a high recurrence rate, most of the time after a relatively short interval following resection.[6-8]

Although local and/or distant disease recurrence occur in up to 80% of patients following PDAC resection, the impact of follow-up and early detection of recurrent disease on survival and quality of life is not well established.[9-10] As a result, current recommendations regarding surveillance strategies after resection of PDAC are based on low-level evidence and expert opinion.[11] Consequently, a global controversy on this subject currently prevails, with various national and international guidelines proposing different follow-up strategies.[10,12-17] The search for the most appropriate follow-up approach to detect recurrent pancreatic cancer is ongoing.[18,19]

Occult PDAC recurrence can be suspected in case of recurrence-associated symptoms or a sudden elevation of serum tumor markers such as carbohydrate antigen (CA) 19-9.[20] However, to provide further information on the extent and location of recurrent disease, radiological information frequently determines patient eligibility for any additional treatment or inclusion in clinical trials.[21-23] To this purpose, imaging modalities such as CT, MRI and FDG PET-CT are used, with CT imaging commonly favored.[24] Unfortunately, radiological assessment after pancreatectomy is challenging. Especially distinguishing local tumor recurrence from post-operative fibrosis is difficult.[21,22]

This systematic review and meta-analysis aim to provide a comprehensive overview of the literature on the diagnostic performance of different imaging modalities for detection of recurrent disease after resection of PDAC.

Methods

Systematic Literature Search

A systematic search was performed in PubMed, EMBASE and Cochrane Library up to 20 December 2017, following the Preferred Reporting Items for Systematic Review and Meta-

Table 1. Search syntax

Database	*Search syntax*
Pubmed	(((((((recurrent[Title/Abstract]) OR recurrence[Title/Abstract]) OR relaps[Title/Abstract]) OR recrudescence[Title/Abstract]) OR recurrence[MeSH Terms])) AND (((((((pancreas[Title/Abstract]) OR pancreatic[Title/Abstract]) OR pancreas[MeSH Terms])) AND ((((duct[Title/Abstract]) OR ductal[Title/Abstract])) AND cell[Title/Abstract])) AND ((((((cancer[Title/Abstract]) OR adenocarcinoma[Title/Abstract]) OR carcinoma[Title/Abstract]) OR tumor[Title/Abstract]) OR malignancy[Title/Abstract]) OR neoplasm[Title/Abstract]))) OR ((((((pancreas[Title/Abstract]) OR pancreatic[Title/Abstract]) OR pancreas[MeSH Terms])) AND ((((((cancer[Title/Abstract]) OR adenocarcinoma[Title/Abstract]) OR carcinoma[Title/Abstract]) OR tumor[Title/Abstract]) OR malignancy[Title/Abstract]) OR neoplasm[Title/Abstract])))) AND (((((((diagnostic imaging[MeSH Terms]) OR ((diagnostic[Title/Abstract]) AND imaging[Title/Abstract])) OR ((((computed[Title/Abstract]) AND tomography[Title/Abstract])) OR tomography[MeSH Terms])) OR ((ultrasonography[Title/Abstract]) OR ultrasonography[MeSH Terms])) OR (((magnetic[Title/Abstract]) AND resonance[Title/Abstract]) AND imaging[Title/Abstract])) OR (((((((positron[Title/Abstract]) AND emission[Title/Abstract]) AND tomography[Title/Abstract])) OR positron emission tomography[MeSH Terms]) OR PET[Title/Abstract]) OR PET-CT[Title/Abstract])) OR imaging[Title/Abstract])))
EMBASE	``(recurrent:ti,ab OR recurrence:ti,ab OR relaps:ti,ab OR recrudescence:ti,ab OR 'recurrence'/exp OR 'recurrence') AND ((pancreas:ti,ab OR pancreatic:ti,ab OR 'pancreas'/exp OR 'pancreas') AND (duct:ti,ab OR ductal:ti,ab) AND cell:ti,ab AND (cancer:ti,ab OR adenocarcinoma:ti,ab OR carcinoma:ti,ab OR tumor:ti,ab OR malignancy:ti,ab OR neoplasm:ti,ab) OR ((pancreas:ti,ab OR pancreatic:ti,ab OR 'pancreas'/exp OR 'pancreas') AND (cancer:ti,ab OR adenocarcinoma:ti,ab OR carcinoma:ti,ab OR tumor:ti,ab OR malignancy:ti,ab OR neoplasm:ti,ab))) AND ('diagnostic imaging'/exp OR 'diagnostic imaging' OR (diagnostic:ti,ab AND imaging:ti,ab) OR (computed:ti,ab AND tomography:ti,ab) OR 'tomography'/exp OR 'tomography' OR ultrasonography:ti,ab OR 'ultrasonography'/exp OR 'ultrasonography' OR (magnetic:ti,ab AND resonance:ti,ab AND imaging:ti,ab) OR (positron:ti,ab AND emission:ti,ab AND tomography:ti,ab) OR 'positron emission tomography'/exp OR 'positron emission tomography' OR pet:ti,ab OR 'pet ct':ti,ab OR imaging:ti,ab)
Cochrane Library	((((((recurrent:ti,ab) OR recurrence:ti,ab) OR relaps:ti,ab) OR recrudescence:ti,ab)) AND (((((((pancreas:ti,ab) OR pancreatic:ti,ab)) AND ((((duct:ti,ab) OR ductal:ti,ab)) AND cell:ti,ab)) AND ((((((cancer:ti,ab) OR adenocarcinoma:ti,ab) OR carcinoma:ti,ab) OR tumor:ti,ab) OR malignancy:ti,ab) OR neoplasm:ti,ab))) OR ((((pancreas:ti,ab) OR pancreatic:ti,ab)) AND ((((((cancer:ti,ab) OR adenocarcinoma:ti,ab) OR carcinoma:ti,ab) OR tumor:ti,ab) OR malignancy:ti,ab) OR neoplasm:ti,ab)))) AND (((((((((diagnostic:ti,ab) AND imaging:ti,ab)) OR (((computed:ti,ab) AND tomography:ti,ab))) OR (ultrasonography:ti,ab)) OR (((magnetic:ti,ab) AND resonance:ti,ab) AND imaging:ti,ab)) OR ((((((positron:ti,ab) AND emission:ti,ab) AND tomography:ti,ab)) OR PET:ti,ab) OR PET-CT:ti,ab)) OR imaging:ti,ab)

Analyses of diagnostic test accuracy studies (Table 1).[25] The search was restricted to title and abstract as well as to modern time-period (2000-2017). Relevance of articles was assessed by two authors according to predefined in- and exclusion criteria. Disagreement was settled by discussion and consensus.

Eligibility Criteria

Studies were found to be eligible when reporting on the diagnostic value of imaging modalities for the detection of local and/or distant disease recurrence during follow-up after resection of

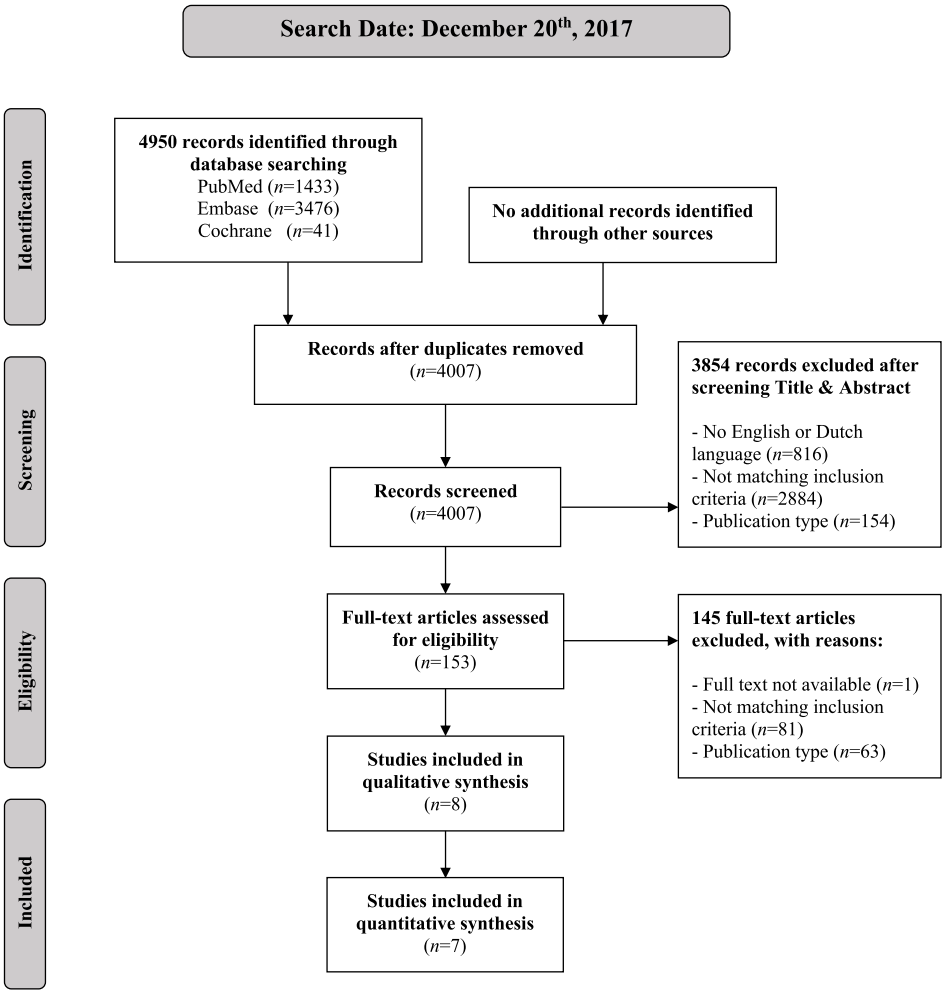

Figure 1. Article selection flowchart.

histopathological proven primary PDAC. If results of PDAC recurrence were undistinguishable from results of primary PDAC or from results representing treatment response after chemo- or radiotherapy, these studies were excluded. Articles specifically including patients with post-operative tissue formation were excluded as well, as these outcomes represent the ability to distinguish benign post-operative changes from PDAC recurrence instead of the diagnostic accuracy for detecting recurrence. Furthermore, articles reporting less than 10 patients were excluded. Both histologic confirmation of recurrence, following biopsy or re-resection, and clinical confirmation by disease progression on follow-up imaging were suitable reference standards. References of included articles were crosschecked for relevant studies.

Assessment of Methodological Quality

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool was used for critical appraisal of methodological quality by two authors independently.[26] Disagreement was settled by discussion and consensus.

Data Extraction

Study characteristics extracted were country, study interval, type of study, number of patients, age, gender, use of (neo)adjuvant treatment, follow-up strategy, follow-up duration, time to detection of recurrence, site of recurrence and value of CA 19-9. Data on index and reference tests, imaging specifications, radiologic criteria for PDAC recurrence, and number of patients with PDAC recurrence were collected additionally. Furthermore, results of diagnostic accuracy, i.e. sensitivity, specificity, PPV and NPV, and their corresponding 95% confidence intervals were extracted, preferably on a per-patient basis. If these values were not reported, they were calculated based on true-positive, false-positive, true-negative and false-negative results.

Statistical Analysis

Descriptive statistics were presented as in the original article. A bivariate random-effects model was carried out to calculate pooled estimates of sensitivity and specificity. Using this approach, any potential correlation between sensitivity and specificity is incorporated, since pairs of the logit-transformed sensitivity and specificity of each study are modeled simultaneously. Furthermore, the random-effects model incorporates heterogeneity beyond chance due to clinical and methodological differences between the included studies.[27] Subgroup analysis was carried out for imaging procedures performed in the context of a routine follow-up. Statistical analysis was performed using R 3.3.1 open-source software (http://www.R-project.org; "lme4" package for fitting nonlinear mixed-effects models). Results were presented in forest plots, created by using GraphPad Prism 7.04 software (GraphPad Software, La Jolla, California, USA).

Results

Search Results

Seven studies with 333 relevant patients were included for data extraction (Figure 1, Table 2 and 3).[23,24,28-32] The study of Ruf et al. was excluded for further analysis, since results of contrast-enhanced CT were indistinguishable from results of contrast-enhanced MRI. Herewith, the applicability of this study to our research question was doubtful.[33]

Table 2. Assessment of methodological quality using the QUADAS-2 tool

	Risk of Bias				*Applicability Concerns*		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference standard
Rayamajhi (2017)[27]	◐	●	●	●	●	●	●
Jung (2016)[23]	●	○	●	●	●	◐	●
Peti (2014)[28]	●	●	●	●	●	◐	●
Sperti (2010)[29]	●	◐	●	◐	●	●	●
Kitajima (2009)[22]	◐	●	●	◐	●	●	●
Ruf (2005)[32]	◐	●	●	●	●	○	●
Jadvar (2001)[30]	○	○	●	◐	●	●	●
Mortele (2000)[31]	●	◐	●	●	●	◐	○

●, consistent with criteria, low risk of bias; ◐, partly consistent with criteria, unknown risk of bias; ○, not consistent with criteria or not reported, high risk of bias.

Study and Patient Characteristics

The study and patient characteristics of the included studies are presented in Table 3; radiologic criteria to define PDAC recurrence are presented in Table 4. Seven studies evaluated the diagnostic accuracy of contrast-enhanced CT imaging for the detection of recurrent PDAC.[23,24,28-32] Five studies reported on the diagnostic value of FDG PET-CT.[23,24,28-30] Overall, FDG PET-CT was performed in patients who had a clinical suspicion of recurrent disease with equivocal or negative CT findings. Three studies also focused on the diagnostic performance of FDG PET-CT combined with contrast-enhanced CT.[23,24,29] Three studies described the use of imaging procedures as part of a standardized follow-up, with regular intervals of 3-6 months during the first two years.[24,30,32] One additional study also described a regular follow-up, although a time interval was not mentioned.[23] Data on follow-up duration, recurrence patterns and time to detection of recurrence were infrequently documented.

Methodological Quality

Overall, the methodological quality was acceptable. All studies performed analyses on retrospectively collected data.[23,24,28-32] In one study it was unclear whether patients were referred for FDG PET imaging in the context of standard tumor surveillance or following detection of an equivocal lesion with other imaging modalities. Therefore, the risk of selection bias in their study-population was considered high.[31]

Table 3. Study characteristics

Author (year)	*Country*	*Study interval*	*Type of study*	*Patients (n)*	*Age (years)* Median (range)	*Age (years)* Mean (range)	*Gender (% male)*	*(Neo)adjuvant treatment*
Rayamajhi (2017)[27]	Nepal, USA & India	NR	Retrospective	39		64 (55-78)	51	Number of patients with or without (neo)adjuvant therapy unknown
Jung (2016)[23]	South Korea	2004-2012	Retrospective	110	62 (35-84)		57	Adjuvant therapy (not further specified): n = 91 (83%)
Peti (2014)[28]	USA	2005-2008	Retrospective	24 (97 lesions[a])	64 (NR)		50	NR
Sperti (2010)[29]	Italy	1998-2007	Retrospective	72		64 (35-84)	47	Adjuvant therapy: n = 49 (68%) - chemotherapy (n = 20) - chemoradiotherapy (n = 29) No neoadjuvant therapy was used
Kitajima (2009)[22]	Japan	2005-2008	Retrospective	45		58 (45-81)	NR	Adjuvant therapy: n = 31 (69%) - chemotherapy (n = 28) - chemoradiotherapy (n = 3) Neoadjuvant therapy NR
Jadvar (2001)[30]	USA	1997-1999	Retrospective	11		61 (40-76)	60	NR
Mortele (2000)[31]	Belgium	1988-1995	Retrospective	32		59 (32-82)	44	NR

[a] Lesion-based analysis

CA 19-9, *carbohydrate antigen 19-9*; CEA, *carcinoembryonic antigen*; NR, *not reported*.

Table 3. Study characteristics (continued)

Author (year)	*Follow-up strategy after resection of pancreatic cancer*	*Follow-up duration (months)*	*Time to detection of recurrence (median months (range))*
Rayamajhi (2017)[27]	NR	NR	NR
Jung (2016)[23]	Serum CA 19-9 and abdominal CT at 3, 6, and 12 months and every 6 months thereafter. Most patients underwent PET-CT once yearly, as well as when other follow-up tests suggested tumor recurrence	26 (9-81)	11 (1-54)
Peti (2014)[28]	NR	6 (1-16)	NR
Sperti (2010)[29]	Physical examination, laboratory tests, CA 19-9 and CEA determination, abdominal US, CT, chest X-rays and/or MRI every 3 months for the first 2 years and then every 6 months + at least one PET-CT	22 (5-104)	11 (3-66)
Kitajima (2009)[22]	Clinical follow-up including close surveillance, periodic evaluation of CA 19-9 levels, and the use of several imaging modalities such as US, CT and MRI.	NR	18 (6-26)
Jadvar (2001)[30]	NR	NR (5-48)	NR (5-48)
Mortele (2000)[31]	CT imaging at regular intervals (3-6 months)	NR	11 (1-36)

[a] Lesion-based analysis.

CA 19-9, *carbohydrate antigen 19-9*; CEA, *carcinoembryonic antigen*; NR, *not reported*.

Two studies reported that the CT procedure contained scans of thorax, abdomen and pelvis.[23,28] In the other studies, just the abdomen was depicted by CT.[24,29-31] Only one of these studies performed a subgroup analysis on the diagnostic values of abdominal CT versus FDG PET-CT by site of recurrence (i.e. local recurrence versus distant recurrence).[24] Overall, a proper comparison of abdominal CT with full-body FDG PET-CT imaging was therefore questionable. Most studies described a time interval of less than four weeks between the procedures.[23,24,29-31] There was considerable variation in the CT-scanning protocols and relevant specifications (e.g. kV, mAs, scan phase) were not uniformly reported.

Table 4. Radiologic criteria to define pancreatic ductal adenocarcinoma recurrence

Author (year)	*CT*	*^{18}F-FDG PET-CT*
Rayamajhi (2017)[27]	Positive: LNs with a short-axis diameter of ≥10mm; a central unenhanced area suggesting central necrosis Equivocal: LNs with a short-axis diameter of 6mm and 9mm Negative: LNs with a short-axis diameter of 5mm; low attenuation centrally suggestive of a fatty hilum (<0 HU) with a LN, regardless of node size	Positive: any increased focal FDG uptake on PET images; LNs with increased FDG uptake, even if their short-axis diameter is <10mm Negative: LNs with no detectable tracer uptake even if their short-axis diameter is >10mm
Jung (2016)[23]	NR	NR
Peti (2014)[28, 42]	Mass/growth in the primary region, enlarged LNs, and hepatic and other distant metastases	The presence of increased focal FDG uptake in a region outside the sites of physiologic uptake
Sperti (2010)[29]	NR	A focal uptake with a SUV>2.5.
Kitajima (2009)[22]	Positive: nodular, plaque-like, or infiltrative soft tissue lesions with abnormal enhancement in the peritoneal fat or on the peritoneal surface (peritoneal implantation); LNs with a short-axis diameter >10mm; the presence of a central un-enhancing area suggesting central necrosis Negative: the presence of peripheral low attenuation suggesting a fatty hilum within a LN, regardless of node size	Positive: abnormal focal FDG uptake observed on PET images, corresponding to an abnormal mass on CT; LNs with increased glucose uptake, even if their short-axis diameter is <10mm Negative: LNs with no detectable tracer uptake were deemed negative for metastatic spread, even if they were >10mm in short-axis diameter
Jadvar (2001)[30]	Either discrete low-attenuation pancreatic mass or diffuse pancreatic enlargement with hepatic or distant metastatic lesions	Tracer accumulation in the pancreatic bed or by lesions identified on CT greater than background hepatic uptake
Mortele (2000)[31]	Local disease recurrence: the presence of a soft tissue mass in the surgical bed Metastatic lymphadenopathy: presence of progressively enlarging nodes on follow-up imaging	NA

HU, *Hounsfield unit;* LN, *lymph node;* NR, *not reported;* NA, *not applicable*; SUV, *standardized uptake value*.

The radiological definition of tumor recurrence for each imaging modality was well described in most studies.[23,28,29,32,33] Some of the studies used a standardized 2-, 3-, or 5-point scale to describe findings.[28,29,32,34] In most studies, scans were independently evaluated by two or more observers, with the final results based on consensus. In two studies, information on the imaging analysis was lacking.[24,31] Blinding was carried out in four studies.[23,28-30] One study described recurrence confirmation by clinical follow-up, although the methods to detect or criteria to define recurrence were not specified.[32]

Diagnostic Accuracy of Imaging Modalities for the Detection of Recurrent Pancreatic Cancer

Considering the test results of contrast-enhanced CT imaging, sensitivities varied between 56-88% and specificities ranged from 67-100% (Table 5).[23,24,28-30,34] The pooled estimates for sensitivity and specificity were 0.70 (95% CI 0.61-0.78) and 0.80 (95% CI 0.69-0.88), respectively (Figure 2). Data on diagnostic accuracy of FDG PET-CT showed sensitivities of 80-97% and specificities of 85-100%, with a pooled estimate for sensitivity of 0.88 (95% CI 0.81-0.93) and for specificity of 0.89 (95% CI 0.80-0.94).[23,24,28-30] For FDG PET-CT combined with contrast-enhanced CT imaging, sensitivities and specificities ranged from 92-98% and 65-95%, respectively.[23,24,29] The pooled estimate for sensitivity was 0.95 (95% CI 0.88-0.98) and for specificity 0.81 (95% CI 0.63-0.92). In the context of a routine follow-up, subgroup analyses showed a pooled estimate for sensitivity and specificity of 0.71 (95% CI 0.60-0.80) and 0.82 (95% CI 0.68-0.90) for CT, and 0.90 (95% CI 0.81-0.95) and 0.88 (95% CI 0.76-0.95) for FDG PET-CT. Due to the limited data, subgroup analysis for FDG PET-CT combined with contrast-enhanced CT was not performed.

Discussion

This systematic review provides an overview of the literature on the diagnostic accuracy of various imaging modalities for the detection of recurrent PDAC during follow-up after pancreatic resection. According to the reviewed literature, contrast-enhanced CT has a moderate diagnostic value with a sensitivity of 70% and specificity of 80%; FDG PET-CT showed a sensitivity and specificity of 88% and 89%, respectively, and for FDG PET-CT combined with contrast-enhanced CT, a sensitivity of 95% and specificity of 81% was found. In the context of routine radiologic surveillance, diagnostic accuracy values of CT and FDG PET-CT were similar compared to imaging performed in case of suspected PDAC recurrence.

During both pre-operative assessment and post-operative follow-up, CT imaging is stated to be the preferred imaging modality.[13,21,35] One common difficulty in post-operative CT

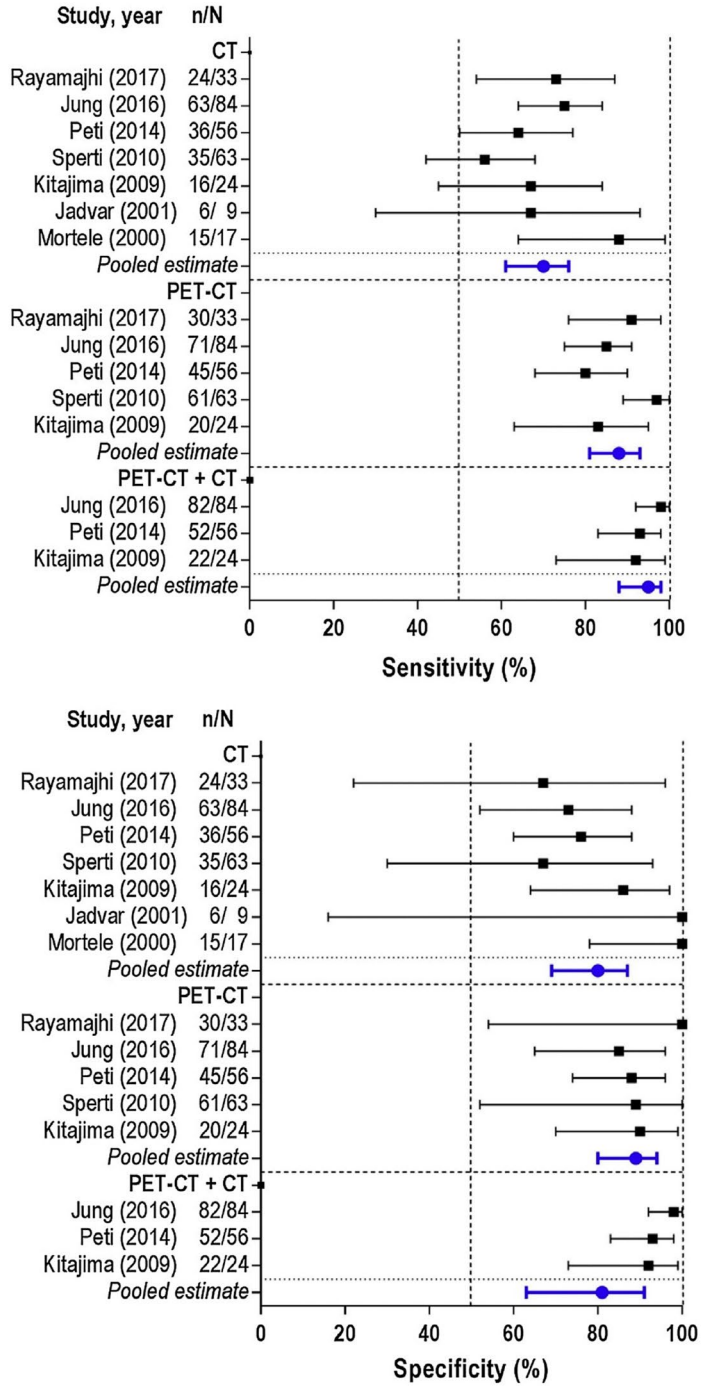

Figure 2. Forest plots of the sensitivity and specificity of CT, FDG PET-CT and FDG PET-CT + CT in detecting PDAC recurrence.

Table 5. Results

Author (year)	*Index test*	*Imaging specifications*	*Reference test*	*Recurrence n (%)*	*Site of recurrence*
	CT of abdomen, pelvis & thorax	125 mL intravenous contrast at 2.5 mL/s with a 10-s monitoring delay and a 45-s diagnostic delay; slice thickness 5mm; reconstruction thickness 2.5mm			
Rayamajhi (2017)[28]	^{18}F-FDG PET-CT	Intravenous FDG (185-370 MBq/injection; 5-10 mCi/injection); slice thickness 3.75mm; 120 kVp; 300 mAs	Pathology (*n*=11) or FU imaging (*n*=28)	33 (85)	Local and distant
	Abdominal CT	- CT: arterial and venous phases; 90 mL intravenous contrast at a rate of 3.0-4.0 mL/s; slice thickness 3-5mm			
	^{18}F-FDG PET-CT				
Jung (2016)[24]	^{18}F-FDG PET-CT + CT	- PET: intravenous FDG 5.18 MBq/kg	Pathology (*n*=31) or FU imaging (*n*=53)	84 (76)	Local and distant
	Abdominal CT	- CT: 120 kV; 150-380 mA; 8mm slice collimation; 5mm reconstruction interval; triple-phase scans incl. a pre-contrast (liver) and an arterial phase scan (abdomen); 120 ml intravenous contrast (injection rate 3 ml/s)			
	^{18}F-FDG PET-CT				
Peti (2014)[29, 43]	^{18}F-FDG PET-CT + CT	- PET: following a standard protocol[48]	Pathology or FU imaging	56 (58)[a]	Local and distant
	Abdominal CT	High resolution, thin slice helical CT			
Sperti (2010)[30]	^{18}F-FDG PET-CT	- CT: 120 kEV, 80 mAs - PET: 7-8 beds (2-3 min/bed) ; intravenous injection of 185-370 MBq of FDG	Pathology (*n*=17) or FU imaging (*n*=46)	63 (88)	Local and distant
	CT neck to pelvis	140 kV; 230 mA; slice thickness 2mm; 150 mL or 2 mL/kg contrast at a rate of 2.5 mL/s	Pathology (*n*=19) or FU imaging + serum tumor marker testing (*n*=24)		
	^{18}F-FDG PET-CT	- CT: 140 kV; 40 mA - PET: interslice spacing 3.75mm; 6-7 beds; 3min/bed; intravenous injection of 4.0 MBq/kg of FDG			
Kitajima (2009)[23]	^{18}F-FDG PET-CT + CT	Combination of the above procedures		24 (53)	Local and distant
	Abdominal CT	40-s after intravenous administration of 140 mL contrast at a rate of 4 mL/s; 5mm collimation; reconstruction thickness 5mm			
Jadvar (2001)[31]	^{18}F-FDG PET-CT[b]	15 contiguous sliced of 6.5mm separation; reconstruction resolution 7mm FWHM	Pathology (*n*=2) or FU imaging (*n*=9)	9 (82)	NR
Mortele (2000)[32]	Abdominal CT	- Conventional scans (n = 59); 8±10mm slices - Spiral scans (n = 25); collimation width 8mm, reconstruction interval 8mm	Pathology (*n*=2) or clinical FU (*n*=15)	17 (53)	Local and distant areas of the abdomen

[a] lesion-based analysis.

[b] interpretation performed in close correlation with contrast-enhanced CT.

^{18}F-FDG, *18F-fluorodeoxyglucose;* FWHM, *full-width half-maximum;* FU, *follow-up;* NA, *not applicable;* NPV, *negative predictive value;* PPV, *positive predictive value*.

Table 5. Results (continued)

Author (year)	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Accuracy*
	73% (24/33)	67% (4/6)	92% (24/26)	31% (4/13)	72% (28/39)
Rayamajhi (2017)[28]	91% (30/33)	100% (6/6)	100% (30/30)	67% (6/9)	92% (36/39)
	75% (63/84)	73% (19/26)	90% (63/70)	48% (19/40)	75% (82/110)
	85% (71/84)	85% (22/26)	95% (71/75)	63% (22/35)	85% (93/110)
Jung (2016)[24]	98% (82/84)	65% (17/26)	90% (82/91)	90% (17/19)	90% (99/110)
	64% (36/56)	76% (31/41)	78% (36/46)	61% (31/51)	69% (67/97)
	80% (45/56)	88% (36/41)	90% (45/50)	77% (36/47)	84% (81/97)
Peti (2014)[29, 43]	93% (52/56)	81% (33/41)	87% (52/60)	89% (33/37)	88% (85/97)
	55% (35/63)	67% (6/9)	92% (35/38)	18% (6/34)	57% (41/72)
Sperti (2010)[30]	97% (61/63)	89% (8/9)	98% (61/62)	80% (8/10)	96% (69/72)
	67% (16/24)	86% (18/21)	84% (16/19)	69% (18/26)	76% (34/45)
	83% (20/24)	91% (19/21)	91% (20/22)	83% (19/23)	87% (39/45)
Kitajima (2009)[23]	92% (22/24)	95% (20/21)	96% (22/23)	91% (20/22)	93% (42/45)
	67% (6/9)	100% (2/2)	100% (6/6)	40% (2/5)	73% (8/11)
Jadvar (2001)[31]	100% (9/9)	50% (1/2)	90% (9/10)	100% (1/1)	91% (10/11)
Mortele (2000)[32]	88% (15/17)	100% (15/15)	NA	NA	94% (30/32)

[a] lesion-based analysis.

[b] interpretation performed in close correlation with contrast-enhanced CT.

^{18}F-FDG, *18F-fluorodeoxyglucose;* FWHM, *full-width half-maximum;* FU, *follow-up;* NA, *not applicable;* NPV, *negative predictive value;* PPV, *positive predictive value*.

assessment is to differentiate local tumor recurrence from non-specific post-operative changes.[21,24] Particularly after neo-adjuvant or adjuvant radiotherapy, which can lead to additional localized fibrosis at the pancreatectomy site. However, sequential post-operative CT scans allow for assessment of local tissue growth, making diagnosis of recurrence more conclusive. Additionally, longitudinal serum tumor marker testing can provide useful information, especially when changes on imaging can be correlated with a spike in tumor marker values.[20-22]

Various studies demonstrated that FDG PET-CT can be helpful in differentiating and has the potential to accurately detect and localize PDAC recurrence.[23,28,36] As shown by this meta-analysis, FDG PET-CT alone or combined with contrast-enhanced CT seems to improve the detection of recurrent PDAC, reflected by a higher sensitivity compared to contrast-enhanced CT alone. An important reason for this superiority is the fact that FDG PET-CT provides more conclusive evidence on the malignancy of questionable lesions found on regular CT imaging. On the other hand, lesions have to be 5-10 mm to become detectable with FDG PET-CT, possibly reducing the utility in discovering smaller lesions in case of early recurrence. Nevertheless, the combination of FDG PET-CT and contrast-enhanced CT does not seem to improve the specificity compared to contrast-enhanced CT alone, as the number of false-positives found with CT is not decreased when evaluated with integrated FDG PET-CT imaging. Considering the current evidence, FDG PET-CT seems to be of additional value particularly when PDAC recurrence is suspected despite negative or equivocal CT findings. As no studies did report outcomes on the diagnostic performance of MRI separately, the additional value of MRI remains unclear.

The role of routine imaging for the early detection of recurrent PDAC is not well established.[9] As shown in this review, just four of the included studies performed scans as part of a standardized surveillance. A recent study by our institution showed that even in the case of symptomatic follow-up without standardized imaging, the majority of patients underwent additional imaging procedures for the detection of recurrence at least once.[37] However, although patients underwent an average of three scans during follow-up, most recurrences were detected at a late stage, after the manifestation of clinical symptoms. This potentially limits patients to undergo additional treatment for recurrence.

Few studies published on the consequences of radiologic surveillance on survival and quality of life. Witkowski et al. found that annual follow-up imaging did not lead to a significant survival benefit.[38] Furthermore, Tzeng et al. showed that routine CT imaging does not result in clinically significant survival benefits and the cost-effectiveness was herewith argued.[39]

Conversely, others support a surveillance strategy with regular CT imaging. Two observational studies focusing on systematic CT-based follow-up showed that patients with asymptomatic recurrence had improved survival and received oncological treatment more frequently.[40,41] In both studies, it was stated that the detection of asymptomatic recurrence following CT surveillance might identify patients with good performance status and tumor biology that are most likely to benefit from subsequent treatment. Detection of recurrent disease by CT may furthermore facilitate patient inclusion in clinical studies.[40] Another study showed that early PDAC recurrence was detected in the first year in 69% of patients, with an average of 1.5 scans (range 1-3). Herewith, it was suggested that routine CT monitoring during the first year after surgery, using a regular interval of three months, may be appropriate to detect early recurrence and provide additional treatment.[21] A recent study found that routine imaging surveillance, compared to symptom-oriented imaging surveillance, was associated with a prolonged survival in a multivariable model (30.4 months vs. 17.1 months; P=0.002).[42] The mean interval for diagnosis of recurrent PDAC was significantly shorter in the routine imaging surveillance group and more patients received chemotherapy for recurrent disease. The possibility for more aggressive treatment at an early stage could therefore have contributed to improved survival rates. They suggested that CT-imaging at 3-4 months interval can be beneficial.[42]

Although current evidence for additional imaging and tumor marker testing during follow-up is limited and sometimes contradictory, recent advancements in therapeutic options, such as FOLFIRINOX (fluorouracil, leucovorin, irinotecan and oxaliplatin) and stereotactic body radiation therapy, seem to warrant early diagnosis of cancer recurrence. Since it can be difficult to obtain histological evidence of PDAC recurrence, the combination of a rise in serum tumor markers and suspicious, progressive findings on follow-up imaging using CT and/or FDG PET-CT could aid in the early clinical diagnosis. Treatment of recurrence before deterioration of the patient's general condition has the potential to improve disease control and survival rates. However, these possible beneficial consequences should be deliberated in the context of both the possible economic and emotional burden of intensive follow-up. These questions highlight the necessity for future prospective studies to not only define the actual clinical merit but also the psychosocial implications and cost-effectiveness of follow-up approaches after resection of PDAC.

A limitation of this systematic review and meta-analysis was that only seven studies were found to be eligible, with a relatively small number of patients in most studies and all reporting on retrospectively collected data. Selection and information bias may have led to overestimation of the sensitivity and specificity of FDG PET-CT. Furthermore, the included studies had

considerably heterogeneous study designs and not all studies could be included for meta-analyses. Additionally, the diagnostic performance for the detection of distant metastasis could not be compared fairly, since most of the included studies performed abdominal CT only, whilst FDG PET-CT depicts the whole body. For a better comparison, CT images should preferably encompass the thorax, abdomen and pelvis. Given the considerable variation in CT-scanning protocols, no conclusions can be made on the optimal scan protocol. Moreover, the exactness of the presented diagnostic accuracy values could be questioned, since a golden standard reference test for the detection of PDAC recurrence is missing and histological evidence of recurrence was available in only 27% (n=82/309) of patients. No studies on imaging modalities other than CT and FDG PET-CT, such as MRI, were found to be eligible for inclusion. Lastly, as different reference standards were used for the confirmation of disease recurrence, differential verification bias was of concern in most of the included studies.

Conclusion

Evidence supporting radiological surveillance after resection of PDAC is limited. According to the current literature, post-operative contrast-enhanced CT imaging has a moderate diagnostic accuracy for detecting PDAC recurrence and could contribute to the early detection of recurrent disease. Most of the studies performed on the diagnostic accuracy of FDG PET-CT for the detection of recurrent PDAC showed an additional value of FDG PET-CT particularly in case of suspected recurrence with equivocal or negative CT findings. Further optimization of surveillance strategies for the post-operative detection of recurrent PDAC is necessary. Therefore, studies on the diagnostic performance of imaging modalities during follow-up and the consequences of early diagnosis of recurrent disease on survival and quality of life outcomes are needed.

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CHAPTER 11

Use of Imaging During Symptomatic Follow-Up after Resection of Pancreatic Ductal Adenocarcinoma

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Abstract

Objective

Controversy exists whether follow-up after resection of pancreatic ductal adenocarcinoma (PDAC) should include standardized imaging for the detection of disease recurrence. The purpose of this study was to evaluate how often patients undergo imaging in a setting where routine imaging is not performed. Secondly, the pattern, timing and treatment of recurrent PDAC were assessed.

Methods

This was a post-hoc analysis of a prospective database of all consecutive patients undergoing pancreatic resection of PDAC between January 2011 and January 2015. Data on imaging procedures during follow-up, recurrence location and treatment for recurrence were extracted and analyzed. Associations between clinical characteristics and post-recurrence survival were assessed with the log-rank test and Cox univariable and multivariable proportional hazards models.

Results

A total of 85 patients were included. Seventy-four patients (87%) underwent imaging procedures during follow-up at least once, with a mean amount of 3.1 ± 1.9 imaging procedures during the entire follow-up period. Sixty-eight patients (80%) were diagnosed with recurrence, 58 (85%) of whom after the manifestation of clinical symptoms. Additional tumor specific treatment was administered in 17 of 68 of patients (25%) with recurrence. Patients with isolated local recurrence, treatment after recurrence and a recurrence-free survival >10 months had longer post-recurrence survival.

Conclusion

Even though a symptomatic follow-up strategy does not include routine imaging, the majority of patients with resected PDAC underwent additional imaging procedures during their follow-up period. Further prospective studies are needed to determine the actual clinical value, psychosocial implications and cost-effectiveness of different forms of follow-up after resection of PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer related mortality in Europe, with more than 43.000 deaths predicted for 2017.[1,2] Due to late onset of symptoms, up to 80% of patients present with locally advanced or metastatic disease.[3] Most prospective studies for advanced pancreatic cancer involve both locally advanced disease, primary metastatic disease and recurrent disease after surgery.[4] It has been recently acknowledged, however, that survival outcomes for patients with recurrent disease can be superior when compared to locally advanced and primary metastatic disease, possibly warranting additional treatment for these patients.[4,5]

Management of recurrent PDAC is less well established as it is for other stages of PDAC. Furthermore, controversy exists as to whether follow-up after pancreatic resection should include imaging testing for the diagnosis of recurrence.[6,7] The European Society for Medical Oncology, for instance, only recommends symptomatic follow-up that concentrates on symptoms, nutrition and psychosocial support without additional diagnostics for diagnosing recurrence.[8]

Adhering to the European guidelines, follow-up at our institution consists of regular check-ups focusing on post-operative symptoms without imaging for the detection of recurrence. The purpose of this study was to evaluate how often patients undergo imaging in daily practice based on clinical suspicion of disease recurrence in a setting where routine imaging is not performed. Secondly, the pattern, timing and treatment of recurrent PDAC resulting from symptomatic follow-up were assessed. Lastly, potential predictive factors for extended survival after recurrence were evaluated.

Methods

Patient Selection

The primary source of information for this study was the prospective pancreatic surgery database of the UMC Utrecht Cancer Center department of Surgery, which has been approved by our Institutional Review Board for data acquisition and query. Only patients undergoing pancreatic resection of PDAC between January 2011 and January 2015 were included for analysis. Patients with 30-day perioperative mortality were excluded.

Primary Treatment

Resectability and staging were established using pancreatic protocol CT and were discussed

in a multidisciplinary setting. A team specialized in hepatobiliary and pancreatic surgery performed all pancreatic resections. If their performance status allowed it, patients were offered adjuvant chemotherapy in the form of Gemcitabine (1000mg/m^2) every 4 weeks for 6 cycles following Dutch national guidelines based on the ESPAC and CONKO trials.[9] All patients included in this study were considered resectable or borderline resectable and underwent upfront surgery. Neoadjuvant therapy for resectable/borderline/locally advanced PDAC is a relatively recent development in the Netherlands and is generally only performed in the setting of current multi-institutional and national trials. Cancer stage was defined according to the 7th edition of the American Joint Committee on Cancer staging system.[10]

Follow-Up

Patient follow-up occurred at the outpatient clinic of our institution to a standardized schedule of visits. Following completion of all therapy, regular follow-up visits were scheduled at 3, 6, 9, 12, 18 and 24 months, followed by yearly check-ups up to 5 years. After 5 years, patients were discharged from follow-up. The follow-up was performed by either an HPB surgeon or a medical oncologist and consisted mainly of an inquiry concerning post-operative symptoms and signs of endocrine- and exocrine pancreatic insufficiency, followed by a physical examination and weight measurement. Imaging was explicitly not part of our standard follow-up protocol.

For this study, the actual use of imaging procedures was assessed to evaluate the results and implications of this follow-up protocol. Only those examinations ordered with a clear inquiry regarding tumor recurrence were included. Radiological or histological evidence was required for the diagnosis of recurrent disease. Only the first site was documented, using four mutually exclusive categories. Local recurrence was defined as recurrence in the remnant pancreas or in the surgical bed, such as soft tissue along the celiac or superior mesenteric artery, aorta or around the pancreatojejunostomy site. Distant recurrence was defined as recurrence restricted to a single organ or site. When both isolated local recurrence and isolated distant recurrence were revealed simultaneously, recurrence was defined as "local + distant" recurrence. When multiple distant sites and/or carcinomatosis peritonei were revealed, recurrence was recorded as multiple. Records were further analyzed to determine the type of treatment received after detection of recurrence. Symptomatic recurrences were defined as those discovered due to a significant patient-initiated complaint that was new or had increased in severity or frequency.

Statistical Analysis

Summary statistics were obtained using established methods. Patients were dichotomized

whether recurrence occurred fewer or greater than 10 months following surgery. This used cut-off point of 10 months RFS was found to be the most significant value for showing differences in post-recurrence survival when using a minimum p-value approach to analyze our data and was similar proposed cut-off in previously published surgical data.[11,12] The proportion of patients receiving imaging procedures for each month of follow-up was calculated by dividing the amount of patients receiving one or more imaging procedure(s) for the detection of recurrence by the amount of uncensored patients. Censoring occurred at date of recurrence, death or last follow-up if recurrence did not occur. Also, the median interval between surveillance imaging procedures was calculated for each patient from day of surgery to the date of recurrence by dividing this interval with the number of imaging procedures actually performed in that period.

Overall survival (OS) and post-recurrence survival were defined as the time from the date of operation (OS) or the date of recurrence (post-recurrence survival) to either death from any

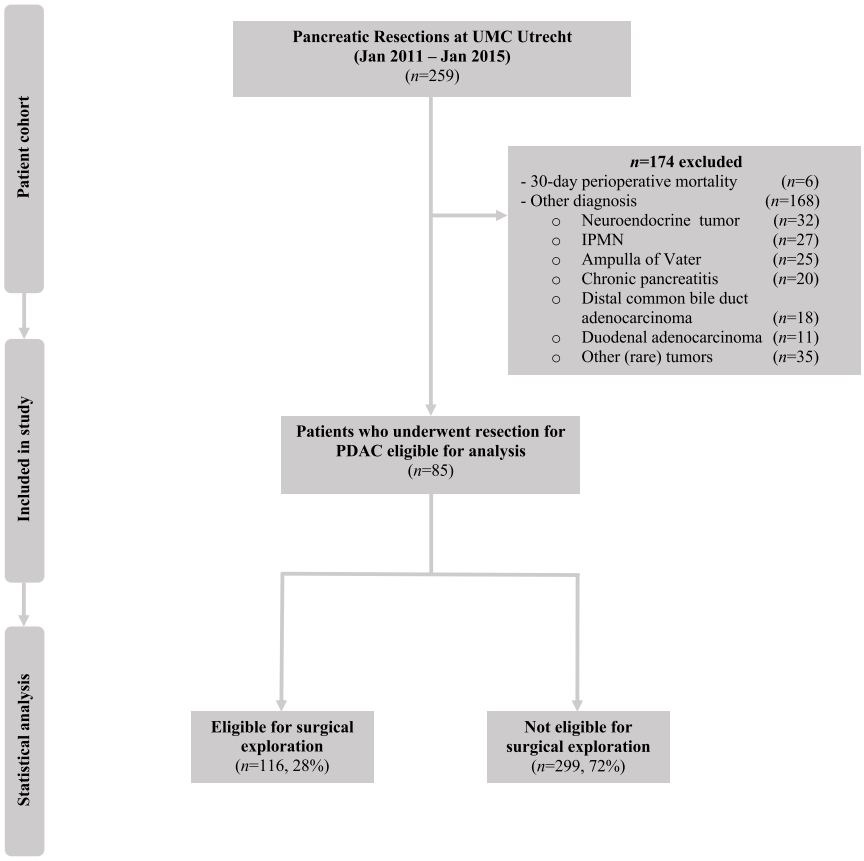

Figure 1. Flowchart of patient selection.

cause or last follow-up. Recurrence-free survival was defined as the time interval between the date of the operation to either date of recurrence or last follow-up if recurrence was not observed. Kaplan-Meier curves were used to estimate median OS, recurrence-free survival and post-recurrence survival with corresponding 95% confidence intervals (CI). The log-rank test was performed to compare between subgroups. Univariable and multivariable analysis were performed using Cox proportional hazard models to identify potential predictive factors for post-recurrence survival. A two-tailed P-value of <0.05 was considered to indicate statistical significance. Values are expressed as median and range, unless specified otherwise. Statistical analysis was performed with SPSS statistical software v22.0 (IBM Corporation, Armonk, NY, USA)

Results

Overall Survival in 85 patients

A total of 85 patients of 259 consecutive patients undergoing pancreatic resection from January 2011 to January 2015 were included (Figure 1). Follow-up data were complete for all patients (100%) with a median duration of follow-up of 16 months (2-45 months). Median OS for all 85 patients was 17 months (95% CI 14-20). Seventeen patients (20%) without proven recurrence had a median follow-up of 19 months (3-33 months) and a median OS of 25 months (95% CI 8-41). Seven patients (8%) are currently alive without any signs of recurrence after a median follow-up of 22 months (13-33 months).

Imaging Procedures During Follow-Up

During follow-up, 74 of the included patients (87%) underwent additional imaging procedures for the detection of recurrence at least once. Among these 74 patients, a total of 227 imaging procedures were performed. CT scans constituted 175, or 77%, of all procedures. The remainder consisted of abdominal ultrasounds (n=36, 16%), MRI scans (n=13, 6%) and FDG-PET scans (n=3, 1%). For the diagnosis of recurrent disease, first examination of choice was a CT scan in 62 patients (84%), followed by ultrasound (n=9, 12%) and MRI (n=3, 4%). Median interval between operation and first imaging procedure for the detection of recurrence was 5 months (1-29 months).

Table 1 shows utilization during follow-up for all imaging modalities and CT separately. Only 3 patients (4%) did not undergo a CT-scan during follow-up. Patients received a mean amount of 3.1 ± 1.9 procedures during the entire follow-up period. Before diagnosis of recurrence, the mean number of examinations was 2.1 ± 1.2 compared to 0.7 ± 1.0 procedures for the monitoring of recurrence. The median interval between examinations was 5 months (1-30

Table 1. Utilization of imaging procedures in patients by follow-up period

	Entire follow-up period		*Diagnosing recurrence*		*Monitoring recurrence*	
Imaging use	***All modalities***	***CT scan***	***All modalities***	***CT scan***	***All modalities***	***CT scan***
Total imaging procedures, *n*	227	175	152	129	75	46
No. of procedures, *n* (%)						
0 procedures	0 (0%)	3 (4%)	0 (0%)	3 (4%)	37 (50%)	48 (65%)
1 procedure	18 (24%)	22 (30%)	31 (42%)	39 (53%)	18 (24%)	13 (18%)
2 procedures	17 (23%)	23 (31%)	22 (30%)	16 (22%)	8 (11%)	6 (8%)
3 procedures	14 (19%)	10 (14%)	12 (16%)	10 (13%)	5 (7%)	7 (9%)
4 procedures	9 (12%)	6 (8%)	4 (5%)	2 (3%)	4 (5%)	0 (0%)
5 procedures	7 (10%)	7 (9%)	5 (7%)	4 (5%)	2 (3%)	0 (0%)
>5 procedures	9 (12%)	3 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mean no. of procedures (SD)	3.1 (1.9)	2.5 (1.5)	2.1 (1.2)	1.8 (1.1)	1.0 (1.4)	0.7 (1.0)
Median time interval, *months* (range)	5 (1-30)	6 (1-30)	4.9 (1-30)	5.1 (1-30)	3.1 (1-16)	3.8 (0-20)

SD, *standard deviation*.

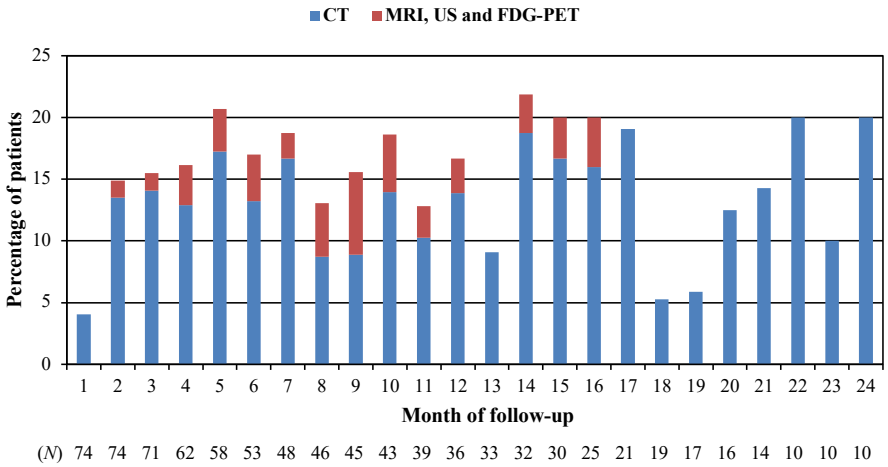

Figure 2. Percentage of patients who received CT, MRI, US or FDG-PET for the detection of recurrence per month of follow-up. Patients who recurred or died were censored at the date of event. N indicates the number of patients who survived uncensored at the end of the month.

months). Figure 2 shows the proportion of patients who underwent imaging for the detection of recurrence during each month of follow-up. The denominator is the number of uncensored patients at the end of each month (N). The graph shows no evidence of periodicity in the use of imaging for recurrence (i.e., spikes every 6 months), confirming the lack of routine imaging during standard follow-up schemes.

Recurrence Characteristics and Survival

Proven recurrence was documented in 68 of 85 patients (80%) at last follow-up. Complete demographic, clinicopathological and primary treatment characteristics are summarized in Table 2. Dichotomization showed that initial patient, tumor and treatment characteristics did not differ significantly between patients with a recurrence-free survival <10 months or >10 months, except for the administration of adjuvant Gemcitabine (P=0.024). The majority of recurrences (n=58, 85%) were diagnosed after the manifestation of clinical symptoms. In the 10 asymptomatic patients, recurrence was detected by imaging performed at the explicit wish of the patient. Symptoms leading to detection of recurrence included abdominal and/or back pain in 40 (69%) and/or weight loss in 10 of 58 (17%) symptomatic patients. Further symptoms, including overall malaise and obstipation amongst others, were seen in 19 patients (33%).

At the time of diagnosis of recurrence, most patients had either recurrence at an isolated distant site (n=22, 32%) or at multiple sites (n=23, 34%) (Table 2). Isolated distant sites included the liver (n=12), lung (n=4), lymph nodes (n=3) and bone (n=3). Local recurrence occurred after a significantly longer median recurrence-free survival (20 months, 95% CI 12-27 months) when compared to isolated distant recurrence (8 months, 95% CI 3-14 months; P=0.021) and recurrence at multiple sites (10 months, 95% CI 4-15 months; P=0.009). Median recurrence-free survival before local + distant recurrence was 11 months (95% CI 0-22 months) and did not differ significantly from local recurrence (P=0.076). Median post-recurrence survival was longer after isolated local recurrence (11 months, 95% CI 5-17 months) as compared to local + distant recurrence (3 months, 95% CI 2-3 months; P<0.001) or recurrence at multiple sites (4 months, 95% CI 1-6 months; P=0.003), but did not differ significantly from isolated distant recurrence (7 months, 95% CI 3-10 months; P=0.066).

Treatment of Recurrence

Median OS for the 68 patients with recurrence was 15 months (95% CI 10-20 months), including a median post-recurrence survival of 4 months (95% CI 4-5 months). Further tumor specific treatment was administered in 17 of 68 of patients (25%) with recurrence while all other patients received best supportive care. Reasons for withholding further treatment were poor performance status of patient (n=29), patient wish (n=12), no treatment offered to patient (n=5) or unknown (n=5). Chemotherapy was the treatment of choice for most patients with recurrence, with FOLFIRONOX (n=8), Gemcitabine/Nab-Paclitaxel (n=2) and Capetacibine (n=2) being the regimens of choice. Other treatments included conventional radiotherapy (n=2), stereotactic body radiation therapy (n=2) and a new phase 1 trial (n=1).

Table 2. Clinicopathological and treatment characteristics of patients with recurrence

Variable	*All patients with recurrence*		*RFS < 10 months*		*RFS > 10 months*		*P-Value*
Recurrence, *n* (%)	68		35	(51%)	33	(49%)	
Male, *n* (%)	42	(62%)	23	(66%)	19	(58%)	0.490
Age (years), mean (SD)	66.6	(8.9)	67.1	(10.3)	66.0	(7.2)	0.625
CA 19-9 (U/ml)[a]							0.722
<37 U/mL, *n* (%)	9	(20%)	4	(17%)	5	(22%)	
>37 U/mL, *n* (%)	36	(80%)	19	(83%)	17	(78%)	
Median (IQR)	170	(41-450)	170	(38-540)	175	(42-440)	0.716
Operation procedure, *n* (%)							0.322
PPPD	52	(77%)	24	(69%)	28	(85%)	
Whipple	7	(10%)	5	(14%)	2	(6%)	
Distal pancreatectomy	5	(7%)	4	(11%)	1	(3%)	
Total pancreatectomy	3	(4%)	2	(6%)	1	(3%)	
Modified Appleby	1	(2%)	0	(0%)	1	(3%)	
Adjuvant Gemcitabine, *n* (%)	40	(58%)	16	(46%)	24	(73%)	**0.024**
Resection margin, *n* (%)							0.190
R0	22	(32%)	10	(29%)	12	(36%)	
R1	29	(43%)	13	(37%)	16	(49%)	
R2	17	(25%)	12	(34%)	5	(15%)	
Tumor differentiation, *n* (%)							0.220
Well/Moderate	55	(81%)	26	(74%)	29	(88%)	
Poor	13	(19%)	9	(26%)	4	(12%)	
Tumor size (cm), median (IQR)	3.7	(2.8-4.5)	4.0	(3.0-4.5)	3.5	(2.4-4.2)	0.052
T-stage, *n* (%)							0.140
2	13	(19%)	4	(11%)	9	(27%)	
3	44	(65%)	23	(66%)	21	(64%)	
4	11	(16%)	8	(23%)	3	(9%)	
Positive lymph nodes, *n* (%)	53	(78%)	28	(80%)	25	(76%)	0.673
Microscopic perineural invasion, *n* (%)	62	(91%)	32	(91%)	30	(91%)	1.000
Microscopic perilymphatic invasion, *n* (%)	28	(41%)	15	(43%)	13	(39%)	0.772
Microscopic vascular invasion, *n* (%)	41	(60%)	20	(57%)	21	(64%)	0.584
Symptomatic recurrence, *n* (%)	58	(85%)	29	(83%)	29	(88%)	0.735
Recurrence site, *n* (%)							0.755
Local	10	(15%)	4	(11%)	6	(18%)	
Local + distant	13	(19%)	6	(17%)	7	(21%)	
Distant	22	(32%)	13	(37%)	9	(27%)	
Multiple	23	(34%)	12	(34%)	11	(33%)	
Treatment after recurrence, *n* (%)	17	(25%)	8	(23%)	9	(27%)	0.674

[a] 34% (*n*=23) not sampled.

CA, *carbohydrate antigen*; RFS, *recurrence-free survival*; PPPD, *pylorus-preserving pancreatoduodenectomy*; SD, *standard deviation*.

Table 3. Factors associated with post-recurrence survival

	Univariable analysis			*Multivariable analysis*		
Clinical characteristics	***HR***	***95% CI***	***P-value***	***HR***	***95% CI***	***P-value***
Resection margin						
R1 vs. R0	0.54	0.29-1.02	**0.059**	0.50	0.23-1.06	0.071
R2 vs. R0	1.19	0.61-2.31	0.610	1.39	0.66-2.96	0.387
T-stage						
3 vs. 2	2.03	0.94-4.35	**0.070**	2.34	1.01-5.41	**0.048**
4 vs. 2	2.22	0.86-5.78	0.101	1.73	0.63-4.76	0.287
Recurrence site						
Local + distant vs. Local	5.31	1.79-15.71	**0.003**	8.20	2.37-28.33	**0.001**
Distant vs. Local	2.47	0.92-6.66	**0.073**	3.77	1.34-10.54	**0.012**
Multiple vs. Local	3.57	1.35-9.44	**0.010**	8.98	2.99-26.97	**<0.001**
Recurrence-free survival						
>10 months vs. <10 months	0.52	0.30-0.88	**0.016**	0.42	0.23-0.76	**0.004**
Treatment after recurrence						
Yes vs. No	0.47	0.25-0.88	**0.019**	0.32	0.16-0.65	**0.002**

All factors with a *P*-value of <0.10 in univariable analysis were included as a covariate in multivariable Cox proportional hazard model.
Clinical characteristics evaluated and insignificant on both univariable and multivariable analysis: age, gender, pre-operative serum CA 19-9 level, type of operative procedure, adjuvant Gemcitabine, tumor size, N-stage, tumor differentiation grade, microscopic ingrowth in neural, lymphatic or vascular structures and symptomatic at recurrence.
CI, *confidence interval;* HR, *hazard ratio*.

Median survival after start of treatment for recurrence was 5 months (95% CI 3-7 months). Median post-recurrence survival for the treatment group (n=17) was 7 months (95% CI 6-8 months), significantly longer (P=0.016) than the median post-recurrence survival of 3 months (95% CI 2-4 months) found for the group receiving best supportive care (n=51) (Figure 3A). Median recurrence-free survival did not differ (P=0.142) between the treatment and best supportive care group (11 months vs. 10 months, respectively). Patients who recurred more than 10 months following surgery had a longer median post-recurrence survival (5 months, 95% CI 2-8 months) when compared to patients who recurred within 10 months (3 months, 95% CI 1-4 months; P=0.013) (Figure 3B). Nine patients out of 68 patients with recurrence (13%) were alive after a median follow-up of 26 months (14-38 months).

Factors associated with Post-Recurrence Survival

Univariable analyses identified five factors associated with longer post-recurrence survival (Table 3). After adjusting for the other covariates in the multivariable analysis, a recurrence-free survival >10 months (Hazard Ratio (HR) 0.42, 95% CI 0.23-0.76, P=0.004) and administration of further treatment for recurrence (HR 0.32, 95% CI 0.16-0.65, P=0.002) were significantly associated with a longer post-recurrence survival. Furthermore, isolated local recurrence was

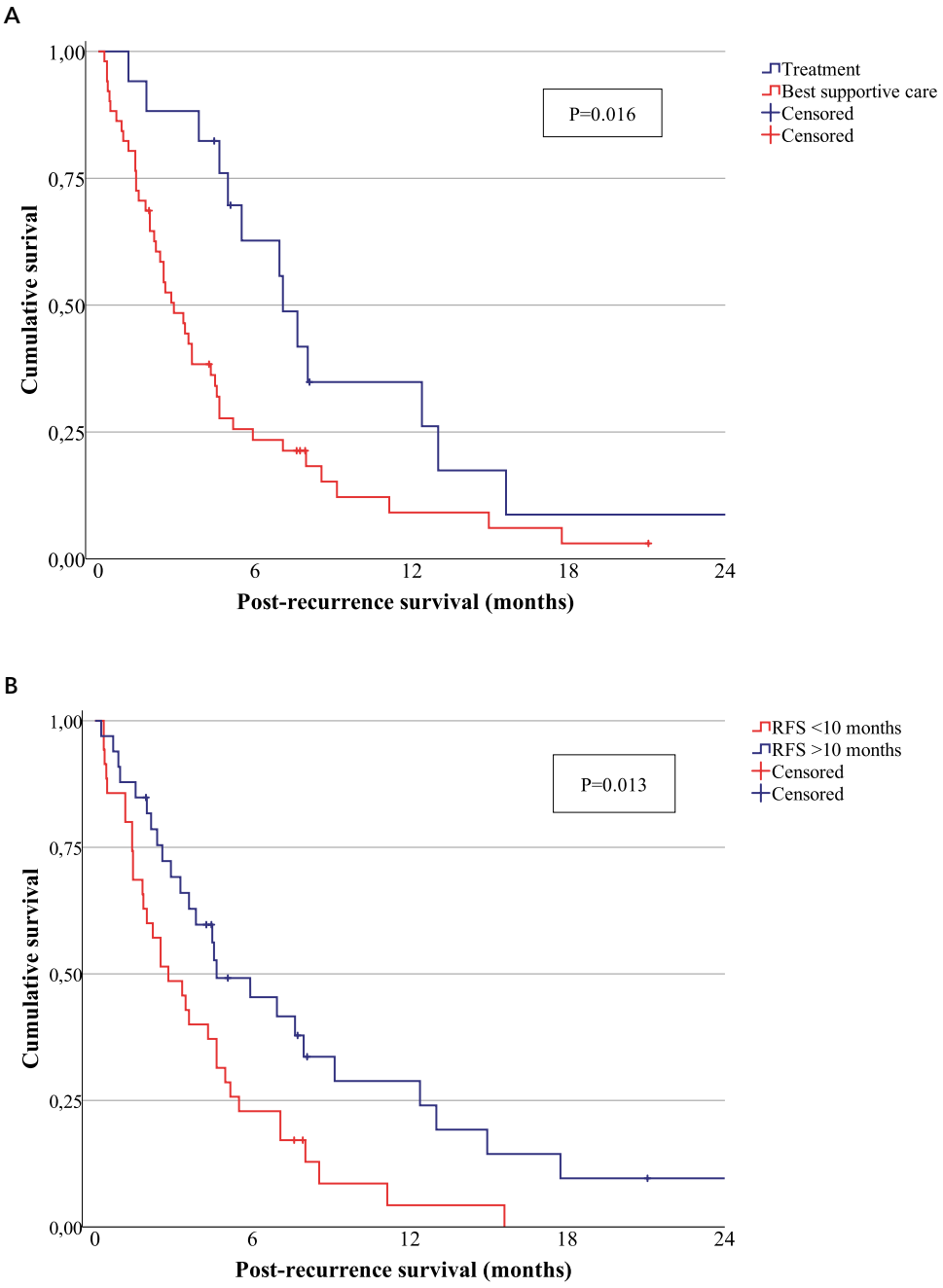

Figure 3. Kaplan-Meier plots showing that (A) post-recurrence survival was longer (P=0.016) for patients receiving treatment as compared to best supportive care (BSC) and (B) post-recurrence survival was longer (P=0.013) for patients with recurrence-free survival of more than ten months.

associated with longer survival than local + distant recurrence (HR 8.20, 95% CI 2.37-28.33, P=0.001), isolated distant recurrence (HR 3.77, 95% CI 1.34-10.54, P=0.012) or recurrence at multiple sites (HR 8.98, 95% CI 2.99-26.97, P<0.001).

Discussion

Although imaging was explicitly not part of our symptomatic follow-up protocol, the majority of patients (87%) in this study underwent additional imaging procedures for the detection of recurrence at least once. Furthermore, patients underwent an average of 3 imaging procedures during the entire follow-up period. Most of the recurrences (85%) were diagnosed at a late stage and after the manifestation of clinical symptoms, resulting in only 17 patients (25%) receiving additional treatment for their recurrence.

The primary objective of this study was to evaluate how often patients undergo imaging during a symptomatic follow-up approach based on European guidelines.[8] Although our single-institutional, non-randomized study did not allow for direct comparison of symptomatic surveillance and follow-up with routine imaging, our results did reveal interesting results that differ from studies reporting on diagnostic follow-up. For instance, in our cohort 85% of recurrences were diagnosed after the manifestation of clinical symptoms. Plausibly, studies employing more intensive follow-up have reported lower rates of symptomatic recurrence (20%-74%).[13-15] Furthermore, only 25% (n=17) of patients in our study received additional treatment for their recurrence, primarily due to advanced disease and poor performance status upon diagnosis of recurrence. This was considerably less than the 44-84% reported by other studies using regular imaging testing for the detection of recurrence.[13-16] A credible explanation for this difference is provided by Tzeng et al., as their study found that additional treatment was less frequently administered to symptomatic patients in whom recurrence was detected at a later stage (91% vs. 61%; P<0.001).[13] However, these findings could also be partly explained by the differences in the approach to treatment of recurrence between different centers.[17] When considering the more intensive follow-up of some major North-American, European and Asian institutions compared to those who follow current European guidelines, it is reasonable to assume they will also promote additional treatment to their patients in a more active manner.

Above mentioned differences between institutions might further explain some results presented in our study. Firstly, we found a relatively high R2 resection rate of 25% in our cohort. The reporting of positive microscopic margin status varies considerably in the literature, from 22% to 85%.[18] In our institution, a uniform method for margin analysis was used by the

surgeon and the pathologist. This standardized protocol used specimen inking, including assessing the pancreatic neck margin, the portal vein groove, the uncinate margin and the posterior pancreatic margin. Using this protocol, R2 rates of around 20% are not uncommon in Dutch or European pancreatic cancer centres. This percentage might be further inflated due to the lack of neoadjuvant chemo(radio)therapy in this cohort, as use of neoadjuvant chemoradiotherapy has been shown to result in higher rates of negative resection margins.[19] When looking at the association between resection margin and development of recurrence, respective groups of R0, R1 and R2 were too small to perform significant analysis. Secondly, only 58% of patients went on to receive adjuvant chemotherapy with Gemcitabine after resection. In our experience, Dutch medical oncologist and PDAC patients tend to be less persistent in pursuing adjuvant chemotherapy than some of their international colleagues, possibly explaining this relatively low percentage. A significant percentage of patients might have chosen to forgo adjuvant therapy and focus instead on quality of life. Also, systemic therapy started after early recurrence was scored as "treatment for recurrence" and not as adjuvant therapy. Patients with very early recurrence whom consequently received treatment are thus not included in the 58% receiving adjuvant treatment.

The secondary objective of this study was to analyze the timing, pattern and treatment of recurrence along with the identification of predictors associated with prolonged post-recurrence survival. In this study, isolated local recurrence was significantly associated with a longer post-recurrence survival when compared to other recurrence sites. Recent studies have focused on patients with isolated local recurrence as a possible subgroup benefitting from additional therapy.[20] Only 15% of patients were diagnosed with isolated local recurrence in our cohort, compared to percentages of 24-37% found in recurrence studies using follow-up with standardized imaging testing.[21-24] Again, the possible explanation for this disparity can be found in the follow-up methodology, with regular imaging procedures increasing the chance of detecting local recurrence before any distant spread.

Apart from an isolated recurrence location and a median recurrence-free survival >10 months, additional treatment for recurrence was an independent predictor for prolonged survival after recurrence. A similar advantage was seen in two other studies comparing patients with recurrence receiving either additional treatment or best supportive care. Meyers et al. divided patients with proven recurrence into three groups: those who received treatment after recurrence (n=45, 64%), those who were not offered treatment or refused (n=9, 13%) and those with poor performance status not receiving treatment (n=16, 23%).[16] Median survival after recurrence for these groups were 10 months, 6 months and 1 month respectively ($P<0.0001$). Likewise, Tzeng et al. compared the post-recurrence survival of

157 (73%) patients with recurrence receiving treatment with untreated patients and found a significantly extended post-recurrence survival of 12 months vs. 3 months (P<0.001).[13]

From the above, it could be argued that instead of postponing imaging until recurrence-related symptoms occur, the average of 3 imaging procedures could be used more effectively when used in 3- or 6-monthly intervals. A more standardized follow-up in this manner could possibly detect asymptomatic recurrence at an earlier stage when patients have a higher chance of being able to receive additional treatment. Also, early detection of recurrence may facilitate patient recruitment to investigational studies and trials. However, these possible beneficial results should be considered in the context of both the possible monetary and emotional burden of intensive follow-up for the patient.[7] These questions highlight the need of future prospective studies to not only determine the actual clinical merit, but also the psychosocial implications and cost-effectiveness of the different approaches to follow-up after resection of PDAC.

The presented results suggest a favorable view on the implementation of a follow-up strategy with regular imaging to identify those patients who might benefit from additional treatment. However, the interpretation our data should be done cautiously. Firstly, the non-randomized design of this study does not allow for direct comparison between different follow-up strategies. Secondly, lead time bias, described as the possibility that the increase in survival time following detection by a surveillance test is a result of earlier diagnosis and does not expose an actual extended survival, could have led to differences in reported survival.[13] However, the median time interval between imaging procedures did not significantly differ for patients with a longer recurrence-free survival or for patients treated for recurrence, implying that this bias was not responsible for the found differences in survival. Furthermore, a prolonged recurrence-free survival may reflect an indolent or even second primary tumor instead of true tumor recurrence, consequently raising the possibility that the longer survival after recurrence is in great part due to tumor biology instead of applied treatment. However, the median recurrence-free survival in the treated and untreated groups of patients were almost identical, limiting the effect of this bias. Lastly, the addition of a quality of life and cost-effective analysis would add an extra dimension to the comparison of symptomatic and regular follow-up and would strengthen any argument for or against symptomatic follow-up.

Conclusion

Established and evidence-based guidelines concerning the follow-up of patients after resection of PDAC are lacking. Furthermore, there are limited data available to guide

treatment decisions for patients with recurrence. The current study is, to our knowledge, the first to evaluate implications of symptomatic follow-up according to European guidelines. In conclusion, we found that even though imaging testing was not part of symptomatic follow-up strategy, the majority of patients underwent additional imaging procedures for the detection of recurrence at least once. Furthermore, while patients underwent an average of 3 imaging procedures during follow-up, most recurrences were detected at a late stage and after the manifestation of clinical symptoms, potentially limiting patients to undergo additional treatment for recurrence. Further prospective studies are needed to determine the actual clinical value, psychosocial implications and cost-effectiveness of different forms of follow-up after resection of PDAC.

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PART IIIb

Biomarkers and Liquid Biopsies

CHAPTER 12

Systematic Review on the Role of Serum Tumor Markers in the Detection of Recurrent Pancreatic Cancer

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Abstract

Objective

Biomarker testing can be helpful to monitor disease progression after resection of pancreatic cancer. This systematic review aims to give an overview of the literature on the diagnostic value of serum tumor markers for the detection of recurrent pancreatic cancer during follow-up.

Methods

A systematic search was performed to 2 October 2017. All studies reporting on the diagnostic value of post-operatively measured serum biomarkers for the detection of pancreatic cancer recurrence were included. Data on diagnostic accuracy of tumor markers were extracted. Forest plots and pooled values of sensitivity and specificity were calculated.

Results

Four articles described test results of CA 19-9. A pooled sensitivity and specificity of respectively 0.73 (95% CI 0.66-0.80) and 0.83 (95% CI 0.73-0.91) were calculated. One article reported on CEA, showing a sensitivity of 50% and specificity of 65%. No other serum tumor markers were discussed for surveillance purposes in the current literature.

Conclusion

Although testing of serum CA 19-9 has considerable limitations, CA 19-9 remains the most used serum tumor marker for surveillance after surgical resection of pancreatic cancer. Further studies are needed to assess the role of serum tumor marker testing in the detection of recurrent pancreatic cancer and to optimize surveillance strategies.

Introduction

Pancreatic cancer is the fourth leading cause of cancer related mortality in Europe for both men and women.[1] For patients with resectable localized disease, radical resection still offers the best chances for long-term survival.[2] However, up to 80% of patients will experience local and/or distant disease recurrence, mostly within two years after surgery.[3] Even resectable pancreatic cancer therefore continues to be associated with a poor 5-year survival of approximately 20%.[4]

Due to limited treatment options for recurrent pancreatic cancer and unclear survival benefits, a hesitant attitude towards recurrence-focused follow-up after resection prevails in Europe.[5] For instance, the European Society for Medical Oncology recommends that post-operative surveillance should focus on symptoms, nutrition and psychosocial support.[6] In contrast, American guidelines from the National Comprehensive Cancer Network advocate an active follow-up approach. This approach consists of routine testing of serum carbohydrate antigen (CA) 19-9 levels and computed tomography (CT) imaging of the abdomen and pelvis every 3-6 months for the first 2 years after surgery.[7] Nevertheless, current guidelines are based on low level evidence and different surveillance strategies remain controversial.[6-10]

The development of new promising treatment options for pancreatic cancer, such as FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan and oxaliplatin), stereotactic body radiotherapy (SBRT), radiofrequency ablation (RFA) and irreversible electroporation (Nanoknife®) has led to a rising interest in early diagnosis and treatment of recurrent pancreatic cancer.[11-13] Therefore, a standardized surveillance strategy is increasingly recommended.[14-17] However, the search for the most appropriate follow-up approach to detect disease recurrence is ongoing. Radiographic surveillance alone is unlikely to detect recurrence in the earliest stages, since lesions have to be more than 1cm in diameter to become radiologically manifest.[18] As occult cancer recurrence can be suspected by a sudden elevation of serum tumor markers, the addition of biomarker testing can help monitor disease progression after surgery.[19] To date, it is unclear which serum tumor markers are best suitable for the early detection of recurrent pancreatic cancer during follow-up. Consequently, the clinical use of biomarkers for surveillance purposes after resection of pancreatic cancer remains limited.

This systematic review aims to give an overview of the literature on the diagnostic value of post-operatively measured serum tumor markers for the detection of recurrent pancreatic cancer.

Methods

A systematic search of the literature was performed in PubMed, EMBASE and Cochrane Library up to 2 October 2017. Synonyms, medical subject headings (MeSH) terms and keywords for "pancreatic cancer recurrence" were combined with different terms for "serum tumor marker", restricted to title and abstract (Online Supplementary eTable S1). The Preferred Reporting Items for Systematic review and Meta-Analyses (PRISMA) guidelines were followed.[20] All titles and abstracts were screened for relevance by two authors (LAD, VPG), based on predefined in- and exclusion criteria. Subsequently, both authors evaluated all full-text articles. Disagreement on eligibility was addressed by discussion and consensus.

Eligibility Criteria

Studies were found to be eligible when reporting on the diagnostic value of serum tumor markers for the detection of disease recurrence as measured during follow-up after resection of pancreatic cancer. Articles in languages other than English or Dutch were excluded. References of included articles were crosschecked, and relevant studies were included if appropriate.

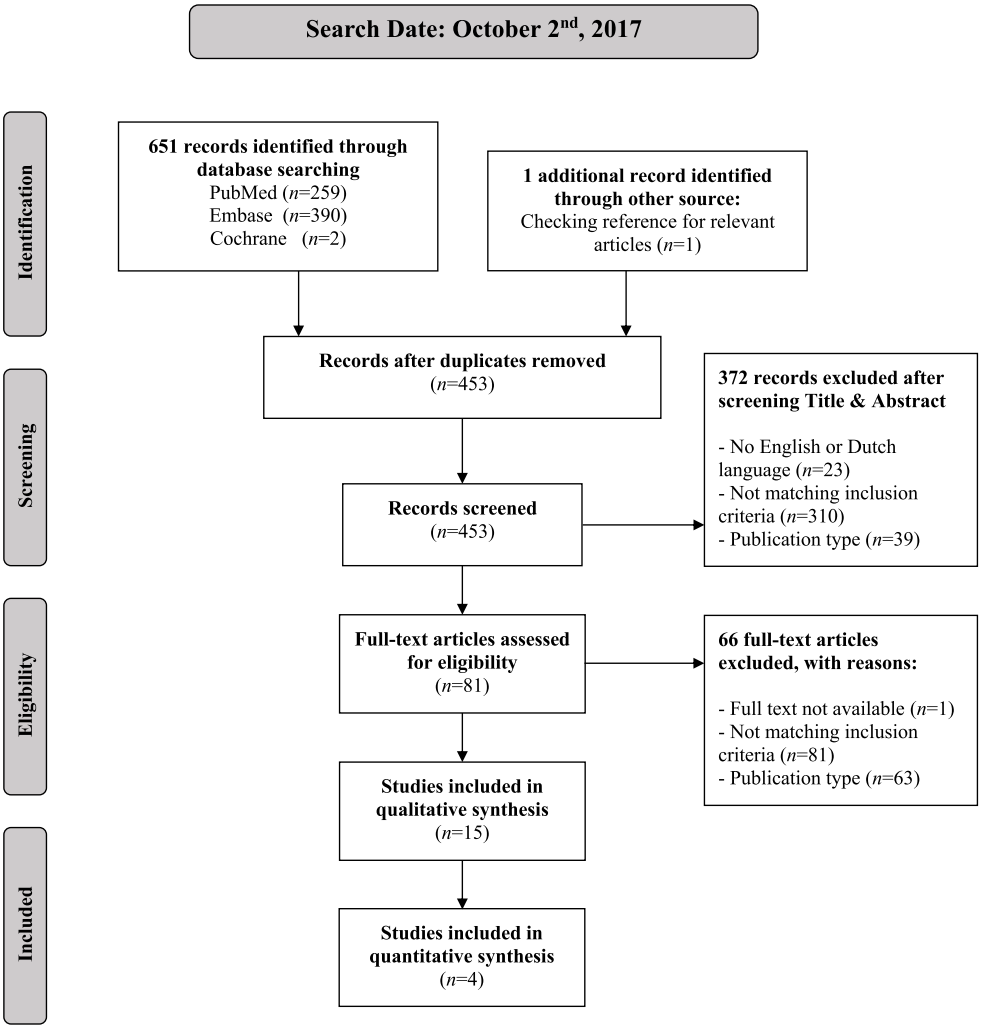
Assessment of Methodological Quality

Critical appraisal of methodological quality was performed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool.[21] Using this tool, the risk of bias and applicability to the research question of each study was evaluated according to four key domains: patient selection, index test, reference standard, and flow and timing.

A study-population of patients with pancreatic cancer undergoing resection with curative intent was deemed representative. Outcomes of patients with different disease entities (i.e. peri-ampullary tumors, biliary-pancreatic cancer) were only used if these results were not separable from outcomes of patients with pancreatic cancer. Furthermore, this systematic review questioned the diagnostic accuracy of serum tumor markers during follow-up in all patients who underwent resection for pancreatic cancer. Studies performing analyses on Lewis antigen α- and β-positive individuals only were therefore excluded. Also, studies were excluded if the diagnostic value of the investigated serum tumor marker could not be established, for instance when the tumor marker was measured after diagnosis of disease recurrence and not at frequent intervals during follow-up.

Both CT and PET-CT were considered as suitable reference standards. CT is generally the

preferred imaging modality for the diagnosis of pancreatic cancer and surveillance with follow-up CT scans is recommended by the NCCN.[7,22] Positron emission tomography-computed tomography (PET-CT) seems to be of additional value in the detection and staging of pancreatic cancer.[23-25] Clinical or sonographic detection of recurrence was considered less appropriate. Furthermore, if variable methods of imaging were used in different study-subjects, the methodological quality of the study was disputed.



Figuur 1. Flowchart of article selection.

Data Extraction

Study characteristics extracted from the selected articles were study period, age, gender, follow-up strategy after resection of pancreatic cancer, follow-up duration and definition of recurrence. Data on recurrence patterns and time to detection of recurrence were collected additionally. Furthermore, results on diagnostic accuracy of each investigated serum tumor marker, i.e. sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), were extracted.

Statistical Analysis

Description statistics were presented as in the original article. Sensitivity, specificity, positive and negative predictive values of serum tumor markers with their corresponding 95% confidence intervals were shown. Forest plots and pooled values of sensitivity and specificity were calculated using Meta-DiSc 1.4 (Hospital Ramon y Cajal and Universidad Complutense, Madrid).

Results

A total of four studies with 318 relevant patients were ultimately found to be eligible for data extraction (Figure 1, Online Supplementary eTable S2).[26-29]

Methodological Quality

Overall, the methodological quality of the included studies was reasonable. Both the studies of Jung et al. and Sperti et al. were originally designed to assess the clinical usefulness of PET-CT in follow-up of patients after radical resection of pancreatic cancer.[26,27] Mataki et al. investigated "the clinical significance of molecular detection of circulating cancer cells in the blood as an early indicator of relapse during follow-up of patients who underwent a curative operation for biliary-pancreatic cancer". In this study, explicit data on diagnostic accuracy of tumor marker testing for pancreatic cancer alone was not distinguishable.[28] Safi et al. aimed to determine the sensitivity and specificity of CA 19-9 in diagnosing pancreatic cancer, to predict resectability rates, and to investigate the value of CA 19-9 levels as a prognostic indicator and for the detection of disease recurrence to monitor patients who have undergone treatment for pancreatic cancer.[29] In three of the four included articles, the definition of recurrent pancreatic cancer was clearly described (Table 1).[26-28] However, disease recurrence was not well defined in the study of Safi et al.[29] Intervals of both the index and reference tests were standardized in all four finally included studies; nevertheless, a cut-off value of serum CA 19-9 was not mentioned in the study of Jung et al.[27]

Study and Patient Characteristics

In all selected studies, routine serum tumor marker testing and additional imaging was performed every 3-6 months during the first two years of follow-up. The four included articles described diagnostic accuracy of CA 19-9, whereas one of them also reported on serum CEA values.[28] A cut-off value of 37 U/ml was maintained for CA 19-9 and 5 ng/ml for CEA. In all studies, diagnostic accuracy values were calculated based on elevation of CA 19-9 above the cut-off value. A time factor was allowed in three of the four included studies, meaning that a rise in serum tumor markers was also considered positive when disease recurrence was confirmed on subsequent follow-up. Safi et al. reported that elevation of CA 19-9 preceded clinical or radiographical changes with a median time ranging from 4-20 weeks.[29] Mataki et al. found suspected changes in serum CEA, serum CA 19-9 and imaging modalities after respectively 303 ± 169, 237 ± 172 and 375 ± 268 days.[28] In the study of Sperti et al., tumor marker positivity was not well defined. However, it was described that PET-CT was performed in 51 patients because of a rise in CA 19-9, i.e. a change in CA 19-9 over time, although this was not precisely mentioned.[26] In Jung et al., recurrent pancreatic cancer could be clinically suspected with abnormal findings on CT and/or PET-CT and/or elevation of serum CA 19-9. Since suspected recurrence had to be confirmed on subsequent follow-up, a time factor was included in their clinical definition of disease recurrence.[27] Recurrent pancreatic cancer was clinically and/or radiologically confirmed in most patients. Two studies described pathological confirmation of disease recurrence by tumor resection, tumor sampling or needle biopsy in 37% and 27% of patients, respectively.[26,27] Follow-up duration varied from one to 104 months, whilst disease recurrence was detected between one and 66 months. In 267 of 318 participants (84%), recurrent pancreatic cancer was diagnosed. Thirty-two of 154 (21%) study-subjects had isolated local recurrence, whereas distant metastases were found in 122 participants (79%) (Table 1).

Diagnostic Accuracy of Serum Tumor Markers for the Detection of Recurrent Pancreatic Cancer

Overall, considering test results of CA 19-9 from four articles, sensitivities ranged from 68-89% and specificities varied between 77-89% (Table 2).[26-29] A pooled sensitivity of 0.73 (95% CI 0.66-0.80, P=0.183, I2=41.2%), and a pooled specificity of 0.83 (95% CI 0.73-0.91, P=0.652, I2=0,0%) was calculated (Figure 2). Due to the limited amount of data, a formal meta-analysis was not performed. Only one article reported on diagnostic accuracy of CEA, showing a sensitivity of 50% and specificity of 65%.[28]

Table 1. Study characteristics

Study (year)	*Study interval*	*FU strategy after re-section of pancreatic cancer*	*FU duration (months)*	*Definition of recurrence*	*Recurrence (n/n total)*	*Time to recurrence (mos)*	
						Median (range)	*Mean ± SD*
Jung (2015)	2004-2012	CA 19-9 + abdominal CT at 3, 6 and 12 months and every 6months thereafter; PET-CT once yearly and when other follow-up tests suggested tumor recurrence	26 (9-81)	Pathological confirmation (*n*=31; 37%) Clinical confirmation: suspected on abdominal CT and/or PET-CT and/or elevation of serum CA 19-9, confirmed as recurrence on subsequent FU (*n*=53; 63%)	84/110 - ILR: 19 - Distant: 65	11 (1-54)	
Sperti (2010)	1998-2007	Physical examination, laboratory tests, CEA and CA 19-9, abdominal US and CT + chest X-rays, every 3 months for the first 2 years and every 6 months thereafter + at least one PET-CT	22 (5-104)	Pathological confirmation (*n*=17; 27%): tumor resection (*n*=8; 13%) or tumor sampling (*n*=9; 14%) Imaging and/or tumor progression (*n*=46; 73%)	63/72 - ILR: 11 - Distant: 53	11 (2-66)	
Mataki (2004)	1996-2001	CEA mRNA, CEA, CA 19-9 + CT, MRI and/or US every 3 months	49 (24-89)	Changes on any imaging modality, confirmed by another type of imaging and/or needle biopsy	7/20 - ILR: 2 - Distant: 5		12 ± 9
Safi (1997)	NR	Clinical examination, chemical and pharmacologic profiles + CT scan at 3 months; in case of CA 19-9 elevation, a search for a metastatic source was conducted independent of the FU procedures	6 (1-42)	NR	113/116	6 (1-42)	

CT, *computed tomography;* FU, *follow-up;* ILR, *isolated local recurrence;* MRI, *magnetic resonance imaging;* NR, *not reported* PET-CT, *positron emission tomography-computed tomography;* US, *ultrasonography;* SD, *standard deviation*.

Table 2. Results

Study (year)	*Tumor marker*	*Cut-off value*	*Reference test*	*Patients (n)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Accuracy*
Jung (2016)	CA 19-9	NR	CT or PET-CT	110	68% (57/84)	89% (23/26)	95% (57/60)	46% (23/50)	73% (80/110)
Sperti (2010)	CA 19-9	37 U/ml	CT or PET-CT	72	81% (51/63)	77% (7/9)	96% (51/53)	37% (7/19)	80% (58/72)
	CA 19-9	37 U/ml	Imaging (CT, MRI and/or US)		69% (11/16)	81% (30/37)	38% (11/18)	75% (30/35)	77% (41/53)
Mataki (2004)	CEA	5 ng/ml		53*	50% (8/16)	65% (24/37)	61% (8/21)	86% (24/32)	60% (32/53)
Safi (1997)	CA 19-9	37 U/ml	CT or clinical examination	116	89% (81/90)	NR	NR	NR	NR

* Patients with biliary-pancreatic cancer.

CT, *computed tomography*; NPV, *negative predictive value*; MRI, *magnetic resonance imaging*; NR, *not reported*; PET-CT, *positron emission tomography-computed tomography*; PPV, *positive predictive value*; *US, ultrasonography*.

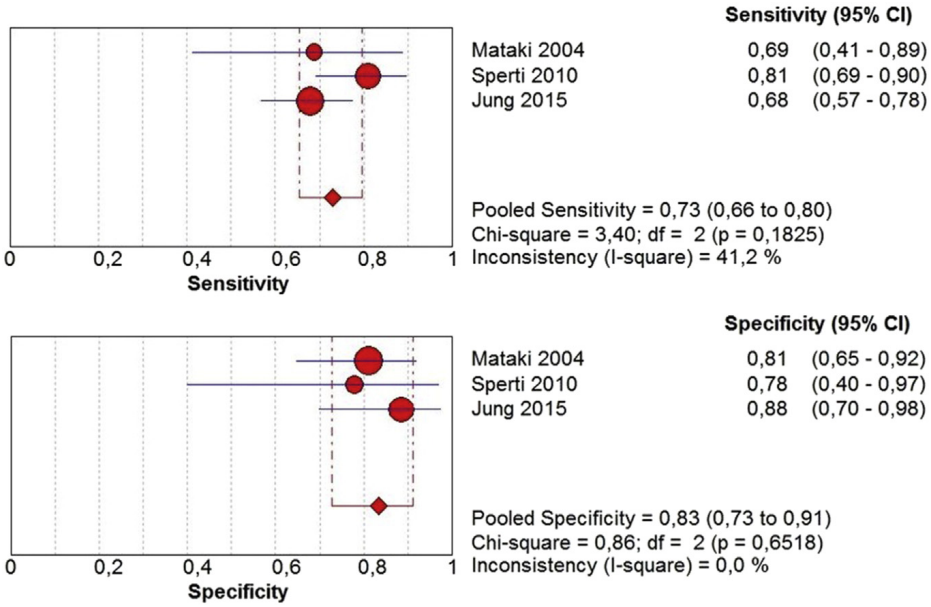


Figure 2. Forest plots of the sensitivity and specificity of CA 19-9.

Discussion

This systematic review provides an overview of the existing literature on the diagnostic accuracy of serum tumor markers for the detection of disease recurrence in the follow-up after resection of pancreatic cancer. According to the reviewed literature, routine testing of serum CA 19-9 during follow-up has a moderate value with a sensitivity of 68-89% and a specificity of 77-89% for detecting recurrence. Only one study reported on the diagnostic accuracy of CEA, showing a low sensitivity and specificity of 50% and 65%, respectively. No other serum tumor markers were investigated for surveillance purposes after surgery for pancreatic cancer in the current literature.

In line with the findings of this literature study, CA 19-9 is the most widely assessed serum biomarker during follow-up after resection of pancreatic cancer in clinical practice.[30,31] Before CA 19-9 was introduced as an effective tumor marker for pancreatic cancer, carcinoembryonic antigen (CEA) was used in the diagnostic workup of pancreatic cancer. However, CEA has been shown to be inferior to CA 19-9 for monitoring disease burden and surveillance following radical resection of pancreatic cancer.[28] Therefore, CEA has mostly been replaced by CA 19-9.

Numerous studies have been published on the prognostic value of pre- and perioperative measured serum tumor markers as predictors of pancreatic cancer recurrence after resection, mainly focusing on CA 19-9. Several of these studies reported a significant additional value of pre-operative CA 19-9 levels >100 U/ml, CA 19-9 levels >50 U/ml adjusted to the serum bilirubin levels, elevated post-operative CA 19-9 levels and post-operative CA 19-9 velocity >95 U/ml/4-weeks in predicting disease recurrence.[18,32,33] As shown in this systematic review, however, only a few studies were performed on the diagnostic accuracy of longitudinal serum CA 19-9 testing during follow-up for detection of disease recurrence, using imaging studies as reference standard.[26-29] Additionally, several other studies found that serum CA 19-9 rises a considerable period of time before tumor recurrence becomes radiologically or clinically manifest, ranging from one to nine months.[19,34-38]

A significant limitation of the clinical use of CA 19-9 as a tumor marker for the detection of pancreatic cancer recurrence is the relatedness of CA 19-9 to the Lewis blood group antigens. About 90-95% of patients belong to Lewis blood groups (i.e. Le (α-β+) or Le (α+β-)) that express the CA 19-9 antigen.[30,39,40] Currently, the search for other more generalizable biomarkers is therefore ongoing. In 2009, a compendium of potential pancreatic cancer tumor markers following an extended review of the literature was published by Harsha et al.[41] Most of these potential useful biomarkers are still being investigated, in particular for

prognostic purposes, to optimize patient selection for resection of pancreatic cancer.[43] Up until now, the evidence on the availability and diagnostic value of accurate biomarkers for detecting disease recurrence in an early stage is lacking.[32]

To date, studies on other potential serum tumor markers, such as s-pancreas antigen-1 (SPan-1) and duke pancreatic monoclonal antigen type 2 (DU-PAN 2), have only focused on pre-operative measured values for the detection of pancreatic cancer or prognostic outcomes.[43-48] According to these studies, SPan-1 is reliable for the detection of pancreatic cancer, with a sensitivity of 81-92% and specificity of 76-85%, and is particularly useful in Lewis antigen α- and β-negative individuals.[45-47,49] SPan-1 therefore may be a useful biomarker for patients who are unable to synthesize CA 19-9, with a prognostic value comparable to that of CA 19-9 when pre-operatively measured. DU-PAN 2 can be detected in Lewis α- and β-negative individuals as well, with a sensitivity of 48-72% and a specificity of 85-94%, according to the current literature.[50-52] Two studies furthermore reported that a pre-operative SPan-1 concentration of >41 U/ml[44] or >37 U/ml[43], and a DU-PAN 2 value of >150 U/ml[43] predicts early disease recurrence. Nevertheless, none of these studies did report on the additional value of post-operative serum SPan-1 and DU-PAN 2 levels during follow-up.

The ideal tumor marker for surveillance after surgical resection of pancreatic cancer has (1) a high sensitivity and positive predictive value, identifying all patients with recurrent pancreatic cancer preferably in an early stage; (2) a high specificity, detecting only patients with pancreatic cancer recurrence and not with benign diseases or without any disease; (3) correlates with the tumor burden; (4) provides prognostic and predictive information; and is (5) affordable and easy to obtain.[53,54] To date, however, no such tumor marker has been identified. In this absence, a combination of regular serum CA 19-9 testing and routinely performed imaging studies, such as CT or PET-CT, continues to be the most used surveillance strategy to assess early (asymptomatic) disease recurrence and to adapt adjuvant treatment as necessary.[55,56] Differentiating local pancreatic cancer recurrence from post-operative fibrosis during radiographic surveillance can be challenging.[55] Moreover, it can be difficult to obtain histological evidence of local disease recurrence. Although the value of current serum biomarkers by itself may be limited, the combination of a rise in tumor markers with suspicious, progressive findings on follow-up imaging using CT and/or PET-CT could aid in the clinical diagnosis of recurrent pancreatic cancer. Depending on the imaging modality used, sensitivities of 50-98% and specificities of 65-100% are reported with this combined strategy.[26,27] However, a prospective, randomized study is needed to validate this approach.

A limitation of this systematic review was the limited data available for statistical analysis. In particular, no prospective studies were found. Nevertheless, the methodological quality of the included studies was reasonable. However, in the study of Jung et al., elevation of serum tumor markers on subsequent follow-up was used for clinical confirmation of recurrence, possibly leading to incorporation bias and overestimation of test accuracy.[27] Future well-designed prospective studies are needed to determine whether detection of asymptomatic recurrence can lead to improved survival and quality of life. Further research on this subject should lead to the implementation of evidence-based recommendations on structured follow-up in national and international guidelines on pancreatic cancer.

Conclusion

Although testing of serum CA 19-9 has some considerable limitations, CA 19-9 remains the most used serum tumor marker for surveillance after surgical resection of pancreatic cancer in current clinical practice. Further studies are needed on the diagnostic accuracy of these and other promising pancreatic cancer tumor markers during follow-up, to assess the role of serum tumor marker testing in the post-operative detection of recurrent pancreatic cancer and optimize surveillance strategies.

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Supplemental Material to Chapter 12

- **Supplemental eTable S1.** Search strategy
- **Supplemental eTable S2.** Assessment of methodological quality using the QUADAS-2 tool

Supplemental material can be consulted online per the website of the journal and/or publisher.

CHAPTER 13

Circulating Tumor Cells Dynamics in Pancreatic Adenocarcinoma Correlate with Disease Status: Results of the Prospective CLUSTER Study

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Abstract

Objective

Previous retrospective studies demonstrated that circulating tumor cell (CTCs) subtypes correlate with overall survival in patients with pancreatic ductal adenocarcinoma (PDAC). The purpose of this study is to assess whether circulating tumor cell dynamics in patients with pancreatic cancer correlate with disease status and reflect progression of disease or response to treatment

Methods

The CLUSTER study is a prospective longitudinal study on PDAC CTCs dynamics (NCT02974764). Multiple peripheral blood samples were collected from 200 consecutively enrolled patients with presumed PDAC diagnosis. CTCs were isolated and characterized by immunofluorescence.

Results

Two major CTCs subtypes were identified in all patients: epithelial CTCs (eCTCs) and mesenchymal CTCs (mCTCs). Patients who received neoadjuvant chemotherapy had significantly lower total CTCs (tCTCs, P=0.007), eCTCs (P=0.007), and mCTCs (P=0.034), compared to untreated patients eligible for upfront resection. Surgical resection of the primary tumor resulted in significant reduction, but not disappearance, of CTCs burden across all cell subtypes (P<0.001). In multivariable logistic regression analysis, pre-operative numbers of all CTCs subpopulations were the only predictors of early recurrence within 12 months from surgery in both chemo-naïve and post-neoadjuvant patients (OR 5.9-11.0). Alterations in CTCs were also observed longitudinally, prior to disease recurrence. A risk assessment score based on the difference of tCTCs increase accurately identified disease recurrence within the next two months, with an accuracy of 75% and 84% for chemo-naïve and post-neoadjuvant patients respectively.

Conclusion

We report novel findings regarding CTCs from a large prospective cohort of PDAC patients. CTCs dynamics reflect progression of disease and response to treatment, providing important information on clinical outcomes, not available by current tumor markers and imaging.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is historically characterized by increased mortality rates.[1] It is currently predicted to become the second leading cause of cancer-related deaths in the United States by 2030.[2] This unfavorable prognosis is mainly associated with the advanced stage of disease at the time of diagnosis, due to a combination of late presentation and early metastasis.[3] The absence of biomarkers with high diagnostic sensitivity and specificity in pancreatic cancer makes early identification and monitoring of disease progression challenging.[4] However, the in-depth genetic characterization of pancreatic tumors[5] and the introduction of possible molecular biomarkers for systemic disease assessment[6] introduce a new potential in identification and treatment of PDAC.

The implication of circulating tumor cells (CTCs) in the metastatic mechanism has been proposed since the introduction of the "seed and soil" hypothesis by Stephen Paget in breast cancer.[7] The hypothesis suggests that primary tumor cells invade adjacent vasculature, disseminate through circulation to colonize distant sites, and give rise to secondary metastases.[8,9] A basic mechanism that allows CTCs to endure the dissemination process is epithelial to mesenchymal transition (EMT).[10] EMT plays a major role in embryonic tissue differentiation and pathologic response to tissue injury.[11] In cancer, epithelial cells respond to EMT-inducing signals from the tumor microenvironment and acquire mesenchymal characteristics and, subsequently, tumor-initiating potential.[12] Involvement of CTCs in the metastatic process has been identified in the majority of solid tumors.[13-16] Additionally, recent studies in PDAC have reported on the potential of CTCs as a molecular biomarker of disease status by identifying an association of their number and phenotype with early disease recurrence, overall survival, and presence of occult metastases.[17-19]

CTCs dynamics, or fluctuations of CTCs burden in circulation, is a promising longitudinal indicator of disease status. In metastatic breast cancer, CTCs dynamics have been identified as a potential biomarker of treatment response and disease progression.[20-22] Similar data regarding utilization of CTCs dynamics in evaluating treatment effect in PDAC are limited. We have designed and conducted a prospective longitudinal cohort study on CTCs dynamics (NCT02974764), in which 200 enrolled patients with PDAC were followed with sequential phlebotomy along the course of their disease. The primary study endpoint was to identify and characterize the association between CTCs dynamics and disease status. Secondary study goals included the assessment of the predictive capacity of CTCs regarding early recurrence and disease-specific mortality, and the identification of CTCs dynamics potential as a biomarker of disease recurrence.

Methods

Patient Cohort

All participating PDAC patients in the CLUSTER study (CircuLating tUmor cellS in pancreaTic cancER) were enrolled consecutively between March 2016 and March 2018. Since most patients with resectable PDAC undergo surgery based on clinical presentation, a biopsy-proven malignancy was not mandatory prior to enrollment. Patients with a personal history of concomitant non-pancreatic malignancy, prior pancreatectomy for PDAC, or age <18 years were excluded. All enrolled patients signed a detailed informed consent and were followed from the time of diagnosis or presentation to the clinic until patient death. The Institutional Review Board has approved this prospective study.

Data Collection

The study design is available in Supplemental Figure S1. Peripheral blood sample collection was performed in prespecified time points, during each phase of patient care. Patients scheduled for surgery had their first blood sample drawn prior to incision and the next one between post-operative days 4 and 6. Blood draws would repeat in two or three-month intervals, often in conjunction with scheduled treatment plans, until the patient's death, loss of follow-up, or consent revocation.

Patient demographic data were collected from the prospectively maintained institutional database in combination with clinical notes. A detailed documentation of clinical presentation at diagnosis and patient performance status according to the American Society of Anesthesiologists classification was performed, together with assessment of pre-operative invasive procedures and imaging modalities. When patients received neoadjuvant therapy, information about the type of regimen and duration of therapy were collected. All surgical resections were performed by the same team of pancreatic surgeons. Perioperative data, including type and duration of surgery, length of stay, 30-day morbidity (per Clavien-Dindo classification)[23], and 90-day mortality were noted. Pathology reports were utilized to assess tumor stage and grade, resection margin status, perineural and lymphovascular invasion, and regional nodal status. Presence of malignant cells within 1mm (≤1mm) from the surgical margin was characterized as R1 resection. In the post-operative setting, all patients who underwent resection were followed at the outpatient surgical clinic every 3 months for the first 6 months after surgery, and every 6 months after that. Adjuvant chemotherapy treatment was decided based on medical oncologist recommendations and patient preference. Post-operative imaging for recurrence monitoring included abdominal/pelvic and thoracic multi-

detector computed tomography (MDCT) every 3-6 months for the first two years and yearly thereafter, or earlier when indicated. Imaging evidence of metastatic or local disease progression were accounted as disease recurrence. Patient date of death was retrieved from institutional medical records, online obituaries, or the Social Security Death Index. All data were collected, de-identified, and analyzed in prospective fashion by an independent reviewer. In this observational prospective study, all clinical decisions were independent of CTCs analysis results.

CTCs Isolation and Characterization

For CTCs isolation and enrichment, we collected 10mL of peripheral blood in ethylenediaminetetraacetic acid (EDTA) vacutainers and processed it with the Isolation by Size of Epithelial Tumor Cells assay (ISET, Rarecells)[24] within four hours. The ISET assay allows entrapment of cells of interest with size >8μm. Control cell-spiking experiments in healthy individual blood samples using commercially available pancreatic cancer cell-lines were also performed to assess the ISET cell isolation capacity. Additionally, blood samples from five healthy volunteers were also filtered and stained as controls, to assess the presence (or lack thereof) of circulating cells with epithelial phenotype. Finally, all isolated cells were fixed on the membrane and preserved in -20°C for further downstream applications (Supplemental Methods).

CTCs enumeration and phenotype characterization were performed with an optimized immunofluorescent staining protocol (Supplemental Methods): a combination of pan-cytokeratin and vimentin antibodies were utilized to assess epithelial and mesenchymal cell traits, respectively. Additionally, in order to ensure a high degree of specificity in CTCs assessment, cells were stained with an antibody "cocktail" (anti-CD45, CD11b, CD14, CD34) to separate different white blood cells populations from CTCs. CTCs were stratified as epithelial-type (pan-cytokeratin+, vimentin-, CD-), mesenchymal type (vimentin+, pan-cytokeratin-, CD-), and epithelial/mesenchymal-type (pan-cytokeratin+, vimentin+, CD-), as previously described.[17] Detected cells were additionally assessed for morphology characteristics (nuclei and cytoplasmic structures). Restrictions in cell size were not applied. CTCs enumeration and characterization were performed by a single reviewer, who was blinded to patient clinical data and sample collection time points. Reported results are in CTCs per mL of blood.

Definitions and Statistical Analysis

Continuous variables were summarized with means or medians and standard deviations or ranges, respectively, and compared with unpaired t-test or Wilcoxon signed-rank tests

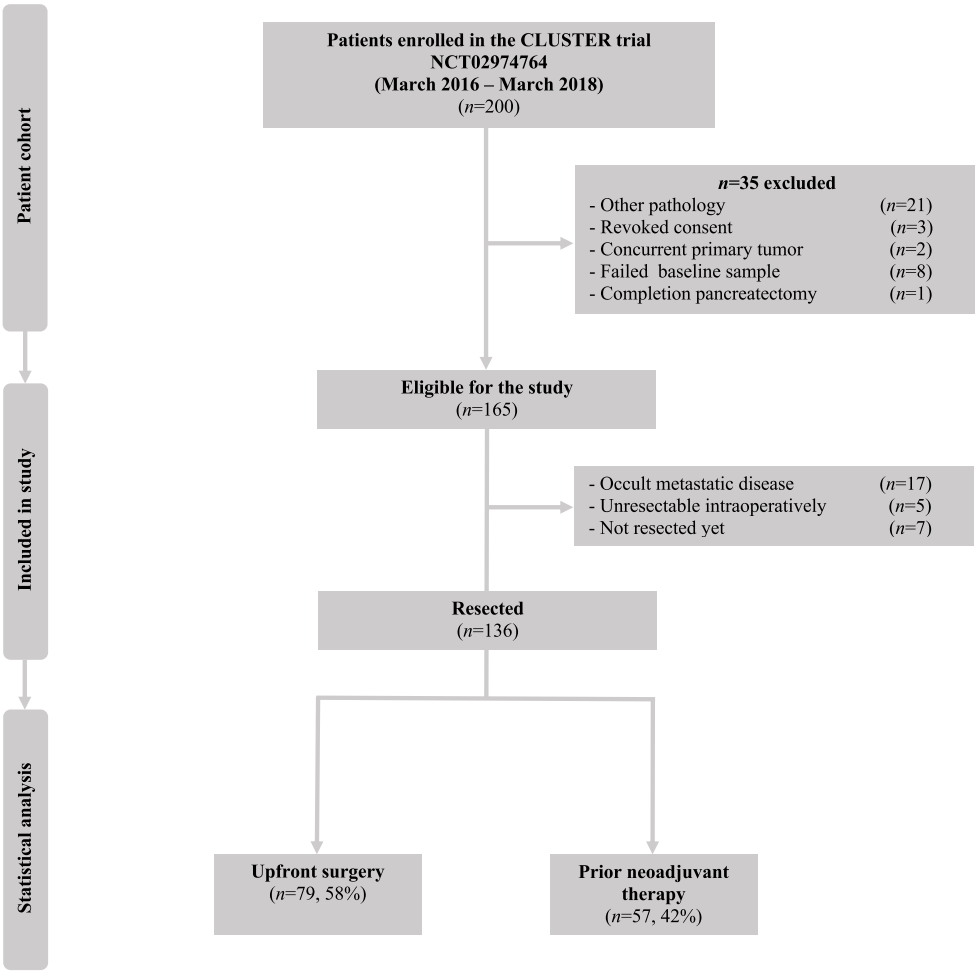


Figure 1. Patient selection flowchart.

as appropriate. Categorical variables were summarized using proportions and counts and compared with chi-square or Fisher's exact test. Recurrence-free survival (RFS) was defined as the time interval between date of operation and either date of recurrence or death, which came first, or censored at last follow-up. Early recurrence was defined as recurrence within one year in patients who had at least one-year follow-up after operation.[25] Overall survival (OS) and post-resection survival were defined as the time from date of diagnosis (OS) or resection (post-resection survival) to either death or censored at last follow-up. Optimal cut-off of CTCs for early recurrence prediction were calculated with Youden Index, which was also used to choose optimal cut-off values for alterations in CTCs dynamics. Multivariable logistic regression was used to estimate the odds ratio (OR) of CTCs for early recurrence,

while adjusting for other covariates. Missing data less than 20% were imputed using multiple imputation (5 permutations). Kaplan-Meier curves were used to estimate median survival with corresponding 95% confidence intervals (CIs). The log-rank test was utilized for subgroup comparison. A P-value <0.05 was considered statistically significant. Statistical analysis was performed with R software (v3.4.1).

Results

Demographic and perioperative data

In total, 200 consecutive patients were enrolled prospectively in the CLUSTER study. The patient selection flowchart and exclusion criteria are available in Figure 1. The final cohort of 136 resected patients was stratified into two groups: chemo-naïve patients eligible for upfront surgical resection (n=79, 58%) and patients who had undergone neoadjuvant therapy pre-operatively (n=57, 42%). The included patients (Table 1) had a median age of 68 years (IQR 59-74) and were predominantly males (n=71, 52%). Most tumors were located in the right pancreas (head and/or uncinate process, n=102, 75%), and the median tumor size was 30mm (IQR 22-42). Margin-negative resection (R0) was achieved in 85% of patients.

Circulating Tumor Cells Characteristics

As previously described[17], we predominantly identified two distinct CTCs populations in the peripheral blood of patients with PDAC: cells that express an epithelial phenotype (eCTCs: pan-cytokeratin+, vimentin-, CD-) and cells that express a combined epithelial/ mesenchymal phenotype (mCTCs: pan-cytokeratin+, vimentin+, CD-; Supplemental Figure S2). A third population of cells with purely mesenchymal phenotype (pan-cytokeratin-, vimentin+, CD-) was not recognized in any of the patient samples. Morphologically, detected CTCs had a median diameter of 13µm (IQR 10-15), without significant size differences between the two phenotypes. This size was comparable to neighboring white blood cells (WBCs: pan-cytokeratin-, vimentin-, CD+), that measured between 10 and 18µm. However, distinct differences were noted in the nuclear structure: CTCs had a characteristically large homogeneous nucleus covering >80% of the cell surface in two-dimensional imaging, and WBCs were characterized by a large segmented and/or lobulated nucleus. CTCs were most often recognized as single cells, and in very few samples (n=4, 1%) small clusters of 3-5 cells were identified (Supplemental Figure S3). Genetic sequencing in a small subset of patients confirmed that identified CTCs harbored mutations in three driver PDAC genes (*KRAS*, *TP53*, and *SMAD4*). Circulating cells with epithelial and/or mesenchymal phenotype were not identified in any of the samples from healthy volunteers (Supplemental Figure S4).

Table 1. Demographics, perioperative outcomes and clinicopathological data of resected patients

Variable	*All patients (n=136)*	*Chemo-naïve (n=79)*	*Post-neoadjuvant (n=57)*	*P*-value
Female, n (%)	65 (48)	30 (38)	35 (61)	**0.006**
Age, years median (IQR)	68 (59-74)	70 (60-75)	66 (56-70)	**0.010**
Pre-operative CA19-9 level U/mL, Median (IQR)	62 (25-299)	109 (32-376)	40 (18-96)	**0.050**
Clinical presentation, n (%)				0.108
Abdominal pain	79 (58)	47 (59)	32 (56)	
Jaundice	63 (46)	37 (47)	26 (46)	
Weight loss	60 (44)	19 (24)	21 (37)	
Type of neoadjuvant treatment, n (%)				N/A
Chemotherapy	24 (18)	N/A	24 (42)	
Chemoradiotherapy	33 (24)	N/A	33 (58)	
Operation type, n (%)				**0.024**
Pancreatoduodenectomy	102 (75)	56 (71)	46 (81)	
Distal pancreatectomy	24 (18)	18 (23)	6 (11)	
DP-CAR	3 (2)	0 (0)	3 (5)	
Total	7 (5)	5 (6)	2 (4)	
Tumor size, mm median (IQR)	30 (22-42)	32 (25-43)	25 (20-40)	0.066
Tumor differentiation, n (%)				0.156
Well/Moderate	78 (58)	47 (59)	31 (56)	
Poor/Undifferentiated	38 (28)	28 (35)	10 (18)	
Positive resection margin (R1), n (%)	21 (15)	14 (18)	7 (12)	0.301
Positive lymph nodes (N1), n (%)	82 (60)	61 (77)	21 (37)	**<0.001**
Microscopic perineural invasion, n (%)				**0.009**
No	29 (21)	9 (11)	20 (35)	
Yes	107 (79)	70 (89)	37 (65)	
Microscopic lymphovascular invasion, n (%)				**0.002**
No	79 (58)	36 (45)	42 (76)	
Yes	57 (42)	44 (55)	13 (24)	
AJCC stage (7th edition), n (%)				**0.001**
<IIA	47 (35)	18 (23)	29 (51)	
>IIB	79 (58)	61 (77)	28 (49)	

AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; DP-CAR, *distal pancreatectomy with celiac axis resection*; IQR, *interquartile range*; NA, *not applicable*.

Treatment Modalities and Alteration of CTCs Dynamics

A total number of 516 peripheral blood samples were collected from the 135 eligible patients (median n=4, IQR 2-5). CTCs were detected in the pre-operative samples of 131 patients (97%), regardless of their pre-operative treatment status. Chemo-naïve patients who were eligible for upfront resection had a median number of 11 total CTCs/mL of blood (tCTCs, IQR 6-15), breaking down to 9 eCTCs/mL of blood (IQR 5-12) and 2 mCTCs/mL of blood

Table 2. CTCs numbers of included patients

Treatment status	*tCTCs Median (IQR)*	*P-value*	*eCTCs Median (IQR)*	*P-value*	*mCTCs Median (IQR)*	*P-value*
Neoadjuvant status		**0.007**		**0.007**		**0.034**
Chemo-naïve (*n*=77)	11 (6-15)		9 (5-12)		2 (1-3)	
Post-neoadjuvant (*n*=48)	7 (3-10)		6 (5-9)		1 (0-2)	
Resection status chemo-naïve cohort		**<0.001**		**<0.001**		**<0.001**
Pre-operative (*n*=77)	11 (6-15)		9 (5-12)		2 (1-3)	
Post-operative (*n*=70)	2 (1-4)		2 (1-3)		0 (0-1)	
Resection status neoadjuvant cohort		**<0.001**		**<0.001**		**0.023**
Pre-operative (*n*=48)	7 (3-10)		6 (5-9)		1 (0-2)	
Post-operative (*n*=44)	2 (1-4)		2 (1-3)		0 (0-1)	
Intraoperative status		**<0.001**		**<0.001**		**<0.001**
OMD patients (*n*=15)	20 (12-24)		15 (9-18)		3 (2-6)	
Non-OMD resected patients (*n*=125)	9 (4-14)		7 (4-11)		1 (0-3)	

eCTCs, *epithelial phenotype CTCs/mL of blood*; IQR, *interquartile range*; mCTCs, *epithelial/mesenchymal phenotype CTCs/mL of blood*; OMD, *intraoperatively-diagnosed occult metastatic disease*; tCTCs, *total CTCs/mL of blood*.

(IQR 1-3) in the pre-operative samples (Table 2). Patients who had previously undergone neoadjuvant therapy were found to have significantly lower median CTCs numbers across all identified populations: 7 tCTCs/mL of blood (IQR 3-10, P=0.007, Figure 2A), 5 eCTCs/mL of blood (IQR 5-9, P=0.007, Supplemental Figure S5A), and 1 mCTC/mL of blood (IQR 0-2, P=0.034, Supplemental Figure S5B) in the pre-operative samples. Statistical correlations between the number of CTCs (tCTCs, eCTCs, and mCTCs) and tumor stage, size, location, and differentiation grade were not identified (P-value varied between 0.19 and 0.68).

In chemo-naïve patients, surgical resection of the primary tumor resulted in significant decrease of CTCs (Table 2), with post-operative median numbers of 2 tCTCs/mL of blood (IQR 1-4, P<0.001, Figure 2B), 2 eCTCs/mL of blood (IQR 1-3, P<0.001, Supplemental Figure S6A), and 0 mCTCs/mL of blood (IQR 0-1, P<0.001, Supplemental Figure S6B). A similar significant CTCs population reduction was observed in neoadjuvant patients measuring post-operatively at 2 tCTCs/mL of blood (IQR 1-4, P<0.001, Figure 2C), 2 eCTCs/mL of blood (IQR 1-3, P<0.001, Supplemental Figure S7A), and 0 mCTC/mL of blood (IQR 0-1, P=0.023, Supplemental Figure S7B).

Additionally, patients who were explored and found to have occult abdominal metastatic disease (OMD) intraoperatively had significantly higher CTCs numbers compared to patients who underwent surgical resection (Table 2): 20 tCTCs/mL of blood (IQR 12-24, P<0.001,

Table 3. Demographics and clinicopathological data comparison between patients who presented with recurrence within 12 months from surgery and patients who did not

Variable	***All patients* (n=100)***	***No-recurrence (n=41)***	***Early-recurrence (n=59)***	***P*-value**
Female, *n* (%)	47 (47)	19 (46)	28 (47)	0.914
Age, years median (IQR)	67 (59-75)	67 (56-75)	68 (59-74)	0.559
Pre-operative CA19-9 level U/mL**, Median (IQR)	56 (25-293)	61 (26-354)	56 (25-266)	0.470
Pre-operative CTCs (*n*/mL of blood)				
tCTCs	10 (4-15)	5 (3-11)	11 (8-16)	**<0.001**
eCTCs	8 (4-11)	5 (2-10)	9 (5-13)	**0.002**
mCTCs	1 (0-3)	1 (0-1)	2 (1-3)	**0.005**
Pre-operative $\frac{\text{mCTCs}}{\text{eCTCs}}$ ratio	0.20 (0-0.31)	0.10 (0-0.22)	0.23 (0.14-0.33)	**0.008**
Post-operative CTCs (*n*/mL of blood)				
tCTCs	2 (1-4)	1 (0-3)	3 (1-5)	**0.044**
eCTCs	2 (1-4)	0 (1-3)	2 (1-4)	0.089
mCTCs	0 (0-1)	0 (0-0)	0 (0-1)	**0.017**
Clinical presentation, *n* (%)				
Abdominal pain	57 (57)	17 (41)	40 (68)	**0.009**
Jaundice	60 (60)	35 (85)	25 (42)	**<0.001**
Weight loss	55 (55)	23 (56)	32 (54)	0.856
Neoadjuvant therapy, *n* (%)				0.609
Yes	53 (53)	23 (56)	30 (51)	
Tumor size, mm median (IQR)	30 (22-43)	25 (22-40)	35 (22-44)	0.502
AJCC stage dichotomous (7th edition), *n* (%)				**0.009**
<IIA	43 (43)	24 (59)	19 (32)	
≥IIB	57 (57)	17 (41)	40 (68)	
Tumor differentiation, *n* (%)				0.798
Well/Moderate	57 (57)	24 (59)	33 (56)	
Poor/Undifferentiated	43 (43)	17 (41)	26 (44)	
Positive resection margin (R1), *n* (%)	11 (11)	2 (5)	9 (15)	0.105
Positive lymph nodes (N1), *n* (%)	57 (57)	17 (41)	40 (68)	**0.009**
Microscopic perineural invasion, *n* (%)	76 (76)	26 (63)	50 (85)	**0.005**
Microscopic lymphovascular invasion, *n* (%)	39 (39)	11 (27)	28 (47)	**0.038**
Adjuvant therapy, *n* (%)	71 (71)	29 (71)	41 (69)	0.895

*Patients with recurrence within 12mo or no recurrence and follow-up >12mo.
**CA19-9 vales available in 73 patients.
AJCC, *American Joint Committee on Cancer*; CA, *carbohydrate antigen*; eCTCs, *epithelial phenotype CTCs/mL of blood*; IQR, *interquartile range*; mCTCs, *epithelial/mesenchymal phenotype CTCs/mL of blood*; tCTCs, *total CTCs/mL of blood*.

Supplemental Figure S8A), 15 eCTCs/mL of blood (IQR 9-18, P<0.001, Supplemental Figure S8B), and 3 mCTC/mL of blood (IQR 2-6, P<0.001, Supplemental Figure S8C). In patients with OMD and patients whose tumor was not resected due to local extension and major vessel involvement, a significant difference between pre- and post-exploration CTCs was not identified (tCTCs P=0.16, eCTCs P=0.09, mCTCs P=0.10).

Table 4. Univariable and multivariable logistic regression analysis for associations between pre-operative risk factors and early recurrence of pancreatic ductal adenocarcinoma within 12 months from resection

Chemo-naïve patients (n=79)	*Univariable*		*Multivariable (tCTCs)*		*Multivariable (eCTCs)*		*Multivariable (mCTCs)*	
Pre-operative risk factors	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*
Age	1 (0.99-1.02)	0.63	1.03 (0.94-1.12)	0.47	1.03 (0.94-1.12)	0.53	1.04 (0.94-1.15)	0.42
Gender: female vs. male	0.96 (0.69-1.31)	0.78	1.01 (0.20-4.98)	0.99	1.54 (0.30-7.87)	0.59	1.18 (0.21-6.55)	0.85
History of EtOH consumption: yes vs. no	1.17 (0.77-1.77)	0.47	1.95 (0.09-42.82)	0.66	1.86 (0.12-30.11)	0.65	1 (0.07-15.36)	1
Smoking: past/current vs. never	1.03 (0.88-1.12)	0.69	0.91 (0.39-2.11)	0.81	1.06 (0.48-2.37)	0.88	0.99 (0.40-2.41)	0.98
BMI	1 (0.98-1.03)	0.79	0.98 (0.85-1.13)	0.75	0.99 (0.87-1.12)	0.84	0.95 (0.82-1.11)	0.52
Tumor location: body/tail vs. head/uncinate	1 (0.73-1.36)	0.99	3.84 (0.60-24.74)	0.15	2.25 (0.36-14.02)	0.38	6.13 (0.7-54)	0.10
Pre-op CA19-9: ≥178 U/ml vs. <178 U/ml	1.18 (0.86-1.62)	0.30	2.53 (0.43-15.03)	0.30	2.62 (0.42-16.13)	0.29	4.25 (0.46-39.08)	0.19
Tumor size: >4 cm vs. ≤4 cm	1.48 (0.99-2.18)	0.06	6.41 (0.67-61.76)	0.10	7.12 (0.72-70.39)	0.09	9.41 (0.71-125.15)	0.09
tCTCs/mL of blood: ≥16 vs. <16	**1.39 (1.04-1.86)**	**0.03**	**5.92 (1.08-32.44)**	**0.04**	-		-	
eCTCs/mL of blood: ≥5 vs. <5	**1.52 (1.09-2.13)**	**0.02**	-		**8 (1.08-59.35)**	**0.04**	-	
mCTCs/mL of blood: ≥3 vs. <3	**1.44 (1.08-1.91)**	**0.02**	-		-		**11.02 (1.54-79.06)**	**0.02**

BMI, *body mass index*; CA, *carbohydrate antigen*; eCTCs, *epithelial phenotype CTCs/mL of blood*; EtOH, *alcohol*; mCTCs, *epithelial/mesenchymal phenotype CTCs/mL of blood*; tCTCs, *total CTCs/mL of blood*.

Neoadjuvant therapy patients (n=57)	*Univariable*		*Multivariable (tCTCs)*		*Multivariable (eCTCs)*		*Multivariable (mCTCs)*	
Pre-operative risk factors	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*	*Odds Ratio (95% CI)*	*P-value*
Age	1.01 (0.99-1.02)	0.63	1.03 (0.95-1.12)	0.42	1.03 (0.95-1.11)	0.49	1.05 (0.97-1.13)	0.22
Gender: female vs. male	1 (0.74-1.37)	0.78	0.88 (0.14-5.62)	0.89	1.28 (0.22-7.66)	0.78	1.77 (0.30-10.54)	0.99
Smoking: past/current vs. never	1.25 (0.96-1.29)	0.69	2.68 (0.49-14.79)	0.25	2.23 (0.43-11.51)	0.33	4.21 (0.71-24.93)	0.13
BMI	1 (0.99-1.01)	0.79	0.99 (0.94-1.06)	0.86	0.99 (0.93-1.06)	0.85	0.99 (0.92-1.06)	0.94
Tumor location: body/tail vs. head/uncinate	0.73 (0.52-1.04)	0.99	0.13 (0.01-2.03)	0.14	0.16 (0.01-2.51)	0.18	0.09 (0.01-1.67)	0.08
Tumor size: >4 cm vs. ≤4 cm	1.05 (0.78-1.84)	0.06	1.45 (0.11-19.25)	0.77	2.48 (0.17-36.75)	0.50	1.63 (0.11-24.29)	0.78
tCTCs/mL of blood: ≥6 vs. <6	**1.55 (1.20-2.01)**	**0.002**	**9.8 (1.90-50.65)**	**0.01**	-		-	
eCTCs/mL of blood: ≥6 vs. <6	**1.49 (1.15-1.93)**	**0.005**	-		**8.19 (1.38-48.46)**	**0.02**	-	
mCTCs/mL of blood: ≥1 vs. <1	**1.40 (1.06-1.84)**	**0.02**	-		-		**8.70 (1.55-49.01)**	**0.02**

BMI, *body mass index*; CA, *carbohydrate antigen*; eCTCs, *epithelial phenotype CTCs/mL of blood*; EtOH, *alcohol*; mCTCs, *epithelial/mesenchymal phenotype CTCs/mL of blood*; tCTCs, *total CTCs/mL of blood*.

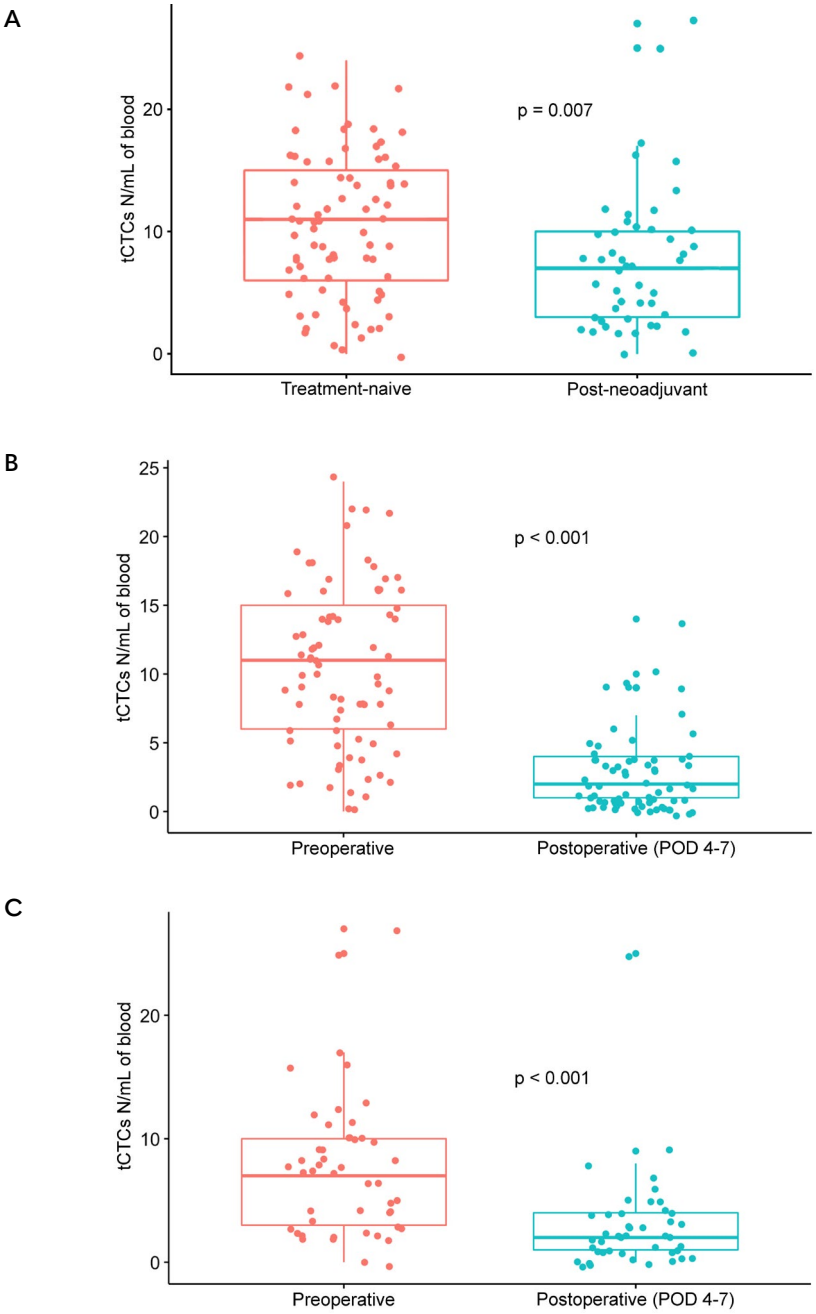


Figure 2. Boxplots of comparison between total CTCs/mL of blood of (A) pre-operative blood samples in chemo-naïve patients and patients who underwent neoadjuvant therapy, (B) pre-operative and post-operative samples in chemo-naïve patients, and (C) pre-operative and post-operative samples in patients who underwent neoadjuvant therapy.

CTCs as a Biomarker for 1-Year Disease Recurrence and Mortality

Fifty-nine patients (44%) presented with disease recurrence within one year from surgery. Multiple-site metastases was the most common pattern (n=23, 39%), followed by local recurrence (n=15, 25%) and liver metastases (n=11, 19%). Pre-operative CTCs were significantly higher in the recurrence cohort (tCTCs P<0.001, eCTCs P=0.002, mCTCs P=0.005; Table 3). The difference was also significant in the post-operative CTCs populations. In order to clearly identify the proportion of mesenchymal phenotype cells in the samples we calculated an mCTCs-to-eCTCs ratio (mCTCs/eCTCs): it was significantly higher in the pre-operative samples of patients who presented with early recurrence (median 0.23, IQR 0.14-0.33, P=0.008) compared to patients who did not recur within a year (median 0.10, IQR 0-0.22).

We further sought to identify pre-operative factors, including demographic and clinical parameters combined with CTCs dynamics, which will allow identification of aggressive disease biology and increased probability of early recurrence. To increase the accuracy of the predictive model, we performed a distinct univariable and multivariable logistic regression analysis for chemo-naïve and neoadjuvant patients (Table 4). Additionally, the multivariable analysis was performed separately for each of two mutually exclusive CTCs populations (eCTCs and mCTCs) and the total number of CTCs (tCTCs). On univariable analysis for the chemo-naïve cohort, CA19-9, which is the primary clinical biomarker for PDAC, was not predictive of one-year recurrence at an optimal cut-off value of 178 U/mL (OR 1.18 [95% CI 0.86-1.62], P=0.30). On the contrary, all three different CTCs populations were independently associated with increased risk of early-recurrence in their separate multivariable models: mCTCs had the highest predictive ability with an area under the curve (AUC) of 0.69, 52% sensitivity, and 82% specificity (Supplemental Figure S9). Similarly, in patients who underwent neoadjuvant therapy, all three CTCs populations were the only independent parameters associated with early recurrence: in this cohort, the tCTCs predictive model had the highest accuracy with an AUC of 0.75, 72% sensitivity, and 75% specificity.

In the studied cohort, mortality within one year from surgery was 31% (n=51). In chemo-naïve patients, pre-operative or post-operative eCTCs and mCTCs numbers were not associated with 1-year disease-specific mortality (P=0.65, P=0.89 and P=0.33, P=0.43, respectively). However, in the neoadjuvant cohort, the predictive role of CTCs was present: post-operative numbers (POD 4-7) of all three populations (tCTCs ≥4/mL, eCTCs ≥3/mL, and mCTCs ≥1/mL of blood) were associated with early death from PDAC (P=0.006, P=0.009, and P=0.018, respectively - Supplemental Figure S10).

CTCs Longitudinal Dynamics and Prediction of Disease Recurrence

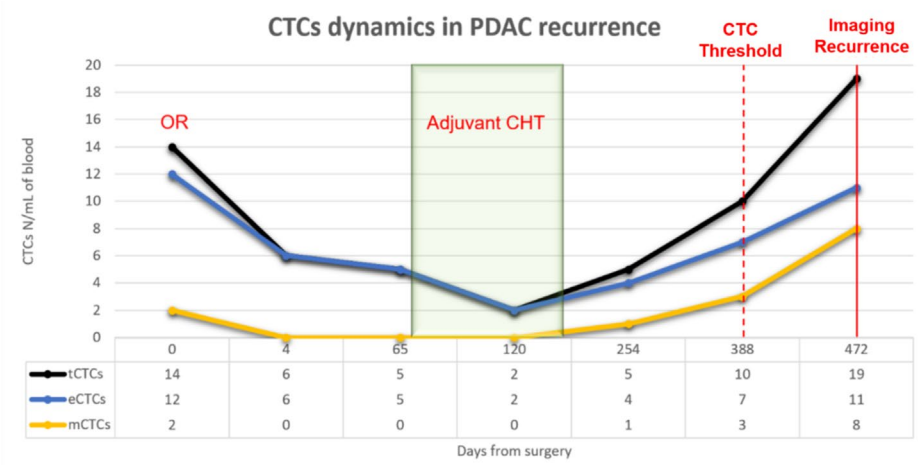
CTCs dynamics were assessed based on changes in CTCs numbers and phenotype distribution in combination with clinical reports and imaging data, along the course of patient disease. All patients with at least three sampling time points were included in the developed prediction model: a baseline pre-operative sample, a post-operative inpatient sample or prior to initiation of adjuvant therapy, and a sample acquired 3-6 months after surgery. Therefore, we retrospectively reviewed sequential CTCs dynamics in 114 patients, including 44 who had undergone neoadjuvant chemotherapy. In each time point, CTCs populations were compared to the baseline sample for identification of alterations.

Alterations in CTCs dynamics, that preceded imaging evidence, were documented in patients with disease recurrence (Figure 3A). This pattern was consistent across the majority of those patients who were longitudinally followed (n=45, 76%). In the developed prediction model, chemo-naïve and neoadjuvant patients were separated into separate groups and an increase cut-off value of 4 and 5 tCTCs/mL of blood was identified, respectively, for prediction of recurrence within 2 months. The generated AUC was 0.871 for chemo-naïve patients (82% sensitivity, 85% specificity), and 0.684 in neoadjuvant patients (67% sensitivity, 83% specificity). On the contrary, an increase in CTCs populations was not observed in patients who did not present with disease recurrence (Figure 3B). Additionally, regardless of pre-operative treatment status, patients who recurred had a disproportionate increase in mCTCs. More specifically, the median pre-operative mCTCs/eCTCs ratio was 0.23 (IQR 0.14-0.33), whereas at the time of recurrence the median ratio value was 0.42 (IQR 0.29-0.57, $P<0.001$). However, the mCTCs/eCTCs ratio was not specific enough to distinguish patients who presented with local versus distant metastases ($P=0.303$).

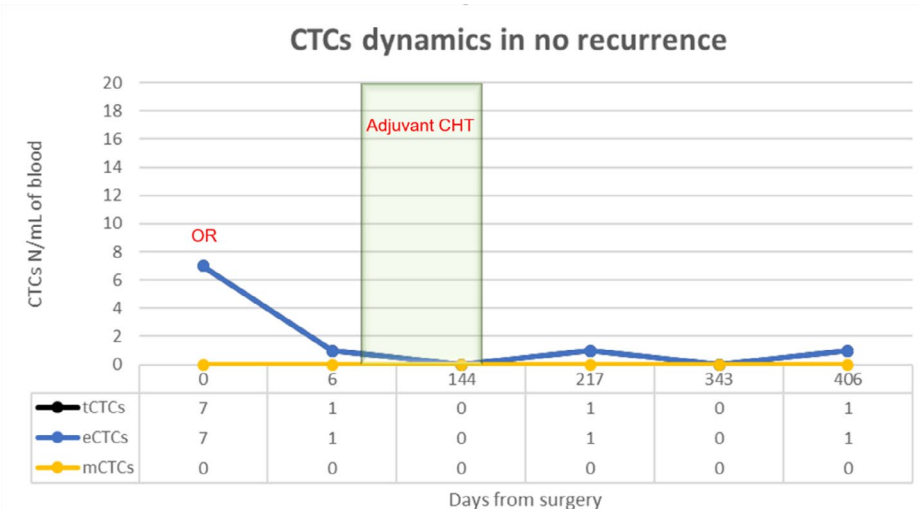
Discussion

The utilization of CTCs as a "liquid biopsy" to assess the tumor burden and guide clinical decisions has developed into an emerging field of study.[26] In pancreatic cancer patients, CTCs have been previously introduced as possible biomarkers for recurrence and survival.[18,27] However, biological hurdles, such as extensive cell heterogeneity and physical challenges associated with cell kinetics within circulation limit that potential.[28-29] In this prospective study, we attempted to characterize the physical attributes and phenotypes of CTCs in PDAC patients, longitudinally assess their dynamics along the course of the disease and develop a comprehensive model for their potential clinical significance. The two main pillars of our study were a two-step isolation technique (size-based positive selection and immunofluorescent biomarker staining) and the stratification of identified cells into an epithelial and mesenchymal phenotype.

A



B



CHT, chemotherapy; eCTCs, epithelial phenotype CTCs/mL of blood; mCTCs, epithelial/mesenchymal phenotype CTCs/mL of blood; OR, operation; tCTCs, total CTCs/mL of blood.

Figure 3. (A) Alterations in CTCs dynamics in a 74-year old treatment-naïve patient with PDAC. The patient underwent a pancreatoduodenectomy on Day 0 (OR) and a significant reduction in all CTCs populations was documented. A further decrease during adjuvant chemotherapy (Gemcitabine/Capecitabine, green frame) is also present. An initial increase in CTCs is observed on Day 254. However, our predictive model recognizes Day 388 as the time point of significant CTCs increase (red dotted vertical line). A CT-scan on Day 388 showed no signs of disease recurrence. The patient was diagnosed with local recurrence and pulmonary metastases in a follow-up CT on Day 472 (red solid vertical line). A significant increase in the mCTCs population fraction is also noted: in the pre-operative sample the mCTCs/eCTCs ratio was 0.17 and at recurrence 0.72. (B) CTCs dynamics in a 71-year old male with PDAC, who had neoadjuvant therapy (modified FFX). An initial decrease was noted with surgical resection (distal pancreatectomy on Day 0) and CTC numbers remained low along the patient's follow-up. No imaging evidence of disease recurrence exists by Day 406.

In the studied cohort, more than 95% of resectable PDAC patients had at least one identified CTC/mL of peripheral blood, whereas the median number of total CTCs across all pre-operative samples was 11/mL of blood. Experimental models have calculated the cell dissemination rate from a tumor to vary between 3-4x10^6 per gram/day[30], yet most are passively shed and less than 0.01% have metastatic potential.[27] Based on our observations, the median number of CTCs in the peripheral circulation of a patient with resectable PDAC can be estimated at approximately 50,000 at any given time. Additionally, most of the CTCs were isolated as single cells with a median size comparable to that of WBCs. Previous studies have identified the presence of CTCs clusters or micro-emboli in patients with PDAC.[18] We observed small clusters of <5 cells in 1% of the peripheral blood samples. In PDAC, disseminated cells travel through the portal vein into the liver, and subsequently enter the right-sided and later the peripheral circulation. Even though CTCs clusters are able to pass through capillary-sized vessels[31], it is unlikely that they make it to the periphery, due to extreme hydrodynamic flow forces and shear stress.[32] The occurrence of EMT and the loss of epithelial marker expression, such as E-cadherin, which sustains cell adhesions has an additional negative effect. However, it is possible that large cohesive cell cohorts disseminating from PDAC become trapped in the hepatic lobule network and may eventually give rise to liver metastases, according to the mechanism of collective migration.[33] Identification of CTCs clusters in the portal vein can support this hypothesis and provide an explanation for the increased incidence and aggressiveness of liver metastatic disease in PDAC.[34]

Most identified CTCs expressed exclusively an epithelial phenotype and most likely were the result of tumor apoptosis in the circulation. A variable number of CTCs expressing a mixed epithelial/mesenchymal phenotype (mCTCs) was also present in pre-operative samples, yet in a smaller percentage (n=94, 70%). On the contrary, CTCs with purely mesenchymal traits were not identified in any of the patients. These findings concede with the recently introduced "partial EMT" concept, where CTCs acquire mesenchymal traits, while retaining specific epithelial characteristics.[13,35] This mixed phenotype allows a cellular plasticity that appears to be critical in the establishment of metastatic colonies.[36-37] An ongoing study for genetic characterization of CTCs from our group will provide further insight on the genetic link between the primary tumor and metastatic lesions. *KRAS* mutations have been previously identified in PDAC CTCs verifying their malignant origin[38-39], however, comprehensive DNA sequencing on a single cell level continues to be a challenging task.[40]

Alterations in CTCs dynamics were observed along the course of the patients' disease. Initially, a positive effect of neoadjuvant chemotherapy and surgical resection of the primary tumor on CTCs population was recognized. More specifically, patients who had received neoadjuvant

treatment had significantly lower numbers of CTCs across all phenotypes compared to the chemo-naïve cohort eligible for upfront surgery (P<0.001). The beneficial effect of systemic treatment on CTCs dynamics has been previously identified in breast and prostate cancer, and is an emerging field in PDAC.[16,41] In our study, no correlation between CTCs and tumor pathological characteristics, such as size, site and differentiation grade was identified. We account this observed non-correlation to two main reasons: (1) the relatively small cohort of patients and (2) to the fact that we stratified into chemo-naïve and neoadjuvant patients. However, these results appear to strengthen the hypothesis that CTCs may disseminate very early in tumor development.[42]

Surgical resection of the primary tumor had the largest effect on CTCs dynamics. When the main source of CTCs was removed, their numbers reduced significantly (P<0.001), but in most patients they did not decrease to zero (79%). Currently, all data on CTCs kinetics within the circulation come from experimental models and show a limited half-life of approximately few hours.[43] We hypothesized that 4-7 days after surgery would be a reasonable time interval to assess the effect of surgical resection on CTCs dynamics. The fact that CTCs did not disappear post-operatively in most patients may suggest that collateral sources function as dormant tumor cells (DTCs) reservoirs very early in the disease.[44]

The biophysical attributes of CTCs and their dynamics translated into significant biomarker potential. Regardless of the pre-operative treatment status, patients who presented with early disease recurrence (within 12 months from surgery) had significantly higher tCTCs and mCTCs in both the pre-operative and the post-operative samples. To further assess the significance of mCTCs we calculated an mCTCs/eCTCs ratio: patients who recurred early had significantly higher ratio compared to those who did not (P=0.008). Moreover, patients who were found to have occult metastases also had significantly higher CTCs and mCTCs/eCTCs ratio (both P<0.001), concurring with recently reported results by Court et al.[19] Our group has previously identified CA19-9 as a strong predictor for early recurrence in PDAC[25], however, in this prospective study, pre-operative CA19-9 was not suggestive of early recurrence, most likely because patients who underwent neoadjuvant therapy were included in the comparison. Nonetheless, CTCs proved a reliable biomarker for early recurrence, regardless of the pre-operative patient status. These data indicate that aggressive tumor biology may be reflected in CTCs dynamics.

This hypothesis is further supported by the longitudinal changes in CTCs dynamics. We identified a consistent increase in CTCs numbers in patients who presented with recurrence, prior to imaging evidence of disease. Moreover, the mCTCs/eCTCs ratio at the time of

recurrence was significantly higher compared to the pre-operative sample, suggesting a more aggressive biology, likely associated with treatment resistance and clonal drift.[45] In current clinical practice, disease surveillance is performed with repeated imaging and CA19-9 assessment. A limited number of studies have evaluated CA19-9 as a longitudinal follow-up biomarker. CA19-9 appears to have a mediocre sensitivity and specificity and is not useful for patients who do not express the Lewis antigen.[4] Our developed CTCs model can identify recurrence with an accuracy of 80%, more than two months earlier from imaging evidence of disease. However, specificity was not high enough to predict the site of recurrence (local vs. distant).

Scientific interest on the importance of circulating biomarkers has erupted in recent years, primarily regarding the role of circulating tumor DNA (ctDNA). Recent studies have shown the significant potential of ctDNA on early cancer detection and follow-up of disease.[6,46] However, ctDNA represents collectively all indolent or aggressive clones shedding in the circulation from CTCs, the primary tumor, or metastases. On the contrary, each CTC represents a unique clone and may provide direct insight on the clonal prevalence in the metastatic mechanism. Data from this study suggest that significant potential for CTCs as a PDAC biomarker is present. However, since cell kinetics and survival are significantly affected within the circulation, the only way to overcome these obstacles is to develop optimal isolation and enrichment assays.[47] Longitudinal assessment of ctDNA in PDAC is currently underway in our institution and we will soon be able to make a direct comparison between the two biomarkers or combine them for increased sensitivity and specificity.

This prospective observational study has several limitations. Firstly, use of an isolation by size assay for CTCs identification may result in possible loss of smaller cells (<8μm). Additionally, even though we reviewed cellular morphology and utilized multiple antibodies for phenotypic characterization of CTCs, it is possible that false positives could be present, due to heterogeneous expression of epithelial and mesenchymal markers in blood cells or non-specific staining of dead cells. Moreover, vimentin is a widely used mesenchymal marker for cell characterization, yet, other mesenchymal markers are available and were not utilized in this study. Subsequent genetic analysis was only performed in a very small subset of patients. Additionally, since follow-up of patients who were enrolled later in the study is not yet completed, there is a possibility for small changes in the reported results. Finally, most patients were diagnosed in outside institutions and were treated and followed locally after resection, which resulted in limited data for: (1) pre-neoadjuvant treatment CTCs values, (2) longitudinal CA19-9 values, and (3) CTCs dynamics during salvation chemotherapy after disease recurrence. However, the CLUSTER study is the first to provide a detailed longitudinal insight

on the systemic component of pancreatic cancer through enumeration and phenotypical characterization of CTCs. The next step is description of these cells with DNA and RNA sequencing, and protein expression analysis. Moreover, the major goal is to achieve ex vivo culture of CTCs for further genetic analysis and pharmacotyping for tumor response. Culture of CTCs has proven very challenging with traditional culture methods, due to the rarity and the complex nature of these cells.[48-49] Novel techniques, such as 3-dimensional culture with organoids may prove useful for profiling patient CTCs in the near future.[50]

Conclusion

In the CLUSTER study we came across a detailed macroscopic view of the systemic nature of PDAC and its aggressive metastatic potential by longitudinally observing CTCs dynamics. Systemic therapy was associated with lower CTCs numbers and surgical resection of the primary tumor resulted in significant decrease in CTCs populations. Increased pre-operative CTCs numbers and especially cells with mesenchymal traits were predictive of early recurrence after surgical resection. Additionally, an increase in CTCs was documented in PDAC recurrence, approximately two months before new imaging evidence of disease. The potential of CTCs dynamics as a biomarker is evident and further in-depth analysis of their cellular profile is justified.

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Supplemental Materials to Chapter 13

Supplemental Methods

CTCs Isolation and Enrichment with the ISET Assay

The acquired blood sample was placed on a rotator in room temperature until the isolation process (t<4 hours). For the initial step, the 10mL sample was diluted in the ISET provided buffer (three different powder buffers mixed in sterile water with 1M of NaOH for a final pH of 7.2-7.4) on a 1:10 volume-to-volume (v/v) ratio, and was split into two 50mL Eppendorf tubes, each of them also containing 900μL of 37% formaldehyde solution for cell fixation purposes. Preparation during this step promotes extensive red cell lysis, which will allow filtration of the sample and avoid membrane clogging. The ISET cartridge was preconditioned with 10mL of 1X Phosphate-buffered saline (PBS) solution prior to sample processing. The diluted sample was filtered through the 8μm-pore membrane with a suction pressure of -8 to -11kPa. A second 1X PBS washing step was performed and the membrane was separated from the cartridge and left to dry in room temperature for 30 minutes. Storage of the membrane was done in the dark at -20°C, until further analysis.

Immunofluorescent Staining

For immunofluorescent (IF) staining, commercially available conjugated primary antibodies or a combination of unconjugated primary and a secondary antibody were utilized. More specifically, a list of IF monoclonal antibodies used in this experiment is: pan-cytokeratin conjugated antibody (FITC, ThermoFisher), vimentin conjugated antibody (Alexa Fluor 594, ThermoFisher), CD45 conjugated antibody (Alexa Fluor 647, ThermoFisher), CD11b (Alexa Fluor 647, ThermoFisher), CD34 (Alexa Fluor 647, ThermoFisher), and CD14 unconjugated primary with a secondary antibody (Alexa Fluor 647, ThermoFisher). Antibodies were stored in 4°C per manufacturer's instructions and were all used in a 1% concentration for sample staining. The staining protocol was performed as follows: one core of each sample membrane was cut and re-hydrated in 1mL of 1X tris-buffered saline (TBS) for 10 minutes. It was then sunk in 500μL of 1X TBS with 0.2% Triton-X for 5-6 minutes to achieve adequate cell membrane permeabilization for staining of targeted cytoplasmic elements. After two 5-minute 1X TBS washing steps, the membrane core was incubated in 1mL of 5% milk-based blocking buffer (lab-scientific non-fat dry milk diluted in 5% w/v of 1X TBS with 2% Tween) for 1 hour. This preconditioning step promotes down-regulation of cell surface Fc receptors and blocking of nonspecific monoclonal antibody binding, whereas the addition of Tween prevents non-specific protein interactions. The membrane core is then incubated in the prepared antibody cocktail

(all antibodies in 1% concentration diluted in the milk-based blocking buffer) for 2 hours and washed twice before placed on a glass microscope slide (Fisherbrand™ Superfrost™). A glycerol-based antifade mount ant (ThermoFisher) containing DAPI or NucStain for nuclear staining was applied directly and the membrane core was covered with a coverslip and stored at 4°C overnight prior to review. After review, all slides were stored in the dark at -20°C.

Hematoxylin-Eosin (H&E) Staining Protocol

A separate membrane core was initially re-hydrated in 1X PBS for 5 minutes. It was then submerged sequentially in hematoxylin for 3 minutes, deionized water, eosin for 1 minute and washed again in deionized water. The membrane core was then affixed on a glass microscope slide with a 10% glycerol solution.

CTCs Immunofluorescent Review and Characterization

Review of the stained membrane cores was performed using the Nikon Ti-E inverted microscope system (Nikon, Japan). The membrane core has an approximate surface of 28mm^2 and combined scanning with all IF filters (FITC, DAPI, Alexa Fluor 594, and Alexa Fluor 647) was performed, together with a phase-contrast microscopy of the detected cells. Cell identification was done with 20X and/or 40X magnification and cells were photographed with the Nikon NIS Elements imaging software (version 4.20.02, build 988-64 bit). Exposure times for each filter were 100ms for DAPI, 500ms for Alexa Fluor 594 (Vimentin), 1 second for FITC (pan-cytokeratin), and 2 seconds for Alexa Fluor 647 (CD antibodies). Captured images for each wavelength were combined and saved for future reference and re-evaluation.

Pancreatic Cancer Cell Line Spiking Experiments

A series of different spiking experiments was performed prior to patient sample processing to assess two major experimental parameters: (1) the sensitivity and specificity of IF antibodies to the targeted cell populations, and (2) the capacity of ISET to capture cells of interest. For both control experiments, the AsPC-1 human pancreatic cancer cell line (CRL-1682TM, American Type Culture Collection ATCC) was used (Supplemental Figure S11). Briefly, culture conditions were followed based on the manufacturer's recommendations: RPMI-1640 (with L-Glutamine, Gibco, Life Technologies) with 10% v/v Fetal Bovine Serum (FBS) and 0.5% v/v Penicillin-Streptomycin (Sigma Aldrich). Cells were harvested by adding 0.25% v/v trypsin/EDTA solution and incubating at 37°C for 5 minutes. Finally, the cells were stained with Trypan-Blue for viability assessment and counted with the TC20 Automated Cell Counter (Bio-Rad).

Initially, AsPC-1 and CAF cell lines were stained for pan-cytokeratin and vimentin antibodies, respectively, using the immunofluorescent staining protocol and were reviewed under the IF microscope. A consistent expression of epithelial and mesenchymal markers was observed, whereas none of the cell lines were positive for CD staining.

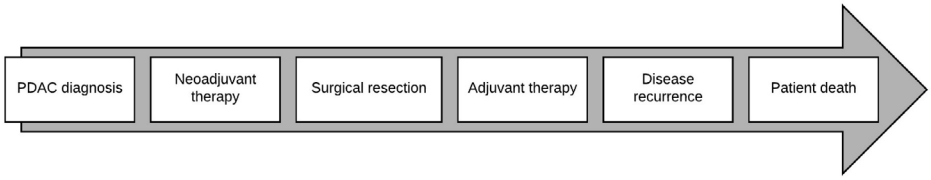
For validation of ISET capacity for cell capture, two different experiments were performed in which 50, and 100 viable AsPC-1 cells per mL of blood were spiked in 5mL of peripheral blood from a healthy volunteer. The recovery rates after processing the spiked samples with the standardized ISET protocol were 81% (202 cells) and 79% (397 cells), respectively (Supplementary Figure 11).

Flow-Assisted Cell Sorting (FACS)

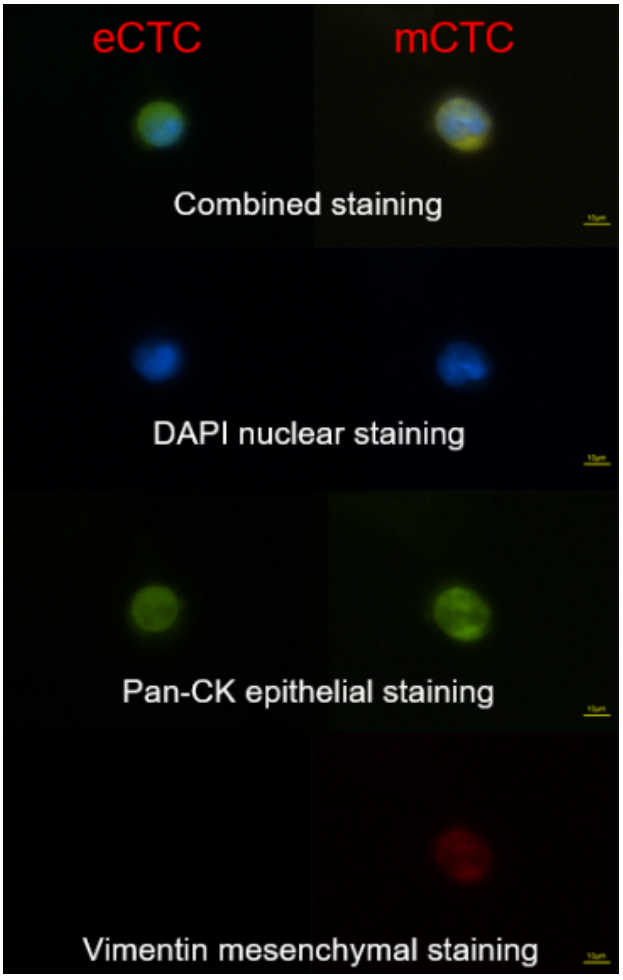
For FACS, an additional sample of 5mL of peripheral blood was separately drawn. An initial negative-selection step was performed using the RosetteSep™ Human CD45 Depletion Cocktail (Stemcell Technologies, Cambridge MA, USA). Red blood cells and white blood cells expressing CD66b and CD45 proteins were marked with antibody complexes and depleted with gradient centrifugation over a buoyant density medium (1200g for 20 minutes). After centrifugation, the targeted cell population is highly-enriched between the plasma and the medium ("buffy coat") and is transferred to a separate Eppendorf tube. Two washing steps follow with HBSS buffer (Hank's Balanced Salt Solution with 2% FBS) and centrifugation at 300g for 10 minutes. After removal of the supernatant, the pellet (approximately 300μL) was stained for epithelial cell surface markers (EpCAM, MUC-1, CD61 - integrin alpha V/ beta 3; all antibodies conjugated with phycoerythrin – PE, Abcam) and CD45 for exclusion of leftover white blood cells, conjugated with allophycocyanin (APC, Biolegend) at a 1% v/v concentration. The stained pellet is washed once more with HBSS buffer after incubation in the dark for 30 minutes and kept in ice until flow cytometry. Prior to sorting, cells are stained with Propidium Iodide (PI) for viability assessment. FACS was performed using with the MoFlo XDP Cell Sorter (Beckman Coulter Life Sciences, Indianapolis, USA). Prior to FACS, instrument tuning is performed with negative and positive control samples from peripheral blood of a healthy volunteer (CD45+, EpCAM-) and the AsPC-1 cell line (CD45-, EpCAM+), respectively. Targeted cells were sorted in a 500μL Eppendorf tube and stored in -80°C. In a predetermined number of enrolled patients (n=30), additional blood was drawn for separate flow-assisted cell sorting (FACS) analysis. After FACS, the sorted cells were immediately stored in -80°C.

CTCs DNA Extraction and Quantification

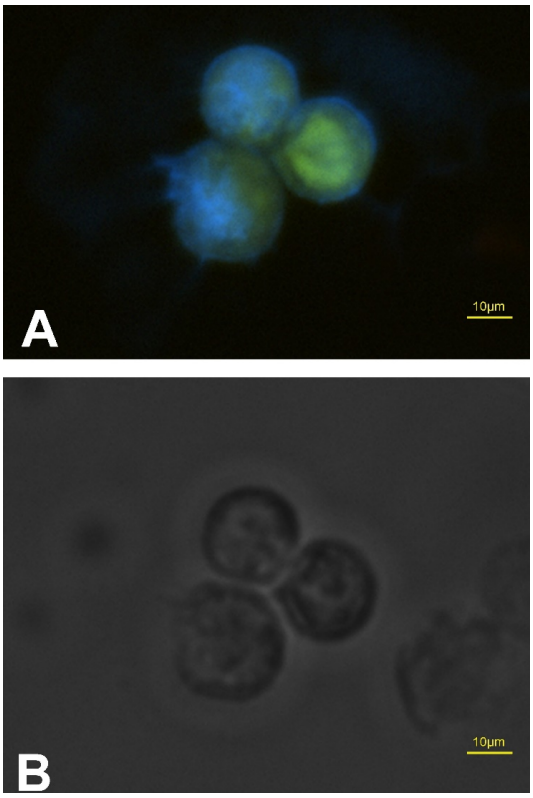
For downstream genetic analysis of isolated CTCs, three cores of each membrane were used. The cores were incubated in a Proteinase-K buffer in 65°C for 16 hours and then placed in a heating block at 95°C for 10 minutes, according to the Arcturus® PicoPure® DNA Extraction Kit protocol (ThermoFisher Scientific). A next step of DNA purification followed using the Agencourt AMPure XP beads (Beckman Coulter, Life Sciences) and the purified DNA sample was quantified with the Qubit® 3.0 Fluorometer and the Quantifiler® Human DNA Quantification Kit (ThermoFisher Scientific). An Ampliseq® custom panel was designed targeting three driver genes in PDAC (*KRAS*, *TP53*, and *DPC4*) with a minimum sequencing library depth of 500X. Digital next-generation sequencing was performed with the Ion Torrent system (Life Technologies).



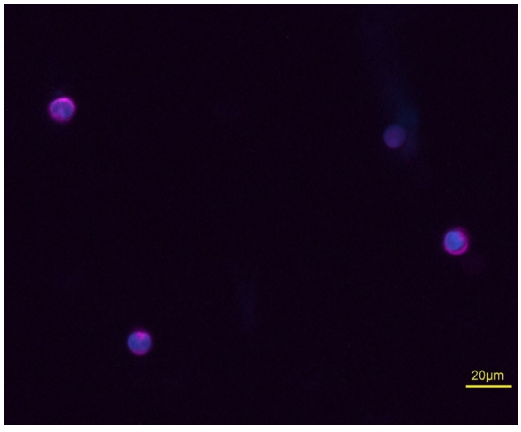
Supplemental Figure S1. Study design illustration.



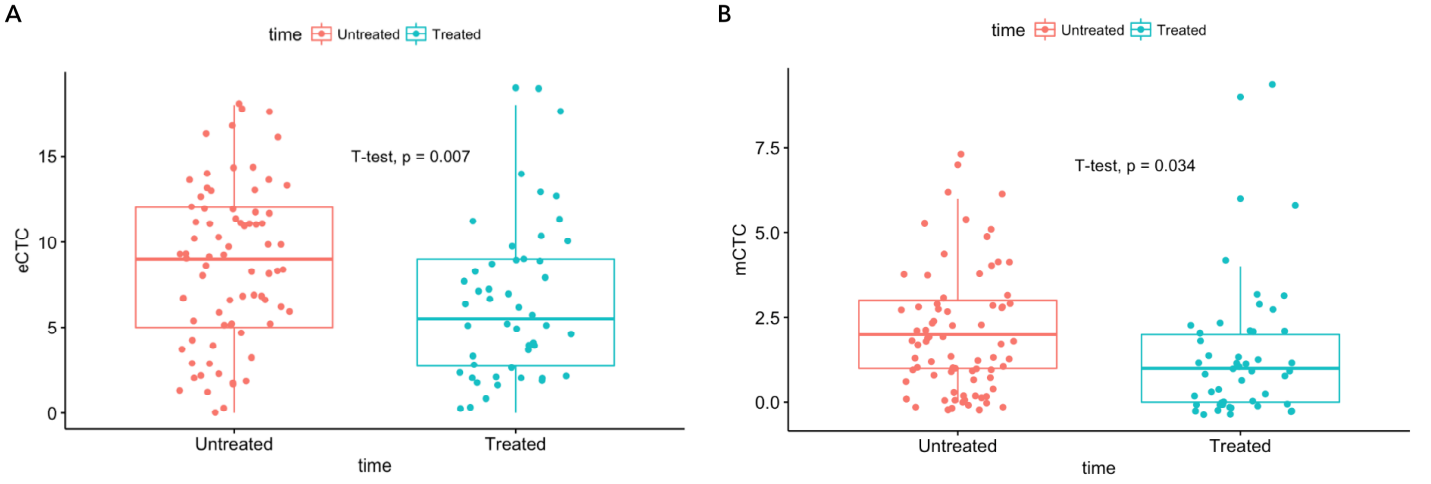
Supplemental Figure S2. Immunofluorescent staining of circulating tumor cells in patients with pancreatic adenocarcinoma. The left column illustrates a cell that expresses an epithelial phenotype (eCTC) and is positive for pan-cytokeratin only. The right column illustrates a cell that expresses a combined epithelial/mesenchymal phenotype (mCTC) and is positive for pan-cytokeratin and vimentin.



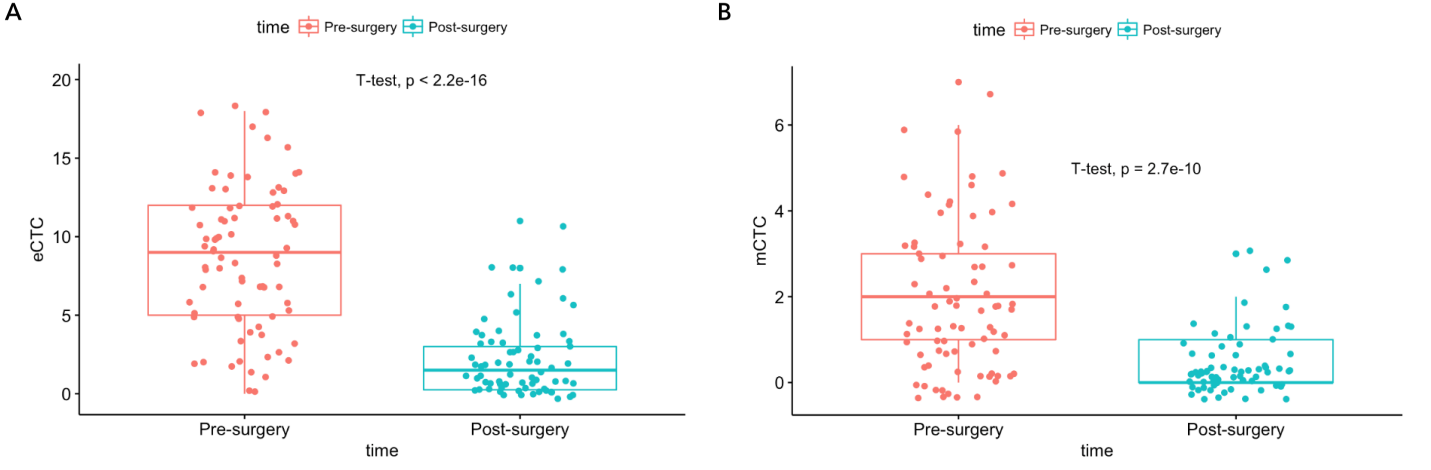
Supplemental Figure S3. Isolated cluster of 3 CTCs. (A) Immunofluorescent staining expressing only pan-cytokeratin (eCTCs phenotype). (B) Photographic image of the identified CTC cluster.



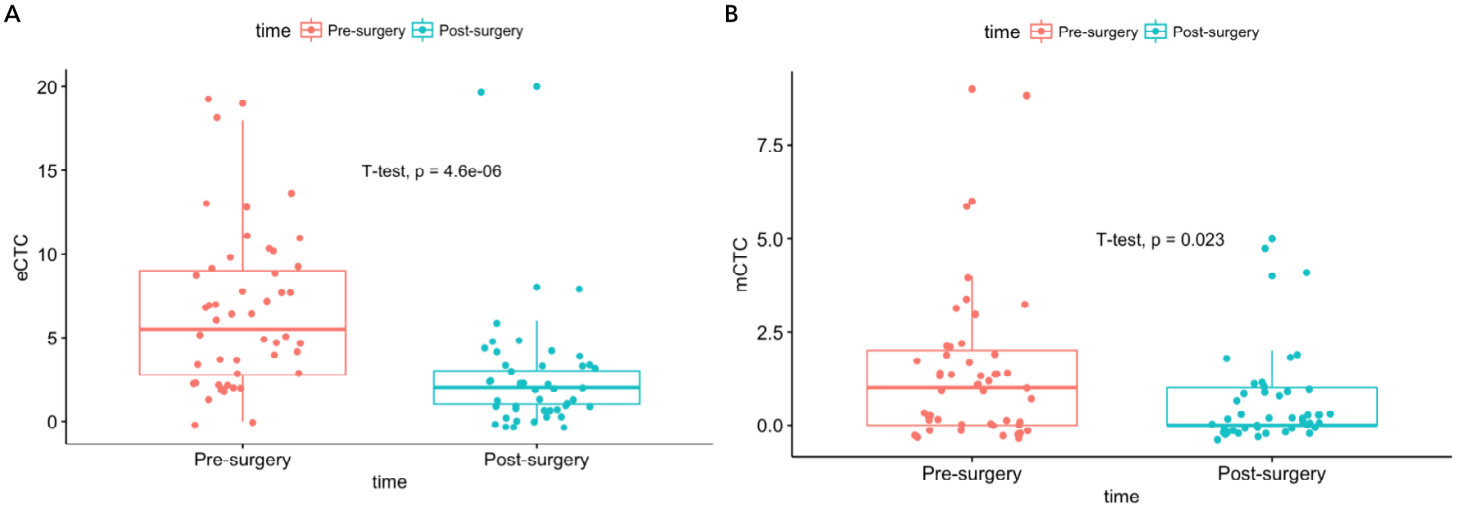
Supplemental Figure S4. Immunofluorescent staining of white blood cells from healthy control specimen. The cells are expressing a CD phenotype (purple color). No cells expressing an epithelial of mesenchymal phenotype were identified in the peripheral blood of healthy controls.



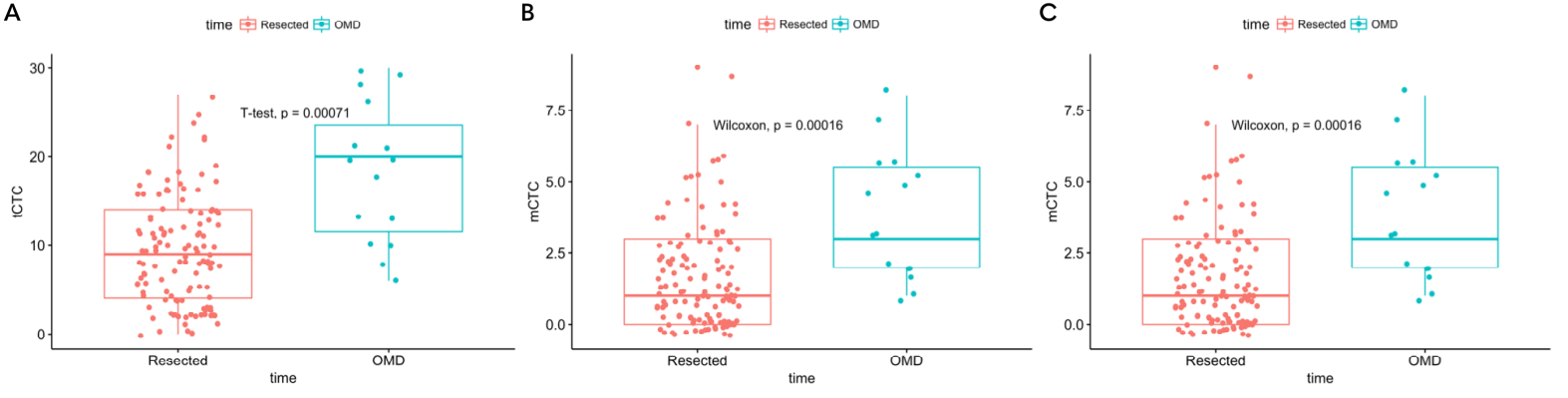
Supplemental Figure S5. Boxplots of comparison between eCTCs (A) and mCTCs (B) between preoperative blood samples in treatment-naive patients and patients who underwent neoadjuvant therapy. *eCTCs: epithelial CTCs/mL of blood, mCTCs mesenchymal CTCs/mL of blood.*



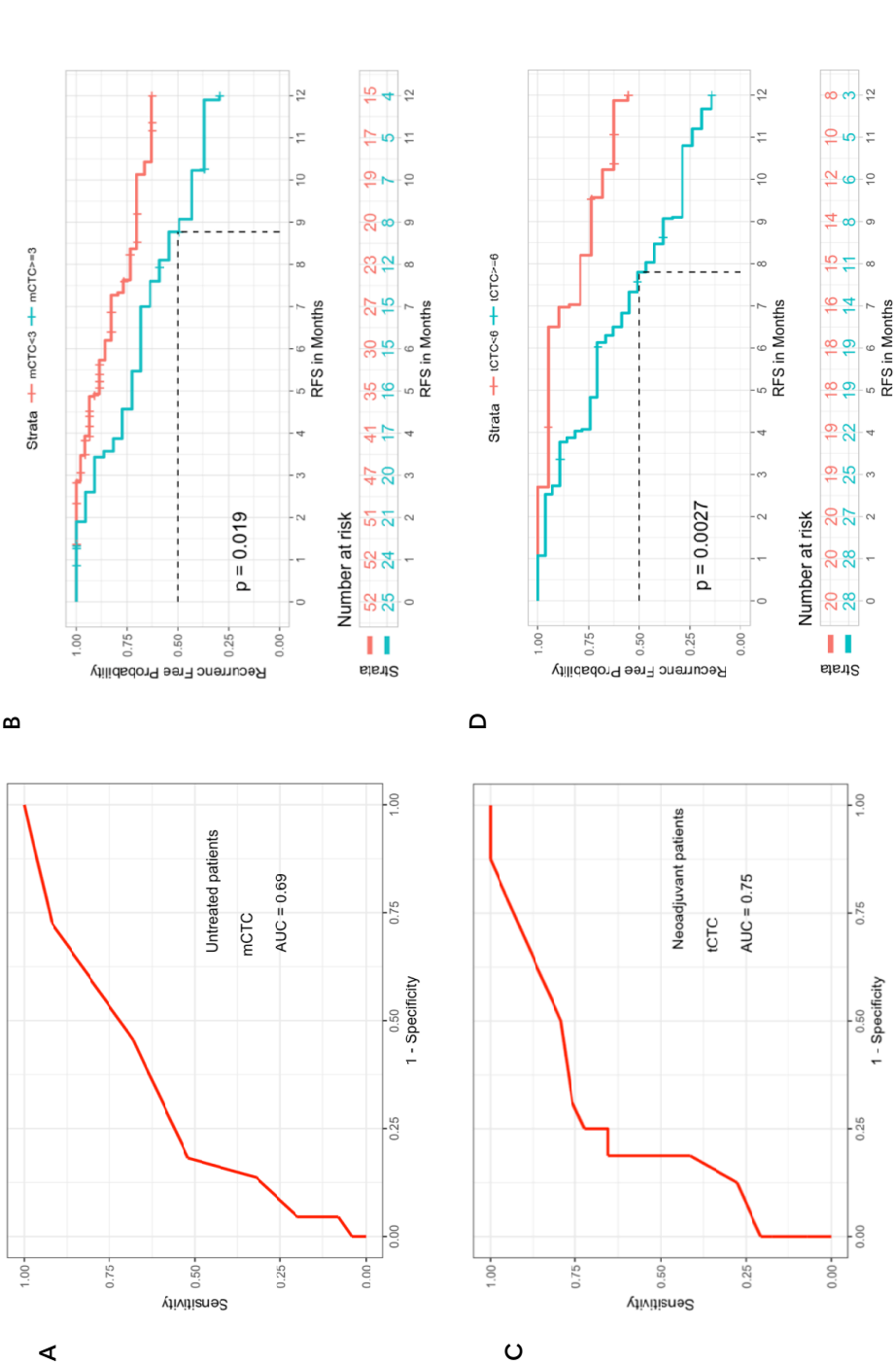
Supplemental Figure S6. Boxplots of comparison between eCTCs (A) and mCTCs (B) of preoperative and postoperative (POD 4-7) blood samples in treatment-naive patients. *eCTCs: epithelial CTCs/mL of blood, mCTCs mesenchymal CTCs/mL of blood, POD: postoperative day.*



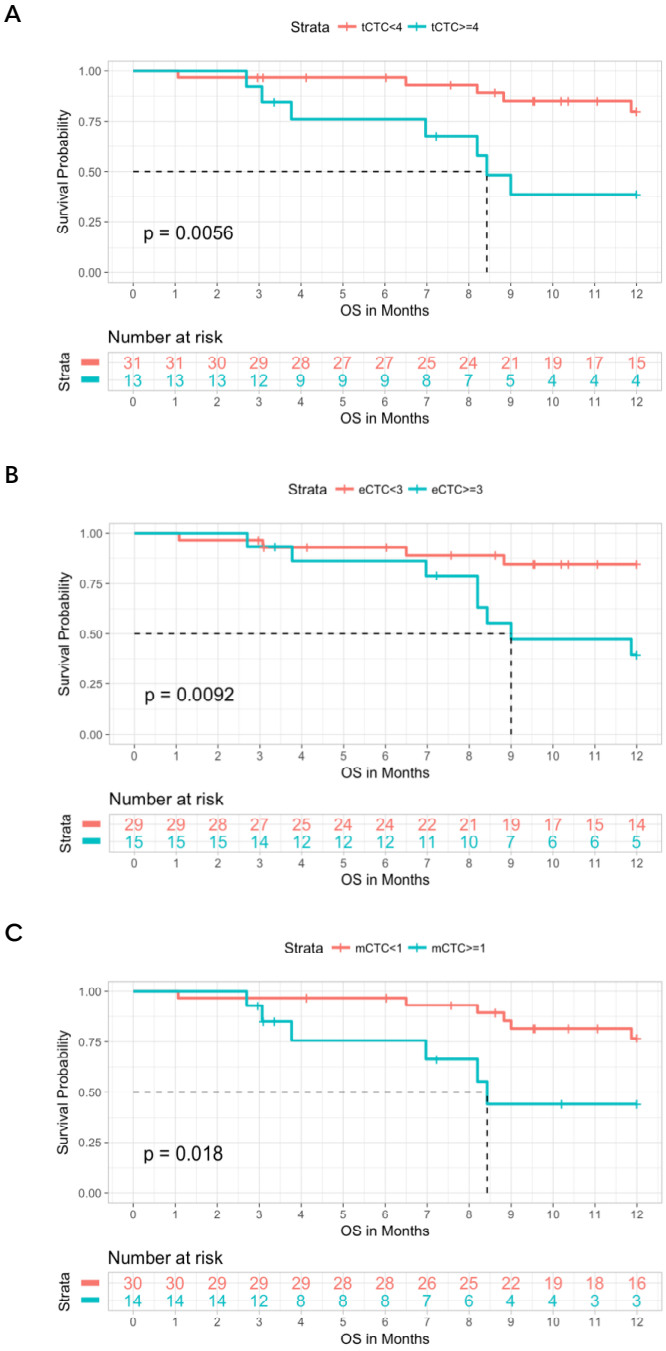
Supplemental Figure S7. Boxplots of comparison between eCTCs (A) and mCTCs (B) of preoperative and postoperative (POD 4-7) blood samples in patients who underwent neoadjuvant treatment. *eCTCs: epithelial CTCs/mL of blood, mCTCs mesenchymal CTCs/mL of blood, POD: postoperative day.*



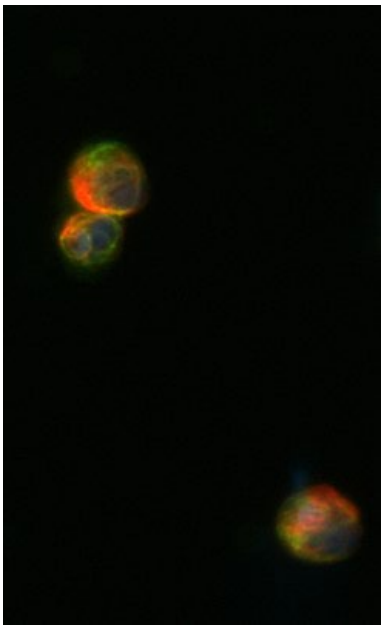
Supplemental Figure S8. .Boxplots of comparison between tCTCs (A), eCTCs (B) and mCTCs (C) of OMD and resected non-OMD patients. *eCTCs: epithelial CTCs/mL of blood, mCTCs mesenchymal CTCs/mL of blood, OMD: intraoperatively-discovered occult metastatic disease.*



Supplemental Figure S9. (A) ROC curve (area under the curve 0.690) for preoperative mCTCs with an optimal cut-off of 3 cells/mL of blood for predicting early recurrence in treatment-naive patients (<12 months, 52% sensitivity, 82% specificity) and (B) associated Kaplan-Meier curve for post-operative 1-year recurrence. (C) ROC curve (area under the curve 0.750) for pre-operative tCTCs with an optimal cut-off of 6 cells/mL of blood for predicting early recurrence in patients who underwent neoadjuvant therapy (<12 months, 72% sensitivity, 75% specificity) and (D) associated Kaplan-Meier curve for post-operative 1-year recurrence. *tCTCs: total CTCs/mL of blood, mCTCs: mesenchymal CTCs/mL of blood, ROC: receiver operating characteristic.*



Supplemental Figure S10. Kaplan-Meier curves for 1-year disease-specific mortality associated with postoperative (post-operative day 4-7) (A) tCTCs (≥4/mL), (B) eCTCs (≥3/mL), and (C) mCTCs (≥1/mL) in patients who underwent neoadjuvant therapy. *tCTCs: total CTCs/mL of blood, eCTCs: epithelial CTCs/mL of blood, mCTCs: mesenchymal CTCs/mL of blood.*



Supplemental Figure S11. Combined immuno-fluorescent staining (DAPI, pan-cytokeratin, vimentin) of AsPC-1 cancer cells spiked in human blood and isolated using the ISET assay.

CHAPTER 14

Circulating Tumor DNA as a Clinical Test in Resected Pancreatic Cancer

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Clinical Cancer Research 2019

Abstract

Objective

In research settings, circulating tumor DNA (ctDNA) shows promise as a tumor-specific biomarker for pancreatic ductal adenocarcinoma (PDAC). This study aimed to perform analytical and clinical validation of a *KRAS* ctDNA assay in a Clinical Laboratory Improvement Amendments certified and College of American Pathology inspected clinical laboratory.

Methods

Digital-droplet PCR (ddPCR) was used to detect the major PDAC-associated somatic *KRAS* mutations (G12D, G12V, G12R and Q61H) in liquid biopsies. For clinical validation, 290 pre-operative and longitudinal post-operative plasma samples were collected from 59 PDAC patients. The utility of ctDNA status to predict PDAC recurrence during follow-up was assessed.

Results

ctDNA was detected pre-operatively in 29 (49%) patients and was an independent predictor of decreased recurrence-free (RFS) and overall survival (OS). Patients who had neoadjuvant chemotherapy were significantly less likely to have pre-operative ctDNA than were chemo-naïve patients (21% vs. 69%; $P<0.001$). ctDNA levels dropped significantly after tumor resection. Persistence of ctDNA in the immediate post-operative period was associated with a high rate of recurrence and poor median RFS (5 months). ctDNA detected during follow-up predicted clinical recurrence (sensitivity 90% (95% CI 74-98%), specificity 88% (95% CI 62-98%)) with a median lead time of 84 days (IQR 25-146). The presence of ctDNA during post-pancreatectomy follow-up was associated with a median OS of 17 months, while median OS was not yet reached at 30 months for patients without ctDNA ($P=0.011$).

Conclusions

Measurement of *KRAS* ctDNA in a CLIA laboratory setting can be used to predict recurrence and survival in patients with PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) continues to be a devastating disease, with 133,300 estimated deaths for the past year in the United States and Europe alone.[1,2] Poor survival is mostly attributed to a lack of effective screening methods, late diagnosis, propensity for early metastatic spread, and minimally effective systemic therapies.[3-6] Even after resection of a localized tumor, up to 80% of patients will develop disease recurrence.[7]

Currently, nine different chemotherapeutic drugs are approved by the United States Food and Drug Administration (FDA). These are increasingly administered in more effective multi-drug regimens, such as FOLFIRINOX and Gemcitabine/Nab-Paclitaxel.[8-11] The optimal evaluation of current and future treatment options emphasizes the need for biomarkers that can reliably identify residual or progressive disease, so that refractory patients can be started or switched on to second- or third-line regimens in a timely manner.[5,12] Currently used protein serum markers have several limitations that render them of limited use to help guide treatment decisions.[13,14] Carbohydrate antigen (CA) 19-9, for instance, can be elevated in extra-pancreatic malignancies and benign conditions.[13] Furthermore, approximately 6% of the Caucasian population and 22% of the African-American population in the United States are Lewis-antigen negative and do not produce CA 19-9.[15]

Cell-free circulating tumor DNA (ctDNA) has shown promise as a biomarker to improve early tumor detection, prognostic stratification and monitoring of tumor dynamics.[16-19] In addition, ctDNA could be useful clinically to detect PDAC recurrence during post-pancreatectomy follow-up.[17,20,21] A robust method for quantifying low-abundance point mutations in cell-free circulating DNA is digital-droplet polymerase chain reaction (ddPCR).[17,22] Of the major PDAC driver genes[3], *KRAS* is mutated in more than 90% of PDACs, and over 95% of these mutations are at the hot spots G12D, G12V, G12R or Q61H.[23,24] Several research studies have reported on the prognostic significance of *KRAS* ctDNA detected in the metastatic and peri-operative setting (Online Supplemental eTable S1).[25,26] However, few if any ctDNA assays for PDAC have been validated in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Online Supplemental eTable S1).[27]

In this prospective study, we performed analytical and clinical validation of *KRAS* ctDNA as a prognostic marker using ddPCR in a CLIA laboratory setting as part of a prospective longitudinal single-institution study (NCT02974764)[28], of patients undergoing resection of PDAC.

Methods

Study Design and Population

Patients with localized PDAC eligible for pancreatectomy were recruited from a prospective single-institution cohort study (NCT02974764).[28] The Johns Hopkins Medicine Institutional Review Board approved this prospective study. This study was conducted and presented according to the most recent REporting recommendations for tumor MARKer prognostic studies (REMARK).[29]

Patients were consented and blood samples for ctDNA analysis were drawn prior to surgical incision and post-operatively in a longitudinal fashion during regular clinical post-operative follow-up appointments. At each time-point, 40mL of peripheral blood was collected in 10mL ethylenediaminetetraacetic acid (EDTA) vacutainers (BD, Franklin Lakes (NY), USA). Follow-up was performed in accordance with National Comprehensive Cancer Network guidelines.[30] Methods and definitions of diagnosing clinical PDAC recurrence after pancreatectomy at the Johns Hopkins Hospital have been described previously.[6,7,31] Relevant demographics, clinicopathologic and treatment data of included patients were collected prospectively. Decisions regarding adjuvant treatment or treatment recurrence were made by the local or institutional treating clinician, who were blinded to the ctDNA results.

Next-Generation Sequencing of Primary Tumor

To determine *KRAS* mutational status, the surgically resected primary tumor was sequenced using next-generation sequencing (NGS) (see Supplemental Methods).

Circulating Tumor DNA Extraction

Thirteen different extraction kits from various companies were tested to compare yields of ctDNA (see Supplemental Methods). The manual QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) resulted in the highest yield of DNA and was thus used for this study. Within 6 hours of collection, blood samples were centrifuged with a first run at 2000g x 10min and a second run at 4000g x 10min in the CLIA-certified molecular diagnostics laboratory of the Johns Hopkins Hospital. All isolated plasma was aliquoted and stored in -80 °C for later use.

Digital Droplet PCR and Analysis

Emulsion-based ddPCR was performed using the RainDrop Digital PCR system (RainDance

Technologies, Billerica (MA) USA) (see Supplemental Methods). Each assay uses a single primer set and two TaqMan probes, one specific to the wild-type allele and a second specific to the mutant allele. RainDrop Analyst II (RainDance Technologies) was used to determine a positive signal for either wild-type (VIC) or mutant *KRAS* (FAM). Positive control (cell line mixtures at 1:1000 and 1:100), negative control (gDNA), negative control (plasma), and non-template control were run additionally on each ddPCR chip. Results were presented as mutant *KRAS* droplets per 10,000 total genomic droplets (per 10k).

Analytical Validation of *KRAS* ddPCR

Four *KRAS* assays for G12V, G12D, G12R and Q61H (c.183A>C) were developed using ddPCR on the Raindance system (see Supplemental Methods). A linearity study was performed using DNA mixtures from cell lines containing known mutations[32,33], serially diluted into wild-type DNA, to measure accuracy and determine the limit of detection. Mutant droplets were consistently detected in the 1:10,000 samples for all four cell line mixes, consistent with analytic limit of detection of 1:10,000.

Baseline noise was determined using a set of de-identified normal plasma samples from healthy donors (FFP, from donors age less than 25, and frozen within 8 hours of collection) and discarded plasma-24 (PF24, frozen within 24 hours) from the Johns Hopkins Hospital. Using these normal plasma samples, 50 assays were performed sampling >50,000 wild-type droplets for each assay. The baseline noise was exceedingly low for G12V and Q61H, slightly higher for G12R and bases susceptible to cytosine deamination such as G12.[34] Qualitatively similar findings were found with male normal plasma sampled in 171 separate runs to a total depth of more than 9 million wild-type droplets.

We required 3 (G12V, Q61H) or 4 (G12D, G12R) positive droplets for a sample to be called positive and since most plasma samples tested had ~5,000-20,000 amplifiable *KRAS* molecules, the limit of detection for clinical samples was ~1:2,000-1:6,000, depending on the number of genomes tested and the specific mutation detected

Definitions and Statistical Analysis

Continuous variables were reported as median (interquartile range [IQR]) and compared with the Mann-Whitney rank-sum test. Categorical variables were summarized as frequency (%) and compared with the Chi-square or Fisher's exact tests as appropriate. Sensitivity, specificity, positive predictive value, and negative predictive value of *KRAS* circulating tumor DNA (ctDNA) detection for prediction of recurrence throughout the follow-up period were

calculated using contingency tables. Disease recurrence was characterized using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines.

To assess the prognostic value of ctDNA, recurrence-free survival (RFS) and overall survival (OS) from the time of surgery were the primary endpoints of interest. RFS was defined as the time interval between the date of surgery and the date of recurrence. Patients were censored at the date of last cross-sectional imaging if recurrence did not occur. OS was defined as the time from the date of surgery to death from any cause. Patients were censored at the date of the last follow-up visit if no date of death was available. Kaplan-Meier curves were used to estimate median survival with corresponding 95% confidence intervals (CIs). The log-rank test was utilized for subgroup comparison. Univariable Cox proportional hazard analysis was performed to identify potential pre-operative predictors for RFS and OS. All factors with a P-value of <0.10 in univariable analysis were included as a covariate in a multivariable regression model to identify independent prognostic factors with corresponding Hazard Ratios (HRs). Statistical analysis was performed using SPSS v25.0 (IBM Corporation, Armonk, NY, USA) and R version v3.3.3 (R Foundation, Vienna, Austria).

Results

Analytical Validation of *KRAS* ddPCR

Analytic validation of ddPCR for ultrasensitive detection of the most common *KRAS* mutations in PDAC, G12V, G12D, G12R and Q61H was performed. Normal plasma was used to define baseline noise, and used cell line mixes to determine the accuracy, limit of detection, and precision. Baseline noise was higher for G12D and G12R, however patient samples with 1-2 droplets could be followed by a negative result or a high positive result. Accordingly, samples were categorized into three categories: negative, borderline (one or two positive droplets) and positive (see Supplemental Methods).

Study Cohort and Primary Tumor *KRAS* status

A CONSORT diagram showing study design, patient selection and cohort analysis is presented in Online Supplemental eFigure S1. The final case cohort consisted of 59 patients with resected PDAC. Primary tumor *KRAS* status was G12V in 28/59 (48%), G12D in 16/59 (27%), G12R in 12/59 (20%) and Q16H in 3/59 (5%) of patients. Baseline demographics, clinicopathologic and treatment characteristics are given in Table 1.

Pre-Operative ctDNA Status and Association with Clinicopathological Features

Prior to incision, 49% of patients (n=29) had detectable ctDNA, consisting of either a borderline (n=13, 22%) or a positive sample (n=16, 27%). In these 29 patients, median number of total *KRAS* mutant droplets was 4 (IQR 2-13) and median number of mutant *KRAS* droplets per 10k was 1.7 (IQR 0.8-8.5). Several significant associations were observed between presence of pre-operative ctDNA and historic clinicopathologic risk factors such as larger tumor size, lymph node positivity, presence of microscopic lymphovascular invasion and elevated pre-operative CA 19-9 (Table 1).

Pre-operative ctDNA as Predictor for Recurrence and Survival

After a median follow-up of 16 months (95% CI 13-19 months), disease recurrence was observed in 41 (70%) of the 59 patients and 26/59 (44%) had died. For the entire cohort, median RFS was 10 months (95% CI 8-11 months) while median OS was 22 months (95% CI 0-not reached). Patients with detectable pre-operative ctDNA had significantly decreased median RFS (8 months; 26/29 (90%) recurred) when compared to patients negative for ctDNA (19 months; 15/30 (50%) recurred) P<0.001; Figure 1A).

Similarly, median OS was not yet reached in the ctDNA-negative group (8/30 (27%) died), compared to a median OS of 14 months (18/29 (62%) died) for patients with pre- (operative borderline or positive ctDNA (P<0.001; Figure 1B). In multivariable Cox regression models, detection of ctDNA was a pre-operative predictor for both RFS (HR 2.67; P=0.011) and OS (HR 2.37; P=0.048) (Online Supplemental eTable S2).

Impact of Treatment on ctDNA Status

Several interactions between treatment for localized PDAC and ctDNA dynamics were observed. For instance, patients who had prior neoadjuvant chemotherapy were significantly less likely to have pre-operative ctDNA than were chemo-naïve patients (21% vs. 69%; P<0.001). Also, both pre-operative median number of mutant *KRAS* droplets per 10k (0 (IQR 0-0.5) vs. 1.2 (IQR 0-5.4); P=0.002) and median number of total *KRAS* mutant droplets (0 (IQR 0-1) vs. 2 (IQR 0-8); P=0.003) were significantly lower in post-neoadjuvant patients than in untreated patients (Online Supplemental eFigure S2A-B). Outcomes were relatively poor for the five patients with detectable ctDNA after neoadjuvant therapy: all recurred after surgery (median RFS 5 months) and two of the five were deceased after a median follow-up of 12 months following surgery. Anecdotally, one patient with extensive post-neoadjuvant treatment effect seen in the surgical specimen (College of American Pathologists (CAP) score 1) was negative for *KRAS* ctDNA in both the pre- and post-operative sample.

Table 1. Demographic, clinicopathologic, and treatment characteristics of included patients

Variable	*All patients (n=59)*	*ctDNA negative (n=30)*	*ctDNA borderline & positive (n=29)*	*P*-value
Male, *n* (%)	31 (53%)	19 (63%)	12 (41%)	0.091
Caucasian, *n* (%)	49 (83%)	24 (80%)	25 (86%)	0.731
Age, median years (IQR)	70 (61-77)	65 (59-72)	71 (66-80)	**0.014**
Treatment status, *n* (%)				**<0.001**
Chemo-naïve surgery	35 (59%)	11 (37%)	24 (83%)	
Post-neoadjuvant surgery	24 (41%)	19 (63%)	5 (17%)	
Pre-surgery CA 19-9, median U/ml (IQR)[a]	95 (29-244)	63 (26-128)	200 (61-609)	**0.007**
Type of operation, *n* (%)				NA
Pancreatoduodenectomy	39 (66%)	23 (77%)	16 (55%)	
Distal pancreatectomy	10 (17%)	3 (10%)	7 (24%)	
Total pancreatectomy	7 (12%)	1 (3%)	6 (21%)	
DP-CAR	3 (5%)	3 (10%)	0 (0%)	
Location of tumor, n *(%)*				0.211
Head/uncinate	43 (73%)	24 (80%)	19 (66%)	
Body/tail	16 (27%)	6 (20%)	10 (35%)	
R0 margin (>1 mm), *n* (%)	46 (78%)	24 (80%)	22 (76%)	0.701
Tumor size, median cm (IQR)	3.0 (2.5-4.0)	2.5 (2.1-3.5)	3.5 (2.7-5.1)	**0.002**
T-stage (AJCC 8th edition), *n* (%)				**0.015**
T1-T2	43 (73%)	26 (87%)	17 (59%)	
T3-T4	16 (27%)	4 (13%)	12 (41%)	
N-stage (AJCC 8th edition), *n* (%)				**<0.001**
N0-status	23 (39%)	19 (63%)	4 (14%)	
N1-status	20 (34%)	6 (20%)	14 (48%)	
N2-status	16 (27%)	5 (17%)	11 (38%)	
Lymph node positive, *n* (%)	37 (63%)	12 (40%)	25 (86%)	**<0.001**
Tumor differentiation, *n* (%)				0.138
Well/Moderate	40 (68%)	23 (77%)	17 (59%)	
Poor	19 (32%)	7 (23%)	12 (41%)	
Microscopic perineural invasion, *n* (%)	49 (83%)	23 (77%)	26 (90%)	0.184
Microscopic lymphovascular invasion, *n* (%)	29 (49%)	10 (35%)	19 (66%)	**0.002**
Tumor cellularity on NGS, median %	11.7 (6.7-21.0)	8.3 (3.4-16.0)	18.2 (10.9-26.9)	**<0.001**
KRAS mutation				0.928
G12V	28 (48%)	15 (50%)	13 (45%)	
G12D	16 (27%)	8 (27%)	8 (28%)	
G12R	12 (20%)	6 (20%)	6 (21%)	
Q61H	3 (5%)	1 (3%)	2 (7%)	

[a] 47 patients had documented pre-surgery CA 19-9 values, of whom 4 patients were non-expressors of CA 19-9 and excluded from analysis.

AJCC, *American Joint Committee on Cancer;* CA, *carbohydrate antigen;* CAP, *College of American Pathologists;* CI, *confidence interval;* DP-CAR, *distal pancreatectomy with celiac axis resection;* IQR, *interquartile range;* NA, *not applicable;* NGS, *next-generation sequencing*.

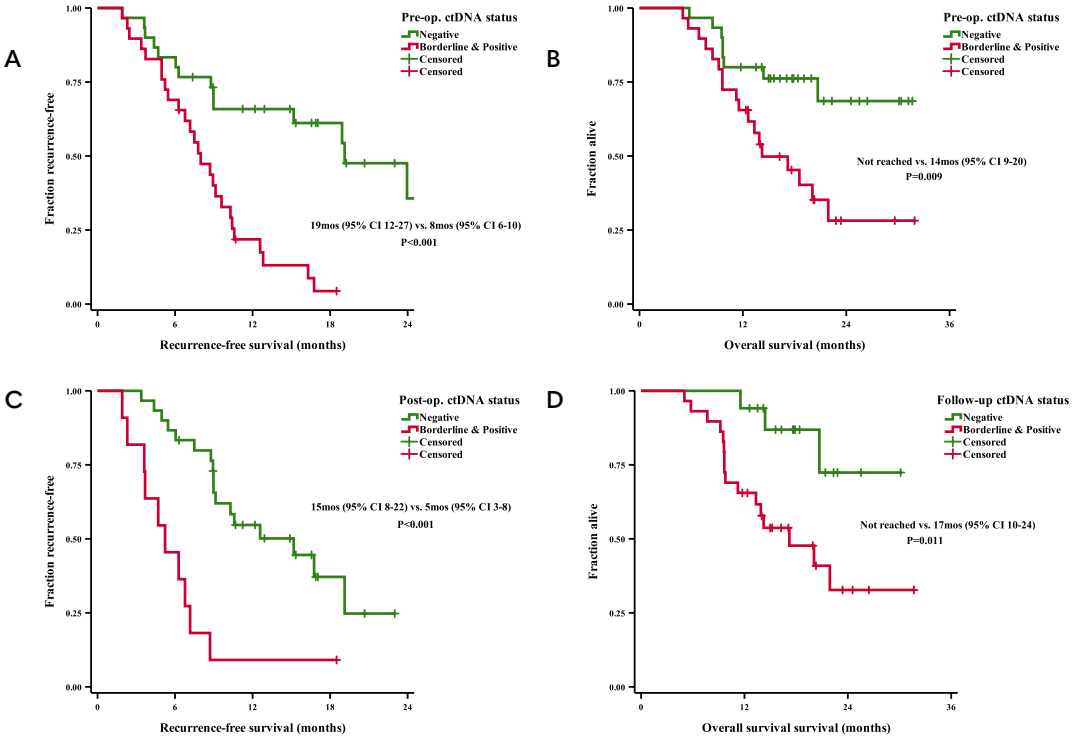


Figure 1. (A-B) Patients with pre-operative detectable KRAS ctDNA had decreased recurrence-free and overall survival. (C) Patients with detectable KRAS ctDNA in the immediate post-operative period had decreased recurrence-free survival. (D) Patient with detectable KRAS ctDNA during follow-up had decreased overall survival.

Forty-one patients had a post-operative sample available before the start of adjuvant treatment (sample at median post-operative day 24). In patients with detectable pre-operative ctDNA, surgery resulted in a drop of median number of mutant *KRAS* droplets per 10k from 1.0 (IQR 0.7-8.3) to 0 (IQR 0-1.3; P<0.001), and a drop of median number of total *KRAS* mutant droplets from 3 (IQR 2-30) to 0 (IQR 0-3; P<0.001) (Figure 2 & Figure 3). Persistence or emergence of ctDNA in the immediate post-operative period was detected in 11 patients (Figure 2D). Patients with ctDNA detected shortly after surgery prior to adjuvant treatment were highly likely to recur (10/11, 91%) and had a median RFS of only 5 months, significantly shorter than the 15 months (P<0.001) for patients without detectable post-operative ctDNA (17/30 (57%) recurred) (Figure 1C).

Longitudinal ctDNA Monitoring for Prediction of Recurrence

To assess the clinical utility of ctDNA for monitoring patients with PDAC for recurrence after their surgery, serial follow-up plasma samples were collected. At total of 277 samples from 46

patients were analyzed (median 5 samples per patient). Median follow-up for this subcohort of patients was 15 months, during which 30 of the 46 (65%) patients recurred and 19/46 (41%) died. Table 2 shows that 27 of the 30 (90%) patients that recurred at the time of last follow-up had detectable ctDNA during post-operative follow-up (Figure 4A-B). By contrast, 14 of the 16 (88%) patients free of recurrence never had ctDNA detected in any follow-up sample (Figure 4C-D). In these 16 patients without recurrence, pre-operative samples were positive in 1 patient, borderline in 2 and negative in 13 patients. The sensitivity of any detectable ctDNA during post-pancreatectomy follow-up for predicting recurrence was 90% (95% CI 74-98%) while specificity for predicting disease recurrence was 88% (95% CI 62-98%) (positive predictive value was 93% (95% CI 79-98%) and negative predictive value 82% (95% CI 61-93%)). The diagnostic accuracy of the ctDNA assay for predicting post-operative recurrence was 89% (95% CI 76-96%).

Figure 5 shows that in the majority of the 27 patients in whom ctDNA correctly predicted recurrence of their PDAC, the detection of ctDNA preceded the detection of disease

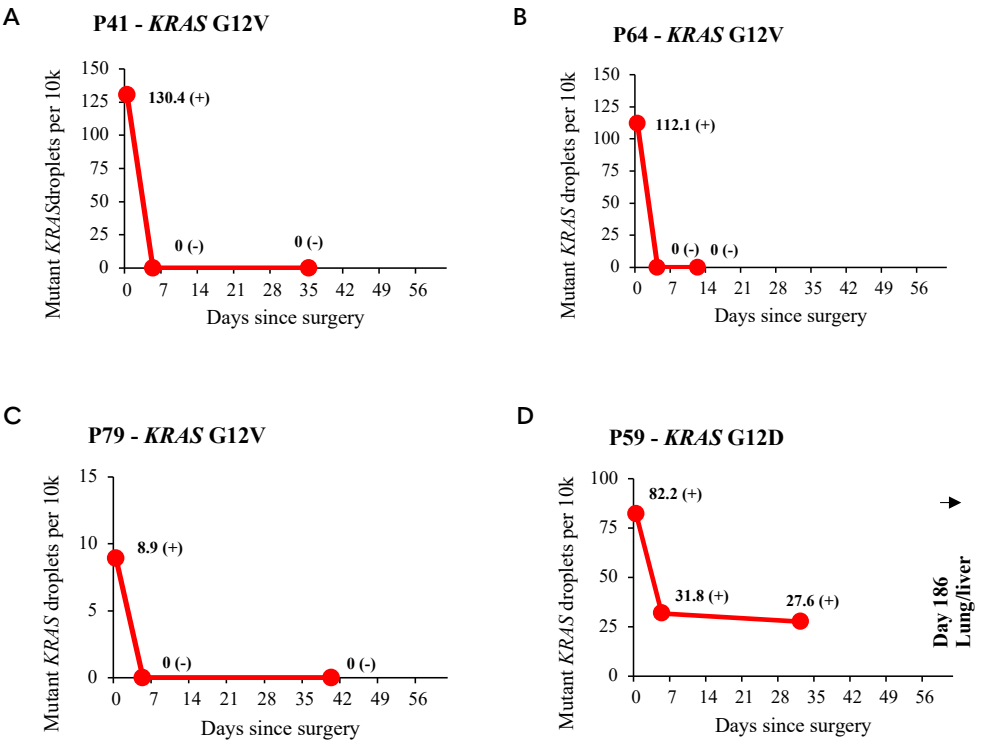


Figure 2. Examples of individual patient graphs showing (A-C) disappearance or (D) persistence of detectable *KRAS* ctDNA in the 2-month period following surgical resection of the primary tumor.

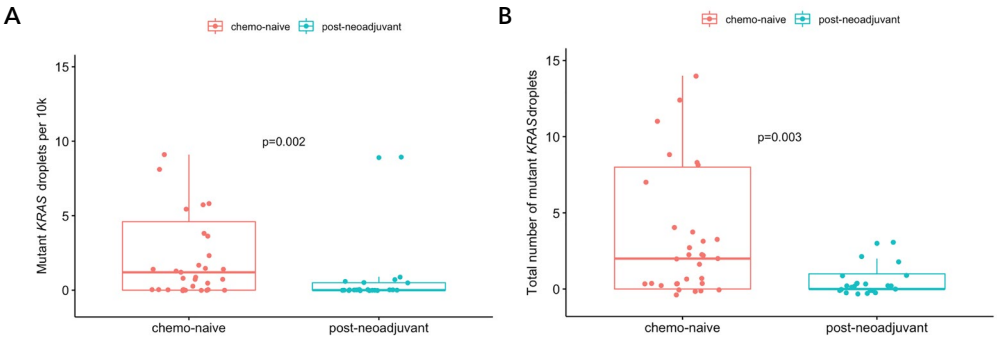


Figure 3. Examples of individual patient graphs showing (A-C) disappearance or (D) persistence of detectable *KRAS* ctDNA in the 2-month period following surgical resection of the primary tumor.

Table 2. Contingency table comparing *KRAS* ctDNA detection during post-pancreatectomy follow-up to clinical disease recurrence

	No disease recurrence	***Disease recurrence***	***Total***
KRAS ctDNA negative during follow-up	14 (11, 23, 26, 27, 44, 47, 51, 66, 68, 72, 77, 78, 84, 87)	3 (60, 85, 86)	17 (37%)
KRAS ctDNA borderline during follow-up	2 (61, 88)	5 (19, 22, 74, 81, 83)	7 (15%)
KRAS ctDNA positive during follow-up	0 ()	22 (5, 10, 15 20, 25, 29, 32, 34, 35, 38, 40, 41, 46, 59, 63, 64, 65, 75, 79, 89, 90, 91)	22 (48%)
Total	16 (35%)	30 (65%)	46 (100%)
Individual patient numbers displayed in brackets			

recurrence by imaging (22/27 (81%)). The median lead time of ctDNA relative to imaging was 84 days (IQR 25-146). Although CA 19-9 was not collected as part of this study, several patients had serial clinical CA 19-9 values available. In some patients, the dynamics of CA 19-9 levels and ctDNA had matching dynamics (Figure 6A-B). In other patients, the additional value of ctDNA was evident when CA 19-9 remained low despite disease recurrence (Figure 6C), or when CA 19-9 was undetectable (Figure 6D).

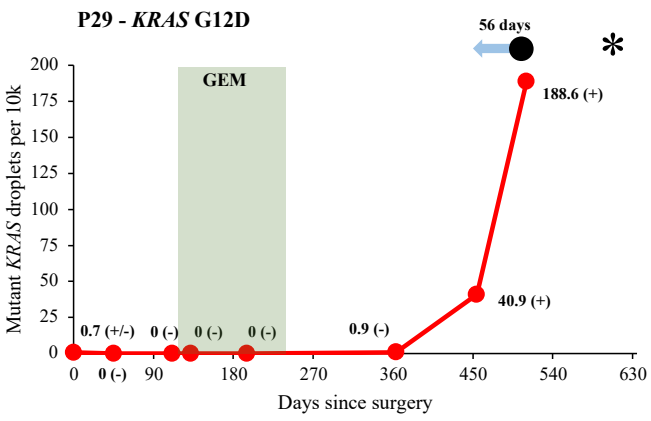
Detection of ctDNA during follow-up was associated with a median OS of 17 months, while median OS was not yet reached at 30 months for patients without ctDNA during follow-up (P=0.011) (Figure 1D). At the conclusion of the study period, 16/29 (55%) patients with detectable ctDNA during follow-up had died (Figure 4A-B), compared to 3/17 (18%) patients who had consistently negative plasma samples (P=0.013) (Figure 4C-D).

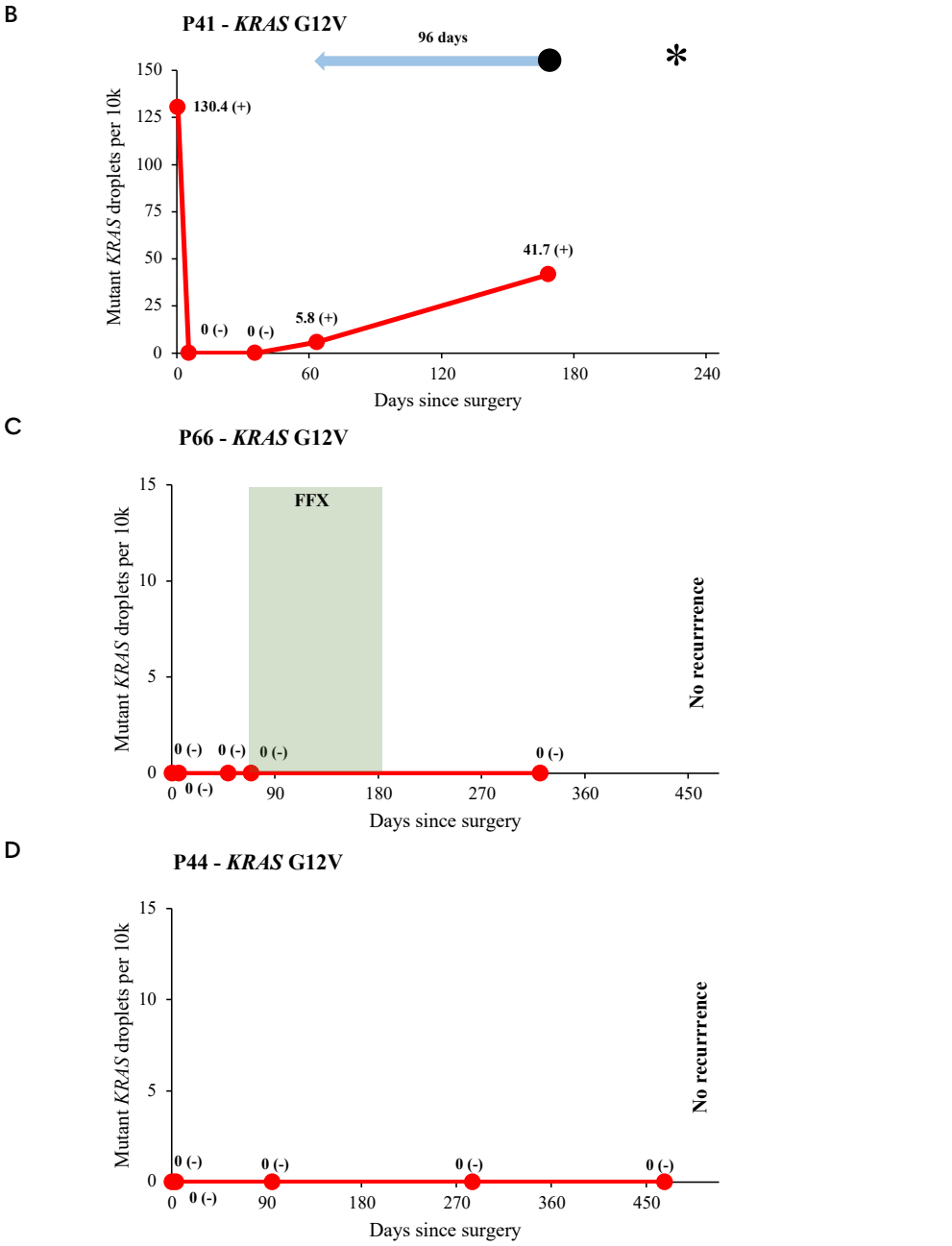
ctDNA Dynamics

ctDNA dynamics correlated with a response to treatment of PDAC recurrence in several patients. For instance, patient 34 developed several hepatic metastases 138 days after pancreatoduodenectomy (Figure 6E). FOLFIRINOX was started and the metastases showed a dramatic response on CT imaging. Correspondingly, *KRAS* ctDNA became undetectable and the patient currently continues on chemotherapy with stable disease on imaging. In another example, a patient developed local recurrence and was subsequently treated with FOLFIRINOX followed by radiotherapy (Figure 6F). A radiographic response was observed, consistent with a decrease in both ctDNA and CA 19-9 levels.

In this observational study, treatment decisions were made independent from ctDNA results. Patient 91 illustrates instances where information on ctDNA might have made a difference in a patient's treatment (Figure 6G). This patient, initially diagnosed with borderline resectable PDAC, was treated with neoadjuvant Gemcitabine/Nab-Paclitaxel followed by a pancreatoduodenectomy. An increase in both ctDNA and CA 19-9 was observed on post-operative day 383, coinciding with questionable signs of local recurrence on CT. Repeat imaging performed 3 months later definitively showed local recurrence and new metastases in both lungs. Palliative Gemcitabine/Nab-Paclitaxel was initiated with an observed drop in CA 19-9 and ctDNA. Patient 91 currently remains on treatment with stable disease on imaging. However, the patient's latest plasma sample showed a sudden increase in ctDNA. In this patient alone, additional evidence provided by ctDNA could have led to an earlier diagnosis of recurrence, and subsequent start of palliative treatment by 3 months. Additionally, after being on palliative Gemcitabine/Nab-Paclitaxel for an extended period of time, the recent spike in ctDNA might indicate refractory disease and the need to switch to a different chemotherapeutic regimen.

A





Signifies chemotherapy treatment. FFX, *FOLFIRINOX*; GEM, *Gemcitabine*.
Signifies lead time of *KRAS* ctDNA detection relative to clinical recurrence.
● signifies clinical disease recurrence; * signifies patient death; (-) signifies a negative *KRAS* ctDNA result; (+/-) signifies a borderline *KRAS* ctDNA result; (+) signifies a positive *KRAS* ctDNA result.
Sample at time point 0 represents the pre-operative sample.

Figure 4. Examples of individual patient graphs showing (A-B) detectable *KRAS* ctDNA during follow-up in patients with recurrence, and (C-D) no *KRAS* ctDNA detected during follow-up in patients without recurrence.

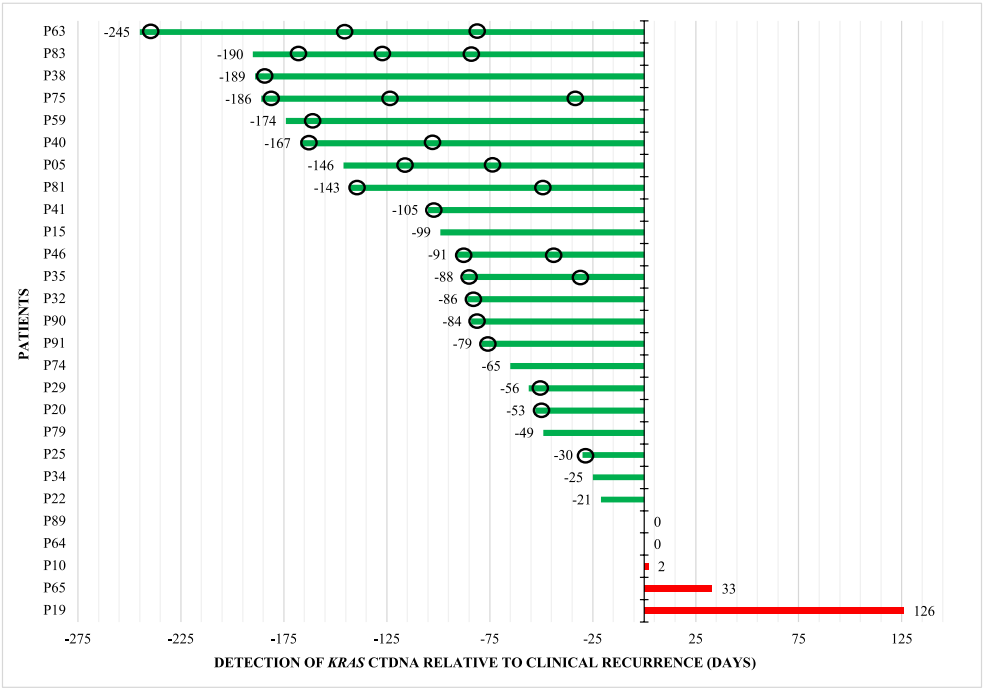
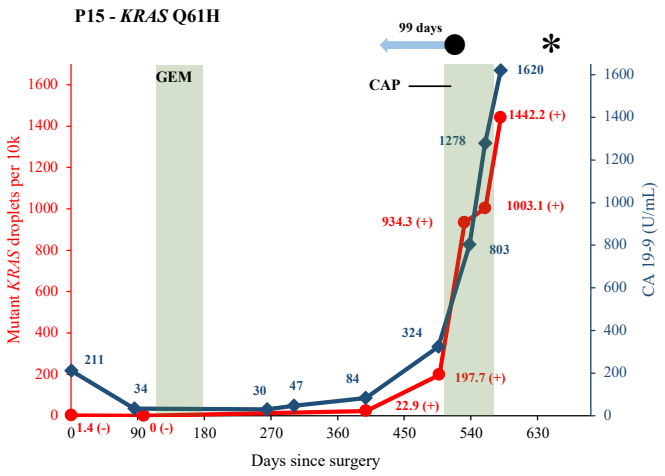


Figure 5. Plot showing timing of detection of KRAS ctDNA relative to manifestation of clinical recurrence in 27 patients with detectable KRAS ctDNA and recurrence during post-pancreatectomy follow-up. O signifies imaging without disease recurrence.

A



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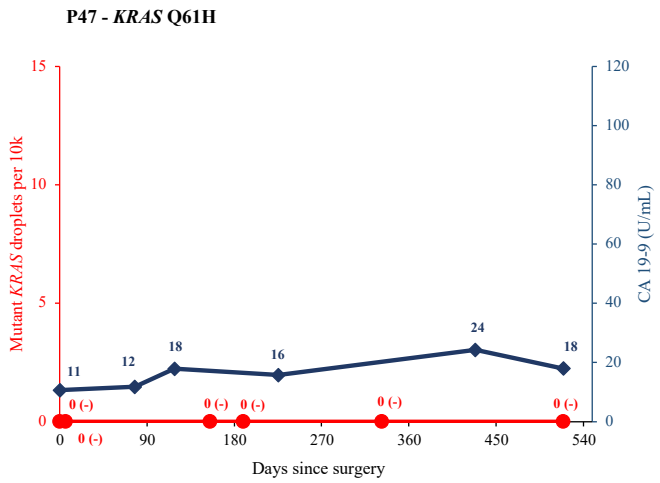
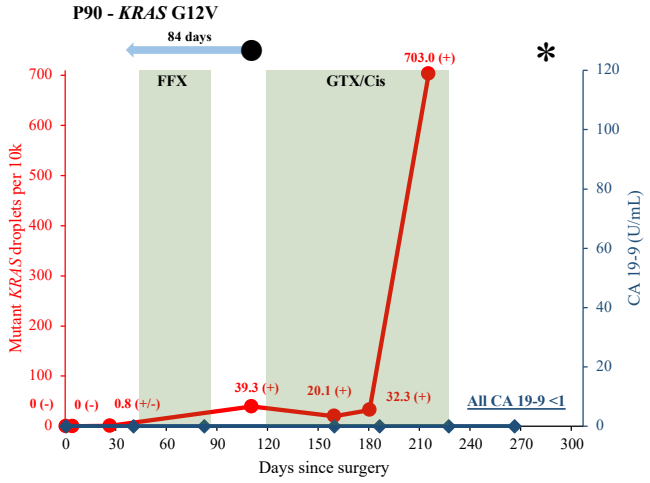
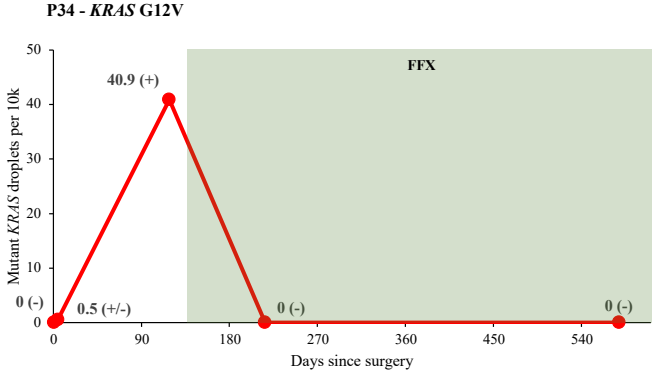

P47 - KRAS Q61H
Mutant KRAS droplets per 10k
CA 19-9 (U/mL)
Days since surgery
11
12
18
16
24
18
0 (-)
0 (-)
0 (-)
0 (-)
0 (-)
0 (-)
0 (-)

C


P90 - KRAS G12V
84 days
FFX
GTX/Cis
*
Mutant KRAS droplets per 10k
CA 19-9 (U/mL)
Days since surgery
703.0 (+)
39.3 (+)
20.1 (+)
32.3 (+)
0.8 (+/-)
0 (-)
0 (-)
All CA 19-9 <1

D


P34 - KRAS G12V
FFX
Mutant KRAS droplets per 10k
Days since surgery
40.9 (+)
0.5 (+/-)
0 (-)
0 (-)
0 (-)

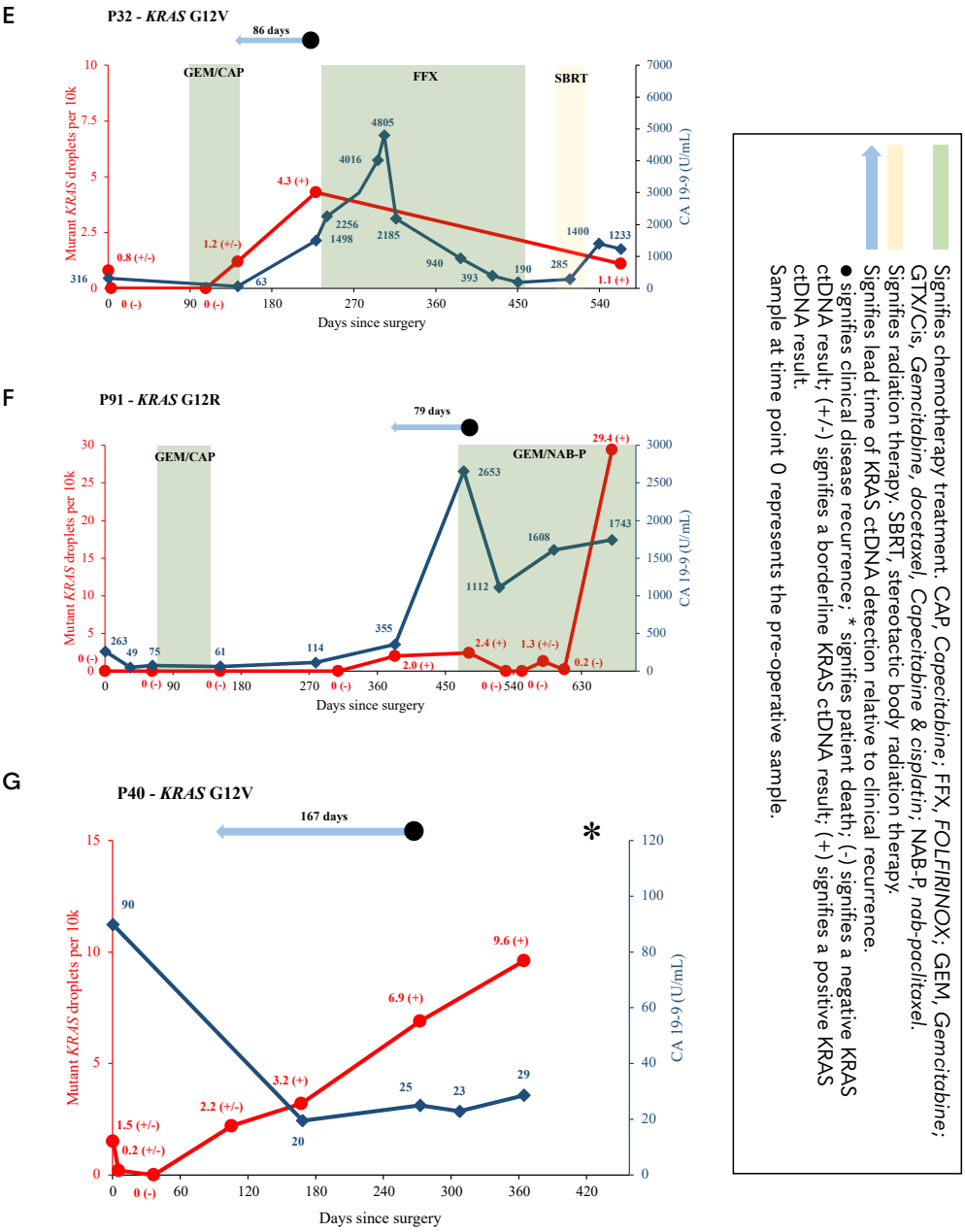


Figure 5. Examples of individual patient graphs showing (A) matching rise in CA 19-9 and KRAS ctDNA in a patient with recurrence, (B) similar stability in CA 19-9 and KRAS ctDNA in a patient without recurrence, (C) rise in KRAS ctDNA with low and stable CA 19-9 in a patient with recurrence, (D) rise in KRAS ctDNA in non-expressor of CA 19-9 with recurrence, E-F) KRAS ctDNA response to treatment and (G) a patient in whom KRAS ctDNA might have helped clinical decision-making.

Discussion

ctDNA testing offers the potential to significantly improve clinical decision making for patients with PDAC.[35] However, patients with PDAC often have very low levels of ctDNA (often in the range of 0.1% mutated DNA relative to wild-type DNA) necessitating ultrasensitive and reproducible approaches for clinical testing.[16,17,36] In this prospective study performed in a CLIA-laboratory setting, analytical validation of a ddPCR *KRAS* ctDNA assay showed a high degree of accuracy, sensitivity, and precision, with sufficiently low background noise and limit of detection. Additionally, this study confirmed the promise of ctDNA as a clinically useful prognostic biomarker in three clinical situations: (1) detectable ctDNA levels as a bad prognostic marker in either pre-surgery samples or (2) when persisting in the immediate post-operative period, and (3) detection of ctDNA during post-pancreatectomy follow-up as an accurate predictor of PDAC recurrence. As a result, the *KRAS* ctDNA assay is now being offered as an in-house clinical test in the Johns Hopkins Hospital.

Several research studies have established the detrimental prognostic impact of detecting pre-treatment ctDNA in patients with advanced PDAC.[25] One recent report of 105 resected PDAC patients found that the pre-operative detection of *KRAS* ctDNA by ddPCR was associated with both decreased median RFS (6 vs. 16 months; $P<0.001$) and decreased OS (14 vs. 28 months; $P<0.0001$).[37] In recent years, it has been argued that selected patients with resectable PDAC might benefit from a neoadjuvant chemotherapy-first approach.[38,39] However, currently available pre-operative risk factors for predicting early PDAC recurrence after resection, such as CA 19-9, have relatively poor predictive value.[6] ctDNA analysis might help identify those patients with a high likelihood of early recurrence, which could greatly aid physicians and patients alike in selecting the appropriate sequence of therapies.[26]

Surgical resection resulted in a drop of ctDNA in most patients, indicating that most pre-operative ctDNA in patients with early-stage PDAC originates from the primary tumor. In a subpopulation of patients however, ctDNA persisted in the immediate post-operative period. These patients had a recurrence rate of 91% and median RFS of only 5 months, providing evidence of ctDNA reflecting clinically occult residual disease after pancreatectomy. This strong association between post-resection ctDNA and disease recurrence has also been shown in recent studies on resected colon and rectal cancer by Tie et al.[21,40] Based on those results, the DYNAMIC trial is currently being conducted, randomizing patients with stage II colon or rectal cancer to adjuvant or no adjuvant therapy based on post-resection ctDNA results (ACTRN12615000381583). Adjuvant therapy is an essential part of the standard of care for patients with resected PDAC and adequate performance status. Patients with post-operative

detectable ctDNA appear to represent a group for whom systemic therapy is necessary.

Post-pancreatectomy surveillance for the detection of recurrent PDAC currently includes serial assessment using CT imaging and CA 19-9 level.[30] However, recent meta-analyses have shown that CT and CA 19-9 only have moderate diagnostic accuracy for the diagnosis of recurrent PDAC.[14,41] This study found that post-operative follow-up with *KRAS* ctDNA predicts recurrence with high sensitivity (90%) and specificity (88%) and predicts recurrence before imaging with a median lead time of almost 3 months. Similarly, a recent study from our institution found that detection of epithelial and mesenchymal circulating tumor cells during post-pancreatectomy follow-up accurately identified disease recurrence within the next 2 months.[42] These results indicate that the addition of *KRAS* ctDNA and other liquid biopsies can facilitate accurate and early recognition of PDAC recurrence post-pancreatectomy. The theoretical advantage of early detection of disease recurrence is that treatment can be initiated at a time when tumor burden is still limited, possibly resulting in eradication of recurrent disease or delay of progression.[27]

Dynamic monitoring of therapeutic response to cancer therapies is another use for ctDNA assays. Evidence of correlations between changes in ctDNA levels and tumor responses or outcomes have been demonstrated in other cancers, such as breast cancer[43,44], lung cancer[20,45], and colorectal cancer.[46,47] Although this study was not designed to assess the value of *KRAS* ctDNA for monitoring of therapeutic response, observed ctDNA dynamics in several patients suggested a correlation between ctDNA levels and response to cancer treatment. Current evidence for the use of ctDNA for therapeutic tracking in PDAC is limited to several smaller series in patients with metastatic disease (Online Supplemental eTable S1). For instance, in a recent study of patients with metastatic PDAC treated with FOLIRINOX, serial testing found concomitantly reduced ctDNA for 12 patients with partial response or stable disease.[48] Another study showed that reduction in *KRAS* ctDNA was associated with tumor responses observed by CT scan in 10/13 patients with metastatic PDAC.[49] Future, large prospective interventional trials are needed to demonstrate if changing treatment based on ctDNA dynamics can improve patient outcomes. In PDAC, an example of such a trial could encompass starting patients on either FOLFIRINOX or Gemcitabine/Nab-Paclitaxel, and switching to the other regimen following a sustained spike in ctDNA indicating refractory disease.

The results of this prospective study should be interpreted within the context of its limitations. One limitation is that direct comparison of CA19-9 with ctDNA in simultaneously collected samples was not possible in the present study. Another important limitation is that this *KRAS*

ctDNA assay can only be used to measure tumor dynamics in the 90+% of patients with a *KRAS* mutated PDAC and could potentially underestimate tumor burden if subclones harboring a different mutation start to expand. However, a recent study that included PDAC showed that there is minimal functional driver gene heterogeneity among metastases.[50] Furthermore, the analytic limit of detection of 1:10,000 that was established for this *KRAS* ddPCR, is difficult to achieve with current multi-gene panel or NGS approaches.[35] Finally, direct comparison of CA19-9 with ctDNA in simultaneously collected samples was not possible in the present study.

Despite these limitations, this study had important strengths that should also be acknowledged. First, this was a prospective study conducted in a CLIA-certified laboratory setting. Second, a robust analytical validation was performed, including determining the background noise in plasma samples from healthy donors. Finally, a relatively large number of longitudinally collected plasma samples was available for clinical validation.

Conclusion

KRAS ctDNA measured in a clinical laboratory provides useful prognostic information for patients with PDAC undergoing pre-operative evaluation and post-operative surveillance. Clinical trials using ctDNA dynamics to evaluate treatment decisions for patients with PDAC will need to be conducted to determine if it can improve patient outcomes.

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Supplementary Material to Chapter 14

Analytic Validation of a *KRAS* DDPCR Assay
Materials & Methods

Genomic DNA Preparation

Genomic DNA was isolated from pancreatic cell lines (Panc 502 harboring *KRAS* p.G12D mutation, Panc 504 harboring *KRAS* p.G12V mutation, TS0129 harboring p.G12R mutation, and A6L harboring p.Q61H (c.183A>C mutation), normal male blood sample, GM12878 and GM19240 cells using the Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia (CA) USA) and stored at -20°C. Concentrations were quantified using Qubit Fluormeter (ThermoFisher Scientific (CA) USA). Genomic DNA samples were sheared to 1500bp using the Covaris M220 focused ultrasonicator (Covaris, Inc., Woburn (MA) USA), and serial dilutions were made of the four pancreatic cell lines into the normal wild-type DNA at 1:100, 1:300, 1:1000, 1:3000, and 1:10,000.

Next-Generation Sequencing of Primary Tumor

To determine *KRAS* mutational status, the surgically resected primary tumor was sequenced using next-generation sequencing (NGS). In short, the tumor was identified, circled, estimated for neoplastic nuclear percentage on H&E stained slides, and macro-dissected from unstained slides using 5µm sections. DNA was isolated using the Tissue Preparation System (Siemens Healthineers, Erlangen, Germany) with Versant Tissue Preparation reagents (Siemans Healthineers) and DNA concentration was assessed with Qubit (ThermoFisher Scientific). NGS was performed using 1ng to 30ng genomic DNA the 50-gene AmpliSeq Cancer Hotspot Panel (v2), the Ion Chef (520/530 Chef Reagents kit) for emulsion PCR and template preparation, and sequenced on the Ion 530 Chip on the Ion S5-XL (Life Technologies Carlsbad (CA) USA). Up to 24 specimens were barcoded using Ion Xpress Barcode Adapters (Life Technologies) for each chip. To obtain *KRAS* status the Ion Torrent Variant Caller Plugin (v5.2.1.39) was utilized in the Torrent Suite software (v5.2) with a limit of detection set at 2%. Specimens in which no *KRAS* mutation was detected by the variant caller were visually inspected in Integrative Genomics Viewer (IGV) to identify the *KRAS* mutation.

Plasma bank and Isolation Kit Comparison

We obtained 46 Plasma-24 samples (PF24, frozen within 24 hours, typically 200-300mLs) from the Johns Hopkins Hospital was discarded for various reasons (outdated after thawing, broken bags) and generously provided by the transfusion medicine division. We also obtained 10 FFP

samples collected from donors less than 25 years old (frozen within 8 hours of collection) kindly provided by The Community Blood Center (Dayton, Ohio). The cutoff age of 25 years was based on the notion that healthy donors below that age would be very unlikely to have a fully developed malignancy or even a precursor lesion such as a colon adenoma or pancreatic intra-epithelial neoplasm. We tested a series of ctDNA isolation kits comparing each to the manual Qiagen kit (Online Supplemental eTable S3). The Qiagen manual kit was superior to all of them (data not shown).

ctDNA Isolation

Peripheral blood (40mLs EDTA) was processed within 6 hours of collection. Purple top tubes were centrifuged (Sorvall centrifuge) at 2000g x 10 mins, plasma was collected, transferred to new tubes, centrifuged at 4000g x 10 mins, transferred to new tubes and frozen at minus 80°C for processing. ctDNA was isolated using the manual QIAamp circulating nucleic acid kit (#55114) per manufacturer's protocol with the exception that all the plasma from a single patient was serially loaded onto one column and eluted in 60 H2O. Samples were vacuum dessicated (Savant) to 25µL, and DNA quantified using Qubit (ThermoFisher Scientific) per manufacturer's instructions, and converted to haploid genome equivalents assuming 3pg per haploid genome. Cell-free DNA (cfDNA) and FFPE DNA were not sheared.

Digital Droplet PCR

Emulsion-based digital droplet PCR (ddPCR) was performed using the RainDrop Digital PCR system from RainDance Technologies (Billerica (MA) USA). Primers and probes shown in Online Supplemental eTable S4 were purchased from ABI-Life Technologies. Probes were designed to be minor grove binding (MGB) where the wild-type probes contained VIC fluorophore while mutant probes contained 6FAM fluorophore. A 50µL ddPCR reaction was prepared using 1X TaqMan Genotyping Master Mix (Life Technologies), 900nM of forward and reverse target primers, 200nM wild-type and mutant target probes, and 300ng of sheared (genomic DNA from cell lines) or 60ng unsheared (ctDNA or FFPE) DNA. Additionally, 1X proprietary hydrofluorinated droplet stabilizer (RainDance Technologies, Billerica, MA, USA) was added to aid in emulsion formation. Emulsions for each reaction were prepared on the RainDrop Source instrument (RainDance Technologies) to produce 10 million, 5pL volume droplets per 50µL reaction. Thereafter, the emulsions were placed on the Bioer LifeTouch, Life Pro thermal cycler (Bioer Technology, Hangzhou, China). PCR conditions for amplification consisted of an activation step at 95°C for 10 minutes followed by 40 cycles of amplification at 95°C for 15 seconds, 64°C for 15 seconds, and 60°C for 45 seconds with a ramp rate of 0.5C/sec from denature to annealing, a polymerase inactivation step of 98°C for 10 minutes

and a final hold at 10°C (for *KRAS* G12D, G12V, G12R). The same settings were used for *KRAS* Q61H, with the exception of a 60°C annealing temperature. The amplified droplets were then placed on the RainDrop Sense instrument (RainDance Technologies) for signal detection.

Digital Droplet PCR Analysis

RainDrop Analyst II (RainDance Technologies) was used to determine positive signal for each allele type, either wild-type (VIC) on the Y-axis or mutant (FAM) on the X-axis. Gates were applied to regions of clustered droplets to define positive calls for each allele according to manufacturer's instructions. Data was exported to Microsoft Excel to calculate averages for mutant signals. A positive control (cell line mixtures at 1:1000 or 1:100), negative control (gDNA), negative control (plasma), and non-template control were included on each ddPCR chip.

Results

Linearity Study/Accuracy

We identified cell lines containing each of the *KRAS* mutation, sheared the DNA and made serial dilutions of DNA for each of them into wild-type DNA at 1:100, 1:300, 1:1,000, 1:3,000, and 1:10,000 (see Methods).[1,2] After designing primers and probes for the various reactions, we tested the serial dilutions in the appropriate ddPCR reaction on the RainDance instrument (Online Supplemental eFigure S3), and constructed dilution series curves (Online Supplemental eFigure S4). Each assay was performed in triplicate and Least-squares analysis resulted in R^2 values that exceeded 0.99. The samples at a 1:10,000 dilution were consistently detected for all four assays (Online Supplemental eTable S5). Using the NTC (water) control included on every run, we detected zero mutant droplets and only a total of 14 wild-type droplets among the 154 runs, analyzing a total of 1049 million droplets (Online Supplemental eTable S6).

To further assess accuracy, we compared the variant allele frequency in the primary tumor as measured by NGS to that determined using ddPCR (Online Supplemental eFigure S5). The two assays were highly concordant (R^2=0.97). We were unable to compare the assays for ctDNA samples with lower levels of mutant DNA as our established limit of detection of the NGS assay is 2%.[3]

Predicting Assay Efficacy

We calculated the number of genome equivalents (assayed by Qubit) that were used for

each ddPCR assay. We then compared that number to the number of droplets detected after PCR and sensing (Online Supplemental eFigure S6). Note that the correlation is linear, although the correlation coefficient is low (R^2=0.57). The slope is close to 1 (m=0.70). Using this relationship, and the requirement of a 40mL blood draw (resulting in ~20mLs of plasma) we were able to detect at least 10,000 wild-type droplets from 96% of samples.

Limit of Blank (Background Noise)

To determine the background noise level of the assay we collected a plasma bank composed of discarded plasma-24 and FFP (collected from donors age <25 years old.). We first tested cfDNA from the normal plasma sample from a normal male DNA (Online Supplemental eTable S7). Some reactions were remarkably "clean" such as the G12V where we performed 66 reactions totaling over 3.7 million wild-type droplets and did not detect a single mutant false positive droplet. In contrast, the G12D reaction had a higher baseline noise level (42 mutant droplets per 2.8 million wild-type droplets, equivalent to 0.15 per 10,000 wild-type) as the mutation can be produced from the endogenous process of cytosine deamination.[4,5] Similarly, the G12R reaction also had a slightly higher baseline noise level. We found qualitatively similar results with the normal plasma (Online Supplemental eTable S8). Accordingly, we set 0 or 1 droplet as the limit of blank for these two assays.

Sign-Out Categories

From the male normal and normal plasma testing, 1-2 positive droplets could be noise, especially for some of the assays (G12D and G12R). During the clinical validation, low-level positives (1-2 positive droplets) had about an equal chance of the next sample testing negative or testing high level positive. Accordingly, 1-2 mutant droplets could be clinically meaningful, and yet clinical attendings were uncomfortable signing out one mutant droplet as unqualified "positive", we therefore developed a third category designated "borderline". Sign-out categories and thresholds are summarized in Online Supplemental eTable S9.

Precision

To assess reproducibility, we repeatedly tested the 1:100 and 1:1,000 samples in separate runs (Online Supplemental eTable S10). Coefficients of variation were consistently lower for the 1:100 dilution samples (mean 8.4%, range 6.0 to 11.5%) than the 1:1,000 dilution samples (mean 18.2%, range 10.4% to 33.4%), as expected. We also measured inter-run variation for 27 PDAC specimens that were repeated on different runs and different days, where we compared the number of wild-type droplets and number of mutant droplets (if any, Online

Supplemental eTable S11). The combined wild-type droplets exceeded 10,000 droplets in the majority of samples and the number of mutant droplets was highly consistent. Intra-run repeatability was also highly consistent (data not shown).

Analytic Specificity

To assess potential crosstalk of the probes with different mutations (e.g. a false positive G12V assay when a G12D mutation was present), we tested 7 different combinations of serially diluted controls with a given mutation in an assay designed to detect a different mutation (Online Supplemental eTable S12). Of the 52 tests, all assays were negative with the exception 4 borderline false calls.

Checkerboard Testing

To test for crosstalk between adjacent wells in the strip, we alternated 1:10,000 positive samples with negative samples (example of G12V shown in Online Supplemental eTable S13). We did not detect any crosstalk between positive and negative samples.

Discussion

We conclude the ddPCR assays for the four *KRAS* mutations to be analytically validated. The dose-response curves and the comparison between NGS and ddPCR both demonstrate a high degree of accuracy. Baseline noise is defined and sufficiently low. Based on the results that baseline noise varied according to mutation, a minimum number of droplets was determined to call a sample outright positive and a borderline category was developed to accommodate for the fact that low level positives can likely be false positives as they are followed by a negative result, but they are also commonly followed by a high positive result.

Other methods could have been selected for this work, most notably NGS. NGS is problematic for such work as its error rate is usually higher than the desired limit of detection. Three seminal papers have been published to address this and the common thread to them is the use of barcodes or zipcodes.[6-8] This is most easily thought about as ligating a short piece of random DNA (the barcode or zipcode) onto the molecule prior to amplification. Following amplification, the barcode allows one to identify all of the progeny of that molecule into families and by definition, all family members must be identical, either wild-type or mutant. A wild-type family that contains a few mutant molecules is designated PCR error and discarded. A novel approach, using the first and last bases of the molecule along with a small barcode, has recently been developed.[9]

To avoid the complex bioinformatics issues associated with the NGS approach, we selected ddPCR as the technology to first approach this problem. It is an outstanding ultrasensitive detection strategy capable of reliably detecting variants in the range of 1:10,000.[10-12] Using this technology, the positions of droplets positive for wild-type molecules and droplets positive for mutant molecules are precisely defined and gated. With the RainDance instrument, 10 million droplets are generated, so that the vast majority of droplets (e.g. 99.9% if only 10,000 positive droplets) are empty, thus the probability of a droplet receiving both a wild-type and a mutant molecule is extremely low. In this unlikely event, the double positive droplets fall outside of the gates and are discarded. The absolute worst-case scenario is therefore that a droplet receives a wild-type double stranded molecule, but the polymerase makes an error in the very first cycle of replication producing an apparent G12V mutation. This would result in 4 strands, 3 wild-type and 1 mutant. However, because of the tight gating that is used in the system, a droplet composed of 75% wild-type molecules and 25% mutant molecules would not land in the gate, and therefore would be discarded. While ddPCR is an ultrasensitive and safe technology, each assay must be validated individually, so ctDNA assays will need to be migrated to NGS as the number of targets grow.

Conclusion

In summary, we consider ddPCR for *KRAS* to be analytically validated. Baseline noise levels are defined and low. Precision and accuracy are sufficiently high. The assay shows excellent analytic specificity, and cutoffs are defined.

Supplemental Tables and Figures to Chapter 14

- **Supplemental eTable S1.** Review of ctDNA in pancreatic cancer patients.
- **Supplemental eTable S2.** Univariate and multivariable Cox proportional-hazards regression analysis.
- **Supplemental eTable S3.** List of kits.
- **Supplemental eTable S4.** Primers and probes.
- **Supplemental eTable S5.** Serial dilution data.
- **Supplemental eTable S6.** Results of NTC testing.
- **Supplemental eTable S7.** Results of male normal.
- **Supplemental eTable S8.** Digital-droplet PCR results from normal plasma.
- **Supplemental eTable S9.** Clinical sign-out categories.
- **Supplemental eTable S10.** Precision of 1:100 and 1:1000 samples.
- **Supplemental eTable S11.** Inter-run variation.
- **Supplemental eTable S12.** Analytic specificity.
- **Supplemental eTable S13.** Checkerboard testing.
- **Supplemental eFigure S1.** Flowchart with overview of patient enrollment, sample collection and analysis.
- **Supplemental eFigure S2.** Boxplots of comparison between *KRAS* ctDNA. (A) *KRAS* ctDNA mutant droplets per 10k and (B) total amount of mutant *KRAS* droplets of pre-operative blood samples in chemo-naïve patients and patients who underwent neoadjuvant therapy.
- **Supplemental eFigure S3.** Digital-droplet PCR dilution series. Dilution series was produced for the G12V mutation in Panc504 and tested in the G12V assay.
- **Supplemental eFigure S4.** Dose-response Graphs. Representative dose response curves for G12D (A), G12V (B), G12R (C) and Q61H (D). Least-squares linear regression was R2 >0.99 for all reactions.
- **Supplemental eFigure S5.** Accuracy of digital-droplet PCR compared to next-generation sequencing on primary tumors. We measured the *KRAS* variant allele frequency in primary tumors using the AmpliSeq (Ion Torrrent) 50 gene panel on the S5 instrument and compared the result to the variant allele frequency determined by digital-droplet PCR.
- **Supplemental eFigure S6.** Predicting assay success. Total droplets detected in digital-droplet PCR as a function of the number of haploid genome DNA equivalents used as input for the reaction.

Supplemental tables and figures can be consulted online per the website of the journal and/or publisher.

References of Supplemental Material

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PART IV

Treatment of Recurrence

CHAPTER 15

Systematic Review on the Treatment of Isolated Local Recurrence of Pancreatic Cancer after Surgery; Re-Resection, Chemoradiotherapy and SBRT

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Abstract

Objective

The majority of patients who have undergone a pancreatic resection for pancreatic cancer develop disease recurrence within two years. In around 30% of these patients, isolated local recurrence (ILR) is found. The aim of this study was to systematically review treatment options for this subgroup of patients.

Methods

A systematic search was performed in PubMed, Embase and the Cochrane Library. Studies reporting on the treatment of ILR after initial curative-intent resection of primary pancreatic cancer were included. Primary endpoints were morbidity, mortality and survival after ILR treatment.

Results

After screening 1152 studies, 18 studies reporting on 313 patients undergoing treatment for ILR were included. Treatment options for ILR included surgical re-resection (8 studies, 100 patients), chemoradiotherapy (7 studies, 153 patients) and stereotactic body radiation therapy (SBRT) (4 studies, 60 patients). Morbidity and mortality were reported for re-resection (29% and 1% respectively), chemoradiotherapy (54% and 0%) and SBRT (3% and 1%). Most patients had a prolonged disease-free interval before recurrence. Median survival after treatment of ILR of up to 32, 19 and 16 months was reported for re-resection, chemoradiotherapy and SBRT respectively.

Conclusion

In selected patients, treatment of ILR following pancreatic resection for pancreatic cancer seems safe, feasible and associated with relatively good survival.

Introduction

Pancreatic ductal adenocarcinoma continues to be associated with poor survival.[1] Surgical resection is the only potentially curative option, with prognostic factors and outcomes for pancreatoduodenectomy extensively studied.[2]

Pancreatic cancer is often seen as a systemic disease at time of diagnosis as up to 80% of patients will develop cancer recurrence at either local or distant sites after successful surgical resection of the primary tumor.[3] Both clinical and autopsy studies show that isolated local recurrence (ILR) in the remnant pancreas or locoregional structures occurs in up to 30% of patients.[4-7] Despite these high local recurrence rates, no established strategies exist to deal with ILR.[8-10]

These patients represent a significant subgroup, in which timely diagnosis and treatment of recurrent disease might improve quality of life and survival. Locally destructive tumor growth was shown to be the probable cause of death in up to 30% of patients with pancreatic cancer.[11] Local tumor control could thus offer these selected patients a chance of extended survival. On the other hand, routine treatment of ILR might generate considerable morbidity in patients with an already poor clinical status and limited survival outlook.

The aim of this study was to assess the safety and feasibility of the different treatment modalities and to determine potential survival benefits for patients presenting with ILR following attempted curative treatment for pancreatic ductal adenocarcinoma.

Methods

Systematic Literature Search

Adhering to the 2009 PRISMA guidelines[12], a systematic search was performed in PubMed, Embase and the Cochrane Library for articles published up to 17 September 2016. Different domains of medical subject headings (MeSH) terms and keywords were combined with "AND", and within domains the terms were combined with "OR". The first domain included words related to recurrent pancreatic cancer, whereas the second domain contained terms related to possible treatment options. Terms were restricted to MeSH, title, abstract and keywords; a full description of the search strategy is available in Online Supplemental eTable S1. Two authors screened all titles and abstracts for eligible articles based on predefined eligibility criteria. Full-text manuscripts were evaluated, and references of the identified manuscripts were examined for potentially relevant articles not identified by the initial search.

Eligibility Criteria

Included were studies concerning treatment options for patients with ILR of pancreatic ductal adenocarcinoma after primary curative-intent resection. Only studies reporting on one or more of the following outcomes were included: morbidity, mortality and survival. Excluded were studies reporting on primary or recurrent endocrine tumors and emergency intervention. Also excluded were studies with fewer than 5 relevant patients, studies with only combined results for recurrent, metastatic or locally advanced disease, review articles and published conference abstracts. When the same study group published multiple articles and no difference in study period was described, the most recent article was selected.

Assessment of Methodological Quality

Quality assessment of the included studies was performed according to the Oxford Centre for Evidence-Based Medicine (CEBM) levels of evidence.[13] Risk of bias was therefore assessed using a standardized list of ten potential risks of bias, based on the Oxford CEBM Critical Appraisal Skills Programme appraisal sheets for cohort and case control studies.[14,15]

Data Extraction

All selected studies were evaluated for relevant patients, defined as patients undergoing treatment for ILR of malignant pancreatic cancer after initial curative-intent resection. Data on morbidity, mortality and survival were extracted. Included studies reporting on re-resection used varying definitions of surgical complications and post-operative morbidity. For this review, operative morbidity was reported as the combination of surgical complications and non-surgical 30-day morbidity as recorded by the individual studies. For the studies on chemoradiotherapy and stereotactic body radiation therapy (SBRT), toxicity of grade 3 and higher according to the Common Terminology Criteria for Adverse Events v4.0 guidelines[16] was deemed relevant. Mortality was defined as death within a 30-days post-intervention. Survival from both date of initial operation and date of start of treatment for ILR were reported. Treatment specifics and additional data on follow-up methodology and disease-free interval (DFI) were also extracted if available. Authors of the included studies were contacted when data were not reported separately or were missing. Results including patients with tumors of periampullary origin or other irrelevant patients were only used when deemed illustrative and if data for relevant patients was not obtainable separately.

Statistical Analysis

Results and outcomes in tables are expressed as they were originally reported. No meta-

analysis was performed because of obvious heterogeneity between studies. Hence, no statistical analysis was done owing to variation in the outcomes reported and variation of treatments used.

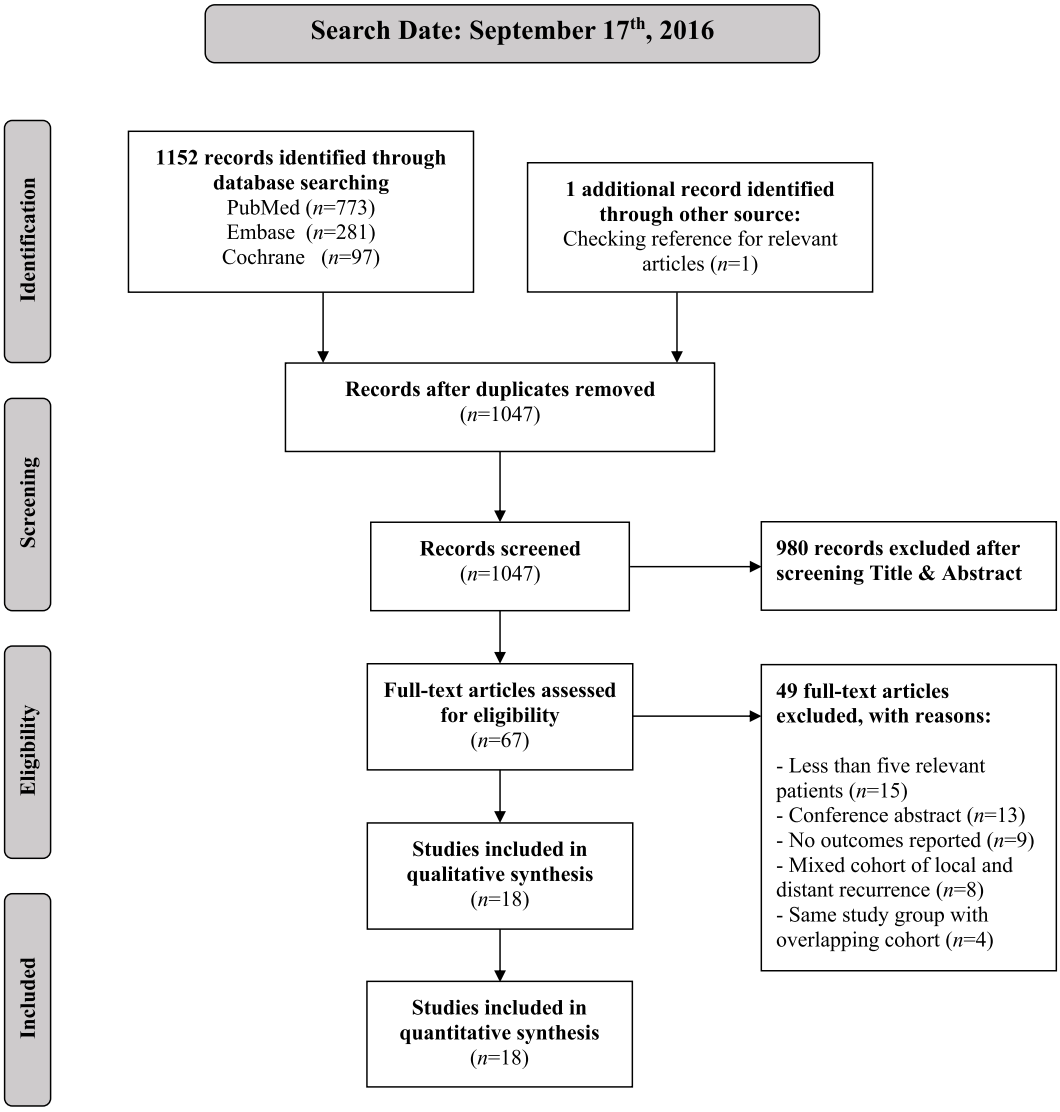

Figure 1. Flow chart of article selection.

Results

After screening of titles and abstracts, 68 full-text articles were assessed for eligibility and ultimately 18 studies[17-34] with 313 relevant patients were included for critical appraisal (Figure 1). Treatment options for ILR included surgical re-resection (8 studies, n=100), chemoradiotherapy (7 studies, n=153) and SBRT (4 studies, n=60). Pancreatic ductal adenocarcinoma was the primary tumor in 99% (n=309) of patients, with periampullary carcinoma (n=4) making up the other 1%. Results are presented per treatment modality, with a final section focusing on follow-up strategies used for diagnosing recurrence and corresponding disease-free interval (DFI).

Surgical Re-Resection

Eight studies[17-24], describing 100 patients undergoing re-resection for ILR, were included (Table 1). These 100 patients formed 2% of a total of 6069 patients undergoing primary resection for pancreatic cancer. Three articles[18,19,21] compared a re-operative group with a control group not selected for reoperation, while Suzuki et al.[24] compared patients receiving either best supportive care, chemotherapy or a reoperation. The other studies reported on single series of patients (Online Supplemental eTable 2).

All initial pancreatic resections were performed with curative-intent (R0 or R1). Lavu et al.17 described the use of adjuvant therapy after the initial operation, while five others[18,20,21,23,24] offered adjuvant therapy after both initial and reoperation. Only Strobel et al.[19] applied neoadjuvant therapy before the reoperation. Chemotherapeutic regimens typically contained Gemcitabine (GEM) and/or tegafur-gimeracil-oteracil potassium (S-1). Re-resection was often performed immediately, with no studies reporting on the use of chemotherapy after detection of ILR to determine whether lesions remain stable prior to reoperation.

In total, 100 patients underwent 116 reoperations to reach adequate resection margins. Thirty-seven % (n=43) of reoperations were pancreas-sparing while completion pancreatectomy was ultimately necessary in 65% (n=65) of the patients. Sixty-nine (69%) patients obtained a R0 status; R1 and R2 margins were seen in 20 (20%) and 11 (11%) patients after re-resection. Complications after re-resection included intra-abdominal abscess (n=7; 8%), pneumonia (n=4; 5%), delayed gastric emptying (n=4; 5%), urinary tract infection (n=3; 3%) and wound infection (n=2; 2%) among others. One patient (1%) died following the direct effects of reoperation.[26] Cause of this single death was not given.

Three studies described survival of patients with resected versus unresected ILR. Miyazaki

Table 1. Study outcomes of surgical re-resection for the treatment of locally recurrent pancreatic cancer

						Median survival		
Reference	*Relevant patients (total)*	*Treatment specifics for isolated local recurrence*	*Operative morbidity*	*Mortality*	*Median DFI (months)*	*Overall (months)*	*After reoperation (months)*	*Location of second recurrence after re-operation*
Lavu *et al.* (2011)[17]	8 (11)	CP (*n*=8)	1	0	35 (2-107)	42.5 (24-114)	16 (2-53)	NR
Thomas *et al.* (2012)[18]	7 (426)	CP (*n*=5), resection bed (*n*=1), abdominal wall resection (*n*=1)	NR	0	41	79	NR	None (*n*=1), peritoneum (*n*=3), local (*n*=2), lung + peritoneum (*n*=1)
Strobel *et al.* (2013)[19]	41 (105)	Pancreas-sparing regional resection (*n*=31), CP (*n*=19), SP (*n*=5)[§]	14	1	NR	NR	26	NR
Hashimoto *et al.* (2014)[20]	7 (10)	CP (*n*=6), SP (*n*=1)	NR	0	33 (21-86)	52 (36-118)	16 (10-85)	NR
Miyazaki *et al.* (2014)[21]	11 (170)	CP (*n*=10), distal pancreatectomy (*n*=1)	3	0	32 (7-89)	78 (17-107)	25 (3-61)	None (*n*=5), peritoneum (*n*=4), peritoneum + liver (*n*=1), lung (*n*=1)
Boone *et al.* (2014)[22]	10 (22)	Resection bed (*n*=3), CP (*n*=2), DPS (*n*=2), gastrectomy (*n*=2), SBR (*n*=1)	2	0	25 (9-61)	59 (19-128)	32 (4-81)	NR
Shima *et al.* (2015)[23]	6 (6)	CP (*n*=8)	0	0	25.5 (12-60)	49 (26-107)	27.5 (6-70)	None (*n*=1), local (*n*=2), lung (*n*=2), liver (*n*=1)
Suzuki *et al.* (2015)[24]	10 (23)	CP (*n*=9), distal pancreatectomy (*n*=1)	5	0	50.5 (15-143)	79.5 (38-154)	30 (1-90)	None (*n*=5), peritoneum (*n*=2), lung (*n*=1), liver (*n*=1), liver + lung (*n*=1)
Total	**100 (773)**	**NA**	**25 of 86 (29%)**	**1 of 100 (1%)**	**NA**	**NA**	**NA**	**NA**

[§] 41 relevant patients underwent 55 reoperations to achieve curative resection status.
CP, *completion pancreatectomy*; DFI, *disease-free interval*; DPS, *distal pancreatosplenectomy*; NA, *not available*; NR, *not reported*; SBR, *small bowel resection*; SP, *segmental pancreatectomy*.

et al.[21] reported that patients with unresected ILR (n=56) had a significant shorter median survival of 8 months compared to a median survival after reoperation for resected patients (n=11) of 25 months (P<0.02). Likewise, Strobel et al.[19] showed that 16 patients with ILR who were not re-resected due to irresectability (median survival 11 months) survived significantly shorter than the 41 patients undergoing re-resection (median survival 26 months) (P=0.01).

Lastly, Suzuki et al.[24] noted a significant shorter median survival of 12 months after receiving best supportive care or chemotherapy as compared to re-resection (P=0.005).

Chemoradiotherapy

A total of 7 retrospective studies were included with 153 patients (Online Supplemental eTable 3). Three studies[25,28,29] described treating ILR after primary resection with chemoradiotherapy (CHRT) and three studies[24,26,30] used chemotherapy (CHT) only (Table 2). Lastly, Zhang et al.[27] described a mixed cohort of patients receiving CHRT (n=15), CHT (n=5) or best supportive care (n=2). Initial resection resulted in R0/R1 status in 105 patients (95%) in the studies[25,27,28,30] reporting on resection margin. In all but two studies[26,30], either neoadjuvant or adjuvant chemotherapy was given surrounding the initial operation. Most regimens were GEM-based, with the use of 5-fluorouracil (5-FU), S-1, Cisplatin and Capecitabine also described. Only one patient received prior radiotherapy.

Treatment specifics of CHRT were available in three studies.25,28,29 Median radiation doses varied from 45 to 60 Gy in 15 to 30 fractions. Concurrent CHT consisted of GEM (n=55; 65%), GEM/Cisplatin (n=8; 9%) or GEM/5-FU (n=6; 7%). Also used were S-1 (n=7; 8%), 5-FU alone (n=5; 6%) or 5-FU/Capecitabine (n=4; 5%). Likewise, GEM-based regimens (n=62; 84%) were preferred as sequential CHT, with S-1 and Capecitabine making up the other 16% (n=12). As part of larger studies using GEM or GEM+S-1, Hashimoto et al.[26] and Xue et al.[30] included subgroups of 12 and 24 patients with ILR respectively.

Reported relevant therapy-related acute toxicity consisted mainly of hematotoxicity in the form of grade ≥3 leukopenia, thrombocytopenia or neutropenia. Experienced gastrointestinal side effects were mild (grade 1-3) and included nausea, vomiting, abdominal pain and diarrhea. Late toxicities included grade 3 ileus and grade 3 gastric bleeding ulcer[29], which were observed in one patient each (2%).

Stereotactic Body Radiation Therapy

Four retrospective case series[31-34] reported on SBRT for the treatment of ILR (Online Supplemental eTable 4). Used linear accelerators were CyberKnife (Accuray, Sunnyvale, CA), Trilogy (Varian Medical Systems, Palo Alto, CA) and Synergy (Elekta, Crawley, UK). SBRT image guidance was based on placed fiducials, existing bile duct stents or location of the spine. In three studies[31-33], all patients had received prior radiation in association with initial surgical resection. Contrastingly, Zeng et al.[34] considered prior radiotherapy as an exclusion criterion.

Table 2. Study outcomes of chemoradiotherapy for the treatment of locally recurrent pancreatic cancer

Reference	Relevant patients (total)	Median radiation dose (Gy) and used chemotherapeutic regimens	Acute toxicity	Late toxicity	Mortality	Median DFI (months)	Median survival	
							Overall (months)	After treatment (months)
Wilkowski *et al.* (2006)[25]	18 (18)	RT: 45 (20-50) CHT: 5-FU, Cisplatin and/or GEM	6	0	0	10 (2-19)	27	17.5
Hashimoto *et al.* (2009)[26]	12 (326)	CHT: GEM	NA	NA	0	NR	NR	19
Zhang *et al.* (2012) [27]	22 (79)	CHRT (*n*=15), CHT (*n*=5) and BSC (*n*=2)	NR	NR	0	14 (3-47)	NR	10 (2-30)
Habermehl *et al.* (2013)[28]	41 (41)	RT: 49.4 (39.6-54.0) CHT: GEM or 5-FU/Capecitabine	22	0	0	17 (3-80)	NR	16
Nakamura *et al.* (2014)[29]	30 (30)	RT: 54 (39-60 Gy) CHT: GEM, S-1 and/or 5-FU	18	2	0	14 (4-80)	36 (12-94)	16
Xue *et al.* (2014)[30]	24 (269)	CHT: GEM or GEM+S-1	NA	NA	0	14 (3-59)	31.5 (8-83)	12 (4-64)
Suzuki *et al.* (2015)[24]	6 (23)	NR	NR	NR	0	83 (29-157)	104 (38-210)	17 (9-53)
Total	**153 (786)**	**NA**	**46 of 89 (52%)**	**2 of 89 (2%)**	**0 (0%)**	**NA**	**NA**	**NA**

5-FU, *5-flourouracil*; BSC, *best supportive care*; CHRT, *chemoradiotherapy*; DFI, *disease-free interval*; GEM, *Gemcitabine*; Gy, *Gray*; NA, *not available*; NR, *not reported*; RT, *radiotherapy*; CHT, *chemotherapy*; S-1, *tegafur-gimeracil-oteracil-potassium*.

Table 3. Study outcomes of stereotactic body radiation therapy for the treatment of locally recurrent pancreatic cancer

Reference	Relevant patients (total)	Median radiation dose (Gy)	Acute toxicity	Late toxicity	Mortality	Median DFI (months)	FFLP rate		Median survival	
							After 6 months	After 12 months	Overall (months)	After SBRT (months)
Rwigema *et al.* (2011) [31]	11 (71)	24 (18-25)	3[§]	0	1[§]	NR	56%	19%	NR	13
Wild *et al.* (2013) [32]	15 (18)	25 (20-27)	0	1	0	13	78%	62%	NR	9 (8-12)
Dagoglu *et al.* (2016) [33]	15 (30)	25 (24-36)	0	0	0	24 (5-84)	72%	72%	42 (10-96)	16 (3-32)
Zeng *et al.* (2016) [34]	19 (24)	45 (42-50)	0	0	0	14 (2-55)	95%	42%	29 (10-73)	12 (4-23)
Total	**60 (143)**	**NA**	**3 of 120 (3%)**	**1 of 63 (2%)**	**1 of 120 (1%)**	**NA**	**NA**	**NA**	**NA**	**13 ± 5**

[§] Separate outcomes not obtainable. Presented results include patients receiving SBRT for isolated local recurrence *n*=11), locally unresectable disease (*n*=40), metastatic disease (*n*=8) and positive margins after primary resection (*n*=12).
DFI, *disease-free interval*; FFLP, *freedom from local progression*; NA, *not applicable*; NR, *not reported*.

Rwigema et al.[31] reported on 71 patients with advanced adenocarcinoma of the pancreas treated with SBRT, 11 of whom (16%) had local recurrence following surgical resection (Table 3). Sixty-four patients (90%) received chemotherapy in combination with SBRT. In all 71 patients, 3 (4%) grade 3 acute toxicities (nausea, abdominal pain and gastroparesis) and no late grade 3 toxicities were seen. In one patient, grade 3 gastroparesis led to hospitalization, severe volume depletion and death. Wild et al.[32] included 15 patients with ILR after surgical resection and 3 patients with local progression after definitive chemoradiotherapy for locally advanced pancreatic cancer. All patients received GEM-based maintenance chemotherapy before SBRT. No grade ≥3 acute toxicity was observed while one patient experienced a grade 3 late toxicity (small bowel obstruction). Of the seven patients presenting with abdominal/back pain prior to SBRT, effective symptom palliation could be achieved in four patients.

Dagoglu et al.[33] described a further 15 patients with ILR treated by SBRT, all of which received some form of chemotherapy prior to or after SBRT. Although eleven patients reported treatment-related mild fatigue, no grade ≥3 toxicity was reported. Furthermore, all 4 patients presenting with pain symptoms experienced alleviation of these symptoms as a result of SBRT. Similarly, Zeng et al.[34] reported relief of abdominal or back pain in 11 out of 14 patients of which 8 patients experienced complete relief of pain and discontinuation of analgesics.

Screening after Initial Operation and Disease-Free Interval

Thirteen out of 18 studies described a standard follow-up protocol after primary resection for the timely diagnosis of local recurrence. All studies used a combination of tumor marker testing and imaging (Table 4).

All but three studies[19,25,30] reported on DFI; the time between primary resection and diagnosis of ILR. DFI for the surgical re-resection group appeared to be longer than the DFI for the chemoradiotherapy and SBRT group, with medians ranging from 25-51 months, 10-83 months and 13-24 months respectively. The significance of DFI when treating ILR was demonstrated by 5 studies. Thomas et al.[18] showed that patients with ILR after a DFI >20 months had a median overall survival of 92 months compared with 31 months for patients with DFI <20 months (P=0.033). Similarly, Boone et al.[22] and Zhang et al.[27] reported significantly longer survival after a DFI >15 months and a DFI >12 months respectively. Nakamura et al.[29] found a DFI value of 19 months to be the sole factor associated with survival. Based on earlier publications, Wild et al.[32] reported a median survival after SBRT of 3 months for patients recurring within 9 months while a median survival of 11 months was seen for a DFI >9 months (P=0.019).

Table 4. Follow-up protocol for the detection of ILR after primary resection and disease-free interval

Reference	*Follow-up for recurrence after initial operation*	*Interval of follow-up*	*Median DFI (months)*	
Lavu *et al.* (2011)[17]	NR	NR	35 (2-107)	
Thomas *et al.* (2012)[18]	Marker: CA 19-9 Imaging: X-chest, CT	0-2 years: 4 months 2-5 years: 6 months >5 years: yearly	41	
Strobel *et al.* (2013)[19]	Marker: CA 19-9, CEA Imaging: CT	Every 3 months	NR	
Hashimoto *et al.* (2014)[20]	Marker: NR Imaging: CT	Every 3 months	33 (21-86)	
Miyazaki *et al.* (2014)[21]	Marker: CA 19-9 Imaging: CT, MRI, PET-CT	Every 2-3 months	32 (7-89)	
Boone *et al.* (2014)[22]	Marker: CA 19-9 Imaging CT, PET-CT	0-2 years: 3 months > 2 years: yearly	25 (9-61)	
Shima *et al.* (2015)[23]	Marker: CA 19-9, CEA Imaging: CT, PET-CT	Every 2-3 months	25.5 (12-60)	
Wilkowski *et al.* (2006)[25]	Marker: CA 19-9 Imaging CT, MRI	NR	10 (2-19)	
Hashimoto *et al.* (2009)[26]	NR	NR	NR	
Zhang *et al.* (2012)[27]	Marker: CA 19-9 Imaging: CT, PET-CT	0-1 year: 3 months 1-2 years: 6 months >2 years: yearly	14 (3-47)	
Habermehl *et al.* (2013)[28]	NR	NR	17 (3-80)	
Nakamura *et al.* (2014)[29]	Marker: CA 19-9 Imaging: CT, MRI, PET-CT	NR	14 (4-80)	
Xue *et al.* (2014)[30]	Marker: CA 19-9 Imaging: CT, PET-CT	NR	14 (3-59)	
Suzuki *et al.* (2015)[24]	Marker: CA 19-9, CEA Imaging: CT, MRI	Every 3-6 months	50.5¶ (15-143)	83¶ (29-157)
Rwigema *et al.* (2011)[31]	NR	NR	NR	
Wild *et al.* (2013)[32]	Marker: CA 19-9, CEA Imaging: CT, MRI, PET-CT	0-2 years: 3 months 2-5 years: 6 months >5 years: yearly	13	
Dagoglu *et al.* (2016)[33]	Marker: CA 19-9 Imaging: CT	0-1 year: 3 months	24 (5-84)	
Zeng *et al.* (2016)[34]	Marker: CA 19-9 Imaging: CT, PET-CT	0-1 year: 3 months > 1 years: 6 months	14 (2-55)	

¶ Median disease-free interval for re-resection (n=10) and chemotherapy (n=6) cohort respectively.
CA, *carbohydrate antigen*; CEA, *carcinoembryonic antigen*; CT, *computed tomography*; MRI, *magnetic resonance imaging*; NR, *not reported*; PET, *positron emission tomography*.

Discussion

Surgical re-resection, chemoradiotherapy and SBRT for the treatment of ILR of pancreatic cancer appear to be safe and feasible in selected patients. Furthermore, the reviewed literature suggests that, in these patients, treatment of ILR resulted in extended survival.

In the studies reporting on re-resection, morbidity rates ranged from 0% to 50% while mortality was low (1%). Historic morbidity rates after primary pancreatic surgery range from 16% to 45% with mortality rates in high-volume centers ranging from 1-4%.[2,35,36] Likewise, chemoradiotherapy was well tolerated with a low late toxicity rate of 2% and no associated mortality. On the other hand, acute toxicity was seen in up to 60% of patients. However, most of these were of hematological nature and were treated sufficiently with best supportive care. Comparable GEM-based chemotherapy for locally advanced pancreatic cancer can generate ≥grade 3 toxicity rates ranging from 33% to 77%.[37-41] These results imply that both re-resection and chemoradiotherapy for ILR can be performed safely and with comparable morbidity and mortality rates to those of initial resection or treatment of locally advanced pancreatic cancer.

However, most patients with ILR cannot undergo re-resection due to technical barriers such as vascular involvement (Figure 2). SBRT is a relatively new treatment option that can deliver radiation more conformally than conventional radiotherapy in just three to five fractions, sparing organs at risk and other normal tissues (Figure 2C). The minimally invasive nature of SBRT is reflected in the found low acute (3%) and late (2%) toxicity rates. Similarly, a review reporting on the use of SBRT for locally advanced pancreatic cancer found that complications rates varied from 0% to 25%.[42] SBRT thus represents a possible effective therapeutical choice that is less drastic than re-resection however offers more local control than chemotherapy with or without conventional radiotherapy.

Sperti et al.[4] reported that 89% of patients developed recurrent disease after initial surgical resection, of which 33% were located solely in the remnant pancreas or locoregional structures. A median DFI of 10 months was seen, with a subsequent median survival of only 7 months without any intervention. Similarly, Hernandez et al.[43] studied the natural history of resected pancreatic cancer and described a median DFI of 7 months and overall survival of 14 months for local recurrence. The published survival after re-resection, chemoradiotherapy or SBRT in this review, ranging from 9 to 32 months, suggests a relative survival benefit for the selected group of patients with ILR that received treatment when compared to the natural course or best supportive care.

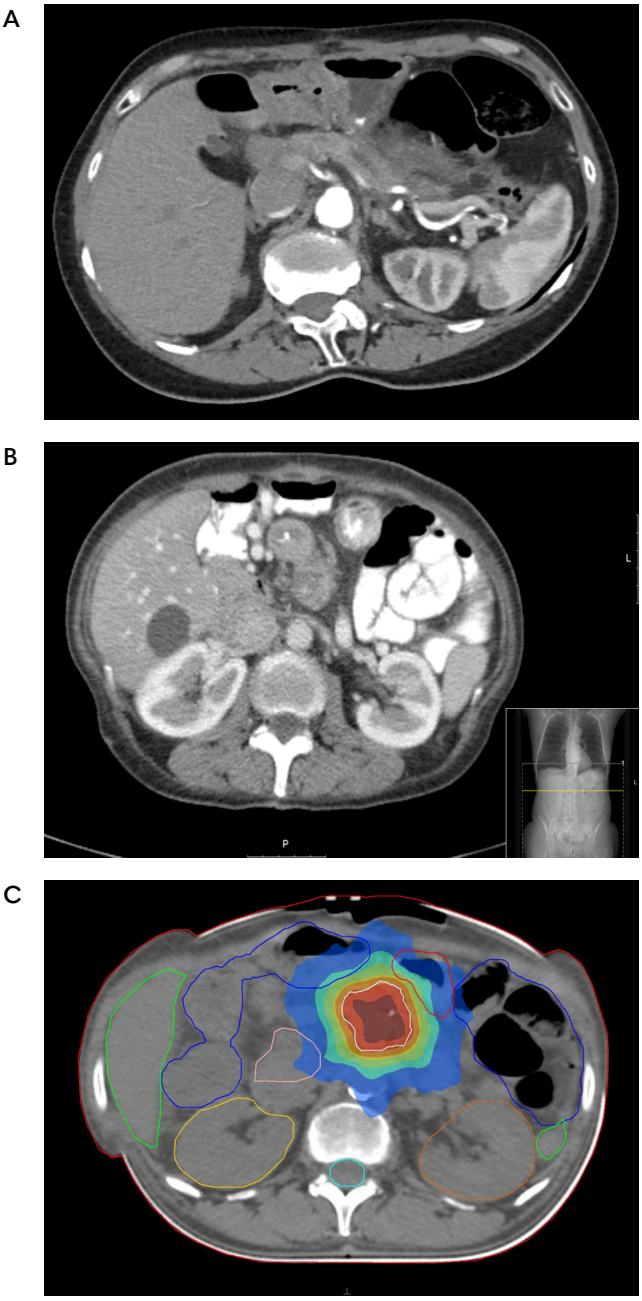

Figure 1. (A) Transversal CT imaging showing resectable isolated local recurrence with dilatation of the pancreatic duct upstream. (B) Transversal CT imaging showing unresectable isolated local recurrence with complete encasement of the arteria mesenterica superior and obliteration of the vena mesenterica superior. (C) Stereotactic body radiation planning for the same patient of Figure 2B with the isolated local recurrence circled in white and organs at risks circled in other colors.

In reference to these survival outcomes, it should be noted that only highly selected patients with good performance status were treated in these studies. This may imply that less-aggressive tumor biology is in part responsible for the found extended survival outcomes. Therefore, resulting survival is probably more favorable than it would have been in an unselected patient group. Apart from this evident selection bias, our review is limited by the relatively small number and heterogeneity of patients included by the retrospective single-institution studies. Also, all studies used different neoadjuvant and/or adjuvant treatment regimens surrounding the primary operation and even within the same study, different chemotherapeutic agents and radiation doses were used for the treatment of ILR. Despite these limitations and the lack of prospective randomized controlled trials in the current review, the presented results and outcomes of all available literature on treatment procedures for ILR allow for a comprehensive understanding and critical evaluation of this approach.

Reported median survival after re-resection (range, 16-32 months) seemed higher when compared to chemoradiotherapy (range, 16-19 months) and SBRT (range, 9-16 months). However, re-resection was not always combined with systemic treatment, making it difficult to determine the impact on survival and effectively compare different treatment modalities. Larger, prospective studies are required before final relative survival benefit for the selected group of patients with ILR that received treatment when compared to the natural course or best supportive care. conclusions can be drawn. Also, a significant number of patients receiving chemoradiotherapy or SBRT would have been deemed unresectable, possibly accounting for the found difference in survival. Furthermore, DFI for patients in the re-resection group appeared to be longer than that for patients receiving chemotherapy and SBRT.

Re-laparotomy obviously has a big impact on patients and their quality of life. Moreover, additional pancreatectomy often results in a total pancreatectomy with poorly regulated diabetes as a consequence, although several recent studies have shown that this diabetes is better manageable in present times.[44,45] The non-invasive character of chemoradiotherapy and SBRT makes them very suitable as salvage treatment. Especially when patients undergo reoperation for suspected ILR, and exploration reveals disseminated disease not seen in the pre-operative workup, the whole procedure of re-resection could be too aggressive of an approach for patients with such limited survival expectations. Furthermore, with current imaging modalities it is often difficult to differentiate true local recurrence from post-operative changes and fibrosis, possibly leading to unnecessary re-laparotomy. Strobel et al. mentioned that in 8 (7.6 %) of 105 patients with suspected local recurrence, all intra-operative biopsies were tumor free. Seven of these patients had received pre-operative chemoradiation prior to re-resection. Additionally, Strobel et al.[19] reported that in 53% of 105 patients who underwent

re-exploration, peritoneal or liver metastases or unresectable locally recurrent disease were found that had not been identified in the pre-operative workup. Most of these patients had to undergo exploration without re-resection. Yet, in the era of laparoscopic pancreatic surgery, a diagnostic laparoscopy might be a valuable approach in patients in whom the primary operation was also performed laparoscopically. Evidently, future prospective studies need to compare a surgical approach with non-invasive approaches and take both survival and quality of life into account.

Due to the aggressive nature of pancreatic cancer and the tendency of metastatic spread, definitive cure seems unlikely even if extended survival is reported. For treatment to be effective, without unnecessary burden for the patient, it is essential to select only those patients with the greatest chance of benefitting from multimodality treatment. DFI could play an important role in this selection process. Five included studies showed that a prolonged DFI is associated with longer survival, advocating intervals ranging from 9 to 20 months. Two theories exist for explaining the impact of DFI on survival. One suggests that slow growing tumors are less aggressive and thus result in longer survival. The other hypothesis, the "two-hit theory", centers on the idea of a second primary tumor forming in the remnant pancreas. In this scenario, chances of disseminated disease are smaller than with true recurrence.[22] Extended survival after a prolonged DFI is probably the result of a combination of the two theories with both hypotheses supporting the indication of further treatment.

When considering DFI, it seems crucial to diagnose ILR before the stage of advanced local growth or metastatic disease. One study group showed that over 50% of patients can be diagnosed with recurrence before onset of clinical symptoms with a surveillance regimen combining carbohydrate antigen (CA 19-9) testing with abdominal CT-scans every 3-4 months, but nonetheless argued that clinical evaluation and CA 19-9 testing every six months after surgery is the most cost-effective strategy.[46,47] The emergence of fluorodeoxyglucose positron emission tomography (FDG-PET) as new imaging modality has made the detection of early ILR more sensitive. One study reported a 96% success rate of detecting local relapse with the use of FDG-PET, compared to 39% with CT/MRI.[48] Presently however, no consensus exists on whether standard surveillance should be offered to patients. American guidelines seem to favor a more aggressive approach to follow-up with CA 19-9 and CT-imaging of the abdomen and pelvis every 3-6 most.[49] Contrarily, the European Society for Medical Oncology argues that surveillance should concentrate on symptoms, nutrition and psychosocial support and not on disease recurrence.[50] These guidelines regarding surveillance and follow-up are based on expert opinion and other low level evidence. Further prospective studies are needed to find a surveillance protocol for selecting patients whom would benefit from treatment of ILR.

Conclusion

For patients with recurrent pancreatic cancer, it is paramount to find multidisciplinary treatment that optimizes patient selection while balancing disease control, toxicity and quality of life.[51] This review shows that treatment of ILR can be performed with acceptable morbidity and mortality rates. As no randomized controlled trials were available, this review cannot recommend re-resection, chemoradiotherapy or SBRT as the best treatment option for patients with ILR. However, compared to best supportive care, treatment of ILR seems to offer a chance of extended survival in selected patients. Specifically, patients with a prolonged DFI seem to benefit most from treatment of ILR. As these findings are based on smaller cohort studies and patient series, further prospective studies should focus on identifying other clinicopathological features predicting which patients benefit most of these therapeutical options and the comparison between the different treatment options. Also, more research is needed to find an accurate and cost-effective surveillance algorithm best suited for identifying patients with ILR before they develop metastatic disease.

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Supplemental Material to Chapter 15

- **Supplemental eTable S1.** Systematic search strategy.
- **Supplemental eTable S2.** Critical appraisal of articles reporting on surgical re-resection for the treatment of isolated local recurrence.
- **Supplemental eTable S3.** Critical appraisal of articles reporting on chemoradiotherapy for the treatment of isolated local recurrence.
- **Supplemental eTable S4.** Critical appraisal of articles reporting on stereotactic body radiation therapy for the treatment of isolated local recurrence.

Supplemental material can be consulted online per the website of the journal and/or publisher.

CHAPTER 16

Stereotactic Body Radiation Therapy for Isolated Local Recurrence After Surgical Resection of Pancreatic Ductal Adenocarcinoma Appears to be Safe and Effective

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Abstract

Objective

A standardized treatment regimen for unresectable isolated local recurrence (ILR) of pancreatic ductal adenocarcinoma has not been established. The purpose of this study was to evaluate the outcomes of patients with ILR treated with stereotactic body radiation therapy (SBRT).

Methods

We retrospectively reviewed the records of patients with ILR treated with SBRT between 2010-2016. Symptom palliation and treatment-related toxicity were recorded. Associations between patient or treatment characteristics and overall survival (OS), progression-free survival (PFS), and local progression-free survival (LPFS) were assessed.

Results

Fifty-one patients who received SBRT for ILR were identified. Twenty-six (51%) had not received radiation therapy prior to SBRT. Median OS was 36 months from diagnosis. From the first day of SBRT, median OS, PFS, and LPFS were 16, 7, and 10 months, respectively. Patients with a recurrence-free interval ≥9 months after surgery had superior OS (P=0.019). Maintenance chemotherapy after SBRT was associated with superior OS (P<0.001) and LPFS (P=0.027). On multivariable analysis, poorly differentiated tumor grade (HR=11.274) positive surgical margins (HR=0.126) and receiving maintenance chemotherapy (HR=0.141) were independently associated with OS. Having positive surgical margins (HR=0.255) and receiving maintenance chemotherapy (HR=0.299) were associated with improved LPFS. Ten of 16 patients (63%) experienced abdominal pain relief following SBRT. Four patients (8%) experienced grade 3 gastrointestinal toxicity. One experienced grade 4 gastrointestinal toxicity.

Conclusion

SBRT for ILR improved pain in a majority of patients with acceptable acute and late toxicity. SBRT is a feasible treatment for select patients with ILR. For those who receive SBRT, maintenance chemotherapy should be considered.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a five-year overall survival (OS) rate of less than 8%.[1] Surgical resection is the only potentially curative treatment for PDAC. However, only 20% of patients are candidates for surgery at the time of diagnosis, and up to 80% of these patients will experience a local recurrence in the tumor bed after resection.[2-4]

Isolated local recurrence (ILR), defined as recurrence in the remnant pancreas and surrounding locoregional structures in the absence of distant metastasis, is less common (~30%).[5-9] At our institution, the frequency of isolated local recurrence was determined to be 23.7% (126 of 531 patients with recurrence) over a 10 year period.[9] Although prognosis is considered to be superior for patients with non-metastatic disease, approximately 30% of PDAC patients die of locally destructive disease.[10] Furthermore, local recurrence can be symptomatic and debilitating.[11,12] Therefore, local tumor control may offer patients with ILR improved quality of life.

Unfortunately, a standardized, evidence-based treatment regimen for patients with ILR has not been established.[13] A promising, minimally invasive treatment that provides good symptom palliation as a primary treatment is stereotactic body radiation therapy (SBRT).[14-19] SBRT delivers radiation more conformally than chemoradiation therapy (CRT), sparing normal tissues, in just three to five fractions.[20] Our institution contributed to a multi-institutional study that suggested that re-irradiation with SBRT appears to be a safe and reasonable option for patients with ILR of PDAC.[21] However, published data on the outcomes of patients receiving SBRT for ILR are limited, and little is known about which patients would benefit from SBRT. The current single institutional study sought to further assess the efficacy and safety of treating ILR of PDAC with SBRT and to identify patient and treatment characteristics associated with patient outcomes.

Methods

Patient selection

With the approval of our Institutional Review Board, all PDAC patients treated with SBRT for ILR after surgical resection at the Johns Hopkins Hospital between 2010 and 2016 were identified. Inclusion criteria included: age ≥18, histologically confirmed PDAC, ILR after resection as determined by an experienced radiologist on pancreas-protocol computed tomography (CT) scan, and treatment of ILR with SBRT with a minimum of 3 months follow-

up. Patients with radiographic evidence of metastatic disease prior to delivery of SBRT were excluded. Selection for treatment with SBRT was based on an established institutional approach. Patients with local recurrence were first assessed for candidacy for surgical re-resection. Patients who were not surgical candidates received chemotherapy. If there was no evidence of disease progression while receiving chemotherapy for at least 2 months, patients received standard CRT if their tumor invaded the small bowel or stomach or SBRT if it did not. SBRT was avoided in patients with invasion of the small bowel or stomach in order to avoid the high risk of treatment-related toxicity that would accompany delivering high doses of radiation to these surrounding structures. Patients received a wide variety of systemic therapies before and after SBRT. Patients were followed with a CT scan and labs every 3-6 months per the NCCN guidelines.[22]

SBRT Planning and Delivery

Patients underwent a simulation CT scan with oral and intravenous contrast while in the supine position in a custom-made immobilization device. Tumor motion during respiration was characterized by a 4-dimensional CT scan. If tumor motion exceeded 3mm, active breathing control (ABC) was used during treatment (n=37). Patients were treated at end-inspiration. If tumor motion was less than 3mm or patient could not tolerate ABC, an internal target volume (ITV) was defined after review of diagnostic CT, respiration-correlated 4-dimensional CT, pancreas protocol CT, and positron emission tomography (PET)/CT scans when available (n=14). Using the simulation scan, a single radiation oncologist defined the gross tumor volume (GTV). The planning treatment volume (PTV) was then defined by a 1- to 3-mm margin expansion of the GTV or ITV depending on the distance of the tumor from the adjacent duodenum, small bowel, or stomach.

Initial patient positioning was based on cone-beam CT with alignment to spine. Volumetric kV-imaging was then used to align fiducial markers (n=23), biliary stents (n=2), surgical clips (n=19), or a combination of these markers (n=3) to the digitally reconstructed radiograph. Four patients were aligned to spine alone due to the inability to place fiducials and/or absence of surgical clips.

Clinical Outcomes

Data concerning demographics, clinicopathologic and treatment characteristics, symptom palliation, treatment-related toxicity, and survival were obtained from patient charts. Positive resection margins were defined by the presence of disease at the tumor resection margins on gross exam (R2 margin) or within 1mm of the resection margin on microscopic exam (R1

margin), as determined by an experienced pancreatic pathologist. Recurrence-free interval (RFI) was defined as the time between date of pancreatic resection and date of local recurrence. Survival was measured from date of histologic diagnosis until date of death, censoring patients at date of last follow-up if no date of death was available. Overall survival (OS), progression-free survival (PFS) and local PFS (LPFS) were measured from date of the first fraction of SBRT until date of death, first progression (local or distant), or local progression, respectively. Patients were censored at the date of last follow-up (OS) or last CT scan (PFS and LPFS). Date of progression was defined as date of the first follow-up cross-sectional imaging study (CT or PET/CT) demonstrating local progression or distant metastasis after SBRT. Distant and local progression were characterized using RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 guidelines. Abdominal pain was evaluated prior to SBRT delivery and again on follow-up evaluation within 3 months of the last day of SBRT. For patients with abdominal pain noted in the pre-SBRT clinical notes (reported by the patient on a scale from 0 to 10 in severity), pain score improvement noted on follow-up within 3 months was considered effective symptom palliation. Treatment-related toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.[23] Acute (within 3 months of SBRT) and late (more than 3 months after SBRT) grade ≥3 treatment-related gastrointestinal toxicity was reported.

Statistical Analysis

Demographic, clinicopathologic, and treatment characteristics were summarized using descriptive statistics. Survival outcomes were estimated using Kaplan-Meier curves, and the log-rank test was used to assess differences between subgroups. Univariable Cox regression analyses were performed to detect associations between patient or treatment characteristics and OS and LPFS. Factors with $P \leq 0.15$ on univariable analysis were used as covariates in a multivariable Cox proportional hazards model for OS and LPFS.[24,25] A two-sided alpha level of ≤ 0.05 was considered statistically significant. Statistical analyses were performed using R, version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

Fifty-one patients with ILR met the inclusion criteria. Baseline clinicopathologic and treatment characteristics are summarized in Tables 1 and 2, respectively. All patients received SBRT for the treatment of ILR following surgical resection. Eleven patients (22%) were initially diagnosed with borderline resectable PDAC and received neoadjuvant CRT (n=9) or SBRT (n=2) prior to resection. The remaining 40 patients (78%) were diagnosed with resectable

Table 1. Demographic and clinicopathologic characteristics

Clinicopathologic variables	***All patients (n=51)***
Gender, *n* (%)	
Male	31 (61%)
Female	20 (39%)
Age, years median (IQR)	65 (55-73)
Pre-SBRT CA19-9 (U/ml)	
Median (IQR)	58.2 (21.0-132.7)
<37 U/ml, *n* (%)	16 (31%)
>37 U/ml, *n* (%)	27 (53%)
CA19-9 data unavailable	8 (16%)
Initial disease stage	
Borderline Resectable	11 (22%)
Resectable	40 (78%)
Operation procedure, *n* (%)	
Pancreatoduodenectomy	42 (82%)
Distal pancreatectomy	9 (18%)
Tumor differentiation, *n* (%)	
Well/moderate	30 (59%)
Poor	20 (39%)
Unknown	1 (2%)
Tumor size, cm median (IQR)	2.7 (2.5-3.5)
Positive lymph node, *n* (%)	25 (49%)
Positive resection margin (R1), *n* (%)	14 (27%)
Microscopic invasion, *n* (%)	
Perineural invasion present	36 (68%)
Lymphovascular invasion present	25 (47%)

CA, *carbohydrate antigen*; IQR, *interquartile range*; SBRT, *stereotactic body radiation therapy*

disease and did not receive neoadjuvant radiation. Fourteen patients (27%) received adjuvant CRT after resection. Twenty-six patients (51%) did not receive any radiotherapy in the neoadjuvant or adjuvant setting prior to salvage SBRT. All patients received adjuvant chemotherapy after initial resection. Median RFI was 13 months (IQR, 6-19). Patients who received SBRT developed local recurrence in the post-resection tumor bed where the head and neck of the pancreas had been removed during pancreatoduodenectomy (n=42, 82%) or where the body and tail of the pancreas had been removed during distal pancreatectomy and splenectomy (n=9, 18%). All tumors were deemed unresectable due to involvement of major vessels (e.g., the superior mesenteric artery or superior mesenteric vein). The locally recurrent tumors did not invade the stomach or small bowel. SBRT was delivered over five fractions to a median total cumulative dose of 25 Gy (IQR, 25-33). Most patients (88%) received induction chemotherapy after developing ILR and before starting SBRT for a median duration of 3 months (IQR, 1.5-5). Induction chemotherapy regimens varied, often based on performance

status, with most patients receiving Gemcitabine (monotherapy or with either Nab-Paclitaxel or Capecitabine and Docetaxel) (n=28), FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) (n=13), a combination of Gemcitabine- or FOLFIRINOX-based regimens (n=3), or other (n=1). Thirty patients (59%) received maintenance chemotherapy after SBRT for a median duration of 4 months (IQR, 1-5.5 months). Maintenance chemotherapies were also varied, with the most common regimens being Gemcitabine- (n=8), FOLFIRINOX- (n=6), and Capecitabine-based (n=4) regimens. Essentially, if patients had received one therapy prior to failure, patients were then often offered an alternative chemotherapy regimen.

Survival

Median duration of follow-up was 30 months from SBRT (range, 3-73 months); at last follow-up, 17 patients (33%) remained alive. Median OS (mOS) was 36 months from date of histologic diagnosis, and mOS was 16 months from the first day of SBRT (Figure 1A-B, Table 3). Median PFS (mPFS) and LPFS (mLPFS) from SBRT were 7 and 10 months, respectively (Figure 1C-D, Table 3).

Differences in mOS, mPFS, and mLPFS between re-irradiated and radiation-naïve patients were not statistically significant (Figure 2A, Table 3). Based on previously published data concerning treatment of ILR,21,26 patients were stratified into two groups: those with an RFI <9 months and those with an RFI ≥9 months. Patients with an RFI ≥9 months showed superior mOS compared with patients who had an RFI <9 months (16 months vs. 6 months, P=0.019) (Figure 2B, Table 3). Differences in mPFS and mLPFS between these groups were not statistically significant (Table 3). To analyze the importance of systemic therapy, patients were stratified by receipt of maintenance chemotherapy after SBRT. Patients receiving maintenance chemotherapy demonstrated superior mOS (21 vs. 8 months, P<0.001) and mLPFS (15 vs. 5 months, P=0.018) compared with patients who did not (Figure 2C, Table 3).

Univariable Cox regression identified 9 and 5 covariates associated (P≤0.15) with OS and LPFS, respectively (Table 4). After adjusting for these covariates, multivariable Cox regression identified 5 independent factors that were significantly associated with OS: (1) age (HR 0.944, 95% CI 0.895-0.995); (2) serum carbohydrate antigen (CA) 19-9 (HR 1.003, 95% CI 1.001-1.006); (3) poorly differentiated tumor grade (HR 11.274, 95% CI 2.831-44.904); (4) positive surgical margins (HR 0.126, 95% CI 0.034-0.471); (5) Eastern Cooperative Oncology Group (ECOG) performance status 0-1 (HR 0.059, 95% CI 0.008-0.425); and (6) maintenance chemotherapy after SBRT (HR 0.141, 95% CI 0.050-0.395) (Table 4). Age (HR 0.915, 95% CI 0.863-0.970), positive surgical margins (HR 0.255, 95% CI 0.070-0.923), and maintenance

Table 2. Characteristics of treatments of primary and recurrent lesions

Treatment characteristics	
(Neo) adjuvant therapy, *n* (%)	
Surgery without radiotherapy	26 (51%)
Surgery + adjuvant chemoradiation therapy	14 (27%)
Neoadjuvant chemoradiation therapy + surgery	9 (18%)
Neoadjuvant SBRT + surgery	2 (4%)
Prior CRT dose (Gy)	
Median (IQR)	50.4 (48.5-54)
Recurrence-free interval, months median (IQR)	13 (6-19)
<9 months, *n* (%)	15 (29%)
≥9 months, *n* (%)	36 (71%)
ECOG PS prior to SBRT, *n* (%)	
ECOG PS 0-1	46 (90%)
ECOG PS 2-3	5 (10%)
Tumor motion control, *n* (%)	
Active breathing control	37 (73%)
Free-breathing (ITV)	14 (27%)
SBRT dose (Gray)	
Median total dose (IQR)	25 (25-33)
Median dose per fraction (IQR)	5 (5-6.6)
Systemic therapy	
Induction chemotherapy before SBRT	45 (88%)
Median duration in months (IQR)	3 (1.5-5)
Maintenance chemotherapy after SBRT	30 (69%)
Median duration in months (IQR)	4 (1-5.5)

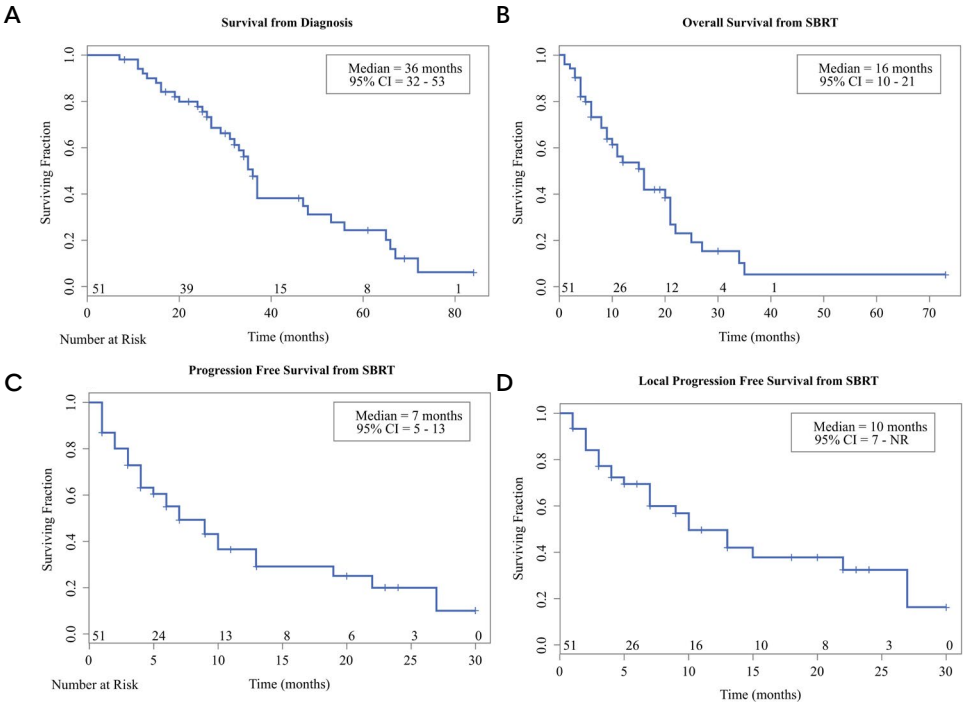
ECOG PS, *Eastern Cooperative Oncology Group performance status*; IQR, *interquartile range*; ITV, *internal target volume*; SBRT, *stereotactic body radiation therapy*.

Table 3. Clinical outcomes: 6- and 12-month survival rates and median overall (OS), progression-free (PFS), and local progression-free (LPFS) survival times by subgroup with *P*-values from log-rank test

		Prior radiotherapy status			***Recurrence-free interval***			***Maintenance chemotherapy***		
Clinical outcomes	***All patients (n=51)***	***Re-irradiated (n=25)***	***RT-naïve (n=26)***	***P-value***	***<9 months (n=15)***	***≥9 months (n=36)***	***P-value***	***No (n=21)***	***Yes (n=30)***	***P-value***
OS										
6-month	73%	78%	68%		46%	85%		54%	86%	
12-month	54%	48%	59%		37%	61%		27%	74%	
Median	16	11	16	0.992	6	16	**0.019**	8	21	**<0.001**
(IQR)	(6-22)	(8-21)	(4-25)		(3-21)	(9-25)		(4-11)	(12-34)	
PFS										
6-month	55%	51%	59%		30%	63%		49%	58%	
12-month	36%	26%	47%		30%	39%		0%	42%	
Median	7	7	10	0.070	4	9	0.811	5	10	0.240
(IQR)	(3-19)	(2-13)	(4-27)		(3-NR)	(3-19)		(2-9)	(4-22)	

LPFS										
6-month	70%	65%	75%		50%	76%		49%	80%	
12-month	47%	37%	62%		50%	50%		0%	61%	
Median (IQR)	10 (4-27)	9 (3-13)	22 (7-27)	0.054	4 (3-NR)	13 (7-27)	0.866	5 (3-9)	15 (7-27)	**0.018**

IQR, *interquartile range*; LPFS, *local progression-free survival*; NR, not reached; PFS, *progression-free survival*; RT, *radiotherapy*; OS, *overall survival*.



The number of patients at risk in each group is indicated above the X-axis. Censored patients are indicated by +.

Figure 1. Kaplan-Meier curves showing (A) overall survival from date of histologic diagnosis and (B) overall survival from stereotactic body radiation therapy (SBRT), (C) progression-free survival, and (D) local progression-free survival from SB.

chemotherapy after SBRT (HR 0.299, 95% CI 0.103-0.871) were also independently associated with LPFS on multivariable analysis (Table 4).

Symptom Palliation and Treatment-related Toxicity

Among 16 patients reporting abdominal pain prior to SBRT delivery, 10 (63%) experienced abdominal pain relief within 3 months of SBRT completion with a median pain score

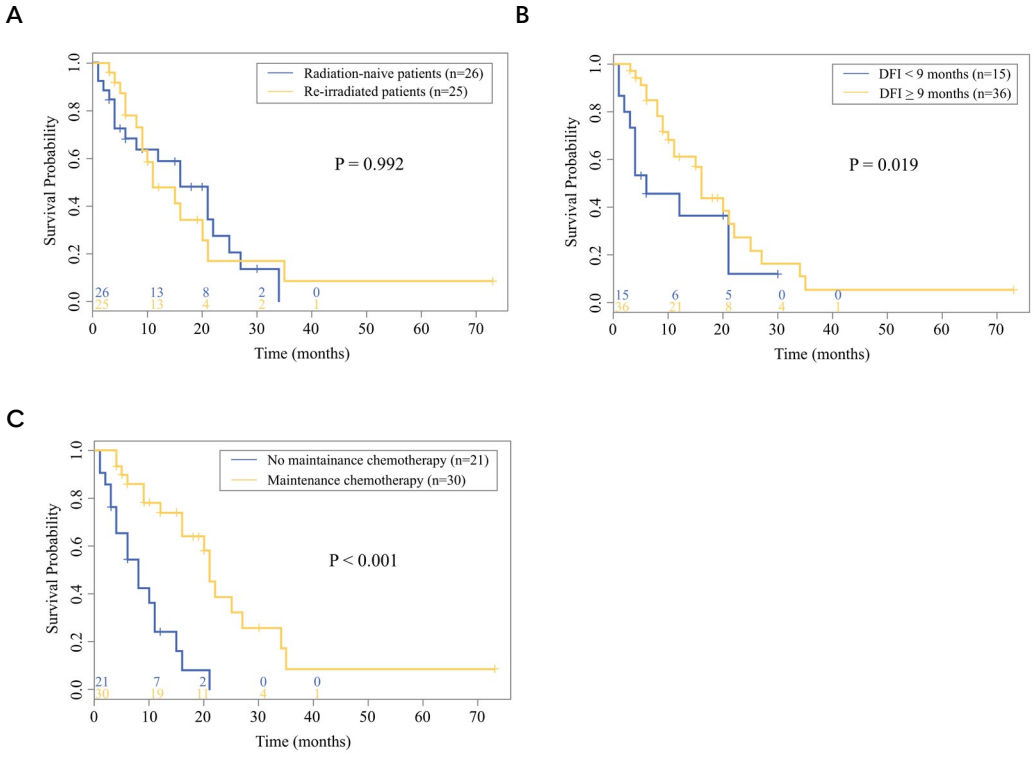
Table 4. Univariable and multivariable associations between patient characteristics and overall survival and local progression-free survival. Bolded univariable associations were selected for multivariable analysis

	OS		***LPFS***	
Univariable Associations	***HR (95% CI)***	***P-value***	***HR (95% CI)***	***P-value***
Age (years)*	0.971 (0.941 - 1.001)	**0.060**	0.964 (0.929 - 1.000)	**0.052**
Gender Female vs. male	0.657 (0.302 - 1.433)	0.291	1.278 (0.552 - 2.963)	0.567
Operation PD vs. DPS	1.153 (0.469 - 2.837)	0.757	0.844 (0.330 - 2.158)	0.723
Max tumor diameter (cm)*	0.880 (0.596 - 1.298)	0.519	0.998 (0.606 - 1.643)	0.993
Histologic grade Poor vs. other	1.755 (0.854 - 3.608)	**0.126**	1.396 (0.606 - 3.215)	0.434
Positive surgical margins R1 (<1mm) vs. R0 (>1mm)	0.477 (0.209 - 1.088)	**0.079**	0.355 (0.119 - 1.058)	**0.063**
Positive lymph nodes Yes vs. no	0.871 (0.443 - 1.715)	0.690	0.697 (0.305 - 1.592)	0.392
Microscopic perineural invasion Yes vs. no	1.017 (0.457 - 2.265)	0.966	0.616 (0.251 - 1.513)	0.290
Microscopic lymphovascular invasion: Yes vs. no	0.689 (0.324 - 1.469)	0.335	0.404 (0.170 - 0.959)	**0.040**
Baseline CA19-9 (U/mL)*	1.002 (1.000 - 1.004)	**0.015**	1.000 (0.998 - 1.003)	0.813
Baseline CA19-9 >37 U/mL vs ≤37 U/mL	1.870 (0.813 - 4.303)	**0.141**	1.284 (0.509 - 3.236)	0.773
Prior radiotherapy Yes vs. no	1.004 (0.500 - 2.016)	0.992	2.242 (0.941 - 5.343)	**0.068**
Recurrence-free interval <9 months vs. ≥ 9 months	0.561 (0.269 - 1.169)	**0.123**	1.088 (0.400 - 2.957)	0.869
ECOG PS 0 to 1 vs. 2 to 3	0.061 (0.019 - 0.194)	**<0.001**	1.998 (0.713 - 5.601)	0.190
Biliary stent Yes vs. no	2.550 (0.860 - 7.562)	**0.091**	2.050 (0.464 - 9.062)	0.344
Induction chemotherapy before SBRT Yes vs. no	1.403 (0.491 - 4.010)	0.528	0.780 (0.259 - 2.354)	0.660
Maintenance chemotherapy after SBRT Yes vs. no	0.217 (0.100 - 0.471)	**<0.001**	0.347 (0.136 - 0.887)	**0.027**
Significant Multivariable Associations				
Age (years)*	0.944 (0.895 - 0.995)	0.032	0.915 (0.863 - 0.970)	0.003
Baseline CA 19-9 (U/mL)*	1.003 (1.001 - 1.006)	0.010	NA	
Histologic grade Poor vs. other	11.274 (2.831 - 44.904)	0.001	NA	
Positive surgical margins R1 (<1mm) vs. R0 (>1mm)	0.126 (0.034 - 0.471)	0.002	0.255 (0.070 - 0.923)	0.037
ECOG PS 0 to 1 vs. 2 to 3	0.059 (0.008 - 0.425)	0.005	NA	

Maintenance chemotherapy after SBRT				
Yes vs. no	0.141 (0.050 - 0.395)	<0.001	0.299 (0.103 - 0.871)	0.027

* Continuous

CA, *carbohydrate antigen*; CI, *confidence interval*; DPS, *distal pancreatectomy and splenectomy*; ECOG PS, *East ern Cooperative Oncology Group performance status*; HR, *hazard ratio*; LPFS, *local progression-free survival*; NA, not applicable; OS, *overall survival*; PD, *pancreatoduodenectomy*; RFI, *recurrence-free interval*; SBRT, *stereotactic body radiation therapy*.



The number of patients at risk in each group is indicated above the X-axis. Censored patients are indicated by +.

Figure 2. Kaplan-Meier curves comparing overall survival from first fraction of stereotactic body radiation therapy (SBRT) between subgroups: (A) radiation-naïve patients (n=26) vs. patients re-irradiated with SBRT (n=25); B) patients with a disease free interval (DFI) <9 months (n=15) vs. ≥9 months (n=36); and C) patients who received maintenance chemotherapy (n=30) vs. those who did not (n=21).

improvement of 5 (IQR, 4-6). Five patients reported no pain at 3-month follow up. The overall rate of grade ≥3 gastrointestinal toxicity was 10%. One patient (2%) experienced acute grade 3 toxicity in the form of small bowel obstruction. This patient presented with abdominal pain and nausea and the obstruction resolved with conservative management not requiring operative intervention. Three patients (6%) experienced late grade 3 toxicity, one (2%) in the form of small bowel obstruction, one (2%) in the form of gastrointestinal bleeding, and one (2%) in the form of sepsis from cholangitis. One patient (2%) experienced late grade 4 small bowel obstruction. Both patients with late small bowel obstruction presented to the hospital with abdominal pain. The patient with grade 4 small bowel obstruction underwent resection of the obstructed bowel two days after admission, while the patient with grade 3 small bowel obstruction was managed conservatively and did not require surgical intervention. No acute or late grade 5 toxicity was observed. Four of the five patients (80%) who experienced grade ≥3 toxicity (all except the case of sepsis) received chemoradiation therapy prior to developing an ILR. The patient who experienced gastrointestinal bleeding and the patient with small bowel obstruction requiring surgical intervention received adjuvant chemoradiation, while the two patients with small bowel obstruction managed conservatively received neoadjuvant chemoradiation. For these patients, the median time interval between initial chemoradiation treatment and re-treatment with SBRT was 20 months (range, 12-24) and median prior radiation dose was 54 Gy (range, 50.4-54 Gy).

Discussion

Despite the frequency of ILR in patients with PDAC, evidence to guide treatment decisions for these patients is limited. We first evaluated SBRT for ILR in 2013, reporting preliminary results in a series of 17 re-irradiated patients at two institutions.[21] The current, larger single-institutional series includes 7 patients who were included in the previous study and builds on that previous work, allowing further evaluation of the efficacy and safety of SBRT in patients with ILR who have or have not previously received radiation prior to SBRT. In addition, using this larger dataset, we were able to identify variables associated with clinical outcomes.

After initial surgery and local recurrence, most patients cannot undergo re-resection due to technical barriers (vascular involvement or post-radiation fibrosis) or medical barriers (poor performance status or medical comorbidities). Even for patients who are re-resection candidates, surgery carries significant risks. The largest study to date on re-resection examined the outcomes of 57 patients with ILR who underwent surgical exploration.[27] Re-resection was achieved in 41 patients (72%) whose mOS was 26 months. For the 16 patients for whom re-resection was aborted, mOS was 10 months. Fourteen of the 57 patients (34%) experienced

operative morbidity, and 1 patient (1%) died due to complications of re-laparotomy. In our study, grade ≥3 acute (2%) and late (8%) toxicity was minimal. Furthermore, while surgery can delay return to systemic therapy for months,[26] patients treated with SBRT in our study returned to chemotherapy within one week. Those who resumed chemotherapy after SBRT had a mOS of 21 months, suggesting that SBRT may be considered as a minimally invasive alternative to surgical re-exploration for selected patients with ILR of PDAC.

Multiple studies have analyzed the efficacy and safety of conventionally fractionated CRT for ILR.[28-34] In the two largest studies to date, Habermehl et al. and Nakamura et al. described the outcomes of 41 and 30 radiation-naïve patients, respectively. In both studies, mOS and mPFS from CRT were 16 and 6 months, respectively. Habermehl et al. reported a mLPFS of 13 months and acute grade 1-3 gastrointestinal toxicity in 54% of patients, while Nakamura et al. reported acute grade ≥3 toxicity in 60% of patients. The 26 radiation-naïve patients in our study had a mOS, mPFS, and mLPFS of 16, 10, and 22 months, respectively. For these patients, the rates of acute and late grade ≥3 toxicity were 4% (1 of 26) and 12% (3 of 26), respectively.

Although direct conclusions concerning the effects of different treatments on survival or disease progression cannot be drawn from our study or by comparison with these small, retrospective studies, our data suggest SBRT may provide palliative benefit and be considered as a safe alternative to re-resection or chemoradiation for radiation-naïve patients with ILR. Furthermore, SBRT requires only 5 days for delivery and may be more cost-effective than CRT, both of which are important considerations for PDAC patients who often face a poor prognosis.[35,36] Conventional CRT is usually not an option for patients who received prior radiotherapy due to dose constraints to previously irradiated normal tissue. In this setting, our analysis agrees with smaller studies that re-irradiation with SBRT is a viable option with reasonable survival, local control, and toxicity outcomes.[21,37-39] In addition, it is important to stress that efforts were made to avoid overlap of the planning treatment volume (area irradiated) with residual small bowel thus explaining the low rate of toxicity despite previous irradiation in nearly half of the cases treated. This was sometimes at the expense of tumor coverage.

Patients treated with SBRT achieved favorable rates of symptom palliation. Data from our study confirms previous findings that SBRT in the setting of ILR can lead to improvement of abdominal pain.[21] Sixty-three percent of patients with abdominal pain experienced significant improvement and 31% reported no pain three months after treatment. Treatment related toxicity was relatively minimal, although there was a higher rate of grade ≥3 gastrointestinal

toxicity among re-irradiated patients (4 of 25 re-irradiated patients (16%)) when compared to radiation-naïve patients (1 of 26 radiation-naïve patients (4%)). However, rates of symptom palliation compare favorably to rates of treatment related toxicity (even among re-irradiated patients), and the palliative benefit of SBRT may outweigh the risk of toxicity.

Furthermore, our study highlights several factors that correlated with survival outcomes. Patients with an RFI <9 months had a poorer prognosis after SBRT than did patients with an RFI ≥9 months.[21] Patients who received maintenance chemotherapy after SBRT had a longer OS and LPFS when compared to patients who did not. It is difficult to draw direct conclusions about the benefit of SBRT or chemotherapy from these results, as the effects of treatment and the effects of other factors, such as tumor biology, on outcomes cannot easily be separated. However, these results suggest directions for future studies comparing treatments directly and suggest that, for select patients, prognosis may be relatively good after SBRT and chemotherapy.

Positive surgical margins after resection were associated with both a better OS and LPFS. Of note, this relationship holds while the RFI is similar for patients with margin-positive and -negative resections (16 months vs. 14 months, respectively, P=0.71). Although this finding alone is difficult to interpret, patients who experienced ILR after margin-negative resection may have more unfavorable tumor biology that resulted in ILR despite negative margins and adjuvant therapy. Patients who experienced ILR after margin-positive resection may have had recurrence primarily due to microscopic residual disease in the remnant pancreas that had not undergone the process of hematogenous metastasis. The importance of tumor biology is further suggested by the finding that poorly differentiated tumor grade was significantly associated with mortality.

The results of this study should be interpreted within the context of its limitations, principal among these being the use of a single-institution, retrospective data set. Importantly, the non-randomized study design and heterogeneity of therapies prior to and following the initial pancreatectomy and SBRT make it difficult to determine the particular effects of different treatments on observed outcomes. Despite these limitations, the strengths of this study should also be acknowledged. To our knowledge, this is the largest study to date evaluating the efficacy and safety of SBRT for ILR of PDAC. Follow-up was relatively long in this patient population, with a median of 30 months. Furthermore, variables were identified that correlated with survival outcomes.

Conclusion

In conclusion, SBRT appears to be a safe and effective treatment for ILR of PDAC. Symptom palliation was achieved in 63% of patients and treatment-related toxicity rates were acceptable. Patients who received maintenance chemotherapy had better survival outcomes than those who did not. Future prospective studies should compare SBRT directly with other treatment options for ILR such as chemotherapy, conventional CRT, and re-resection.

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CHAPTER 17

Isolated Pulmonary Recurrence After Resection of Pancreatic Cancer: The Effect of Patient Factors and Treatment Modalities on Survival

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Abstract

Objective

The literature suggests favorable survival for patients with isolated pulmonary recurrence after resection of pancreatic ductal adenocarcinoma (PDAC) as compared to other recurrence patterns. Within this cohort, it remains unclear what factors are associated with improved survival. The aim of this study was to describe accurately the clinical course and outcomes of patients with isolated pulmonary recurrence after resection of pancreatic cancer

Methods

Patients who developed pulmonary recurrence after pancreatectomy were selected from a prospective database. Predictors for post-recurrence survival (PRS) were analyzed using a multivariable Cox regression model.

Results

Ninety-six patients were included. Median recurrence-free survival, PRS and overall survival (OS) were 16.3, 18.8 and 39.6 months, respectively. Further systemic treatment and/or metastasectomy (n=64, 67%) was associated with significantly improved PRS and OS when compared to best supportive care (n=35, 22%) (26.3 vs. 5.3 and 48.1 vs. 18.4, respectively; both $P<0.001$). Patients who were able to undergo metastasectomy (n=19) achieved a PRS and OS of 35.0 and 68.9 months, respectively. More than 5 pulmonary lesions, symptoms and CA 19-9 ≥100 U/mL at time of recurrence were predictive of decreased PRS. A recurrence-free interval of >16 months and treatment for recurrence were independently associated with improved PRS.

Conclusion

Isolated pulmonary recurrence occurs in 13% of patients with recurrent PDAC and is associated with a median OS of 40 months. Aggressive treatment in highly selected patients was correlated with improved survival.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is associated with dismal survival outcomes. For patients with a localized tumor, a combination of surgical resection and systemic therapy provides the best chance for long-term survival.[1,2] However, even after completion of multimodality therapy, local and/or distant recurrence is common and the principal cause of disease-specific mortality.[3,4] No high quality prospective studies or clinical trials exist to guide the treatment of recurrent PDAC.

Treatment of PDAC recurrence has historically been limited to best supportive care, with salvage chemotherapy generally offered to patients with adequate performance status.[5] However, recent evidence suggests that recurrent PDAC displays diverse behaviors based on recurrence timing and location.[6-10] These differences potentially provide a basis for a more individualized approach to the treatment of recurrence. Isolated pulmonary recurrence in particular has been associated with prolonged survival when compared to recurrence at other sites.[7,11-13] Consequently, patients with isolated pulmonary recurrence could represent a clinical subpopulation with potentially favorable tumor biology, in which a more intensive treatment strategy might be justified.

As of yet, few studies have focused on patients with pulmonary PDAC recurrence. Furthermore, it remains unclear to what degree patient factors, recurrence features (i.e. timing, extent, presentation), and different treatment strategies are related to survival outcomes. Therefore, the primary goal of this study was to accurately describe the clinical course and outcomes of patients with isolated pulmonary recurrence after PDAC resection. Secondary goals were to identify factors associated with survival after pulmonary recurrence. A better understanding of factors and treatment modalities associated with favorable outcomes has the potential to aid clinicians in providing more patient-tailored and improved care in this unique population of PDAC patients.

Methods

Patient Selection

This retrospective cohort study utilized a single-institution database of patients who had upfront pancreatectomy for histologically confirmed PDAC between 2000 and 2013. From patients with comprehensive recurrence and follow-up data available, only those with first recurrence restricted to the lungs were included. Exclusion criteria were neoadjuvant therapy prior to pancreatic resection and simultaneous recurrence in the lungs and other anatomic

sites at time of first recurrence.

Demographic, Clinicopathologic, and Primary Treatment Characteristics

Demographic and clinicopathologic characteristics were extracted from an institutional pancreatectomy database. Resection margin (R) status was defined as R0 when distance of tumor cells to the closest resection margin was >1mm, or R1 when distance was ≤1mm. Tumor staging was reported according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system for pancreatic cancer.[14] Additionally, details regarding the initial treatment of the primary tumor were obtained, including type of pancreatectomy and use of adjuvant therapy.

Follow-Up and Treatment for Recurrence

Follow-up after pancreatectomy was done conform the National Comprehensive Cancer Network (NCCN) guidelines, and generally consisted of at least one clinic visit including a computed tomography (CT) scan of the chest, abdomen, and pelvis every 3-6 months for the first 2 years, and yearly thereafter.[15] Fluorodeoxyglucose positron emission tomography (FDG-PET) was performed if necessary to clarify equivocal CT findings.

During the study period, there was no specific established institutional approach to the treatment of pulmonary PDAC recurrence. Patients with good performance status were commonly offered palliative chemotherapy, or were included in investigative clinical trials for metastatic PDAC patients. In highly selected patients, pulmonary metastasectomy was considered. Signs of indolent tumor biology, such as a relatively long interval to recurrence after resection and stable isolated disease over time, were typically deemed necessary for pulmonary metastasectomy to be contemplated.[16,17] Only following review in a pancreatic multidisciplinary clinic, where patient performance status and tumor biology were taken into account, were select patients referred to a thoracic surgeon for consultation.

Recurrence characteristics were retrospectively collected from patient electronic medical records. These included carbohydrate antigen (CA) 19-9, and number and location of pulmonary lesions on imaging at time of recurrence. Based on a receiver operating characteristics curve, a CA 19-9 cut-off of 100 U/mL at time of recurrence was utilized for statistical analysis. Symptomatic recurrence was defined as a recurrence discovered due to a significant patient-initiated complaint that was new or had increased in severity or frequency. Patient records were further queried for the type of treatment received after detection of pulmonary recurrence. Lastly, date and anatomic location of secondary extra-pulmonary

Table 1. Demographics, clinicopathological and treatment characteristics of included patients

Variable	*All IPR patients (n=96)*	*Early IPR (RFS <16 months) (n=47)*	*Late IPR (RFS >16 months) (n=49)*	*P*-value
Male, *n* (%)	54 (56%)	25 (53%)	29 (59%)	0.554
Caucasian, *n* (%)	88 (92%)	43 (92%)	45 (92%)	0.951
Age at surgery, years median (IQR)	68 (60-74)	68 (60-77)	65 (60-74)	0.416
Operation procedure, *n* (%)				0.339
Pancreatoduodenectomy	77 (80%)	37 (79%)	40 (82%)	
Distal pancreatectomy	14 (15%)	6 (13%)	8 (16%)	
Total pancreatectomy	5 (5%)	4 (9%)	1 (2%)	
R0 resection margin, *n* (%)	66 (69%)	29 (66%)	37 (76%)	0.145
Tumor differentiation, *n* (%)				0.423
Well/Moderate	67 (70%)	31 (67%)	36 (74%)	
Poor	29 (30%)	15 (33%)	13 (27%)	
Tumor size, cm median (IQR)	3.0 (2.4-3.5)	3.0 (2.5-4.0)	2.5 (2.0-3.4)	**0.033**
T-stage (AJCC 8th edition), *n* (%)				**0.041**
T1	19 (20%)	6 (13%)	13 (27%)	
T2	64 (67%)	31 (66%)	33 (67%)	
T3	13 (14%)	10 (21%)	3 (6%)	
N-stage (AJCC 8th edition), *n* (%)				**0.032**
N0	25 (26%)	9 (19%)	16 (33%)	
N1	30 (31%)	12 (26%)	18 (37%)	
N2	41 (43%)	26 (55%)	15 (31%)	
Microscopic perineural invasion, *n* (%)	71 (74%)	43 (92%)	42 (86%)	0.375
Microscopic lymphovascular invasion, *n* (%)	66 (69%)	36 (77%)	30 (61%)	0.150
Adjuvant therapy, *n* (%)	84 (88%)	38 (81%)	46 (94%)	**0.045**
CA 19-9 at time of recurrence[a]				
U/mL median (IQR)	53 (30-196)	56 (30-262)	51 (29-131)	0.814
≥ 100 U/mL, *n* (%)	24 (34%)	13 (39%)	11 (30%)	0.305
Symptomatic recurrence, *n* (%)	18 (19%)	12 (26%)	6 (12%)	0.095
Number of pulmonary lesions, *n* (%)				**0.022**
1	25 (26%)	8 (17%)	17 (35%)	
2-4	13 (14%)	4 (9%)	9 (18%)	
≥5	58 (60%)	35 (75%)	23 (47%)	
Location of lesion(s), *n* (%)				**0.010**
Right lung	10 (10%)	4 (9%)	6 (12%)	
Left lung	22 (23%)	5 (11%)	17 (35%)	
Bilateral	64 (67%)	38 (81%)	26 (53%)	
Treatment of recurrence, *n* (%)				**0.005**
Best supportive care	32 (33%)	18 (38%)	14 (29%)	
Chemo- and radiotherapy	45 (47%)	26 (55%)	19 (39%)	
Pulmonary metastasectomy	19 (20%)	3 (6%)	16 (33%)	
Survival outcomes (months, 95% CI)				
Recurrence-free survival	16.3 (11.7-20.9)	9.3 (8.4-10.3)	34.0 (20.9-47.2)	**<0.001**
Post-recurrence survival	18.8 (14.7-22.9)	12.2 (9.9-14.6)	28.3 (24.9-31.8)	**<0.001**
Overall survival	39.6 (26.3-52.9)	22.2 (17.6-26.9)	66.3 (61.7-70.9)	**<0.001**

[a] 78 patients had documented CA 19-9 values at time of recurrence, of whom 8 patients were Lewis-antigen negative and excluded from analysis.

AJCC, *American joint committee on cancer*; CA, *carbohydrate antigen*; CI, *confidence interval*; IPR, *isolated pulmonary* recurrence; IQR, *interquartile* range.

progression of disease were documented.

Outcomes and Statistical Analysis

Summary statistics were obtained using established methods. Overall survival (OS) and post-recurrence survival (PRS) were defined as the time from the date of pancreatectomy (OS) or the date of recurrence (PRS) to either death from any cause or last follow-up. Recurrence-free survival (RFS) was defined as the time interval between the date of the operation and date of recurrence. Kaplan-Meier curves and the log-rank test were used to estimate median survival with a corresponding 95% confidence interval (CI) and compare between subgroups.

Using the log-rank test and a minimum P-approach as previously described[9,18], the optimal length of RFS to distinguish between early and late pulmonary recurrence, based on subsequent PRS, was established. To identify potential predictive factors for PRS, univariable and multivariable analyses were performed using Cox proportional-hazard regression models. All variables with a P-value of <0.10 in univariable analysis were included as a covariate in the multivariable regression model. A two-tailed P-value of <0.05 was considered to indicate statistical significance. Statistical analysis was performed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA)

Results

A total of 1520 patients had upfront, chemo-naïve pancreatectomy for PDAC at the Johns Hopkins Hospital between January 2000 and December 2013 (see patient flowchart in Online Supplemental eFigure S1). Detailed follow-up data were available for 957 patients, 754 (79%) of whom had disease recurrence at last follow-up. Of these 754 patients with recurrence, 96 (13%) had first recurrence restricted to the lungs and were thus included in the study. In these 96 patients, isolated pulmonary recurrence occurred after a median RFS following initial pancreatectomy of 16.3 months (95% CI 11.7-20.9). Following pulmonary recurrence, median PRS was 18.8 months (95% CI 14.7-22.9), resulting in a median OS of 39.6 months (95% CI 26.3-52.9). Eight of 96 patients (8%) are currently alive after a median follow-up of 96.0 months (95% CI 34.7-157.2) following initial pancreatectomy, and 55.4 months (95% CI 27.1-83.7) following date of recurrence.

Cohort Characteristics

Complete demographics, clinicopathologic, and primary treatment characteristics are summarized in Table 1. Most recurrences (n=78, 81%) were diagnosed on routine follow-

up scans. Eighteen patients (19%) experienced single or multiple recurrence-associated symptoms leading to initial detection of recurrence, including chest and/or back pain in 9, dyspnea in 5, unintentional weight loss in 4 and/or hemoptysis in 4 of 18 symptomatic patients. At the time of recurrence diagnosis, a majority of patients had ≥5 pulmonary lesions (n=58, 60%) and/or recurrent disease in both lungs (n=64, 67%). Median CA 19-9 at time of recurrence was 53 U/mL (interquartile range [IQR] 30-196). Forty-five patients (47%) had pathologic confirmation of pulmonary recurrence with a tissue biopsy while the remaining 51 patients were diagnosed based on imaging evidence alone.

Defining Early and Late Pulmonary Recurrence

Evaluated cut-offs to define early and late pulmonary recurrence based on PRS are shown in Online Supplemental eTable S1. The optimal length of RFS to distinguish between early and late recurrence was 16 months ($P=1.97x10^{-7}$) (Online Supplemental eFigure S2). Dichotomization of the cohort using the found RFS cut-off of 16 months showed that larger primary tumors and more positive lymph nodes on final pathology were associated with early (RFS <16 months) pulmonary recurrence (Table 1). Additionally, early recurrence patients had received adjuvant treatment less frequently when compared to late recurrence patients. Lastly, Table 1 shows that patients with early pulmonary recurrence presented more often with ≥5 pulmonary lesions and recurrence in both lungs at initial observation of recurrence.

Median RFS in the early (<16 months) pulmonary recurrence cohort (n=47, 49%) was 9.3 months (95% CI 8.4-10.3), followed by a median PRS of 12.2 months (95% CI 9.9-14.6). Patients with recurrence after 16 months (n=49, 51%) had a median RFS of 34.0 months (95% CI 20.9-47.2) with a median PRS of 28.3 months (95% CI 24.9-31.8). When assessing PRS rates, patients with early recurrence had 1-, 2-, and 5-year PRS rates of 51%, 17% and 0%, compared to 80%, 65% and 12% for the late recurrence group (all P<0.001). Median OS was significantly longer for patients with late pulmonary recurrence (66.3 months, 95% CI 61.7-70.9) when compared to patients with early recurrence (22.2 months, 95% CI 17.6-26.9; P<0.001).

Treatment for Pulmonary Recurrence

Further recurrence-specific therapy was administered in 64 of 96 patients (67%) while all other patients received best supportive care (Table 2). Reasons for relinquishing further treatment were poor overall performance status of patient (n=11), patient wishes (n=8), specific significant comorbidities precluding treatment (n=4), complications following pulmonary biopsy (n=2) and unknown (n=7). Chemotherapy was the treatment of choice

Table 2. Recurrence characteristics and survival outcomes stratified according to different treatment strategies

Variable	*Best supportive care (n=32)*	*Chemo(radio)-therapy (n=45)*	*Pulmonary metastasectomy (n=19)*	*P*-value
Fraction of total included patients	32/96 (33%)	45/96 (47%)	19/96 (20%)	NA
Age at recurrence, years median (IQR)	70 (63-79)	69 (63-77)	65 (60-74)	0.282
CA 19-9 at time of recurrence[a]				**0.050**[b]
U/mL median (IQR)	75 (45-273)	47 (30-185)	34 (22-68)	
Symptomatic recurrence	10/32	8/45	0/19	**0.021**[c]
Number of pulmonary lesions				**<0.001**[d]
1	4/32	8/45	13/19	
2-4	5/32	5/45	3/19	
≥5	23/32	32/45	3/19	
Location of lesion(s)				**<0.001**[e]
Unilateral	6/32	11/45	15/19	
Bilateral	26/32	34/45	4/19	
Chemo(radio)therapy, *n* (%)				NA
Chemotherapy only	0/32	38/45	6/19	
Chemotherapy and radiotherapy	0/32	7/45	5/19	
None	0/32	0/45	8/19	
Survival outcomes (months, 95% CI)				
Recurrence-free survival	14.2 (11.6-16.9)	13.2 (10.4-16.0)	24.3 (18.8-29.9)	**0.014**[f]
Post-recurrence survival	8.1 (4.4-11.7)	20.2 (17.2-23.2)	35.0 (21.1-48.9)	**<0.001**[g]
Overall survival	24.5 (20.6-28.5)	34.2 (23.4-45.1)	68.9 (45.5-92.3)	**<0.001**[h]

[a] 78 patients had documented CA 19-9 values at time of recurrence, of whom 8 patients were Lewis-antigen negative and excluded from analysis.
[b] Post-hoc analysis: BSC vs. CHRT: *P*=0.146; BSC vs. PM: *P*=0.018; CHRT vs. PM: *P*=0.152.
[c] Post-hoc analysis: BSC vs. CHRT: *P*=0.169; BSC vs. PM: *P*=0.008; CHRT vs. PM: *P*=0.049.
[d] Post-hoc analysis: BSC vs. CHRT: *P*=0.730; BSC vs. PM: *P*<0.001; CHRT vs. PM: *P*<0.001.
[e] Post-hoc analysis: BSC vs. CHRT: *P*=0.553; BSC vs. PM: *P*<0.001; CHRT vs. PM: *P*<0.001.
[f] Post-hoc analysis: BSC vs. CHRT: *P*=0.180; BSC vs. PM: *P*<0.110; CHRT vs. PM: *P*=0.003.
[g] Post-hoc analysis: BSC vs. CHRT: *P*=0.001; BSC vs. PM: *P*<0.001; CHRT vs. PM: *P*=0.002.
[h] Post-hoc analysis: BSC vs. CHRT: *P*=0.386; BSC vs. PM: *P*<0.001; CHRT vs. PM: *P*<0.001.
BSC, *best supportive care*; CHRT, *chemo(radio)therapy*; CA, *carbohydrate antigen*; CI, *confidence interval*; IQR, *interquartile range*; NA, *not applicable*; PM, *pulmonary metastasectomy*.

for most patients with pulmonary recurrence (n=56, 58%). Chemotherapy regimens were chosen at the discretion of the local or institutional medical oncologist, with significant variations noted in received regimens. Radiotherapy was administered less often (n=12, 13%) and included conventional external beam radiotherapy (n=6), stereotactic body radiotherapy (n=5), or intensity-modulated radiotherapy (n=1). No patient received radiotherapy for recurrence without also undergoing prior, concurrent, or subsequent chemotherapy. Lastly, pulmonary metastasectomy was performed in 19 patients (20%), 9 of whom were the subject of an earlier institutional case series.[16]

Median RFS did not differ (P=0.895) between the treatment and best supportive care groups. Following pulmonary recurrence, median survival for patients receiving any treatment for recurrence was 26.3 months (95% CI 20.9-31.7), significantly longer than the median PRS of 8.1 months (95% CI 4.4-11.7) found for best supportive care (P<0.001) (Figure 1A). The association between treatment and improved median PRS was seen in both the early pulmonary recurrence cohort (18.8 vs. 5.3 months; P<0.001) and the late recurrence cohort (35.0 vs. 10.4 months; P=0.002).

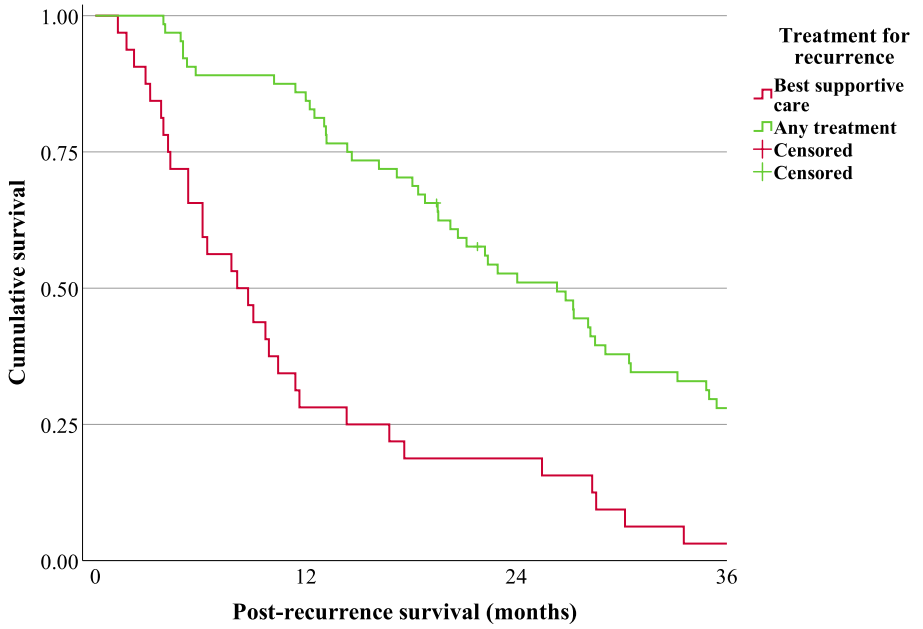
To compare between different treatment strategies, patients were further stratified in best supportive care (n=32, 33%), chemo(radio)therapy (n=45, 47%) and pulmonary metastasectomy (n=19, 20%) cohorts (Table 2). Patients not undergoing further treatment had higher median CA 19-9 at time of recurrence when compared to the pulmonary metastasectomy cohort (P=0.018). However, there was no difference in CA 19-9 between the chemo(radio)therapy and pulmonary metastasectomy cohort (P=0.152). Additionally, patients who had metastasectomy presented less often with recurrence-associated symptoms, ≥5 pulmonary lesions, or recurrence in both lungs when compared to both the chemo(radio) therapy and best supportive care patients (Table 2). Lastly, median RFS was significantly longer in the pulmonary metastasectomy cohort when compared to the chemo(radio)therapy cohort (P=0.003), yet did not differ when compared to the best supportive care cohort (P=0.110).

Kaplan-Meier curves showing PRS of the three treatment cohorts are shown in Figure 1B while median RFS, PRS and OS with pairwise comparisons are listed in Table 2. Pulmonary metastasectomy was associated with significantly improved median PRS (35.0 months, 95% CI 21.1-48.9) when compared to both chemo(radio)therapy (20.2 months, 95% CI 17.2-23.2, P=0.002) and best supportive care (8.1 months, 95% CI 4.4-11.7, P<0.001). Additionally, patients undergoing chemo(radio)therapy had superior median PRS when compared to patients receiving best supportive care (P=0.001). When comparing treatment modalities in the late recurrence cohort, metastasectomy remained significantly associated with improved median PRS when compared to chemo(radio)therapy (47.6 vs. 30.4 months, P=0.038) and best supportive care (47.6 vs. 10.4 months, P=0.001).

Pulmonary Metastasectomy

A detailed overview of the clinical course, performed surgical procedures, and additional treatments of patients who had pulmonary metastasectomy is shown in Table 3. Median interval between pulmonary recurrence and pulmonary metastasectomy was 2.7 months (IQR 1.3-7.1). Two patients had treatment for recurrence prior to pulmonary resection, while 12

A



B

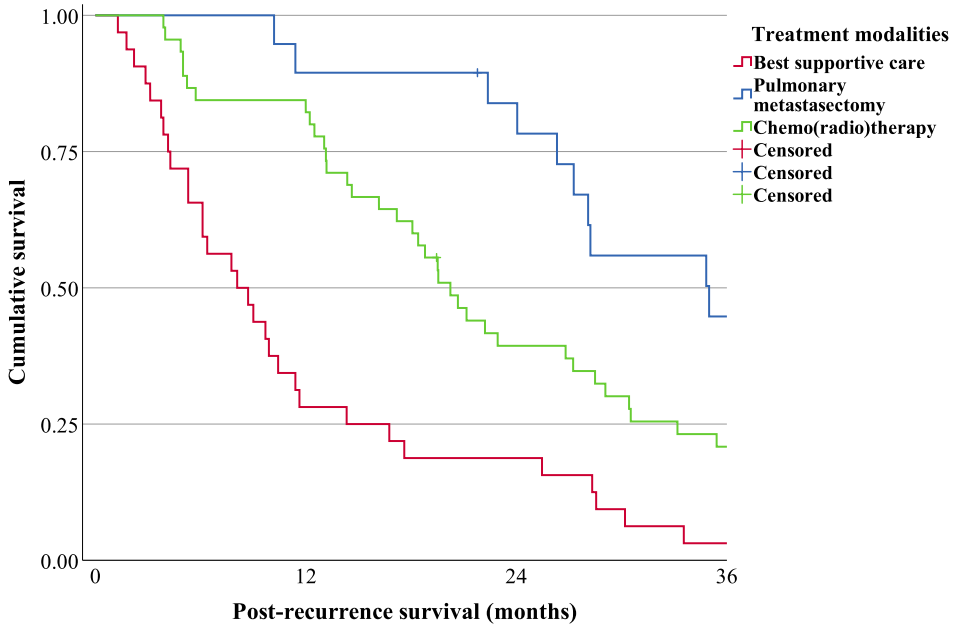


Figure 1. Kaplan-Meier curves showing (A) post-recurrence survival stratified for patients receiving any form of treatment and patients receiving best supportive care and (B) post-recurrence survival stratified for different treatment modalities for isolated pulmonary recurrence.

patients received some form of treatment following metastasectomy. Number and size of resected pulmonary lesions are given in Table 3. Median length of hospital stay following pulmonary metastasectomy was 3 days (IQR 2-4). Three of 19 patients experienced 90-day post-operative morbidity (all Clavien-Dindo[19] grade 2). No surgery-related mortality was observed during the entire follow-up period.

Median survival following primary pancreatectomy and pulmonary resection was 68.9 months (95% CI 45.5-92.3) and 27.1 months (95% CI 23.3-31.0), respectively. Sixteen of 19 resected patients experienced subsequent secondary recurrence after a median interval of 17.7 months (IQR 12.7-23.1). Five patients had secondary recurrence to the lungs yet never developed extra-pulmonary disease progression (Table 3). At last follow-up, five of 19 patients were alive after a median follow-up of 104.7 months and 38.4 months following primary pancreatectomy and pulmonary resection, respectively. Three of those five currently remain disease-free.

Factors Associated with Post-Recurrence Survival

Univariable Cox proportional-hazard regression analyses identified six factors significantly associated with PRS in patients with isolated pulmonary recurrence (Table 4). After adjusting for these covariates in a multivariable Cox regression model, presence of ≥5 pulmonary lesions (Hazard Ratio [HR] 2.63, P=0.013), symptomatic recurrence (HR 2.45, P=0.004) and CA 19-9 ≥100 U/mL (HR 2.58, P=0.001) at time of recurrence were significant independent predictors of decreased PRS. An RFS of ≥16 months prior to recurrence (HR 0.29, P<0.001) and any treatment of recurrence (HR 0.25, P<0.001) were independently associated with improved PRS.

Secondary Disease Progression

Of 96 patients with pulmonary recurrence, eight (8%) had no subsequent imaging performed due to rapid clinical deterioration. In another 21 patients (22%), imaging data after initial recurrence was unavailable due to subsequent follow-up performed at local institutions. Of the remaining 67 patients with imaging data available on secondary disease progression, 28 (42%) did not experience extra-pulmonary disease progression after a median follow-up of 28.3 months (95% CI 20.4-36.3) from initial recurrence. Thirty-nine patients (58%) showed extra-pulmonary disease progression after a median interval of 12.7 months (95% CI 8.7-16.8) from initial recurrence. Location of first progression outside the lungs included local sites (12/39), liver (6/39), peritoneum (6/39), ribs (3/39), spine (1/39), right iliac bone (1/39), brain (1/39) and a combination of multiple sites (9/39).

Table 3. Details on the clinical course of patients with isolated pulmonary recurrence who

No.	*Primary resection + adjuvant*	*RFS1 (months)*	*Treatment prior to pulmonary metastasectomy*	*Interval to surgery [a] (months)*	*Pulmonary surgery procedure*
#1	PD + 5-FU + RT	13.3	Immunotherapy trial (J0206)	6.7	Robotic, LLL lobectomy
#2	PD + 5-FU + RT	24.3	None	1.0	VATS, LLL wedge resection
#3	PD + 5-FU + RT	47.5	None	1.3	Open thoracotomy, LLL lobectomy
#4	PD + 5-FU/CIS + RT	18.4	None	12.3	"Right open thoracotomy with resection metastatic lesion" (outside hospital)
#5	PD + GEM	24.3	None	7.1	VATS, RLL wedge resection
#6	PD + GEM + RT	48.9	None	1.4	VATS, LUL lobectomy
#7	PD + GEM + RT	18.3	None	17.3	VATS, RLL wedge resection (x2)
#8	PD + GEM	16.3	None	0.3	VATS, LLL lobectomy
#9	PD + GEM + RT	16.8	None	2.7	VATS, LLL wedge resection
#10	PD + GEM + RT	26.8	None	4.3	VATS, LLL lobectomy
#11	PD + GEM	40.3	None	24.3	VATS, RLL segmentectomy
#12	PD	9.6	None	0.5	VATS, LLL lobectomy (outside hospital)
#13	DP + GEM + RT	47.2	None	0.6	VATS, RLL wedge resection (2x)
#14	DP + GEM/Oxaliplatin + RT	23.0	1. GEM/Oxaliplatin 2. SBRT LUL lesion	10.1	1. VATS, RUL lobectomy 2. VATS, LUL lobectomy (+2 months)[c]
#15	DP + GEM + RT	54.8	None	2.0	VATS, RLL lobectomy
#16	DP + 5-FU + RT	61.8	None	2.7	Open thoracotomy, LUL lobectomy
#17	DP + GEM + RT	11.4	None	3.9	VATS, LUL wedge resection
#18	DP + GEM + RT	83.0	None	4.6	Robotic, LUL wedge + LLL wedge resection
#19	DP + GEM + RT	35.8	None	2.3	Open, RLL lobectomy

[a] Interval between diagnosis of initial pulmonary recurrence and pulmonary metastasectomy.

[b] 5 years after first pulmonary metastasectomy, patient 8 developed a single pulmonary lesion in the right lung which was also resected. Patient 8 has recently developed peritoneal carcinomatosis, 96 and 25 months after first and second pulmonary metastasectomy, respectively.

[c] Patient 14 had stable bilateral pulmonary lesions after 8 months of GEM/Oxaliplatin followed by SBRT to a large LUL lesion. All lesions were successfully resected with a RUL lobectomy followed by an LUL lobectomy 2 months.

underwent pulmonary metastasectomy

Pathology		Treatment following pulmonary metastasectomy	First site of secondary recurrence	RFS2 (months)	Extra-pulmonary recurrence	Survival (months)		Current status
n	Size (cm)					From PM	OS	
1	2.0	None	Right lung	17.7	-	19.6	39.6	DWD
1	1.1	GEM/CIS	Bilateral lungs + local	13.8	Yes (local)	27.1	52.4	DWD
1	1.0	GEM	Liver + peritoneum	9.5	Yes (liver + peritoneum)	10.1	58.9	DWD
1	?	Unspecified chemotherapy	Local	10.6	Yes (local)	22.6	53.2	DWD
1	0.8	None	Specific site unknown	?	Specific site unknown	16.9	48.3	DWD
1	1.5	1. CIS 2. Carboplatin + RT 3. DTX	Bilateral lungs	33.5	-	83.9	134.1	DWD
2	0.4; 0.4	Immunotherapy trial (J0248)	Local	22.6	Yes (local)	33.2	68.9	DWD
1	2.0	VATS, RUL lobectomy (+5 years) [b]	Right lung	61.6	Yes (peritoneum)	106.5	122.8	AWD
1	0.7	1. GEM 2. RT to brain metastasis	Bilateral lungs	14.1	Yes (brain)	25.5	45.0	DWD
1	0.7	1. GEM/Nab-P 2. FFX 3. GTX/CIS 4. CAP 5. Phase 1 trial	Left lung	23.1	Yes (peritoneum)	43.3	74.4	DWD
1	0.9	None	-	-	-	38.4	103.0	ANED
1	?	None	Bilateral lungs + liver	4.8	Yes (liver)	9.7	19.8	DWD
2	1.0; 2.0	1. RFA LLL lesion 2. RFA + IMRT RUL lesion 3. GTX 4. FFX	Left lung	18.0	Yes (liver)	58.9	106.7	DWD
4 1	0.3-1.0 2.8	GEM/Nab-P	Bilateral lungs	12.7	-	24.9	58.0	DWD
2	0.5; 4.0	1. GEM 2. CAP 3. RT to pleura	Right pleura	17.9	Yes (ribs)	25.3	82.1	DWD
1	1.4	None	-	-	-	71.6	136.1	ANED
1	0.3	1. Immunotherapy trial (J0834) 2. CAP	Bilateral lungs	14.1	-	18.5	33.8	DWD
2	0.3; 1.2	None	-	-	-	17.1	104.7	ANED
1	0.7	Unknown	Bilateral lungs	26.8	-	37.0	75.0	AWD

5-FU, 5-fluorouracil; ANED, alive with no evidence of disease; AWD, alive with disease; CAP, Capecitabine; CIS, cisplatin; DP, distal pancreatectomy; DTX, docetaxel; DWD, death with disease; FFX, FOLFIRINOX; GEM, Gemcitabine; GTX, Gemcitabine/docetaxel/Capecitabine; IMRT, intensity-modulated radiation therapy; LLL, left lower lobe; LUL, left upper lobe; Nab-P, nab-paclitaxel; OS, overall survival from initial pancreatectomy; PD, pancreatoduodenectomy; PM, pulmonary metastasectomy; RFA, radiofrequency ablation; RFS1, interval between pancreatectomy and initial pulmonary recurrence; RFS2, interval between pulmonary metastasectomy and diagnosis of secondary recurrence; RLL, right lower lobe; RT, radiotherapy; RUL, right upper lobe; SBRT; stereotactic body radiation therapy; VATS, video-assisted thoracoscopic surgery.

Table 4. Univariable and multivariable Cox proportional-hazard regression analysis of independent predictors for post-recurrence survival

Clinical characteristics	*Cohort, n (%) n=96*	*Univariable analysis*			*Multivariable analysis*		
		HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Gender							
Male	54 (56%)	Ref.					
Female	42 (44%)	0.78	0.51-1.20	0.247	-		
Race							
Caucasian	88 (92%)	Ref.					
All others	8 (8%)	0.92	0.44-1.92	0.817	-		
Age at time of recurrence							
<70 years	51 (53%)	Ref.					
≥70 years	45 (47%)	0.88	0.68-1.58	0.883	-		
Recurrence-free survival							
<16 months	47 (49%)	Ref.					
≥16 months	49 (51%)	0.31	0.20-0.49	<0.001	**0.29**	**0.17-0.49**	**<0.001**
Number of lesions							
<5 lesions	38 (40%)	Ref.					
≥5 lesions	58 (60%)	1.93	1.24-3.00	0.003	**2.63**	**1.23-5.64**	**0.013**
Location of lesion(s)							
Unilateral	32 (33%)	Ref.					
Bilateral	64 (67%)	1.77	1.10-2.83	0.018	2.24	0.96-5.18	0.061
Symptomatic recurrence							
No	78 (19%)	Ref.					
Yes	18 (81%)	3.60	2.06-6.29	<0.001	**2.45**	**1.34-4.48**	**0.004**
CA 19-9 at time of recurrence[a]							
<100 U/ml	46 (48%)	Ref.					
≥100 U/ml	24 (25%)	2.38	1.40-4.03	0.001	**2.58**	**1.48-4.49**	**0.001**
Unknown/Lewis-ant. negative	26 (27%)	1.74	1.04-2.89	0.034	1.54	0.90-2.62	0.114
Any treatment for recurrence							
No	32 (33%)	Ref.					
Yes	64 (67%)	0.34	0.22-0.53	<0.001	**0.25**	**0.15-0.43**	**<0.001**

Significant values on multivariable analysis are show ($P<0.050$) are shown in bold.
[a] 78 patients had documented CA 19-9 values at time of recurrence, of whom 8 patients were Lewis-antigen negative and excluded from analysis.
Ant., *antigen*; CA, *carbohydrate antigen*; CI, *confidence interval*; HR, *hazard ratio*.

Median survival from date of extra-pulmonary progression was limited (4.7 months, 95% CI 4.0-5.5). Patients who experienced extra-pulmonary progression had decreased median survival from initial pulmonary recurrence when compared to those who did not (20.2 vs. 30.2 months, P=0.013). At last follow-up, one of 39 patients with extra-pulmonary progression was alive, compared to seven of 28 patients who had disease recurrence that remained restricted to the lungs (P=0.008).

Discussion

This study presents a detailed single-institution study of resected PDAC patients with initial recurrence restricted to the lungs. Isolated pulmonary recurrence occurred in 13% (n=96) of recurrent patients and was associated with a relatively favorable median OS of 40 months. This large cohort allowed for multivariable analysis of prognostic factors for survival after recurrence in this unique subpopulation of patients. Several recurrence characteristics were found to be risk factors for decreased PRS, while a prolonged recurrence-free interval and further treatment for recurrence were independently associated with improved PRS. Lastly, pulmonary metastasectomy was performed in a highly selected cohort of 19 patients, leading to a median PRS and OS of 35 and 69 months, respectively. These identified factors and associations have the potential to aid clinicians with prognostic stratification and might help guide decisions regarding further treatment of pulmonary PDAC recurrence.

Several prior studies have described favorable survival associated with pulmonary metastases from PDAC.[11-13,20-24] In a case-control study of 37 patients with lung only metastases matched with 37 patients with liver only metastases, Decoster et al. showed that pulmonary metastases in the primary metastatic setting are associated with better survival (median 32 vs. 9 months, $P<0.001$).[22] Similarly, multiple studies have shown that lung only recurrence after PDAC resection results in superior survival when compared to other recurrence patterns, such as liver only, peritoneal or multiple-site recurrence.[10-12,20] The OS for patients with pulmonary recurrence in these studies (median 3612, 3620, and 4011 months) was remarkably similar to the 40 months found in the current study, further supporting the hypothesis that favorable tumor biology exists in these patients.

Previous autopsy studies on the metastatic spread from PDAC provide insight in how PDAC cells can spread to the lungs via routes that circumvent the liver and other organs. One described pathway involves circulating tumor cells entering the systemic circulation through collateral vessels caused by portal vein or splenic vein obstruction, thus bypassing the portal systemic circulation and the liver.[25] Additionally, a different autopsy study of 42 patients with pulmonary metastases from PDAC identified several potential lymphatic routes: (1) through pleural lymphatics along connective tissue septa and into the alveolar spaces and bronchial walls, (2) through retrograde lymphatic invasion of the lung from the tracheobronchial or mediastinal glands, and (3) through involvement of metastatic lymph nodes of the venous angle.[26] In support of these autopsy findings, a recent study on predictors of specific recurrence locations identified microscopic lymphovascular invasion and a positive lymph node ratio >0.2 to be the only two predictors of lung only recurrence.[4]

A complete understanding of why pulmonary PDAC metastases are associated with relatively favorable RFS and OS remains elusive. One theory assumes that the large capacity of the lungs allows patients to endure a greater metastatic tumor burden leading to extended survival.[12] Another proposed theory argues that both the first recurrence site and tumor aggressiveness are determined by common underlying biologic pathways.[21] Future genetic analyses of both primary PDACs and metastatic lesions might elucidate if distinct genetic signatures lead to these organ-specific metastases. The biologic mechanisms that determine PDAC recurrence patterns and associated differences in survival constitute a fascinating and worthy field of study, since improved understanding has the potential to provide novel therapeutic approaches.

Several recurrence characteristics were risk factors for decreased PRS, such as ≥5 pulmonary lesions, symptoms and CA 19-9 ≥100 U/ml at time of recurrence. Presence of these features at time of recurrence could be interpreted as signs of a more aggressive tumor biology. On the other side, an RFS of ≥16 months prior to recurrence was predictive of improved PRS, possibly indicating more favorable tumor behavior in patients with late recurrence. While controlling for these potential biology-associated risk factors in a multivariable model, further treatment of pulmonary recurrence remained significantly associated with improved PRS. These results suggest that the improved survival in the treatment cohort was not due to selection bias alone, and that the therapeutic efforts might have played a role in the prolonged survival for these patients. However, it is important to note that this was not a randomized clinical trial and found differences in survival should be interpreted cautiously. It is likely that selection bias played a role in the extended survival of patients undergoing additional chemotherapy, and/or pulmonary resection. This was a selected group of patients, and it could be argued that they might have had prolonged survival even without additional therapy.

Patient who underwent pulmonary metastasectomy showed the longest median PRS and OS. In recent years, interest in this approach has increased, with numerable case reports[27-33] and several case series[17,34,35] published mostly from institutions in the United States and Japan. Similar to the current study, previous reports on pulmonary metastasectomy describe low rates of surgery-related morbidity and mortality, while median survival after metastasectomy of up to 47 months was observed.[17] There is precedent for metastasectomy in different neoplasms, especially for liver metastases from colorectal or pancreatic neuroendocrine cancer.[36,37] However, recurrent PDAC is particularly difficult to manage surgically, as it typically is characterized by multifocal and destructive spread. Therefore, pulmonary metastasectomy remains a non-standard and individual treatment approach, for which appropriate selection of patients with favorable tumor biology is paramount. Specifically, pulmonary resection could

be considered in patients with good performance status when pulmonary recurrence occurs after a recurrence-free interval of ≥16 months, and if the disease remains stable over time, or during a period of initial systemic treatment.

The results of this study should be interpreted within the context of its limitations. Importantly, the non-randomized design of this study and heterogeneity of treatments before and after the pulmonary recurrence make it difficult to determine the particular effects of distinct treatments on observed outcomes. Additionally, pulmonary recurrence was based on radiological evidence without pathological confirmation in approximately half of included patients. This might have influenced data on number and location of lesions, as benign pulmonary nodules are seen commonly and are often difficult to differentiate from true pulmonary metastases.[38] Despite these limitations, the current study has several important strengths. The current study is the largest to date to study patients with pulmonary PDAC recurrence and includes the largest series of performed pulmonary metastasectomies reported in the literature. Furthermore, multivariable analysis with comprehensive recurrence and treatment data identified prognostic factors for survival after pulmonary recurrence not previously reported. Lastly, data was available on secondary disease progression, emphasizing that survival from time of extra-pulmonary progression was particularly limited (median 5 months).

Conclusion

Isolated pulmonary recurrence occurs in 13% of patients with recurrence following PDAC resection and is associated with a median OS of 40 months. Within this unique subpopulation, further systemic therapy and/or metastasectomy was associated with improved survival after recurrence in selected patients. To better identify patients who might benefit from more aggressive treatment for recurrence, future research should focus on elucidating underlying biologic mechanisms that might determine PDAC recurrence patterns and associated differences in survival.

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Supplemental Materials to Chapter 17

- **Supplemental eTable S1.** Evaluated cut-off values for defining early and late recurrence based on the prognosis after pulmonary recurrence.
- **Supplemental eFigure S1.** Flowchart of patient inclusion.
- **Supplemental eFigure S2.** Different cut-off values of recurrence-free survival with corresponding P-values (log-rank test) show that the optimal threshold for defining early and late pulmonary recurrence based on the difference of post-recurrence survival is 16 months.

Supplemental material can be consulted online per the website of the journal and/or publisher.

PART V

General Discussion and Future Perspectives

CHAPTER 18

Summary

Summary

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second most common cause of cancer-related mortality within the next decade. Although surgical techniques for pancreatectomy have been perfected, survival statistics for PDAC patients undergoing resection remain grim. Disease recurrence remains the single most important barrier to curing localized PDAC after resection. Comprehensive understanding of why seemingly successful surgery fails is essential in the pursuit of improved outcomes for these patients. As such, this thesis aims to provide insight in varying aspects of recurrent PDAC, including the pattern and timing of recurrence (Part I), novel implications of neoadjuvant therapy (Part II), value of post-pancreatectomy imaging and biomarker surveillance (Part III) and treatment of recurrence (Part IV). In the following chapter, the findings of the studies presented in this thesis are summarized.

PART I. Recurrence Following Primary Resection

Chapter 2. Patterns of Recurrence After Pancreatectomy

A greater understanding of PDAC recurrence can provide valuable insight when contemplating appropriate strategies regarding adjuvant treatment, oncologic surveillance, and management of recurrence. This large and detailed Johns Hopkins Hospital study found that 76% of recurrences occur at a distant site, demonstrating that most patients with PDAC have systemic disease at the time of resection. Furthermore, specific recurrence patterns were shown to have unique recurrence-free survival curves and predictors. All off these findings are highly suggestive of unique biological behaviors of tumors within a distinct recurrence pattern and open the door to identification of tumors for detailed molecular analysis.

Chapter 3. Implications of Recurrence on Survival

The relationship of specific PDAC recurrence locations with overall survival is not well established. This study demonstrated that that overall survival and survival after PDAC recurrence differ based on the anatomical site of first recurrence. Specifically, the current study found that patients with multiple-site recurrence had a limited median survival after recurrence of <5 months, while patients who developed pulmonary metastases had a median survival of >15 months after first diagnosis of recurrence. Also, using two different techniques of multivariable Cox regression analysis, specific recurrence patterns were shown to have distinct independent prognostic values. These finding could help with prognostic stratification and decisions regarding treatment after the diagnosis of recurrence.

Chapter 4. Defining and Predicting Early Recurrence

Resectable patients susceptible to early recurrence constitute a key cohort worthy of further study, as these selected patients may benefit from a neoadjuvant-first approach. This study implied that the optimal cut-off value to differentiate between early and late recurrence, based on subsequent prognosis, is a recurrence-free interval of at least 12 months. Additionally, independent risk factors for the development of early PDAC recurrence after resection were found, including a pre-operative Charlson age-comorbidity index score of ≥4, tumor size on the last pre-operative CT scan >3.0cm and a CA 19-9 level of >210 U/mL. Additionally, pathologic findings of poor tumor differentiation grade, microscopic lymphovascular invasion, a positive lymph node ratio >0.2, and post-operative CA 19-9 level of >37 U/mL were independently associated with early recurrence. These findings suggest that length of recurrence-free survival could be a clinically useful surrogate for appreciating PDAC behavior.

PART II. Recurrence Following Post-Neoadjuvant Recurrence

Chapter 5. Survival After Resection of Locally Advanced Pancreatic Cancer

An increasing number of patients with locally advanced pancreatic cancer (LAPC) who respond favorably to neoadjuvant therapy undergo surgical resection. This study provided detailed insight on the management and outcomes of patients with LAPC and clearly identified a survival advantage in a selected cohort who underwent resection of the primary tumor, compared to those who did not. As previously suggested imaging response alone may be inadequate to declare resectability after neoadjuvant treatment, and this also proves to be the case in the presented series: only 12% of resected patients had partial or complete response according to RECIST criteria. A multidisciplinary approach in high-volume centers is necessary to identify patients who can benefit from an aggressive approach to LAPC.

Chapter 6. Survival After Pathologic Complete Response

A pathological complete response in PDAC is uncommon and observed in only 3-11% of resected specimen treated with neoadjuvant therapy. In this study, 10% of PDAC patients had a pathological complete response after surgery following a neoadjuvant treatment with chemotherapy and chemoradiation. The median survival of these 19 patients was over 60 months, which is unprecedented in pancreatic cancer. By comparison, the median survival of patients with a near complete response (defined as aggregate tumor of <1cm and negative nodes) was 27 months, and that of patients with a more limited response 26 months. These findings lend support to the use of neoadjuvant chemoradiation treatment in patients with

advanced localized PDAC and the use of a pathological complete response as an endpoint for future study.

Chapter 7. Recurrence Patterns After Post-Neoadjuvant Resection

The incidence, timing, and implications of disease recurrence in patients undergoing post-neoadjuvant resection of advanced localized PDAC are not well established. While low rates of R1-margin and lymph node positivity were observed in this study, PDAC recurrence nevertheless occurred in more than 80% of patients. Furthermore, 72.5% of recurrences occurred at distant sites, suggesting that despite a radiographic progression-free period leading to resection, viable micrometastatic PDAC can persist after systemic neoadjuvant treatment. Also, several clinicopathologic and treatment features were found to be associated with distinct recurrence locations and patient survival. This detailed knowledge on the timing, pattern and factors associated with disease failure may help clinicians with important decisions regarding the challenging multimodality care of these patients with advanced localized disease.

PART III. Detection of Recurrence

Chapter 8. Follow-Up After Pancreatic Cancer Resection

Recommendations for follow-up in patients who underwent resection of PDAC vary widely throughout the world, and the importance of a diagnostic post-operative follow-up is heavily debated. This systematic review of the literature demonstrated that the combination of a rise in serum tumor markers and suspicious, progressive findings on follow-up imaging could aid in the early clinical diagnosis of PDAC recurrence. However, current evidence for routine imaging and tumor marker testing during follow-up after resection of PDAC is limited and contradictory. Consequently, high-quality evidence is lacking to provide recommendations for surveillance exists. Future well-designed prospective studies are exceedingly needed to define the clinical merit as well as economical and psychosocial implications of routine diagnostic follow-up after resection of PDAC.

Chapter 9. Survey on Post-Pancreatectomy Follow-Up

The most recent Dutch guidelines for pancreatic cancer care currently do not contain recommendations for follow-up frequency, methodology or focus. This survey reflects the lack of a standardized approach to follow-up after resection of PDAC. Furthermore, it reveals the doubts and uncertainty on the role of imaging and tumor marker testing during follow-up. Also, this study shows that the current follow-up strategies of Dutch pancreatic surgeons, in accordance with European guidelines, mainly consist of regular outpatient visits with physical

examination. However, detection of recurrent PDAC during follow-up will facilitate patient recruitment into investigational studies and recurrence data can benefit novel research. The high motivation among Dutch pancreatic surgeons to participate in a new study for the detection and treatment of recurrence appears to support this, making a national trial achievable.

PART IIIa. Imaging Surveillance

Chapter 10. Imaging Diagnosis of Pancreatic Cancer Recurrence

To provide further information on the extent and location of recurrent disease, radiological information frequently determines patient eligibility for any additional treatment or inclusion in clinical trials. For this purpose, imaging modalities such as CT, MRI and FDG PET-CT are used. This systematic review provides an overview of the literature on the diagnostic accuracy of various imaging modalities. According to the reviewed literature, contrast-enhanced CT has a moderate diagnostic value with a sensitivity of 70% and specificity of 80%; FDG PET-CT showed a sensitivity and specificity of 88% and 89%, respectively, and for FDG PET-CT combined with contrast-enhanced CT, a sensitivity of 95% and specificity of 81% was found. In the context of routine radiologic surveillance, diagnostic accuracy values of CT and FDG PET-CT were similar compared to imaging performed in case of suspected PDAC recurrence. To optimize surveillance strategies, future studies on the diagnostic performance of imaging modalities during follow-up and the consequences of early diagnosis of recurrent disease on survival and quality of life outcomes are needed.

Chapter 11. Use of Imaging During Follow-Up

Adhering to the European guidelines, follow-up at the University Medical Center Utrecht consists of regular check-ups focusing on post-operative symptoms without imaging for the detection of recurrence. This study found that although imaging was explicitly not part of our symptomatic follow-up protocol, the majority of patients (87%) in this study underwent additional imaging procedures for the detection of recurrence at least once. Furthermore, patients underwent an average of 3 imaging procedures during the entire follow-up period. Most of the recurrences (85%) were diagnosed at a late stage and after the manifestation of clinical symptoms, resulting in only 17 patients (25%) receiving additional treatment for their recurrence. It could be argued that instead of postponing imaging until recurrence-related symptoms occur, the average of 3 imaging procedures could be used more effectively when used in 3- or 6-monthly intervals. A more standardized follow-up in this manner could detect asymptomatic recurrence at an earlier stage when patients have a higher chance of being able to receive additional treatment.

PART IIIb. Biomarkers and Liquid Biopsies

Chapter 12. Tumor Markers for Detection of Recurrence

Occult cancer recurrence can be suspected by a sudden elevation of serum tumor markers. This systematic review provides an overview of the existing literature on the diagnostic accuracy of serum tumor markers for the detection of disease recurrence in the follow-up after resection of pancreatic cancer. According to the reviewed literature, routine testing of serum CA 19-9 during follow-up has a moderate value with a sensitivity of 68-89% and a specificity of 77-89% for detecting recurrence. Only one study reported on the diagnostic accuracy of CEA, showing a low sensitivity and specificity of 50% and 65%, respectively. No other serum tumor markers were investigated for surveillance purposes after surgery for pancreatic cancer in the current literature. Further studies are needed on the diagnostic accuracy of pancreatic cancer tumor markers during follow-up and to assess the role of serum tumor marker testing in the post-operative detection of recurrent pancreatic cancer.

Chapter 13. Circulating Tumor Cell Dynamics

Recent studies in PDAC have reported on the potential of circulating tumor cells (CTCs) as a molecular biomarker of disease status by identifying an association of their number and phenotype with early disease recurrence, overall survival, and presence of occult metastases. CTCs dynamics, or fluctuations of CTCs burden in circulation, is a promising longitudinal indicator of disease status. In a prospective longitudinal cohort study on CTCs dynamics (NCT02974764), 200 enrolled patients with PDAC were followed with sequential phlebotomy along the course of their disease. Two major CTCs subtypes were identified: epithelial CTCs and mesenchymal CTCs. Systemic therapy was associated with lower CTCs numbers and surgical resection of the primary tumor resulted in significant decrease in CTCs populations. Increased pre-operative CTCs numbers and especially cells with mesenchymal traits were predictive of early recurrence after surgical resection. Additionally, an increase in CTCs was documented in PDAC recurrence, approximately two months before new imaging evidence of disease. The potential of CTCs dynamics as a biomarker is evident and further in-depth analysis of their cellular profile is justified.

Chapter 14. Clinical Utility of Circulating Tumor DNA

In research settings, circulating tumor DNA (ctDNA) has shown promise as a tumor-specific biomarker. A clinical validation of a *KRAS* ctDNA assay was performed in a Clinical Laboratory Improvement Amendments (CLIA) certified and College of American Pathology (CAP) inspected clinical laboratory. This study confirmed the clinical utility of ctDNA as a

tumor-specific biomarker for prognostic stratification and monitoring of tumor dynamics in patients with pancreatic cancer. First, detection of mutant *KRAS* ctDNA prior to surgery or in the immediate post-operative period predicts a higher likelihood of tumor recurrence and poorer survival. Second, rising mutant *KRAS* ctDNA during post-operative follow-up predicts radiographic/clinical recurrence with a lead time of 84 days. As a result of this study, this *KRAS* ctDNA assay is now live at the Johns Hopkins Hospital as an in-house test (go live date, 4 December 2018). Future prospective and interventional trials will determine if treatment decisions based on ctDNA dynamics can improve patient outcomes.

PART IV. Treatment of Recurrence

Chapter 15. Treatment Options for Local Recurrence

For patients with recurrent pancreatic cancer, it is paramount to find multidisciplinary treatment that optimizes patient selection while balancing disease control, toxicity and quality of life. This review shows that treatment of isolated local recurrence can be performed with acceptable morbidity and mortality rates. As no randomized controlled trials were available, this review cannot recommend re-resection, chemoradiotherapy or stereotactic body radiation therapy as the best treatment option for patients with local recurrence. However, compared to best supportive care, treatment of local recurrence seems to offer a chance of extended survival in selected patients. Specifically, patients with a prolonged disease-free interval seem to benefit most from treatment. As these findings are based on smaller cohort studies and patient series, further prospective studies should focus on identifying other clinicopathological features predicting which patients benefit most of these therapeutical options and the comparison between the different treatment options. Also, more research is needed to find an accurate and cost-effective surveillance algorithm best suited for identifying patients with local recurrence before they develop metastatic disease.

Chapter 16. Stereotactic Body Radiation Therapy for Local Recurrence

Despite the frequency of local recurrence in patients with PDAC, evidence to guide treatment decisions for these patients is limited. Stereotactic body radiation therapy (SBRT) represent a relatively novel treatment option for PDAC that requires only 5 days for delivery and may be more cost-effective than conventional radiotherapy, both of which are important considerations for PDAC patients who often face a poor prognosis. In this study, Symptom palliation was achieved in 63% of patients and treatment-related toxicity rates were acceptable. Furthermore, this study highlights several factors that correlated with survival outcomes. Patients with a disease-free interval of <9 months had a poorer prognosis after SBRT than did patients who recurred later than 9 months. Patients who received maintenance

chemotherapy after SBRT had a longer progression-free and overall survival when compared to patients who did not. These results suggest directions for future studies comparing treatments directly and suggest that, for select patients, prognosis may be relatively good after SBRT and chemotherapy.

Chapter 17. Treatment of Pulmonary Recurrence

The literature suggests favorable survival for patients with isolated pulmonary recurrence after resection of PDAC as compared to other recurrence patterns. Within this cohort, it remains unclear what factors are associated with improved survival. This study presents a detailed single-institution study of resected PDAC patients with initial recurrence restricted to the lungs. Isolated pulmonary recurrence occurred in 13% (n=96) of recurrent patients and was associated with a relatively favorable median survival of 40 months. This large cohort allowed for multivariable analysis of prognostic factors for survival after recurrence in this unique subpopulation of patients. Several recurrence characteristics were found to be risk factors for decreased post-recurrence survival, while a prolonged recurrence-free interval and further treatment for recurrence were independently associated with improved post-recurrence survival. Lastly, pulmonary metastasectomy was performed in a highly selected cohort of 19 patients, leading to a median post-recurrence and overall survival of 35 and 69 months, respectively. These identified factors and associations have the potential to aid clinicians with prognostic stratification and might help guide decisions regarding further treatment of pulmonary PDAC recurrence.

CHAPTER 19

General Discussion and Future Perspectives

Disease recurrence after resection of pancreatic ductal adenocarcinoma (PDAC) unfortunately remains common and marks a critical and emotional time point in the care of PDAC patients. This thesis seeks to provide insight in varying aspects of recurrent PDAC, including the pattern and timing of recurrence (**Part I**), novel implications of neoadjuvant therapy (**Part II**), value of post-pancreatectomy imaging and biomarker surveillance (**Part III**) and treatment of recurrence (**Part IV**). In the ensuing chapter, the scientific and clinical significance of the presented findings are discussed, and future perspectives are offered.

Distinct Behaviors of Recurrence Suggest Unique Biological Heterogeneity of Pancreatic Cancer

For patients with localized PDAC, a combination of resection and systemic therapy offers the best chance for long-term survival.[1] However, even after completion of multimodality therapy, disease recurrence is common and the foremost cause of disease-specific mortality. Evidence presented in Part I of this thesis demonstrates that PDAC recurrences exhibit varying biological behaviors. For instance, **Chapter 2** demonstrates that the majority of resected PDAC patients will develop systemic disease. Yet, a significant proportion of patients will develop isolated or concurrent local recurrence. And even among the patients who develop systemic disease, metastatic spread can be limited to the liver, lung, or peritoneum. Additionally, we have shown that the time to recurrence varies for distinct recurrence locations, with liver only and multiple-site recurrence occurring significantly earlier than local and pulmonary recurrence. Research in **Chapter 3** expands on these findings by establishing significant associations between these specific recurrence patterns and post-recurrence survival. Lastly, we find in **Chapter 4** that the timing of recurrence also greatly impacts post-recurrence survival, with a recurrence-free interval of 12 months as the optimal threshold for differentiating between early and late recurrence, based on subsequent prognosis. All these presented findings have important prognostic implications that can assist patient and physician alike when discussing challenging clinical choices, and the balance between quality of life and further treatment for recurrence. Moreover, since evidently not all PDAC recurrences behave the same, a more individualized approach to the treatment of PDAC recurrence should be considered (Part IV).

The presented results strongly suggest that biologic heterogeneity exists among pancreatic cancer leading to distinct patterns and timing of recurrence. To date, the underlying biological mechanisms causing specific PDAC recurrence patterns and associated survival outcomes remain unexplained. Hopefully, future genetic analyses of both primary tumors and metastases will elucidate if distinct genetic signatures lead to different organ specific recurrences. This possibility seems likely, as molecular profiling has been shown to predict

cancer recurrence in prostate, stomach, and liver cancer.[2-4] In pancreatic cancer, evidence of substantial molecular heterogeneity already exists. Jones et al.[5], for example, identified over 1200 genetic alterations among 96 PDACs, with the average tumor harboring 63 alterations. Similarly, in their genomic study of 99 PDAC samples, Biankin et al.[6] detected 2627 high-confidence genetic mutations. Per patient, an average of 26 mutations was found, ranging from 1 to 116 mutations. Furthermore, mouse models studying metastasis biology in breast and renal carcinoma showed site-specific metastasis could be attributed to gene inactivation or suppression, protein modulators, and tumor- and stroma-supplied proteolytic networks.[7-10]

It is conceivable that both the first recurrence site and tumor aggressiveness are determined by common underlying biologic mechanisms. Evidently, unraveling these biological pathways constitutes an intriguing field of study. Combining clinical factors with genomic information to predict recurrence patterns would facilitate personalized prognostic stratification and treatment approaches, bringing a precision medicine approach to PDAC closer to reality. Apart from translational research efforts, future research should focus on ascertaining the prognostic impact of recurrence characteristics of which the importance currently remains unknown, such as symptoms, tumor burden, and carbohydrate antigen (CA) 19-9 level at the time of recurrence. Lastly, this thesis offers only a cross-section of the natural history of resected PDAC, as just the first site of recurrence was examined. As further disease progression was not accounted for, additional data on secondary disease spread might potentially reveal associations not currently appreciated by the current studies.

Neoadjuvant Treatment Changing the Landscape, Although Recurrence Rates Remain High

Neoadjuvant treatment followed by pancreatectomy for localized PDAC is a relatively novel treatment paradigm. For patients with locally advanced PDAC, which is not amenable to upfront resection, current guidelines recommend palliative chemotherapy through a multidisciplinary approach.[11] However, recent improvements in chemotherapy have emboldened clinicians to attempt curative resection after induction chemotherapy in these patients.[12,13] However, the precise clinical benefit of this strategy remains unclear, as most studies only reported outcomes on resection without commenting on the total denominator of patients including those who did not make it to surgery. In **Chapter 5** we demonstrate that resection was ultimately achieved in 20% of patients with advanced localized PDAC who received neoadjuvant therapy. These selected patients showcase a clear survival benefit compared with patients undergoing neoadjuvant therapy without resection. It is important to note that, as Ferrone et al.[14] have previously suggested, imaging response alone may be inadequate to declare resectability after neoadjuvant treatment. In the presented results, only

12% of resected patients had partial or complete response according to RECIST criteria.[15] Following these findings, it could be argued that surgical exploration should be offered to patients in absence of disease progression following a period of neoadjuvant chemotherapy.

One clear example of the potential beneficial impact of neoadjuvant therapy is the possibility of a complete pathologic response. **Chapter 6** highlights that a pathologic complete response occurs in 10% of patients after neoadjuvant chemotherapy and radiation. Furthermore, a pathologic complete response resulted in an unprecedented median survival of over 60 months, possibly curing a number of these patients, many of whom in the past would not have had surgery. Despite the promising survival, at least 50% of patients with a pathologic complete response developed disease recurrence. Notably, aggressive multidisciplinary therapy for recurrence including chemotherapy, radiotherapies and surgical resection was often pursued, resulting in extended post-recurrence survival. This further suggests the importance of a pathologic complete response as a surrogate marker for tumor biology. Moreover, these patients had a significant initial response to chemotherapy, and appear to continue to have beneficial treatment effect from adjuvant treatment following recurrence prolonging post-recurrence survival. When observing all patients with advanced PDAC undergoing post-neoadjuvant pancreatectomy, **Chapter 7** underlines that still more than 80% of patients experienced disease recurrence. Furthermore, ~70% of these recurrences will occur at distant sites. Interestingly, additional adjuvant treatment delayed recurrence and improved survival. In the current literature, data on adjuvant therapy after neoadjuvant therapy and resection are limited and often contradictory.[16,17] As such, future prospective trials should aim to elucidate the right indications and strategies for adjuvant therapy in these patients.

Many questions regarding the use of neoadjuvant treatment wait to be answered. One question to ask is if a neoadjuvant-first treatment strategy should be considered in patients with upfront resectable PDAC, as currently is the case for esophageal, gastric, and rectal cancer.[18] This topic is currently debated and studied intensely, with the pendulum seemingly swinging more and more towards neoadjuvant treatment. In the near future, randomized controlled studies like the PREOPANC, ALLIANCE and ESPAC-5f trials might expand the indication of neoadjuvant therapy to all patients with localized PDAC.[19,20] Second, it is currently unknown what chemotherapy regimen should be the first choice. Data presented in this and other studies suggest that FOLFIRINOX is more likely to results in resection and improved survival when compared with Gemcitabine-based regimens.[21] However, a substantial amount of patients will fail to respond to FOLFIRINOX, only to show a response after a transition to a combination of Gemcitabine with Capecitabine or Nab-Paclitaxel. Being able to predict which patients will respond to what chemotherapy regimen would obviously have major

implications. Efforts to develop chemo-sensitivity assays are underway, with patient-derived organoid models in particular showing the potential to predict clinical response and enable prospective therapeutic selection.[22,23] Additionally, a recent study emphasizes the promising role liquid biopsies could play in accurate prognostic stratification and appropriate selection of patients for surgery.[24]

Low Quality and Contradictory Evidence Leads to Varying Follow-Up Practices and Guidelines

The hypothesis that routine diagnostic follow-up has survival benefits follows the assumption that detection of disease recurrence at an early stage improves the eligibility to undergo treatment. Consequently, expert and high-volume pancreatic centers worldwide have implemented standardized post-operative surveillance with serum tumor marker testing and routine imaging for resected PDAC patients. As summarized in **Chapter 8**, however, current evidence for routine imaging and tumor marker testing during follow-up after resection of PDAC is limited and contradictory. This distinct lack of high-quality evidence has resulted in international and national guidelines with minimal or even absent recommendations on follow-up (Table 1).[25-31] The literature and available guidelines seem to agree that a standardized approach to follow-up is warranted for the post-surgical care, nutritional, and psychosocial support of patients after pancreatic resection. However, opinions, evidence, and current practice differ when it comes to adding further diagnostic testing for the detection of PDAC recurrence. A survey among Dutch pancreatic surgeons presented in **Chapter 9** illustrates the lack of a standardized approach to post-pancreatectomy follow-up in the Netherlands in the absence of recommendations in the national guidelines on pancreatic cancer.[31] This could potentially deprive certain PDAC patients of meaningful follow-up, while wrongfully harming others.

The role of routine imaging procedures for the early detection of recurrent PDAC is not well established.[32,33] **Chapter 10** underscores that only a few retrospective observational studies with small sample sizes report on the diagnostic value of different imaging modalities or impact of diagnostic follow-up on survival and quality of life. Furthermore, **Chapter 11** demonstrates that even with a symptomatic follow-up strategy without standardized imaging, the majority of patients undergo additional imaging procedures for the detection of recurrence at least once. However, although patients underwent an average of three imaging procedures during follow-up, most recurrences were detected at a late stage and after the manifestation of clinical symptoms. This potentially limits patients to undergo additional treatment for recurrence. Many questions concerning post-pancreatectomy follow-up remain unanswered. Could we extend treatment possibilities for the significant group of patients with PDAC recurrence, by

detecting recurrence at an early stage using a standardized surveillance strategy? Will this improve the poor survival rates for PDAC patients? How will a standardized surveillance affect their quality of life? To provide answers to these questions, the RADAR-PANC (Recurrent diseAse Detection After Resection of PANCreatic adenocarcinoma using a standardized surveillance strategy) study has been designed. The goal of this nationwide cohort multiple randomized controlled trial (cmRCT) is to determine whether an intensive surveillance strategy with serial serum tumor marker testing and routine imaging improves the overall survival in patients after radical resection for PDAC in the Netherlands, compared to current non-standardized follow-up. Furthermore, this study would aim to investigate the acceptance and the impact on the quality of life of an intensive surveillance strategy amongst pancreatic cancer patients.

Prospective Longitudinal Studies Prove the Clinical Utility of Circulating Tumor Cells and DNA

As highlighted repeatedly in this thesis, a high percentage of patients with seemingly successful resection of localized PDAC have occult micrometastases that persist after multimodality treatment. This minimal residual disease reflects the presence of tumor cells disseminated from the primary PDAC to distant sites in patients who lack any radiographic or clinical signs of metastases.[34] Accurate detection, monitoring and characterizing of minimal residual PDAC could facilitate early control of metastatic disease and guide clinical decisions based on real-time treatment resistance information. The ideal tumor marker for surveillance after surgical resection of PDAC has (1) a high sensitivity and positive predictive value, identifying all patients with recurrent PDAC preferably in an early stage; (2) a high specificity, detecting only patients with PDAC recurrence and not with benign diseases or without any disease; (3) correlates with the tumor burden and response to treatment; (4) provides prognostic and predictive information; and is (5) affordable and easy to obtain.[35,36] In **Chapter 12** we conclude that CA 19-9 currently remains the most widely used serum tumor marker for the post-pancreatectomy detection of minimal residual PDAC. However, due to the well-documented limitations of CA 19-9 as a biomarker for PDAC, serial assessment of serum CA only has a moderate diagnostic value for detecting disease recurrence.[37-39] Despite its limits, patient interviews demonstrate that patients perceive detection of relapse through CA-19-9 as the focal point of post-pancreatectomy follow-up, with consultations revolving largely around the CA-19-9 results.[40] As such, there is an important need to develop and validate novel, tumor-specific biomarkers to complement or replace this relatively unprecise marker.

A liquid biopsy can be defined as the analysis of circulating tumors cells or circulating tumor-derived factors, such as cell-free circulating tumor DNA.[34] Research in varying cancer types

suggest that liquid biopsies have many potential clinical applications in the field of precision oncology. In a rapidly evolving field of study, they have shown promise as a valuable tool for early cancer detection, providing insights into tumor heterogeneity, and monitoring tumor dynamics and treatment resistance.[41-44] **Chapter 13** describes a prospective longitudinal study, in which two major circulating tumor cell subtypes were identified in PDAC patients, consistent with an epithelial phenotype and epithelial/mesenchymal phenotype. Additionally, longitudinal assessment of circulating tumor cell dynamics reflected progression of disease and response to treatment, providing important information not available by current tumor markers and imaging. These results suggest significant potential for circulating tumor cells as a new biomarker in PDAC. Moreover, other than just being a biomarker, circulating tumor cells can represent a window into the metastatic process. As each circulating tumor cell represents a unique clone, post-resection detection and analysis potentially provides a direct view of the clonal prevalence of subclinical minimal residual PDAC. Efforts are underway to further characterize the epithelial-to-mesenchymal transition of circulating tumor cells, which might help further understand the role these cells play in the dissemination of metastatic disease.[45,46] Additionally, achieving ex vivo culturing of circulating tumor cells for further genetic analysis and pharmacotyping for tumor response would greatly advance this field of study. However, culture of circulating tumor cells has proven very challenging with traditional culture methods, due to the rarity and the complex nature of these cells.[47,48] Novel techniques, such as three-dimensional culture with organoids, may prove useful for profiling patient circulating tumor cells in the near future.[49,50]

Perhaps no other liquid biopsy has generated more current enthusiasm than cell-free circulating tumor DNA. To date, however, studies on circulating tumor DNA have almost exclusively been performed in research settings. As a result, a joint review by the American Society of Clinical Oncology and College of American Pathologists concludes that the current evidence on the clinical validity and utility of circulating tumor DNA assays is insufficient to endorse the use of circulating tumor DNA in routine clinical practice.[51] To this effect, in **Chapter 14** we performed analytical and clinical validation of a *KRAS* circulating tumor DNA assay using digital-droplet PCR. Data from this longitudinal study (NCT02974764)[52] confirm the promise of circulating tumor DNA as a clinically useful prognostic biomarker in three clinical situations: (1) as a bad prognostic marker in either pre-pancreatectomy samples or (2) when persisting in the immediate post-operative period, and (3) during post-pancreatectomy follow-up as an accurate predictor of PDAC recurrence. Given the enthusing results, and since the research was performed in a Clinical Laboratory Improvement Amendments certified and College of American Pathology inspected clinical laboratory, this *KRAS* circulating tumor DNA assay is now being offered as an in-house clinical test in the Johns Hopkins Hospital (go live date, 4

December 2018).

Although circulating tumor DNA has prognostic value and reflects PDAC burden, future prospective interventional trials are needed to demonstrate if changing treatment based on circulating tumor DNA dynamics can improve patient outcomes. In PDAC, an example of such a trial could encompass starting patients on either FOLFIRINOX or Gemcitabine/Nab-Paclitaxel, and switching to the other regimen following a sustained spike in circulating tumor DNA indicating refractory disease. In the neoadjuvant setting, circulating tumor DNA analysis could help determine the appropriate timing and eligibility for surgery. Given that the presence of pre-pancreatectomy circulating tumor DNA is highly predictive of early recurrence of PDAC, one could envision a study where patients will not be resected until induction chemotherapy has resulted in consistent undetectable circulating tumor DNA. After completion of surgery and systemic therapy, rise of circulating tumor DNA in the absence of clinical or radiological signs likely indicates proliferation of minimal residual PDAC. In these patients, a study could be conducted where emergence of tumor-specific circulating tumor DNA triggers the start of systemic chemotherapy, regardless of a lack of imaging evidence of recurrence. Subsequently, later spikes in circulating tumor DNA or progression on imaging would prompt a switch to a different chemotherapy regimen. Apart from potential clinical studies, efforts should be made to develop and clinically validate multi-gene panel or next-generation sequencing circulating tumor DNA assays, since the current assay is limited to *KRAS* detection only. When considering novel multi-gene assays, it is important that they are able to match or approach the limit of detection of methods like digital-droplet PCR, something current next-generation sequencing methods have failed to accomplish.[42,53]

Differences in Recurrence Behavior Provide A Basis for Personalized Treatment Approaches

Management of recurrent PDAC is less well-established as it is for other stages of PDAC, despite that the majority of patients will experience disease recurrence after pancreatic resection. Currently, no high-quality prospective studies or clinical trials exist to guide the treatment of recurrent PDAC. Treatment of PDAC recurrence has historically been limited to best supportive care, with salvage chemotherapy generally offered to patients with adequate performance status. However, research presented in this thesis demonstrates that recurrent PDAC displays diverse behaviors based on recurrence timing and location. These differences potentially provide a basis for a more individualized approach to the treatment of recurrence. Particularly isolated local or pulmonary recurrence, which are associated with relatively favorable survival, might warrant more aggressive and/or localized additional treatment.

In **Chapter 15**, a systematic review of the literature establishes surgical re-resection,

chemoradiotherapy and stereotactic body radiation therapy as potential treatments of isolated recurrence of PDAC. Furthermore, the reviewed literature suggests that in these patients, treatment of local recurrence resulted in extended survival. **Chapter 16** demonstrates that stereotactic body radiation therapy can achieve pain palliation in more than 60% of patients with local recurrence with acceptable toxicity rates. Stereotactic body radiation therapy can deliver radiation more conformally than conventional radiotherapy in just three to five fractions, sparing organs at risk and other healthy tissues.[54] Furthermore, patients who received concurrent maintenance chemotherapy had better survival outcomes than those who did not. Future comparative studies are needed to assess the additional value of stereotactic body radiation therapy when compared with other treatment options for local recurrence, such as chemotherapy and conventional radiotherapy. Moreover, novel technologies such as MRI guided stereotactic radiotherapy might prove to be more accurate for tumor motion tracking and could further improve outcomes.[55]

Several studies have described favorable survival associated with pulmonary metastases from PDAC when compared with other recurrence sites.[56,57] Within this subpopulation of patients, however, it was unclear what factors are associated with improved survival. With data from the largest cohort to date, **Chapter 17** establishes several recurrence characteristics as risk factors for decreased post-recurrence survival, such as ≥5 pulmonary lesions, symptoms and elevated CA 19-9 ≥100 U/ml at time of recurrence. While controlling for these potential biology-associated risk factors in a multivariable model, further treatment of pulmonary recurrence remained significantly associated with improved post-recurrence survival. These results suggest that the improved survival in the treatment cohort was not due to selection bias alone, and that the therapeutic efforts might have played a role in the prolonged survival of these patients. Furthermore, patients who underwent pulmonary metastasectomy achieved a median survival of almost 70 months. There is precedent for metastasectomy in different neoplasms, especially for liver metastases from colorectal or pancreatic neuroendocrine cancer.[58,59] However, recurrent PDAC is particularly difficult to manage surgically, as it typically is characterized by multifocal and destructive spread. Therefore, pulmonary metastasectomy remains a non-standard and individual treatment approach, for which appropriate selection of patients with favorable tumor biology is paramount. Specifically, pulmonary resection should only be considered in patients with good performance status when pulmonary recurrence occurs after an extended recurrence-free interval, and if disease remains stable over time, or during a period of initial systemic treatment. To better identify patients who might benefit from more aggressive treatment for recurrence, future research should focus on elucidating underlying biologic mechanisms that might determine PDAC recurrence patterns and associated differences in survival.

Main Study Questions and Conclusions

Part I. Recurrence Following Primary Resection

Chapter 2 **What are the pattern, timing, and predictors of disease recurrence after a potentially curative resection for pancreatic cancer?**
Specific recurrence locations have different predictive factors and possess distinct recurrence-free survival curves, supporting the hypothesis that unique biological heterogeneity exists among pancreatic cancer leading to distinct recurrence patterns.

Chapter 3 **Do specific recurrence patterns impact survival of patients with resected pancreatic cancer?**
Specific patterns of recurrence are associated with different survival outcomes and have unique independent predictive values.

Chapter 4 **Does timing of recurrence impact survival outcomes and what is an evidence-based definition of early recurrence after resection of pancreatic cancer?**
Timing of recurrence impacts subsequent prognosis and a recurrence-free interval of 12 months is the optimal threshold for differentiating between early and late recurrence.

Part II. Recurrence Following Post-Neoadjuvant Resection

Chapter 5 **What is the survival of patients with locally advanced pancreatic cancer and how does surgical resection after neoadjuvant therapy impact patient outcomes?**
Surgical resection of locally advanced pancreatic cancer after neoadjuvant therapy is feasible in a highly selected cohort of patients (20%) and is associated with a median survival from diagnosis of 35 months.

Chapter 6 **What is the survival outcome of patients with borderline resectable or locally advanced pancreatic cancer who have a pathologic complete response following neoadjuvant chemoradiation?**
A pathologic complete response after neoadjuvant chemoradiation and resection is associated with a median survival of more than 60 months and is an independent prognostic factor for improved survival.

Chapter 7 **What are the incidence, timing, and implications of recurrence in patients who underwent neoadjuvant treatment and surgical resection of borderline resectable or locally advanced pancreatic cancer?**
Despite neoadjuvant therapy leading to resection and relatively favorable pathologic tumor characteristics in patients advanced localized disease, more than 80% of patients experienced recurrence, 72.5% of which occurs at distant sites.

Part III. Detection of Recurrence

Chapter 8 **What is the best available current evidence for frequency, methodology and focus of follow-up after pancreatectomy for pancreatic?**
Current evidence for frequency, methodology and focus of follow-up after pancreatic cancer resection is limited and contradictory.

Chapter 9 **What is the current practice of follow-up after pancreatectomy for pancreatic cancer among Dutch pancreatic surgeons?**
Lack of guidelines regarding follow-up results in different strategies among pancreatic surgeons in the Netherlands.

Part IIIa. Imaging Surveillance

Chapter 10 **What is the diagnostic performance of different imaging modalities for the detection of recurrent disease after surgery for pancreatic cancer?**
The diagnostic accuracy of post-operative CT imaging for the detection of recurrent disease is moderate. FDG PET-CT imaging could be of additional value when disease recurrence is suspected despite negative or equivocal CT findings.

Chapter 11 **How often patients undergo imaging during post-pancreatectomy follow-up in a setting where routine imaging is not performed?**
The majority of patients with resected pancreatic cancer undergo additional imaging procedures during symptomatic follow-up, even though this strategy does not include routine imaging.

Part IIIb: Biomarkers and Liquid Biopsies

Chapter 12 **What is the diagnostic value of serum tumor markers for the detection of recurrent pancreatic cancer during follow-up?**

Testing of serum CA 19-9 has some considerable limitations yet remains the most used serum tumor marker for follow-up after resection of pancreatic cancer in current clinical practice.

Chapter 13 **Do circulating tumor cell dynamics in patients with pancreatic cancer correlate with disease status and reflect progression of disease or response to treatment?**

Circulating tumor cell dynamics reflect disease status, response to treatment and can detect disease recurrence within two months during post-pancreatectomy patient follow-up.

Chapter 14 **What is the clinical validity and utility of a *KRAS* circulating tumor DNA assay tested in a Clinical Laboratory Improvement Amendments certified and College of American Pathology inspected clinical laboratory?**

Measurement of *KRAS* circulating tumor DNA in a certified laboratory and clinical setting can be used to predict recurrence and survival in patients with pancreatic cancer.

Part IV. Treatment of Recurrence

Chapter 15 **What are the treatment options and associated outcomes for patients with isolated local recurrence after resection of pancreatic cancer?**

In selected patients, re-resection, chemoradiotherapy or stereotactic body radiation therapy of isolated local recurrence following resection of pancreatic cancer are safe, feasible and associated with relatively good survival.

Chapter 16 **Is stereotactic body radiation therapy for patients with isolated local recurrence of pancreatic cancer safe and what are the palliative and survival outcomes?**

Stereotactic body radiation therapy for isolated recurrence therapy can improve pain and survival with acceptable acute and late toxicity. For those who receive stereotactic body radiation therapy, maintenance chemotherapy should be considered.

Chapter 17

What is the clinical course of patients with isolated pulmonary recurrence after resection of pancreatic cancer and what is the effect of patient factors and treatment on survival?

Isolated pulmonary is associated with a median survival of 40 months. Within this unique subpopulation, further systemic therapy and/or metastasectomy is associated with improved survival after recurrence in selected patients.

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APPENDICES

Nederlandstalige Samenvatting

In het komende decennium zullen ductaal adenocarcinomen van het pancreas (PDAC) naar verwachting de op één na meest voorkomende oorzaak van kanker-gerelateerde sterfte worden. Hoewel de chirurgische aspecten van pancreas resecties vrijwel zijn geperfectioneerd, blijven de overlevingsuitkomsten voor PDAC-patiënten die een resectie hebben ondergaan matig. Recidief kanker blijft het belangrijkste obstakel voor het genezen van gelokaliseerde PDAC na resectie. Om betere resultaten te bereiken voor deze patiënten, dienen we te begrijpen waarom een schijnbaar succesvolle operatie op deze manier faalt. Dit proefschrift beoogt inzicht te verschaffen in verschillende aspecten van recidiverende PDAC, inclusief het patroon en de timing van recidieven (Deel I), nieuwe implicaties van neoadjuvante therapie (Deel II), waarde van post-pancreatectomie beeldvorming en surveillance met biomarkers (Deel III) en de behandeling van recidieven (deel IV). Het volgende hoofdstuk bevat de bevindingen van de onderzoeken waaruit dit proefschrift bestaat worden samengevat.

DEEL I. Recidieven na Primaire Resectie

Hoofdstuk 2. Recidief Patronen na Pancreatectomie

Een beter begrip van PDAC-recidieven kan waardevolle inzichten bieden, onder andere bij het overwegen van geschikte strategieën met betrekking tot adjuvante behandeling, oncologische surveillance en het behandelen van een recidief. Uit deze grote en gedetailleerde studie van het Johns Hopkins ziekenhuis blijkt dat 76% van de recidieven zich op een distale locatie voordoet. Deze resultaten suggereren dat de meeste patiënten met PDAC radiologische occulte metastasen hebben op het moment van resectie. Bovendien bleken specifieke recidief patronen unieke ziektevrije overlevingscurves te hebben met specifieke voorspellende factoren. Al deze bevindingen zijn zeer suggestief voor uniek biologisch gedrag van tumoren binnen een bepaald recidief patroon.

Hoofdstuk 3. Gevolgen van een Recidief op de Overleving

De relatie tussen specifieke locaties van PDAC-recidieven en overleving is niet geheel bekend. De studie beschreven in dit hoofdstuk toont aan dat de overleving na resectie en overleving na een PDAC-recidief verschilt op basis van de anatomische locatie van een eerste recidief. Verder toont de huidige studie aan dat patiënten met een recidief op meerdere plekken een beperkte mediane overleving na recidiverende ziekte van <5 maanden hebben, terwijl patiënten die pulmonale metastasen ontwikkelden een mediane overleving hebben van >15 maanden na de eerste diagnose van een recidief. Bovendien is met behulp van twee verschillende multivariabele Cox-regressie analyses aangetoond dat specifieke recidiefpatronen

verschillende onafhankelijke prognostische waarden hebben. Deze bevindingen kunnen helpen bij de prognostische stratificatie en bij het maken van beslissingen over eventuele behandeling van een recidief.

Hoofdstuk 4. Het Definiëren en Voorspelling van Vroege Recidieven

Resectabele patiënten die resectie kunnen ondergaan maar risico lopen op vroege recidieven, vormen een belangrijk en interessant cohort. Deze patiënten hebben namelijk potentieel baat bij een benadering met neoadjuvante therapie. Deze studie laat zien dat de optimale afkapwaarde om te differentiëren tussen vroege en late recidieven, op basis van de daaropvolgende prognose, een ziektevrij interval is van ten minste 12 maanden. Daarnaast zijn onafhankelijke risicofactoren gevonden die de ontwikkeling van vroege PDAC-recidieven na resectie voorspelden, waaronder een pre-operatieve Charlson leeftijd-comorbiditeit index score van ≥4, tumorgrootte op de laatste pre-operatieve CT-scan >3.0cm en een CA 19-9 waarde van >210 U/mL. Bovendien zijn pathologische bevindingen van slechte tumordifferentiatie, microscopische lymfe en vasculaire invasie, een positieve lymfklier-ratio >0.2 en een post-operatieve CA 19-9-waarde van >37 U/mL onafhankelijk geassocieerd met vroege recidieven. Deze bevindingen suggereren dat de lengte van ziektevrije overleving klinisch bruikbaar zou kunnen zijn om het biologische gedrag van PDAC-recidieven te voorspellen.

DEEL II. Recidieven na Post-Neoadjuvantie Resectie

Hoofdstuk 5. Overleving na Resectie van Lokaal Niet Operabele Pancreaskanker

Een toenemend aantal patiënten met lokaal gevorderde pancreaskanker (LAPC) dat gunstig reageert op neoadjuvante therapie ondergaat vervolgens chirurgische resectie. Dit hoofdstuk levert een gedetailleerde kijk op de behandeling van patiënten met LAPC en identificeert een evident overlevingsvoordeel in een geselecteerd cohort dat resectie van de primaire tumor onderging (20%), in vergelijking met degenen bij wie dat niet mogelijk was (80%). Zoals eerder gesuggereerd kan de herbeoordeling van resectabiliteit na neoadjuvante behandeling ontoereikend zijn wanneer die slechts gebaseerd is op de behandelingsrespons op beeldvorming. Dit blijkt ook het geval te zijn in de huidige studie: slechts 12% van de gereseceerde patiënten heeft een gedeeltelijke of volledige respons volgens de RECIST-criteria. Een multidisciplinaire aanpak in grote centra is noodzakelijk om patiënten te identificeren die baat kunnen hebben bij een agressieve benadering van LAPC.

Hoofdstuk 6. Overleving na een Volledige Pathologische Respons

Een complete pathologische respons in PDAC is ongebruikelijk en komt voor in slechts 3-11% van gereseceerde patiënten na behandeling met neoadjuvante therapie. In deze studie heeft 10% van de PDAC-patiënten na een operatie een volledige pathologische respons na een neoadjuvante behandeling met chemotherapie en chemoradiatie. De mediane overleving van deze 19 patiënten is meer dan 60 maanden, wat ongeëvenaard is voor patiënten met PDAC. Ter vergelijking: de mediane overleving van patiënten met een bijna complete respons (gedefinieerd als een tumor van <1cm en negatieve lymfeklieren) is 27 maanden en die van patiënten met een beperktere respons 26 maanden. Deze bevindingen ondersteunen het gebruik van neoadjuvante chemoradiotherapie bij patiënten met lokaal gevorderde PDAC en het gebruik van een complete pathologische respons als eindpunt voor toekomstig onderzoek.

Hoofdstuk 7. Patronen van Recidieven na Post-Neoadjuvante Resectie

De incidentie, timing en implicaties van recidieven zijn nauwelijks beschreven voor patiënten die post-neoadjuvante resectie van lokaal gevorderde PDAC ondergaan. Hoewel in dit onderzoek lage percentages van positieve resectie-marges en lymfeklieren zijn waargenomen, treedt een PDAC-recidief toch op bij meer dan 80% van de patiënten. Verder treedt 72.5% van de recidieven op distale locaties op. Deze bevinding suggereert dat ondanks een radiografische progressievrije periode die tot resectie leidt, micrometastases ook na systemische neoadjuvante behandeling kunnen blijven bestaan. Ook blijken verschillende klinisch-pathologische en behandelingskenmerken geassocieerd te zijn met verschillende recidieflocaties en overleving van de patiënt. Deze gedetailleerde kennis over de timing, het patroon en de factoren die samenhangen met het terugkeren van PDAC na resectie kan clinici helpen met lastige beslissingen over de uitdagende multimodale zorg voor deze patiënten met gevorderde lokale ziekte.

DEEL III. Detectie van Recidieven

Hoofdstuk 8. Follow-Up na Resectie van Pancreaskanker

Aanbevelingen over follow-up na resectie van PDAC verschillen over de hele wereld. Vooral het belang van diagnostische post-operatieve follow-up staat ter discussie. Deze systematische review van de huidige literatuur toont aan dat de combinatie van een stijging van serum tumor markers en verdachte, progressieve bevindingen op beeldvorming zouden kunnen helpen bij de vroege klinische diagnose van een PDAC-recidief. Het huidige bewijs voor routinematige beeldvorming en het testen van tumormarkers tijdens de follow-up na resectie van de PDAC is echter beperkt en tegenstrijdig. Hierdoor ontbreekt kwalitatief hoogstaand bewijs om aanbevelingen te doen voor follow-up. Toekomstige, goed ontworpen prospectieve studies

zijn noodzakelijk om de klinische waarde, en de economische en psychosociale gevolgen van routinematige diagnostische follow-up na resectie van de PDAC te definiëren.

Hoofdstuk 9. Survey over Post-Pancreatectomie Follow-Up

De meest recente Nederlandse richtlijnen voor pancreaskanker bevatten geen aanbevelingen voor follow-up frequentie, methodologie of focus. De resultaten van deze enquête onder Nederlandse pancreaschirurgen tonen aan dat er een gebrek is aan een gestandaardiseerde benadering voor follow-up na resectie van PDAC. Bovendien legt deze studie de twijfels en onzekerheid bloot die bestaan over de rol van beeldvorming en het testen van tumormarkers tijdens de follow-up. Ook laat deze studie zien dat de huidige follow-up strategieën van Nederlandse pancreaschirurgen, in overeenstemming met Europese richtlijnen, voornamelijk bestaan uit regelmatige poliklinische bezoeken met lichamelijk onderzoek. Detectie van recidiverende PDAC tijdens de follow-up kan echter de aanwas van patiënten in nieuwe trials vergemakkelijken. Verder kunnen recidief gegevens nuttig zijn voor nieuw onderzoek. De hoge motivatie van Nederlandse pancreaschirurgen om deel te nemen aan een nieuw onderzoek voor de detectie en behandeling van recidieven lijkt dit te ondersteunen, waardoor een nationale studie haalbaar lijkt.

DEEL IIIa. Surveillance met Beeldvorming

Hoofdstuk 10. Diagnose van Pancreaskanker Recidieven met Beeldvorming

Radiologische informatie over de omvang en de locatie van recidief ziekte bepaalt vaak of een patiënt in aanmerking komt voor een aanvullende behandeling of deelname in klinische trials. Hiervoor worden verschillen typen van beeldvorming ingezet, zoals CT, MRI en FDG PET-CT. Deze systematische review biedt een overzicht van de literatuur over de diagnostische nauwkeurigheid van verschillende beeldvormingstechnieken. Volgens de literatuur heeft een CT met contrast een middelmatige diagnostische waarde met een sensitiviteit van 70% en een specificiteit van 80%; FDG PET-CT heeft een sensitiviteit en specificiteit van respectievelijk 88% en 89%, en voor FDG PET-CT in combinatie met contrast CT, is een sensitiviteit van 95% en specificiteit van 81% gevonden. Bij routinematige radiologisch surveillance waren de diagnostische nauwkeurigheidswaarden van CT en FDG PET-CT vergelijkbaar met nauwkeurigheid van beeldvorming die werd uitgevoerd op het moment dat een PDAC-recidief werd vermoed. Om surveillance strategieën te optimaliseren zijn nieuwe studies nodig die de diagnostische waarde van beeldvorming tijdens de follow-up én de gevolgen van vroege diagnose van een recidief op overleving en kwaliteit van leven beoordelen.

Hoofdstuk 11. Gebruik van Beeldvorming Tijdens Follow-Up

Conform de Europese richtlijnen bestaat de follow-up in het Universitair Medisch Centrum Utrecht uit regelmatige controles gericht op post-operatieve symptomen zonder beeldvorming voor de detectie van een recidief. Uit deze studie blijkt dat alhoewel beeldvorming expliciet geen deel uitmaakte van een symptomatisch follow-up protocol, de meerderheid van de patiënten (87%) in deze studie toch ten minste één keer aanvullende beeldvorming ondergaat voor de detectie van een recidief. Bovendien ondergingen patiënten gemiddeld 3 beeldvormingsprocedures gedurende de volledige follow-up-periode. De meeste recidieven (85%) worden in een laat stadium en na manifestatie van klinische symptomen gediagnosticeerd, waardoor slechts 17 patiënten (25%) aanvullende behandeling voor hun recidief hebben gekregen. In plaats van het uitstellen van beeldvorming totdat recidief-gerelateerde symptomen optreden, zouden deze 3 beeldvormingsprocedures theoretisch effectiever kunnen worden gebruikt in intervallen van 3 of 6 maanden. Een meer gestandaardiseerde follow-up zou asymptomatische recidieven eerder kunnen detecteren zodat patiënten een grotere kans maken om aanvullende behandeling te ondergaan.

DEEL IIIb. Biomarkers en Vloeibare Biopsies

Hoofdstuk 12. Tumormarkers voor Detectie van Recidieven

Een plotselinge verhoging van serum tumor markers kan duiden op een radiologisch occult recidief. Deze systematische review verschaft een overzicht van de bestaande literatuur over de diagnostische nauwkeurigheid van serum tumor markers voor de detectie van recidieven tijdens follow-up na resectie van PDAC. Volgens de bestudeerde literatuur heeft het routinematige testen van serum CA 19-9 tijdens de follow-up een matige effectiviteit voor het detecteren van een recidief met een sensitiviteit van 68-89% en een specificiteit van 77-89%. Slechts één studie rapporteert over de diagnostische nauwkeurigheid van CEA, met een lage sensitiviteit en specificiteit van respectievelijk 50% en 65%. In de literatuur zijn tot dusver geen andere serum tumor markers onderzocht voor follow-up na chirurgie voor pancreaskanker. Verdere studies zijn nodig om de diagnostische nauwkeurigheid van tumor markers voor pancreaskanker tijdens de follow-up en de rol van serum tumor marker testen voor de post-operatieve detectie van recidiverende pancreaskanker te beoordelen.

Hoofdstuk 13. Dynamiek van Circulerende Tumor Cellen

Circulerende tumorcellen (CTCs) hebben potentie als een moleculaire biomarker voor PDAC. Recente studies hebben aantal en fenotype van CTCs geassocieerd met vroege recidieven, overleving en aanwezigheid van occulte metastasen. De dynamiek van CTCs, of fluctuaties

van CTCs gedurende het verloop van tijd, is een veelbelovende longitudinale indicator van de ziektestatus van PDAC-patiënten. In een prospectieve longitudinale cohortstudie naar de dynamiek van CTCs (NCT02974764) zijn 200 patiënten met PDAC gevolgd met sequentiële bloedafnames gedurende hun ziekteverloop en behandeling. Twee belangrijke CTC-subtypes zijn geïdentificeerd: epitheliale CTCs en mesenchymale CTCs. Neoadjuvante systemische chemotherapie is geassocieerd met lagere CTCs aantallen en chirurgische resectie van de primaire tumor resulteerde in een significante afname van CTC-populaties. Verhoogde pre-operatieve CTCs en vooral CTCs met mesenchymale kenmerken zijn voorspellend voor vroege recidieven na resectie. Bovendien is bij PDAC-recidieven een toename van CTCs te zien, ongeveer twee maanden voor radiologisch bewijs van een recidief. Het potentieel van de CTCs als biomarker is evident en verdere diepgaande analyse van hun cellulair profiel is gerechtvaardigd.

Hoofdstuk 14. Klinische Significantie van Circulerend Tumor DNA

De potentie van circulerend tumor DNA (ctDNA) als een tumor-specifieke biomarker is in verscheidene onderzoek settingen gedemonstreerd. Ter voorbereiding op introductie in de kliniek is een klinische validatie van een *KRAS* ctDNA test uitgevoerd in een "Clinical Laboratory Improvement Amendments" (CLIA) gecertificeerd en "College of American Pathology" (CAP) geïnspecteerd klinisch laboratorium. Deze studie bevestigt het klinische nut van ctDNA als een tumor-specifieke biomarker voor prognostische stratificatie en het monitoren van tumorstatus bij patiënten met pancreaskanker. Ten eerste voorspelt detectie van gemuteerd *KRAS* ctDNA voorafgaand aan de operatie of in de onmiddellijke post-operatieve periode een hogere kans op een tumor recidief en een slechtere overleving. Ten tweede voorspelt stijgend gemuteerd *KRAS* ctDNA tijdens post-operatieve follow-up een radiologisch/klinisch recidief met een voorsprong van 84 dagen. Als gevolg van deze studie biedt het Johns Hopkins ziekenhuis deze *KRAS* ctDNA-test nu aan als een interne test (startdatum, 4 december 2018). Toekomstige prospectieve en interventionele studies zullen bepalen of behandelbeslissingen op basis van ctDNA de overleving van de patiënt kunnen verbeteren.

DEEL IV. Behandeling van Recidieven

Hoofdstuk 15. Behandelopties Voor Lokale Recidieven

Voor patiënten met recidiverende pancreaskanker is het van het grootste belang om een multidisciplinaire behandeling te vinden die de selectie van de patiënt optimaliseert, en tegelijkertijd ziektebestrijding, toxiciteit en kwaliteit van leven in balans houdt. Deze review laat zien dat behandeling van een geïsoleerd lokaal recidief kan worden uitgevoerd met aanvaardbare morbiditeits- en mortaliteitscijfers. Omdat gerandomiseerde gecontroleerde

onderzoeken niet bestaan, kan deze review re-resectie, chemoradiotherapie of stereotactische lichaamsradiotherapie (SBRT) niet aanbevelen als de beste behandelingsoptie voor patiënten met een lokaal recidief. In vergelijking met enkel ondersteunende palliatieve zorg lijkt verdere behandeling van een lokaal recidief echter een kans te bieden op verlengde overleving in geselecteerde patiënten. Concreet lijken patiënten met een langdurig ziektevrij interval het meeste gebaat bij een behandeling. Aangezien deze bevindingen zijn gebaseerd op kleinere cohortonderzoeken en patiënten series zouden verdere prospectieve onderzoeken zich moeten richten op het identificeren van andere klinische en pathologische kenmerken die voorspellen welke patiënten het meeste van deze therapeutische opties kunnen profiteren. Ook is meer onderzoek nodig naar een nauwkeurig en kosteneffectief surveillance-algoritme dat geschikt is om patiënten met een lokaal recidief te identificeren voordat ze metastasen ontwikkelen.

Hoofdstuk 16. Stereotactische Lichaamsradiotherapie voor Lokaal Recidief

Ondanks de hoge incidentie van lokale recidieven bij patiënten met PDAC is het bewijs beperkt waarop de behandeling is gebaseerd voor deze patiënten. SBRT vertegenwoordigt een relatief nieuwe behandelingsoptie voor PDAC. SBRT kan in slechts 5 dagen worden gegeven en meer kosteneffectief zijn dan conventionele radiotherapie; beide zijn belangrijke overwegingen voor PDAC-patiënten die vaak een slechte prognose hebben. In deze studie is symptomatische palliatie bereikt in 63% van de patiënten met een acceptabele behandelings-gerelateerde toxiciteit. Deze studie benadrukt ook verschillende factoren die verband houden met overlevingsresultaten. Patiënten met een ziektevrij interval van <9 maanden hebben een slechtere prognose na SBRT dan patiënten die later dan 9 maanden een recidief ontwikkelen. Patiënten die onderhoudschemotherapie naast SBRT krijgen, hebben een langere progressievrije en algehele overleving in vergelijking met patiënten zonder. Deze resultaten bieden aanwijzingen voor nieuwe onderzoeken waarin behandelingen direct met elkaar worden vergeleken. Verder suggereren deze resultaten dat, voor geselecteerde patiënten, de prognose relatief goed kan zijn na behandeling met SBRT en chemotherapie.

Hoofdstuk 17. Behandeling van Pulmonaire Recidieven

De literatuur suggereert een gunstige overleving voor patiënten met geïsoleerde pulmonale recidieven na resectie van PDAC in vergelijking met andere recidiefpatronen. Binnen dit cohort blijft het onduidelijk welke factoren samenhangen met goede uitkomsten. Deze studie presenteert een gedetailleerd onderzoek van gereseceerde PDAC-patiënten met een eerste recidief beperkt tot de longen. Geïsoleerde pulmonale recidieven komen voor bij 13% (n=96) van patiënten met een recidief en zijn geassocieerd met een relatief gunstige mediane

overleving van 40 maanden. Door de grootte van dit cohort is multivariabele analyse van prognostische factoren voor de overleving na recidiverende ziekte mogelijk. Verscheidene kenmerken van recidieven blijken risicofactoren te zijn voor verlaagde overleving na recidiverende ziekte. Daarentegen zijn een verlengd ziektevrij interval en verdere behandeling voor een recidief onafhankelijk geassocieerd met verbeterde overleving. Ten slotte is pulmonale metastasectomie uitgevoerd in een sterk geselecteerd cohort van 19 patiënten met als resultaat een mediane post-recidief en totale overleving van respectievelijk 35 en 69 maanden. Deze geïdentificeerde factoren en associaties hebben het potentieel om clinici te helpen met prognostische stratificatie en met het nemen van beslissingen over verdere behandeling van pulmonale PDAC-recidieven.

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Jaarclub Beluga. I have spent many Friday- and Saturday-evenings in Baltimore wondering what I was missing back home. Luckily, fantastic trips to Indonesia and Banff/Yellowstone/Grand Tetons compensated much. It has been a moving time for all of us, and I am glad to be amongst you again.

To the memory of **Joris Lenselink**. Dear Joris, I miss your laughter and wit every day. You would have appreciated this event, and would be one of the first to congratulate me. I am grateful for our last trip through Canada and the States together, memories which I will treasure forever. – "End? No, the journey doesn't end here. Death is just another path, one that we all must take. The grey rain-curtain of this world rolls back, and all turns to silver glass, and then you see it. White shores, and beyond, a far green country under a swift sunrise." - J.R.R. Tolkien.

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My brother **Olivier**. We are said to be twins yet are 3 years apart. It is not always easy to be the younger sibling, but I admire what you do and am proud of who you are. I have lost count of all the adventures and tennis matches I have had you by my side. You always inspire me to be my best self, and I am grateful to you for that.

Dear **Mom and Dad**, *liefste Mama en Papa*. Without your daily advice, invaluable support and unconditional love, this thesis would not have seen the light of day. You are the best parents Oli and I could wish for. You taught me to love reading, but I never thought that would result in writing my own book. This thesis is for you.

List of Publications

1. Daamen LA, **Groot VP**, Intven M, Besselink MG, Busch OR, Groot Koerkamp B, Haj Mohammad N, van Laarhoven HWM, Nuyttens JJ, Wilmink JW, van Santvoort HC, Molenaar IQ, Stommel MWJ. Postoperative Surveillance of Pancreatic Cancer Patients. *Eur J Surg Oncol*. June 2019. [Epub ahead of Print] doi: 10.1016/j.ejso.2019.05.031.

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3. **Groot VP**, Blair AB, Gemenetzis G, Ding D, Burkhart RA, Yu J, Borel Rinkes IH, Molenaar IQ, Cameron JL, Weiss MJ, Wolfgang CL, He J. Understanding Recurrence After Neoadjuvant Therapy and Resection of Borderline Resectable and Locally Advanced Pancreatic Cancer. *Eur J Surg Oncol*. April 2019 [Epub ahead of Print] doi: 10.1016/j.ejso.2019.04.007.

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Curriculum Vitae Auctoris

Vincent Groot was born on the 20th of April 1990 in The Hague. He spent his childhood in The Hague where he graduated from Gymnasium Haganum in 2008 and represented his year as valedictorian. In the following year, he attended Lewis & Clark College in Portland (Oregon, USA) to study Liberal Arts after receiving a Fulbright Campus Scholarship. During this year, he also played Division III varsity tennis and was selected in the Intercollegiate Tennis Association All-Academic and Northwest Conference Scholar-Athlete Honor Team (2008-2009).

After returning to The Netherlands, he started Medical School at Utrecht University in August 2009. During his studies he was an active member of several student associations. He also did two rotations abroad at "het Groote Schuur Hospital" (Cape Town, South Africa) and "het Wit-Gele Kruis" (Willemstad, Curaçao). As a student, he joined the research group of Prof. dr. I.Q. Molenaar at the Department of Surgical Oncology of the University Medical Center Utrecht, to do research on pancreatic cancer. For his final research project, Vincent moved to Baltimore (Maryland, USA) to work with Prof. dr. C.L. Wolfgang, Chief of Surgical Oncology of the Johns Hopkins Hospital. From June 2016 to December 2018 he coordinated a prospective longitudinal study investigating the dynamics of liquid biopsies in blood samples of patients with resected pancreatic cancer, and also graduated from Medical School (December 2016). During this time, he started a collaboration with Prof. dr. J.R.E. Eshleman, Associate Director of the Molecular Diagnostics Laboratory of the Johns Hopkins Hospital. As a result of this collaboration, a circulating tumor DNA assay was validated and is now being used as an in-house clinical test in the Johns Hopkins Hospital (go live date, 4 December 2018). Vincent was rewarded research grants and awards for his research from several institutions, including the "Prins Bernhard Cultuur Fonds", "Living with Hope Foundation", "Stichting de Drie Lichten", "VSB Fonds" and "Prof. Michaël van Vloten Fonds" among others. He has (co-)authored over 25 peer-reviewed articles and has presented his work at several national and international conferences, recently receiving the "Best Abstract Award" at the International Symposium on Pancreatic Cancer (Baltimore, 2018).

Vincent currently lives in Amsterdam and has started clinical work as a resident not in training at the Department of Surgery of the St. Antonius Hospital in Nieuwegein (April 2019).

Almond Blossom
Vincent van Gogh (1853-1890), Saint-Rémy-de-Provence, February 1890
Van Gogh Museum, Amsterdam

My first encounter with Vincent van Gogh's work was in May 1990 at the age of not yet one month when visiting the retrospective exhibition in Amsterdam and Otterlo to commemorate the centenary of his death. We share our first name but that is coincidence. I was named after both my granddads: Vincent Groot and Paul Wera. We also share our brotherly affection. My little brother Olivier has been and always will be my soul mate. Van Gogh painted his almond blossoms as a gift for his brother Theo on the occasion of the birth of his nephew and namesake. The delicate blossoms symbolize new life, new beginnings, and inspire hope. It is this sparkle of hope that drives all medical research, hope for progress in better health care to the benefit of the patients who put their trust in the medical profession.

The challenges of battling pancreatic cancer continue, and much more work needs to be done. Yet, the landscape appears to be changing, and for the multidisciplinary teams that treats this disease, the prospect of patients living longer and having a higher likelihood of cure is a very welcome sign. I hope that my research and that of my colleagues presented in this thesis will advance the pursuit of overcoming pancreatic cancer.

Utrecht, June 2019