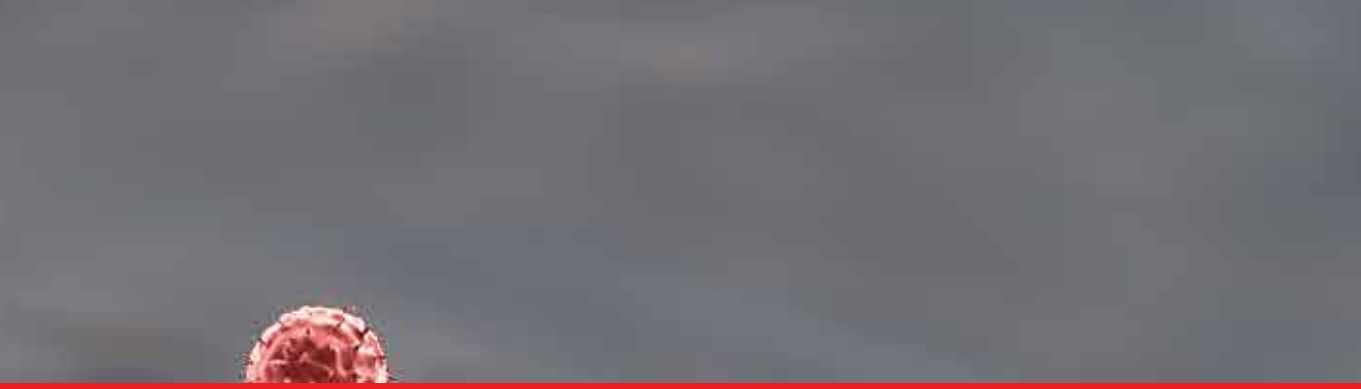

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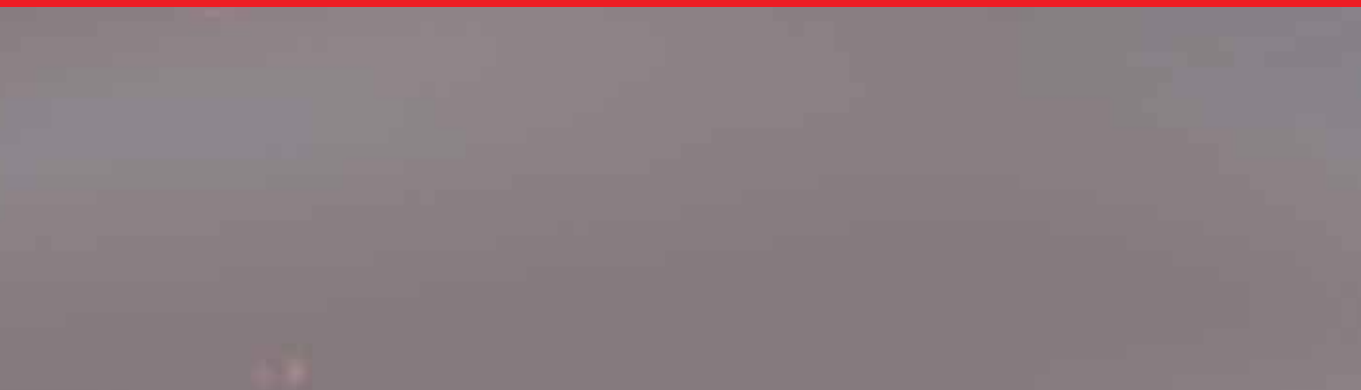

ADVANCES IN PANCREATIC CANCER

Edited by **Luis Rodrigo**

Advances in Pancreatic Cancer
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Meet the editor

Dr. Luis Rodrigo, MD, is an emeritus professor of Medicine at the University of Oviedo (Spain). He has been the chief of the Gastroenterology Service at HUCA in Oviedo for more than 40 years. He obtained his PhD degree in 1975 and developed a long teaching and research career. He has published a total of 575 scientific papers, 293 in English and the rest in Spanish. He participated as the main investigator in a total of 45 clinical trials and directed 40 doctoral theses. He contributed actively in the formation of around 100 specialists in Gastroenterology working in his hospital and other hospitals in Spain and abroad. He has written around 35 book chapters on several subjects and has been the editor of 22 books in his speciality and related diseases.

Contents

Preface

It is a great pleasure and honor for me to present this interesting book about pancreatic cancer, which has been written by an international group of experts, medical doctors, university professors, and basic researchers, all of them are actively working on this subject and of course with a great experience in the field.

This book presents the actual state of the pancreatic tumor, specifically referred to as adenocarcinoma of the pancreas because this is the more common primary malignant tumor and has achieved advances in diagnosis and treatment.

The incidence of pancreatic carcinoma has markedly increased over the past several decades and ranks as the fourth leading cause of cancer death all over the world. Despite the high-mortality rate associated with pancreatic cancer, its etiology is poorly understood. Risk factors for development of this kind of malignant tumor include a family history of pancreatic cancer, heavy cigarette smoking, obesity, chronic pancreatitis of long-standing evolution, and unknown factors.

Pancreatic cancer symptoms depend on the site of the tumor within the pancreas and the degree of tumor involvement. In the early stages of pancreatic cancer, there are not many noticeable symptoms. As the cancer grows, symptoms may include jaundice, light-colored stools or dark urine, pain in the upper or middle abdomen and back, weight loss for unknown reason, loss of appetite, and marked fatigue.

It is a tumor difficult to detect and diagnose for the following reasons. There are no noticeable signs or symptoms in the early stages of pancreatic cancer. The signs when present are like the other found in many other benign illnesses, such as chronic pancreatitis or peptic ulcer. The pancreas is obscured by other organs in the abdomen and is difficult to visualize clearly on imaging tests. To appropriately treat pancreatic cancer, it is crucial to evaluate whether the cancer can be resected.

The use of imaging technology may aid in the diagnosis of pancreatic cancer and in the identification of patients with disease that is not amenable to resection. Imaging tests that may be used include helical computed tomographic (HCT) scan, magnetic resonance imaging (MRI) scan, and endoscopic ultrasound (USE). Minimally invasive techniques, such as laparoscopy and laparoscopic ultrasound, may be used to decrease the use of unnecessary laparotomy.

No tumor-specific markers exist for pancreatic cancer; markers such as serum cancer antigen (CA) 19-9 have a low specificity. Most patients with pancreatic cancer will have an elevated CA 19-9 at diagnosis. Following or during a definitive therapy, an increase in CA 19-9 levels may identify patients with progressive tumor growth. The presence of a normal CA 19-9, however, does not preclude recurrence.

Primary factors that influence prognosis are whether (a) the tumor is localized and can be completely resected and (b) the tumor has spread to lymph nodes or elsewhere. Exocrine pancreatic cancer is rarely curable and has an overall survival (OS) rate of less than 6%. The highest cure rate occurs when the tumor is truly localized to the pancreas; however, this stage of the disease accounts for less than 20% of cases. For patients with localized disease and small cancers (<2 cm) with no lymph node metastases and no extension beyond the capsule of the pancreas, complete surgical resection is associated with an actuarial 5-year survival rate of 18–24%.

Surgical resection is the mainstay of curative treatment and provides a survival benefit in patients with small, localized pancreatic tumors. Patients with unresectable, metastatic, or recurrent disease are unlikely to benefit from surgical resection. Pancreatic tumors are resistant to treatment with chemotherapy and radiation. Patients with any stage of pancreatic cancer can appropriately be considered candidates for clinical trials because of the poor response to chemotherapy, radiation therapy, and surgery as conventionally used.

Palliation of symptoms may be achieved with conventional treatment. Palliative measures that may improve quality of life while not affecting OS include surgical or endoscopic or radiologic biliary decompression, relief of gastric outlet obstruction, pain control, and psychological care to address the potentially disabling psychological events associated with the diagnosis and treatment of pancreatic cancer.

Finally, I would like to thank all the authors for their excellent contributions and the Intech Editorial Team, especially Ms. Marijana Francetic for her continuous support and superb and constant help during the whole editorial process.

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Section 1

Etiopathogenesis and Diagnosis

Chapter 1

Systemic Inflammatory Response in Pancreatic Ductal Adenocarcinoma

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Additional information is available at the end of the chapter

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Abstract

Pancreatic ductal adenocarcinoma induces systemic inflammatory response (SIR), which can be assessed either by ratios between blood cell counts (neutrophil to lymphocyte ratio, NLR; platelet to lymphocyte ratio, PLR) or concentrations of acute phase proteins, clotting factors and albumins. These tests are biologically justified by multiple events including bone marrow activation, development of immune-suppressing immature myeloid cells, generation of pre-metastatic niches and neutrophil extracellular trap formation from externalised DNA network in bidirectional association with platelet activation. Despite biological complexity, clinical assessment of SIR is widely available, patient-friendly and economically feasible. In this chapter, we present a review on NLR, PLR, Glasgow prognostic score and fibrinogen, recently reported to have a prognostic role regarding overall survival, cancer/progression free and cancer-specific survival in early and advanced pancreatic ductal adenocarcinoma. Practical consequences abound, including preference for surgical or combined, active or sparing treatment, as well as prediction of non-resectability or chemotherapy response. In this chapter, we also scrutinise the main controversies including different cut-off levels, hypothetic correlation with tumour burden and morphology, negative findings and discussions on the best marker. Future developments should include elaboration of complex scores as will be described here.

Keywords: pancreatic ductal adenocarcinoma, systemic inflammatory response, neutrophil to lymphocyte ratio, NLR, platelet to lymphocyte ratio, PLR, Glasgow prognostic score, fibrinogen

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is known for notoriously difficult early diagnostics, almost complete lack of well-defined risk groups for targeted surveillance and poor response to treatment in advanced stages. Thus, PDAC remains among the most challenging cancers for medical professionals today. By incidence, pancreatic cancer was estimated to be the 12th most frequent malignant tumour worldwide in the year 2012. However, it ranked seventh in the global estimates of oncological mortality for the same year. The dismal prognosis is reflected in the high mortality-to-incidence ratio reaching 0.98 [1]. Pancreatic cancer encompasses 2.4% of global cancer incidence and 4.0% of cancer-attributable death cases in the world. Even more, it was predicted to be the fourth leading cause of oncological mortality in Europe, comprising 6% of cancer-induced death events in 2017 [2]. Considering the growing incidence, that might be attributable to the epidemic of obesity and metabolic syndrome, and low 5-year survival rate (6%), USA research teams have generated prognosis that pancreatic cancer might become the second most common cause of oncological mortality by the year 2030 [3].

PDAC is responsible for the bulk of pancreatic cancer burden as it is the most common and aggressive pancreatic tumour [4]. The overall survival and long-term survival rates of patients diagnosed with PDAC generally have not improved in last 30 years, despite multiple innovations in the surgery, including resection of multiple organs; anaesthesia; patient referral for surgery in accordance to surgeon's experience and the excellence of medical team/centre; molecular studies and trends towards personalised treatment as well as appearance of new drugs [3–5].

Currently, pTNM is the mainstay for pancreatic cancer staging [4]. Molecular portrait is important to select targets for personalised treatment. It can have a prognostic role as well. However, the current situation forces to look for additional prognostic factors in PDAC, aiming to stratify patient groups by the predicted treatment response or to adjust the necessary treatment intensity in order to improve the survival or life quality.

Recently, systemic inflammatory response (SIR) has been highlighted in different cancers, including PDAC [6–8]. The network of SIR involves cancer microenvironment, bone marrow and metastatic sites, manifesting as the changes of blood cell counts and ratios as well as blood levels of acute phase proteins. SIR encompasses complex interactions between at least three players: the tumour, the innate and adaptive immunity of the host and the distant tissues.

In SIR, the altered functions of bone marrow lead to switches in production and release of inflammatory cells, including neutrophils. Consequently, blood counts of neutrophils increase and immature myeloid derived suppressor cells (MDSC) appear in the peripheral blood.

Neutrophils develop in bone marrow, and 90% of the mature cells remain there until activating stimulus ensures rapid release in appropriate situations. In cancer-induced SIR, neutrophils are ejected from bone marrow in response to colony-stimulating factors that are produced by the malignant cells. In addition, neutrophil response is incited by tissue damage caused by cancer invasion and/ or by tumour necrosis due to hypoxia and insufficient blood supply in the core of growing mass. The colony-stimulating factors influence also the CXCR2/CXCR4 chemokine axis that is responsible for the circulation of neutrophils in

Class	Summary activity	Features and mechanisms	References
N1 neutrophils	Anti-tumour	Cytotoxic, capable to kill cancer cells: high levels of ROS immunostimulatory:	[9]
		• high levels of Fas, TNF alpha, CCL3, ICAM1;	
		• low activity of arginase	
		• lead to activation of T cells	
N2 neutrophils	Pro-tumour	Lack significant cytotoxic activity	[3, 9]
		Imunosuppressive: high activity of arginase	
		Angiogenic: vascular endothelial growth factor (VEGF)	
		Facilitate invasion: matrix metalloproteinases (MMP) 8, MMP9	
M1 macrophages	Pro-inflammatory	Restrict cancer growth	[3]
		Produce pro-inflammatory cytokines TNF alpha, IL-1, IL-6, IL-12, IL-23	[10]
		Express MHC	
		Produce NO synthase	
		Enhance antigen presentation to T lymphocytes	
M2 macrophages	Anti-inflammatory	Promote cancer growth	[3]
		Immunosuppressive: secret IL-10, arginase, transforming growth factor beta	[10]
		Down-regulate MHC class II	
		Facilitate angiogenesis	
		Promote cancer cell migration	

Table 1. The subtypes of neutrophils and macrophages.

accordance to cellular maturity and life cycle: retention of immature myeloid cells in the bone marrow, release of mature cells upon necessity and return of ageing neutrophils that must be destroyed. Consequently, the neutrophil counts in blood of cancer patients increase, and there can be a shift to immature cell release.

In tumour microenvironment, neutrophils can differentiate towards either anti-cancerous N1 or pro-cancerous N2 phenotype (**Table 1**). These subtypes are considered to represent the end points of the activity spectrum, but any neutrophil can exhibit combined traits of both subtypes. Transforming growth factor beta is known as a potent mediator of N2 differentiation [9].

Neutrophils are capable to facilitate the metastatic spread of PDAC. Clusters formed by neutrophils and circulating cells of pancreatic ductal adenocarcinoma have been observed in peritumoural blood vessels. Further, significant relationship was found between neutrophil-characterising blood indices (neutrophil to lymphocyte ratio) and distant metastasis after curative surgery [11]. These clinical observations are explained by a complex network of pathogenetic events. Neutrophils can promote tumour cell proliferation and invasion (see **Figure 1**), as well as enhance angiogenesis and increase vascular permeability. Neutrophils also represent the main cell population involved in the formation of pre-metastatic niche

before malignant cells arrive to the site of metastasis. In the pre-metastatic niches, neutrophils and immature bone marrow-derived cells gather in clusters that ensure tumour cell homing. When circulating tumour cells reach the 'prepared' metastatic site, neutrophils anchor cancer cells to the endothelium, facilitating trans-endothelial migration and invasion. Indeed, malignant cells entrapped in distant organs produce cytokines to attract neutrophils. The classic inflammation-related adhesion molecules, including integrins, can promote cancer cell adhesion [9]. Thus, interleukin-induced expression of ICAM1 has been shown to support the extravasation of malignant cells and pathogenesis of the PDAC metastasis [12]. Leukotrienes, secreted by neutrophils, further promote tumour cell proliferation and growth of the metastasis [9]. To enhance carcinogenesis, neutrophils act in concert with macrophages, similarly to the parallel effects of MDSCs and M2 macrophages. For instance, bone marrow-derived macrophages are involved in the generation of premetastatic niches by pancreatic cancer exosomes [13].

Within the framework of SIR, neutrophils derive unique structures—neutrophil extracellular traps (NETs). NETs represent a mesh of chromatin and nuclear proteins [9]. These structures possibly have evolutionary developed as a mechanism of antimicrobial response. In cancer patient, NETs can wrap a circulating tumour cell, resulting in either reactive oxygen species (ROS)-mediated destruction or facilitated adhesion in a pre-metastatic niche. NETosis evolves in different stressful conditions, including pre-eclampsia, major surgery or surgical infection. Consequently, surgery is not only a mechanical tool to withdraw the tumour from the body, but it can also become a major immunologic switch. Prolonged or complicated surgical intervention might threaten patient's life directly but also through SIR-associated pathways. Indeed, surgical stress or postsurgical infection is shown to facilitate metastatic spread, and NETosis is demonstrated in these conditions [9]. SIR-based molecular events highlight the association between infection or surgery-induced inflammation [14, 15] and recurrence or metastatic spread of the cancer.

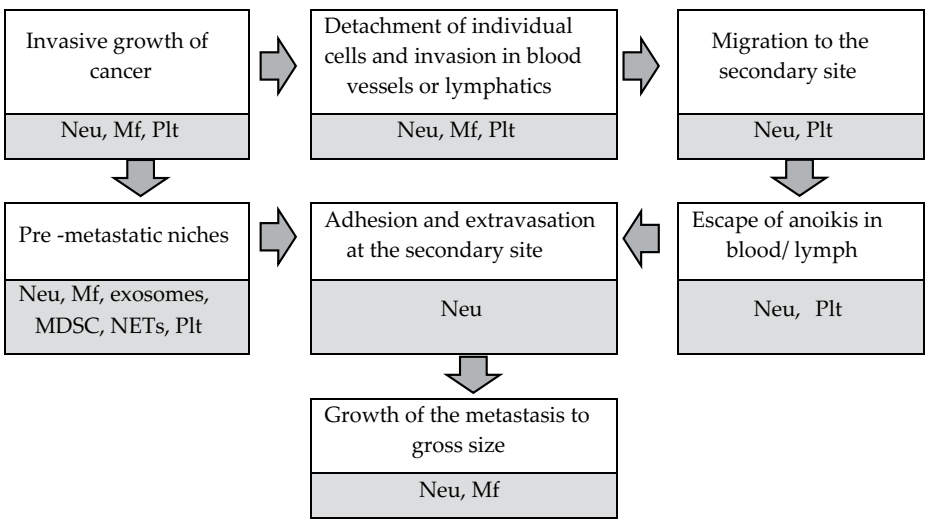

Figure 1. The main pathogenetic events in metastatic dissemination of cancer. Abbreviations: Neu, neutrophils; Mf, macrophages; Plt, platelets; MDSC, myeloid derived suppressor cells; NETs, neutrophil extracellular traps.

Neutrophils along with macrophages and other innate immunity cells are considered to have predominantly pro-tumourous activity, contrasting with adaptive immunity (lymphocytes) having protective role. However, this assumption is not straightforward—N1 neutrophils exhibit contra-cancer activity. Type I interferons can convert neutrophils into anti-tumourous fighters with rich armoury: enhanced production of ROS, suppressed ability to form pre-metastatic niches, upregulated ROS-mediated killing of NET-trapped cancer cells, active direct cytotoxicity (via ROS or antibody-dependent cell-mediated cytotoxicity) and improved capacity to stimulate adaptive immunity [9].

MDSCs represent heterogeneous population of immature cells (namely, the precursors of granulocytes, macrophages, monocytes and dendritic cells) sharing immunosuppressive function and myeloid origin. These cells express wide spectrum of enzymes, inflammatory mediators as well as reactive oxygen species and/or reactive nitrogen intermediates [3]. MDSCs travel via blood from their site of origin in bone marrow to the tumour and to peripheral tissues. In the cancer microenvironment, MDSCs along with M2 macrophages (see **Table 1**) exert immunosuppressive effect [16]. Within the complex immunosuppressive network of events in tumours stroma, MDSCs suppress the activity of CD8-positive T lymphocytes; induce T-cell apoptosis by ROS and nitric oxide derivatives; promote T-cell anergy via regulatory T lymphocytes; inhibit T cell migration via nitration of chemokines and T-cell receptors; block interferon (IFN) gamma pathway and cleave arginine and cysteine via upregulated arginase. The IFN gamma, arginine and cysteine are essential for T lymphocyte activity. In addition to the anti-T cell activities, MDSCs block the M1 phenotype of tumour-infiltrating macrophages. Production of pro-inflammatory interleukin (IL) 6 by MSDCs promote JAK/STAT mediated pathways stimulating cancer cell proliferation, survival and evasion from antigen presentation to dendritic cells [3]. In distant tissues, immature myeloid cells participate in the generation of pre-metastatic niches [14, 17].

The activities of neutrophils and MDSC in tumour stroma are carried out in cooperation with tumour-infiltrating macrophages. Macrophages are recruited by cancer-produced signal molecules, including cytokines and growth factors, as well as by tumour necrosis. In cancer microenvironment, macrophages acquire M2 differentiation and can enhance tumour progression, angiogenesis and metastatic spread [10]. M2 macrophages along with MDSC are immune suppressors in the cancer stroma [16]. In distant tissues, macrophages assist in the creation of premetastatic niches. As noted, this mechanism has been demonstrated in PDAC: bone marrow-derived macrophages are involved in the generation of premetastatic niches by pancreatic cancer exosomes [13].

In turn, lymphocytes mostly play a defensive role against cancer in the whole body and in tumour microenvironment [10].

The pathogenetic association between PDAC and activated blood clotting is acknowledged for centuries, reflected by the historic descriptions of migratory thrombophlebitis, also known as Trousseau syndrome. The related clinical events include thromboembolism and nonbacterial thrombotic endocarditis in cancer patients, occasionally manifesting as the first sign of malignant disease [18]. In peripheral blood, platelet counts increase in response to local cancer invasion causing endothelial damage. The platelet response is also generated by the pro-inflammatory cytokines (IL-1, IL-3 and IL-6) that are produced by the cancer and promote megakaryocyte development [8]. Thrombocytosis has been observed in 15.2% of PDAC patients [19].

Parameter/score	Definition
NLR	Ratio between the absolute counts of neutrophils and lymphocytes in the peripheral blood
PLR	Ratio between the absolute counts of platelets and lymphocytes in the peripheral blood
Glasgow prognostic score	
0	CRP < 10 mg/L AND albumin ≥35 g/L
1	One high-risk finding: CRP ≥ 10 mg/L OR albumin <35 g/L
2	Both high-risk findings: CRP ≥ 10 mg/L AND albumin <35 g/L
Modified Glasgow prognostic score	
0	CRP ≤ 10 mg/L irrespective of albumin level
1	Increased CRP on the background of normal albumin level: CRP > 10 mg/L AND albumin ≥35 g/L
2	Increased CRP and hypoalbuminemia: CRP > 10 mg/L AND albumin <35 g/L

Abbreviation: CRP, C-reactive protein.

Table 2. Parameters of systemic inflammatory reaction.

Locally, platelets promote angiogenesis, invasion, production of growth factors and adhesion molecules [8]. Platelets facilitate metastatic spread by creating clusters with circulating tumour cells and protecting them from immune surveillance, promoting the development of pre-metastatic niches and tumour cell attachment to distant tissues. Along with the metastatic spread, platelets are suggested to have a major role in epithelial-mesenchymal transition—process during which epithelial malignant cells change the phenotype to mesenchymal-like, plastic cells with enhanced capability for invasion into connective tissues, blood and lymphatic vessels as well as metastatic spread [8].

Considering pathogenetic and prognostic role of the interaction between tumour and host inflammatory response, systemic inflammatory response has recently become a hot topic in medical research. Several indices are elaborated to evaluate SIR (**Table 2**). Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and Glasgow prognostic score (GPS) represent the best-known examples.

Although almost all immune and inflammatory cells can have dual effects in cancer, neutrophils mainly act as tumour promoters while lymphocytes represent the protective innate immunity. Thus, NLR represents the balance between pro- and contra-tumourous immune and inflammatory processes of the host. Similarly, activation of blood clotting is associated with burden of invasive tumour, that damages endothelium like 'dozen of sharp knives', and platelets also facilitate the further development and spread of the cancer while lymphocytes exhibit protective action. Hence, PLR is another measure of equilibrium between pro- and contra-tumourous events within SIR. GPS reflects the upregulation of acute phase protein (measured by the prototypic C-reactive protein) and degree of catabolism by hypoalbuminemia. In addition, combined inflammation-based scores have been proposed, derived from combinations of SIR-related factors in order to reach higher prognostic value.

Considering high mortality and poor treatment results of pancreatic ductal adenocarcinoma and the need for prognostic and predictive novelties, this chapter scrutinises the assessment of SIR in PDAC, potential practical implementations and restrictions of those parameters.

2. NLR in pancreatic ductal adenocarcinoma

Neutrophil to lymphocyte ratio is calculated as the ratio between the count of neutrophilic leukocytes and lymphocytes in peripheral blood, using the values detected in a routine full blood count. Hence, the parameter is easily available, especially in carefully examined cancer patients, and economically nondemanding. In fact, sufficient awareness and algorithm for interpretation are the only prerequisites to obtain an additional piece of information from routine blood tests. At present, the association between NLR and different aspects of survival, for example, overall, recurrence free or cancer-specific survival, remains one of the best substantiated aspects in the SIR research in cancer.

2.1. NLR and survival

The prognostic importance of NLR is shown over the whole course of PDAC and is applicable to wide treatment spectrum—from surgically resectable early cases to advanced or metastatic tumours eligible only for non-surgical treatment. Several research teams have demonstrated independent prognostic value of NLR, confirmed by multivariate analysis. In few studies, the association with survival is confirmed by univariate but not multivariate analysis. Some of the reports are on better scores, for example, Glasgow prognostic score had higher informativity in the study performed by Yamada et al. [20].

Although only a minor fraction (around 20%) of pancreatic cancers are amenable to surgery, surgical removal of tumour is highly advisable, if feasible because surgery provides the only definitive cure [5]. Pre-treatment NLR has been evaluated as a prognostic factor for surgically treated PDAC patients, mostly with positive findings. Thus, in a large cohort of 442 patients subjected to pancreatic resection for PDAC, high NLR was associated with significantly lower median survival. The difference was also biologically important: only 12.6 months in those presenting with high NLR (defined in this study by receiver operating characteristics (ROC) curve analysis as ≥5) patients versus 25.7 months in patients having low NLR. Cox proportional hazards analysis confirmed NLR as an independent prognostic factor, associated with hazard ratio (HR) 1.66; 95% confidence interval (CI): 1.12–2.46; $p = 0.012$ [21]. In a small group of 46 patients subjected to pancreaticoduodenectomy, high NLR (≥2.5) was associated with lower overall survival rate. In addition, it predicted surgical complications worse than Clavien-Dindo grade 3 [22]. Among 381 patients treated by curative resection of PDAC, high NLR (≥2) was significantly and independently associated with overall survival [23]. The prognostic value was especially clear in stage I/II [24]. In 110 surgically treated pancreatic cancer patients, high NLR (≥5) was an independent prognostic factor for worse cancer-specific survival, as confirmed by $p < 0.039$ [25].

Standard preoperative assessment of NLR is recommended in cases of borderline resectable pancreatic cancer by consensus statement by the International Study Group of Pancreatic Surgery [26].

In most studies, preoperative NLR has been assessed. However, the patient's immune status after the surgery might be as important. Indeed, postoperative NLR, evaluated 1 month after the surgery, was shown to have a prognostic value for overall and recurrence-free survival. Comparing the patients with NLR ≥ 3 versus NLR < 3.0, the 1-year survival rate was 42.6 versus 81.9% and 3-year survival rate: 7.3 versus 33.9% ($p < 0.001$). Notably, the differences were confirmed to be statistically significant despite relatively small study group comprising 86 patients [27].

However, negative observations have also been reported. In a reliably large study group of 217 surgically treated PDAC patients, overall survival was significantly associated with age, adjuvant treatment, cancer invasion in blood vessels and lymphatics, R1 and pTN while NLR was not predictive of OS [28]. Assessing 379 consecutive patients who underwent curative resection for pancreatic cancer, no significant differences in overall survival were found in patients showing high versus low NLR although another SIR marker, the Glasgow prognostic score was confirmed as a significant predictor of survival [20].

A meta-analysis of eight studies including 1519 patients with resectable pancreatic cancer has been recently carried out by Mowbray et al. [29]. The pooled data confirm association between high NLR and low overall survival: HR = 1.77; 95% CI: 1.45–2.15; $p < 0.01$ [29]. In a systematic review of resectable pancreatic cancer, 10 studies were eligible and 8 had reported NLR. Significant association with survival was found in three of them [5].

The prognostic value of NLR has also been investigated in advanced and metastatic PDAC cases. In a large cohort of patients (497) diagnosed with locally advanced pancreatic cancer and treated by neoadjuvant or definitive chemoradiotherapy, elevated NLR was significantly associated with worse 1-year overall survival and 1-year progression free survival rates. In this study, the median value was selected as the cut-off threshold. Patients presenting with low NLR (<1.89), had 1-year survival rate of 73.2% and 1-year progression free survival rate of 43.9%, contrasting with those having high NLR (≥1.89): 60.8% survived at least 1-year and 31.3% were free of progression at least for 1-year (both $p < 0.001$) as reported by Lee et al., [30]. In advanced pancreatic cancer, high NLR was a significant independent prognostic marker for shorter survival. The median survival was 2.6 versus 8.5 months in patients having NLR ≥ 5 versus NLR < 5 [31]. In 132 patients who underwent chemotherapy for advanced pancreatic cancer, high baseline NLR (>2.78, based on ROC) and high value after two cycles of chemotherapy were associated with lower overall survival. In addition, even worse prognosis was identified in patients who had both these factors. The overall survival was 15.2 months in patients who had low baseline NLR and did not experience the increase of NLR after two cycles of chemotherapy, but only 3.8 months in those having both undesirable factors: high NLR and increase during treatment. If only baseline NLR was high, the survival was 7.6 months. If only NLR increase was observed, the survival was 6.8 months [32]. High NRL retained a prognostic value in elderly (at least 75 years of age) patients who underwent chemotherapy for unresectable PDAC [33].

Interesting findings have been reported on NLR in patients who underwent preoperative chemoradiotherapy followed by complete surgical resection. Such research design allows

morphological evaluation of the response to preoperative treatment. Poor response was associated with higher pre-treatment NLR [34]. To predict efficacy of chemotherapy, both pre-treatment NLR and its dynamics are considered important. Thus, low baseline NLR and low NLR after first-line chemotherapy were associated with higher efficacy of chemotherapy [8] in parallel to the abovementioned study [32].

In patients receiving stereotactic radiotherapy for advanced PDAC, high NLR (>5) was associated with significantly shorter median survival: 6.9 versus 8.5 months; $p = 0.0057$ [35].

Again, the prognostic role of NLR in advanced pancreatic cancer has not always been confirmed. In 122 patients, undergoing chemotherapy for inoperable pancreatic cancer, both high and low NLR was associated with the same median survival of 10 months. In the same study, the dynamics of NLR still predicted outcomes although the biological difference was tiny: the median overall was 10 versus 11 months; $p < 0.001$. The dynamics was assessed as the ratio of pre-treatment NLR versus NLR after first-line chemotherapy. Changes in NLR predicted the efficacy of chemotherapy and the outcome [8].

The prognostic value of NLR is retained in metastatic PDAC. In treatment-naïve patients diagnosed with metastatic PDAC, NLR was significantly associated with survival, and multivariate analysis identified NLR as an independent prognostic factor. In addition, NLR also predicted the efficacy of oxaliplatin treatment [36]. In 39 patients with locally advanced unresectable and metastatic PDAC treated with gemcitabine and paclitaxel, higher NLR was associated with lower overall survival [37]. In similar bur larger study group comprising 261 patients with inoperable pancreatic cancer (both metastatic and locally advanced cases), high NLR (≥5) was an independent prognostic factor for worse cancer specific survival, as confirmed by $p < 0.001$ [25]. High NLR was associated with worse overall survival in the general group of PDAC patients, in metastatic cases and in those who had distant metastasis but also received chemotherapy [38]. Radiofrequency ablation (combined with systemic chemotherapy) for hepatic oligometastatic pancreatic cancer was associated with worse survival in patients having elevated NLR (≥2.5). In this clinical situation, NLR was confirmed as an independent predictive factor, along with cancer location in pancreatic head and diameter of the metastasis [39].

In 306 patients receiving palliative chemotherapy, NLR ≥ 5 was associated with shorter overall survival. In addition, multivariate analysis identified the independent predictive value of NLR [40]. Similarly, prognostic value of NLR in patients receiving palliative chemotherapy was reported by Xue et al., [41]. In patients undergoing gastroenterostomy for advanced pancreatic cancer, high NLR (≥4) was associated with shorter survival: 3.4 versus 9.4 months; $p < 0.001$ [42]. Thus, low NLR might be useful to identify those who have higher benefit from palliative surgery.

Several meta-analyses have been devoted to NLR in pancreatic cancer. Zhou et al. [43] carried out a meta-analysis of 43 cohort studies containing 8252 patients and concluded that high NLR was significantly associated with worse overall survival (hazard ratio (HR) = 1.81; 95% confidence interval (CI): 1.59–2.05; $p < 0.001$) and cancer-free survival: HR = 1.66; 95% CI:1.17–2.35; $p = 0.005$ [43]. Similar findings were reported by Cheng et al. [44].

2.2. NLR and cancer burden

2.2.1. NLR and local tumour features: pT and other traits

If NLR in particular and SIR in general are mostly dictated by the events in cancer stroma, NLR should correlate with cancer burden, reflected by pT or cancer size. However, the data are controversial. Thus, in a study of 442 patients undergoing surgical treatment for pancreatic cancer, there was no association between NLR and tumour size or pT. No correlation was found with perineural invasion, involvement of resection margins, and invasion into blood or lymphatic vessels [21]. In contrast, high NLR (≥2) was significantly associated with pT and grade among 381 patients treated by curative resection [23]. In a recent meta-analysis of 8252 cases, lower NLR was observed in patients having smaller ($p = 0.0007$), better differentiated ($p = 0.003$) tumours at earlier ($p = 0.02$) stage [43].

2.2.2. NLR and regional lymph node involvement: pN

The association between NLR and regional lymph node status also is controversial. While some research teams have observed higher NLR values in patients affected by tumour metastases in regional lymph nodes, other studies have not confirmed these findings. NLR was associated with lymph node metastasis in the study of 159 surgically treated PDAC patients [45]. Similarly, high NLR (≥2) was significantly associated with pN among 381 patients treated by curative resection [23]. In contrast, no correlation was found between NLR and cancer spread to regional lymph nodes reflected by pN or with invasion into lymphatic vessels by Sierzega et al. [21], evaluating 442 surgically treated patients. In Austrian cohort of 110 surgically treated pancreatic cancer patients, NLR lacked correlation with stage [25].

2.2.3. NLR and presence of distant metastasis: pM

In contrast to the previous aspects of tumour burden, namely, pT, size or pN, there is almost general agreement that pM1 is associated with higher NLR.

In patients diagnosed with unresectable pancreatic cancer, high pre-treatment NLR significantly correlated with presence of liver metastases [8]. Distant metastases were significantly more frequently identified in patients presenting with high NLR (>5): 61.6 versus 30.1%; $p < 0.0001$ [38]. In advanced pancreatic cancer (including both metastatic and locally advanced cases), high NLR (≥5) correlated with the presence of metastatic disease [25]. The association between NLR and presence of distant metastases has also been confirmed by a meta-analysis by Yang et al., showing the HR = 1.69; 95% CI: 1.10–2.59; $p = 0.016$ [46].

2.3. Diagnostic role of NLR in pancreatic tumours

The diagnostics of PDAC is frequently a difficult issue. However, the close association between chronic pancreatitis and PDAC significantly limits the applicability of SIR for early diagnostics.

Baseline NLR in unresectable pancreatic cancer has been found to be significantly higher than in healthy controls: 3.81 versus 1.80; $p < 0.001$ [8]. Although the biological difference in the detected levels is remarkable, the comparison between advanced cancer and healthy

persons is not the model to make conclusions on the feasibility of NLR for early diagnostics. Currently, NLR has no role in the primary diagnostic algorithms for PDAC. However, it can assist to solve specific diagnostic questions.

SIR parameters have been proposed as markers of malignancy in pancreatic cystic neoplasms. Thus, in 245 patients with mucinous cystic pancreatic neoplasms, NLR ≥ 1.96 was significantly ($p < 0.001$) associated with invasive carcinoma [47]. In 318 surgically treated patients with pancreatic cystic neoplasms, high NLR was significantly associated with malignant tumour by univariate analysis. However, PLR was found to be superior by multivariate analysis [48].

Regarding intraductal papillary mucinous neoplasms (IPMNs), a trend to higher median NLR was observed in malignant cases: 2.23 versus 2.04 in benign cases. However, because of the rarity of IPMNs, only 60 patients were enrolled in the study, and the difference in medians did not reach statistical significance, reflected by $p = 0.14$. By the cut-off at 3.6, the prediction of malignant behaviour became significant. Still, the sensitivity was only 40% while the specificity reached 93%. By multivariate analysis, enhancement in a solid nodule was found to be superior in comparison with NLR ≥ 3.6 or height of mural nodule ≥11 mm [49]. Assessing 76 patients, higher NLR was reported in malignant than in benign IPMNs: 2.51 versus 2.01 [50]. In a large group of 272 surgically resected IPMNs, NLR exceeding 4.0 was an independent factor (by multivariate analysis), associated with invasive carcinoma. To enhance the predictive value, a nomogram was created incorporating NLR (>4.0) along with cyst size (>3 cm), identification of enhanced solid component, dilation of pancreatic duct (>5 mm) and the presence of jaundice [51]. In PDAC patients, significantly higher NLR has been reported than in case of pancreatic neuroendocrine neoplasms or pancreatic IPMNs [52].

2.4. Confounding factors in NLR assessment

Smoking and a wide spectrum of non-oncological diseases are known to influence NLR. In patients affected by unresectable pancreatic cancer, smoking history significantly ($p = 0.001$) correlated with higher NLR [8].

3. PLR in pancreatic ductal adenocarcinoma

3.1. PLR and survival

PLR is the second best known cellular parameter characterising SIR. Similarly to NLR, most studies have concentrated on the prognostic value of PLR regarding the survival. Two meta-analyses have been published recently (2018), and both teams have reached very similar conclusions on the association between elevated PLR and worse overall survival. In a meta-analysis of 17 studies on PLR in pancreatic cancer, the negative prognostic role of high PLR was confirmed by hazard ratio for worse overall survival HR = 1.28; 95% CI: 1.17–1.40; $p < 0.001$ and worse progression-free survival HR = 1.27; 95% CI = 1.03–1.57; $p = 0.03$ [53]. In another meta-analysis of 17 studies (including 16 reports on association between PLR and overall survival in 3028 patients), high PLR also was found to be associated with worse overall survival HR = 1.22; 95% CI: 1.09–1.36; $p < 0.001$. Interestingly, the prognostic role was confirmed in the subgroup of Asians (HR = 1.22; 95% CI: 1.11–1.34; $p < 0.001$) but not

Caucasians characterised by HR = 1.20; 95% CI: 0.90–1.62; p = 0.22 [54]. The same conclusion, pointing to significant role of PLR in Asia-based studies but not in those carried out in Europe, was reported earlier by Song et al., [55].

Regarding surgically treated PDAC, prognostic role has been ascribed to PLR. In 131 surgically treated PDAC patients, PLR was an independent factor predicting overall and cancer free survival [56]. In a small group of 46 patients treated with pancreaticoduodenectomy for pancreatic cancer, high PLR ≥ 200 was associated with lower overall survival and was the only independent prognostic indicator contrasting with NLR [22]. In borderline resectable PDAC, high PLR (>225) was an independent factor predicting worse survival. The median survival was 10.2 versus 24.7 months in high versus low PLR groups; p = 0.003 [57].

However, these findings have been challenged by a lot of contrary reports. PLR did not predict survival in 217 patients treated for resectable pancreatic cancer [28]. In even larger cohort of 442 pancreatic resections for cancer, there was no association between PLR and survival although NLR was an independent predictor of poor prognosis [21]. In 159 surgically treated patients, PLR lacked prognostic value in regard to overall survival; p = 0.463 although PLR was associated with lymph node metastasis that in turn was independent prognostic factor for overall survival [45]. In 379 consecutive patients who underwent curative resection for pancreatic cancer, PLR was not associated with survival [20]. In 110 surgically treated pancreatic cancer patients, high PLR (≥150) was not associated with cancer-specific survival, as confirmed by p < 0.458 [25].

In a recent meta-analysis, published in 2018, the association between PLR and overall survival was not significant in surgically treated patients (HR = 1.45; 95% CI: 0.84–2.50; p = 0.19) although it was confirmed in the general group of 3028 patients (HR = 1.22; 95% CI: 1.09–1.36; p < 0.001) as well as in subgroups subjected to chemotherapy (HR = 1.18; 95% CI: 1.04–1.35; p = 0.01) or combined treatment (HR = 1.29; 95% CI: 1.07–1.57; p = 0.009). The difference might be attributable to the patient number, constituting only 228 surgically treated cases in contrast to 1313 patients who underwent chemotherapy and 1214—combined treatment [54]. However, another meta-analysis including eight studies, 1904 patients and 823 surgically treated cases, noted the same lack of association with overall survival shown by HR = 1.24; 95% CI: 0.95–1.62; p = 0.11 [55].

In 497 patients with locally advanced pancreatic cancer treated by chemoradiotherapy, elevated pre-treatment PLR (defined by the median as ≥149) was associated with worse 1-year survival rate and 1-year progression-free survival rate: 61.3 and 32.5% in contrast to those presenting with low PLR: 68.1% (p = 0.029) and 37.9% (p = 0.027), respectively [30]. Although in this study, both NLR and PLR were significantly associated with survival, greater biological differences were observed between NLR-defined groups (**Table 3**).

Gao et al. also noted that NLR is more sensitive than PLR in predicting treatment efficacy in unresectable pancreatic cancer [8]. In 88 pancreatic cancer patients treated by combination chemotherapy with gemcitabine and erlotinib, neither progression free survival nor overall survival was predicted by PLR while NLR had a prognostic role [58]. In 56 patients subjected to preoperative chemoradiotherapy and subsequent surgical treatment allowing to evaluate

SIR parameter	1-Year overall survival rate			1-Year progression free survival rate		
	High SIR	Low SIR	p	High SIR	Low SIR	p
NLR	73.2	60.8	<0.001	43.9	31.3	<0.001
PLR	68.1	61.3	0.029	37.9	32.5	0.027

Abbreviations: SIR, systemic inflammatory reaction; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio.

Table 3. Prognostic estimated by PLR versus NLR in advanced pancreatic cancer [30].

the pathologic response, higher mean PLR was observed in poor versus good response group: 172.9 versus 147.3; however, the difference did not reach statistical significance. NLR was superior in this study [34]. In 261 patients with inoperable pancreatic cancer (including both metastatic and locally advanced cases), high PLR (≥150) was not associated with cancer-specific survival, as confirmed by $p < 0.612$ [25].

In contrast, in a small cohort of 66 patients diagnosed with advanced pancreatic cancer, only PLR (not NLR or other SIR parameters) was associated with survival [59]. In advanced pancreatic cancer, high PLR was a significant independent prognostic marker for shorter survival. The median survival was 4.0 versus 9.1 months in patients having PLR ≥ 200 versus PLR < 200 [31].

3.2. PLR and metastatic spread

Several reports indicate an important role of platelet activation in the metastatic cancer spread. PLR was significantly associated with lymph node metastasis; $p < 0.001$ [45]. Anti-platelet treatment, for example, by Clopidogrel, can inhibit the development of metastases [60].

3.3. Diagnostic role of PLR in pancreatic tumours

Differential diagnosis between chronic pancreatitis, presenting with tumour-like mass, and pancreatic ductal adenocarcinoma, can be difficult. Although inflammation is involved in both diseases, thrombocytosis and lymphopenia are more likely to occur in patients harbouring a malignant tumour. Comparing PLR in PDAC and inflammatory masses of pancreatic head, difference was revealed: PLR was 91 (interquartile range (IQR): 77.2–106.6) in patients diagnosed with pseudo-tumorous inflammation and 161.9 (IQR: 117.5–205.6) in PDAC. By ROC analysis, PLR reached area under curve (AUC) value of 88.8%, and the sensitivity and specificity were 79.4 and 92.6%, using cut-off at 113.5 [61]. Another study assessed the diagnostic value of PLR in the distinction between inflammatory pancreatic mass and PDAC in surgically treated patients. The sensitivity and specificity of PLR was comparable to CA 19-9, and combination of both improved the predictive value [62].

In mucinous cystic neoplasms, elevated PLR was shown to be significantly associated with presence of invasive carcinoma in 245 patients from Shanghai [47] and 318 patients from Singapore [48].

4. Classic and modified Glasgow prognostic score in pancreatic ductal adenocarcinoma

Glasgow prognostic score is based on the evaluation of the prototypic acute phase protein, C-reactive protein; and albumin levels in blood serum. CRP is a nonspecific, but sensitive marker of systemic inflammatory reaction, produced in response to pro-inflammatory cytokines (IL-1, IL-6, TNF alpha). Hypoalbuminemia is induced by malnutrition, cancer cachexia or SIR. GPS represents a summary estimate of two crucial pathogenetic processes: the humoral SIR and cancer cachexia therefore it benefits from considerable sensitivity [14].

Two alterations of Glasgow prognostic score are known: the modified GPS and the high sensitivity GPS. In the modified GPS, albumin level influences the score only if CRP is increased. High sensitivity GPS differs from the original GPS by lower cut-off level for CRP [14].

Imaoka et al. evaluated the prognostic value of mGPS across all stages of pancreatic cancer. After adjustment, both mGPS values 1 and 2 showed prognostic significance reflected in the hazard ratios: mGPS of 1 was associated with HR = 1.772; 95% CI: 1.417–2.215, but mGPS of 2 yielded HR = 2.033; 95% CI: 1.284–3.219. However, the biological significance was remarkable between mGPC of 0 and elevated values: the median survival was 15.8 months versus 5.8 versus 4.8 months in those presenting with mGPS 0, 1 or 2, respectively. In this study, the prognostic value of mGPS was not demonstrated in patients who had localised tumours and underwent surgical resection. Instead, mGPS was important in advanced pancreatic cancer [63]. Similarly, in advanced pancreatic cancer, high mGPS was a significant independent prognostic marker for shorter survival. The median survival by mGPS (2 versus 1 versus 0) was 1.8 months versus 9.6 versus 8.3 months [31]. As will be discussed further, most scientists agree on prognostic role of mGPS in advanced pancreatic cancer. The findings in resectable cases are more controversial.

Matsumoto et al reported significant prognostic value of mGPS in resectable pancreatic cancer. However, the role of mGPS in this study was to predict recurrence. According to their findings, mGPS (2 versus 0 or 1) was an independent predictive factor for tumour recurrence within 6 months, along with CA 19-9 (at least 300 U/mL) and tumour diameter (at least 30 mm). To detect survival, the number of these risk factors was significant, as it was associated with significantly ($p < 0.001$) different median survival: 35.5 versus 26.3 versus 15.9 months in those having 0, 1 or 2 or the identified factors, respectively [64].

mGPS (of 2) predicted postoperative pneumonia in 46 patients subjected to pancreaticoduodenectomy for pancreatic cancer. However, mGPS was not associated with overall survival contrasting with NLR and PLR [22].

Still, several researchers and teams have reported on the association between elevated mGPS and survival in surgical PDAC patients. In resectable PDAC, longer overall survival is observed in patients having mGPS of 0: 27–37 months contrasting with less than 18 months in patients with elevated mGPS [65]. In 101 patients treated by pancreatic resection for PDAC,

mGPS of 0, 1 and 2 classified the patients in three distinct groups by overall survival: 37.5 months, 11.5 months and 7.3 months [66].

Elevated classic GPS (0 versus 1 and 2), was not associated with cancer-specific survival in 110 surgically treated pancreatic cancer patients, as confirmed by $p < 0.585$ [25].

Despite the controversies, standard preoperative assessment of mGPS is recommended in cases of borderline resectable pancreatic cancer by consensus statement by the International Study Group of Pancreatic Surgery [26].

In 261 patients with inoperable pancreatic cancer (including both metastatic and locally advanced cases), elevated GPS (0 versus 1 and 2) was significantly associated with worse cancer-specific survival, as confirmed by $p < 0.029$. However, by multivariate analysis, GPS was not an independent prognostic factor in this cohort [25]. Nevertheless, the next level of evidence has been reached: mGPS was an independent prognostic factor in 187 patients with inoperable pancreatic cancer [67]. GPS is associated with survival in patients with unresectable pancreatic cancer treated with gemcitabine [68]. In 96 patients who underwent chemoradiotherapy for histologically confirmed, locally advanced PDAC, Glasgow prognostic score (of 2) was an independent predictor of worse overall survival and progression free survival [69]. In 40 patients undergoing adjuvant chemotherapy by gemcitabine after curative resection, elevated GPS (defined as 1 or 2 in contrast to 0) was associated both with worse disease-free survival ($p = 0.001$) and overall ($p = 0.035$) survival [70].

mGPS shows specific associations with response to treatment. Comparing the efficacy of JAK/STAT inhibitor ruxolitinib or placebo in combination with capecitabine (in both groups) for second-line treatment of metastatic pancreatic cancer, ruxolitinib showed trend to survival benefit only in those patients who had elevated mGPS (1 or 2) or CRP. Thus, in patients who had mGPS of 1–2, treatment by ruxolitinib resulted in hazard ratio HR = 0.60; 95% CI: 0.35–1.03; $p = 0.063$. In contrast, mGPS of 0 was associated with HR = 0.91; 95% CI = 0.46–1.74; $p = 0.77$. The trend reached statistical significance when the groups were compared by C-reactive protein level. Thus, the HR for overall survival in ruxolitinib group was HR = 0.47; 95% CI: 0.26–0.85; $p = 0.011$ in patients whose CRP was above the median value (>13 mg/L) contrasting with HR = 0.89; 95% CI: 0.47–1.65; $p = 0.70$ in those who had CRP ≤ 13 mg/L. The biological differences were minor: the median survival was 2.7 months receiving ruxolitinib versus 1.8 months in controls. The overall survival rates at 3, 6 and 12 months were 48 versus 29%; 42 versus 11% and 11 versus 0% in the ruxolitinib versus placebo groups [71]. In contrast, high mGPS was associated with poor outcome in patients with gemcitabine-refractory advanced pancreatic cancer treated by salvage chemotherapy [72].

Modified Glasgow prognostic score was evaluated in 56 patients who underwent preoperative chemoradiotherapy followed by surgical resection of pancreatic cancer, thus ensuring the option to assess the treatment efficacy by morphology. By this design, mGPS did not predict the response to treatment. However, only five patients presented with mGPS of 1 or 2, while most of the cohort (51 cases) had mGPS of 0. All five patients exhibiting elevated mGPS responded poorly, but this was insufficient to reach statistical significance [34].

5. Fibrinogen and D-dimers in pancreatic ductal adenocarcinoma

Extensive alterations of blood clotting have been demonstrated in pancreatic cancer patients. Sun et al. characterised different coagulation parameters in 139 patients diagnosed with pancreatic cancer and compared the data to forty age- and gender-matched controls. Cancer patients had significantly higher level of fibrinogen ($p < 0.01$), D-dimers ($p < 0.01$), antithrombin III ($p = 0.015$), factor VIII ($p < 0.01$), as well as increased international normalised ratio ($p = 0.022$), longer prothrombin time ($p < 0.01$) and prolonged activated partial thromboplastin time; $p < 0.01$ [73].

Plasma fibrinogen levels are significantly higher in pancreatic cancer than in case of benign pancreatic tumours [74]. Hyperfibrinogenemia has been observed in 24.8% [19]–41.1% of pancreatic cancer patients [74]. In pancreatic cancer patients, levels of fibrinogen and D-dimers are higher before surgery, but significantly lower at the recurrence-free period after surgery; $p < 0.01$ [75]. Fibrinogen level in pancreatic cancer also correlates with NLR and PLR and shows negative correlation with lymphocyte to monocyte ratio [76]. Thus, in pancreatic tumours, hyperfibrinogenemia is associated with malignant course, depends on cancer presence in the body and correlates with SIR parameters. Therefore, elevated fibrinogen level can be considered a component of cancer-induced SIR. It is associated with patient's prognosis.

In 96 patients who underwent chemoradiotherapy for histologically confirmed, locally advanced PDAC, elevated fibrinogen level (≥400 mg/dL) was an independent predictor of worse overall and progression free survival [69]. Similarly, in 321 patients with locally advanced or metastatic pancreatic adenocarcinoma, high plasma fibrinogen was associated with shorter survival. It was confirmed an independent prognostic factor [76]. Wang et al. [19] also noted the association between higher levels of fibrinogen and worse prognosis. However, controversies remain. For instance, elevated preoperative concentrations of D-dimers but not fibrinogen were associated with shorter overall and progression-free survival in the study of Cao et al. [77].

In PDAC, plasma fibrinogen levels increase along with higher stage. In 125 PDAC patients, higher mean fibrinogen concentration was found in stage III/ IV patients compared to those diagnosed at stage I/II. Higher levels of fibrinogen correlated with the presence of distant metastasis [19, 74].

D-dimers represent another blood clotting parameter that is widely studied in pancreatic cancer, including the prognostic role. Thus, elevated preoperative concentrations of D-dimers were associated with shorter overall and progression-free survival [77]. D-dimers also reflect tumour burden. Higher D-dimer levels in plasma were associated with higher stage and grade [73].

Higher concentration of D-dimers predicts shorter survival and non-resectability [75]. The association between non-resectability and elevated D-dimer levels in peripheral blood was also confirmed by Durczynski et al. [78] who assessed 64 patients. The concentration of D-dimers was higher in those who had metastatic cancer in comparison with patients suffering from locally advanced disease [78]. Thus, if the pancreatic tumour seems resectable

by preoperative imaging, high preoperative level of D-dimers might suggest the presence of occult liver metastases or unresectability of other cause, and the surgery should be started with diagnostic laparoscopy in contrast to laparotomy that might turn out to become exploratory laparotomy only.

6. Complex SIR-based scores in pancreatic ductal adenocarcinoma: presence and future

Considering the complexity of carcinogenesis and inflammation, any single parameter has limitations and shortcomings, reflected in the controversial reports. To improve the efficacy of SIR parameters, combinations of those have been tested.

6.1. Combination of baseline and dynamic estimates of NLR

In advanced PDAC, several teams have explored the combination of baseline NLR and dynamics upon the influence of chemotherapy [32]. The baseline value is scored as high or low in regard to threshold level. The cut-offs in SIR studies frequently are identified by ROC analysis or by median value. The dynamics is scored as either increase or decrease in response to the treatment; ratio between NLR in a predefined time point during treatment versus pre-treatment NLR (ratio < 1 is analogous to decrease) or high versus low value (against the threshold) in a predefined time point during treatment. The score is based on the count of adverse prognostic factors: high baseline NLR or increase of NLR upon treatment.

6.2. NLR and other SIR parameters

Combined SIR scores have been generated, including NLR and other SIR parameters. The results might be assessed by the count of adverse prognostic factors, for example high NLR or another parameter that exceeds the cut-off level. Summary score including NLR and PLR is the most obvious option that has been already successfully tested in other cancers, for example, gastric carcinoma [14]. This approach has been fruitful also in PDAC. In patients with locally advanced pancreatic cancer treated by chemoradiotherapy, it was noted that the combination of both elevated NLR and PLR is associated with especially low 1-year survival rate and 1-year progression-free survival rate [30]. Other combinations have been evaluated as well, for example, NLR and blood counts of regulatory T lymphocytes in resectable PDAC [79]. Combined index based on hypoalbuminemia and NLR has been advocated to evaluate the prognosis of gastric cancer [80]. Analogously, in patients receiving stereotactic radiotherapy for advanced PDAC, high NLR (>5) and low albumin levels were associated with shorter median overall survival [35].

6.3. NLR and cancer burden

Currently, there are only few data suggesting dependence of NLR on the tumour burden. The correlations with pT or size have been reported with some authors while corroborated by others.

Survival studies frequently indicate the independent prognostic value of SIR. Hypothetically, SIR is a characteristic of patient's fight, and not a tumour trait. If so, higher informative value could be obtained through complex scores comprising both NLR and an estimate of tumour burden by cancer markers (such as CA 19-9 or CEA), positron emission tomography findings or clinical characteristics of the tumour, for example, the presence of distant metastases or unresectable tumour. All these approaches have been successfully tested in PDAC.

In metastatic pancreatic cancer, a combined score of pre-treatment NLR and CA 19-9 was found to be superior to either parameter alone [81]. In resectable pancreatic cancer, the 2-year overall survival rate was significantly lower in those presenting with high preoperative NLR in combination with high CA 19-9 versus the patients having both values in the low range: 37.5 versus 89.9%, respectively [82]. A complex score including NLR along with metabolic activity detected by positron emission tomography (PET) has been found informative [83]. To predict the overall survival of PDAC patients receiving palliative chemotherapy, NLR ≥ 5 was incorporated in a complex score, designated the prognostic index. The other parameters within the framework of this score were performance status, presence of distant metastases or unresectable tumour, as well as high CEA or CA 19-9 [40].

6.4. Fibrinogen-based complex scores

Similarly to NLR, fibrinogen level has been successfully incorporated in complex scores along with other SIR parameters, for example, GPS, or tumour burden, reflected by stage and/or tumour markers, for example, CA19-9. In cancers of other organs, fibrinogen has also been assessed along with D-dimer levels or NLR [14].

In 96 patients who underwent chemoradiotherapy for histologically confirmed, locally advanced PDAC, Glasgow prognostic score (of 2) and fibrinogen (≥400 mg/dL) were independent predictors of worse overall survival and progression free survival. Complex score based on fibrinogen and GPS had prognostic value [69].

In a large cohort of patients (321 cases) with locally advanced or metastatic pancreatic adenocarcinoma, high plasma fibrinogen was shown to be an independent prognostic factor. It was incorporated in a predictive model along with tumour stage and CA 19-9 level, improving the predictive capability [76].

Prognostic model for overall survival was elaborated on the basis of independent prognostic factors, identified in 125 PDAC patients by the Cox proportional hazard model. These factors comprised plasma fibrinogen, cancer stage and the presence of distant metastasis [19].

6.5. SIR-based complex scores: Future developments in PDAC

Carcinomas of different organs differ markedly by their molecular pathogenesis, prognostic factors and involvement of the inflammation in various stages of carcinogenesis. In addition, even cancers of the same organ are heterogeneous, adding complexity to any cancer research. Nevertheless, the keynotes of SIR-based prognostic scores elaborated in cancers other than

PDAC might yield fruitful research data. Hypothetically, simultaneous assessment of NLR and platelet count, or NLR along with hyperfibrinogenemia might have prognostic value in PDAC, especially, considering the marked tendency to up-regulated blood clotting in pancreatic cancer patients. NLR can also be evaluated along with GPS or mGPS, or patient's somatic, metabolic and/or psychological status.

7. Conclusions

In conclusion, pancreatic ductal adenocarcinoma is associated with systemic inflammatory reaction. The complex pathogenesis of SIR includes ejection of platelets, neutrophils and myeloid-derived suppressor cells from bone marrow, development of neutrophil extracellular traps and pre-metastatic niches as well as upregulated levels of acute phase proteins and blood clotting factors. Despite the biological complexity, SIR can be easily evaluated by patient friendly and cheap blood tests. NLR and PLR are the most frequently used cellular SIR parameters reflecting the balance between pro-tumourous (neutrophils, platelets) and contra-tumourous (lymphocytes) activities. Glasgow prognostic score, levels of fibrinogen and D-dimers characterise proteins that are involved in SIR and thus—in blood clotting. Significant associations with survival have been demonstrated, mostly regarding NLR in surgically treated and advanced cases. PLR is beneficial to estimate prognosis in advanced cases. Both NLR and PLR can improve the preoperative diagnostics of malignancy in pancreatic cystic tumours, while PLR can be helpful to distinguish between pseudo-tumorous chronic pancreatitis and PDAC. Complex SIR-based scores are developing in order to increase the diagnostic accuracy.

Conflict of interest

Authors have no conflicts of interest to declare.

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Chapter 2

Pancreatic Cancer, Leptin, and Chemoresistance: Current Challenges

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Additional information is available at the end of the chapter

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Abstract

Pancreatic cancer (PC) remains a leading cause of cancer-related deaths. Currently, conventional chemotherapies have showed only limited benefits for PC patients. Main factors affecting PC treatment failures are due to late detection, lack of early symptoms and biomarkers, and the development of desmoplasia and chemoresistance. Various mechanisms have been implicated in PC chemoresistance that includes stem cells, epigenetic changes, and alteration of signaling pathways, among others. Obesity is a modifiable factor for PC risk, which is characterized by high levels of the adipokine leptin that is a proinflammatory, proangiogenic, survival factor that affects chemotherapy effectiveness. Here, we will discuss on the mechanisms of PC chemoresistance and the influence of obesity and leptin signaling. Furthermore, the potential use of nontoxic leptin antagonists as a novel sensitization strategy for PC chemotherapeutics will also be discussed.

Keywords: leptin, notch, chemoresistance, pancreatic cancer, obesity

1. Introduction

Pancreatic cancer (PC) is a highly aggressive cancer, characterized by early spread with local diffusion and early metastasis to distant organs. PC is a silent disease, without reliable biomarkers that are commonly detected at an advanced stage. The deep position of the pancreas is an additional factor influencing the late detection of most symptoms of PC, when the disease is at final stages and the tumor size is large enough to interfere with the liver, gallbladder, stomach, or duodenum functions [1]. Patients have rapid disease progression, and few of them survive more than a year. Even for patients with localized disease at the time of diagnosis and

undergoing curative surgical treatment, the median survival remains low, around 18 months. The overall 5-year survival rate is only 8.2% for all stages of PC [2]. Despite the advances in understanding PC biology, survival rates remain unmodified in the past years [3]. The underlying causes for PC dismal prognosis, among others, are the lack of viable methods for patient screening, late detection of specific symptoms, especially in the early stages, and few targeted therapies that remain relatively ineffective [4].

2. Pancreas and pancreatic cancer

2.1. Pancreas: structure and function

The pancreas functions as an accessory gland of the digestive system and is composed anatomically and functionally of a mixed, exocrine, and endocrine component. Most of the pancreatic tissue (99%) is made up of exocrine tissue that is composed of closely packed serous acini that secrete digestive enzymes (proteases, lipases, and amylases). Some of the enzymes (e.g., trypsinogen, chymotrypsinogen, and proelastase) are secreted as inactivated precursors, to prevent pancreatic cell damage, and are activated upon release in the duodenum. Other key digestive enzymes, such as α-amylase and lipase, are present in the pancreas in their active forms. The duct cells secrete a watery, bicarbonate-rich fluid that carries the enzymes and neutralizes the acidity in the small intestine. The endocrine pancreas is composed of islets of Langerhans, clusters of about 3000 cells supported by reticulin fibers, in close contact with fenestrated capillaries. They contain three types of cells that secrete the three pancreatic hormones: α cells secrete glucagon that rises the glucose blood levels, while β cells secrete insulin that decreases the glucose blood levels and Δ cells secrete somatostatin that regulates the endocrine system and affects the neurotransmission and cell proliferation. The islet cells appear paler on hematoxylin and eosin stain (**Figure 1**) [5].

2.2. Pancreatic cancer

The incidence of PC continuously raised in the past years, and it is estimated to become the second leading cause of cancer-related deaths by 2030 [6]. The highest PC incidence occurred in Northern America (7.4 per 100,000 people) and Western Europe (7.3 per 100,000 people), followed by other regions of Europe and Australia (equally about 6.5 per 100,000 people). The lowest rates (about 1.0 per 100,000 people) were observed in Middle Africa and South-Central Asia. More than half of new cases (55.5%) were registered in the more developed regions [7]. PC has been correlated to exposure to risk factors concerning lifestyle, such as obesity, or the environment [8]. The incidence of PC is higher in men than in women [9]. PC is a disease of the elderly, with most of the cases being diagnosed after the age of 55 [10]. African-Americans have the highest incidence rate of PC, that is 28-59% higher than those of other racial/ethnic groups [11].

Most pancreatic tumors are derived from the exocrine tissue. More than 80% of the exocrine PCs are classified as pancreatic adenocarcinomas (PAs). Microscopically, these cancers are characterized by infiltrating small glands that are lined with low-columnar, mucin-containing

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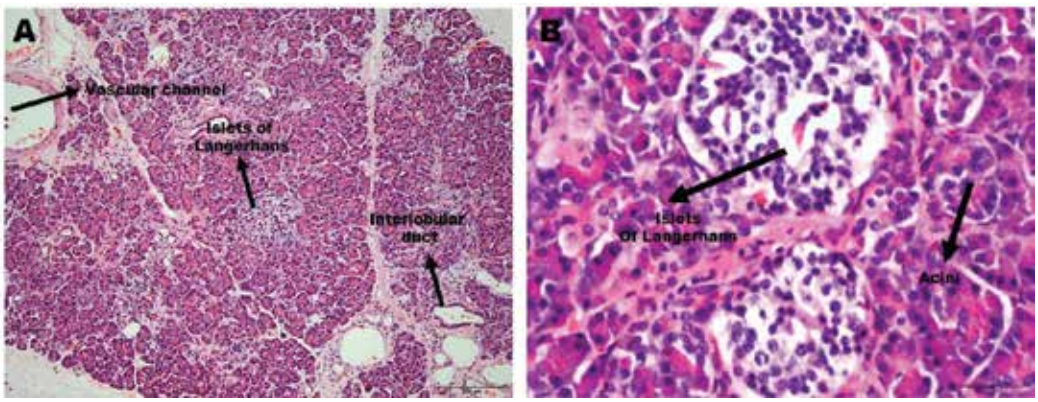

Figure 1. Representative pictures from hematoxylin and eosin staining of pancreatic tissue. (A) Pancreatic parenchyma composed in the vast majority by the exocrine pancreas composed of tightly packed acini that secrete enzymes via a duct system in the duodenum. The endocrine pancreas is composed of islets of Langerhans, which appears as clusters of pale colored cells (10×). (B) High magnification of pancreatic tissue shows exocrine tightly packed acini and endocrine islets of Langerhans. The islets appear pale due to less intracytoplasmic ribosomal content (40×).

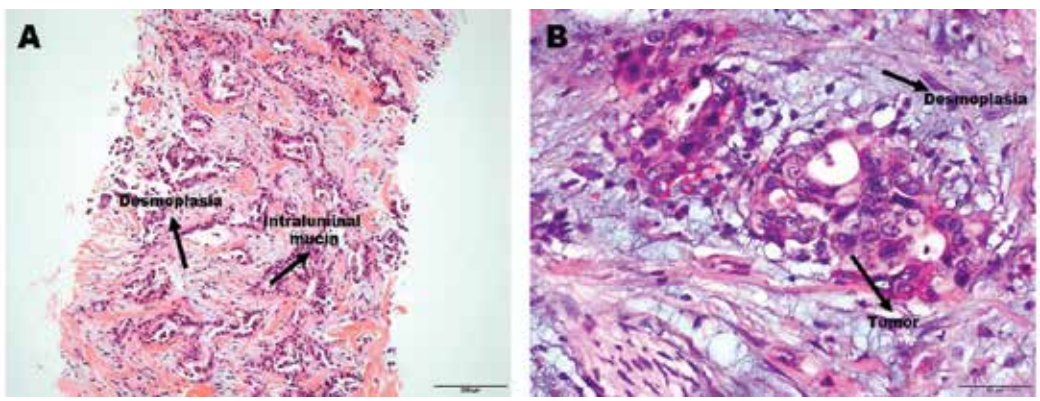

Figure 2. Representative pictures from hematoxylin and eosin staining of PC tissue. (A) Biopsy of pancreatic adenocarcinoma. The malignant glands invade tissue eliciting a strong desmoplastic reaction. Focally intraluminal mucin may be seen (10×). (B) Higher magnification of pancreatic adenocarcinoma shows malignant irregular glands composed of cell with loss of polarity, large nuclei with high nuclear-to-cytoplasmic ratio. The nuclei show irregular shape and are hyperchromatic or vesiculated with prominent nucleoli (40×).

cells. Cell nuclei often show polymorphism, hyperchromasia, loss of polarity, and proeminent nucleoli [12]. PA shows strong desmoplastic reaction that occurs around cancer cells, which is considered a hallmark for this cancer type and may account to up to 90% of the tumor volume (**Figure 2**). The stroma surrounding the cancer cells is actively involved in tumor growth and dissemination. Desmoplastic stroma is composed of extracellular matrix (ECM), cancer-associated fibroblasts, stellate and inflammatory cells, and small blood vessels. Desmoplastic stroma shows high levels of cytokines and growth factors. The desmoplastic stroma creates a barrier for chemotherapeutic drug delivery. Targeted therapies against PC stromal components have so far failed to translate into significant clinical benefits [13].

Pancreatic neuroendocrine tumors (PNETs), representing 1–2% of PC, are commonly called islet cell carcinomas. Functional PNET secretes biologically active hormones (insulin, glucagon, somatostatin, or vasoactive intestinal peptide), causing a clinical syndrome. Nonfunctioning PNET does not cause clinical symptoms [14]. Other types of exocrine PC include acinar cell carcinomas, adenosquamous carcinomas, colloid carcinomas, hepatoid carcinomas, intraductal papillary mucinous neoplasms and pancreatoblastomas [15].

The majority of PC develops silently from pancreatic intraepithelial neoplasia (PanIN) over a long period of time that highlights the importance and the challenge for early diagnosis [16]. Survival of patients with PC depends on the tumor stage at the time of diagnosis. The American Joint Committee on Cancer staging system has defined the relationship of pancreatic tumor with surrounding tissues, lymph nodes, vessels, and distant organs [17]. The first clinical stage of PC refers to tumors that are confined within the pancreas. The second stage involves PC that is spread to the adjacent tissues, especially to the lymph nodes. In Stage 3, the disease has already spread to the blood vessels, while in Stage 4, the metastasis has occurred in distant organs. Unfortunately, at the time of diagnosis, most of the patients have already invasion of vascular, lymphatic, and perineural tissue. The most common sites for distant metastasis are the liver, lung, pleura, peritoneum, and adrenal glands. Surgery may be offered to <20% of patients with PC. An additional challenge is that surgery success rate is gravely limited by the extent of early or occult micro metastases [18].

3. Risk factors for pancreatic cancer

There are several factors that pose high risk for PC, such as obesity, chronic pancreatitis, diabetes, tobacco, and alcohol usage, exposure to chemicals, such as dyes and pesticides, age, and epigenetic changes. High-fat diets activate oncogenic Kras and Cox-2, causing inflammation and fibrosis in the pancreas, leading to PanINs and PC onset. Fat diet that induces pancreatic fatty infiltration could play an important role in PC. Moreover, the presence of PanINs was associated with intralobular fat accumulations [19]. The risk of PC increases with age, more than half of new cases occur in patients over 70 years old. ABO blood types and genetic variants may also influence PC risk [20]. Cigarette smoking increases the risk for PC by 75% when compared with nonsmoking individuals, and the risk persists for 10 years after smoking cessation [7]. Although several risk factors have been identified, the causes of PC are not well known. Understanding the mechanisms through which the risk factors might affect PC progression and survival is the key to develop a prevention strategy for this disease.

3.1. Obesity

Obesity is pandemic in the USA and has been associated with poor prognosis of several malignancies, including prostate, colon, breast, endometrial cancer, and PC. Both general and abdominal obesity are associated with increased PC risk. Moreover, physical inactivity has been linked with increased PC risk [7]. Obesity was linked with increased mortality from PC [21] and the promotion of stromal desmoplasia [22].

The most common method for obesity detection is the determination of the body mass index (BMI) that is calculated based on the relationship between body height and weight (BMI 18.5–24.9, normal; 25.0–29.9, overweight; ≥30, obese). Obesity strongly correlates with body fat levels. Adipose tissue has a very strong endocrine function, secreting various adipokines that are involved in cancer development and progression, and insulin resistance. Leptin, IL-6, and tumor necrosis factor-alpha (TNF-α) are inflammatory factors increased in cancers, but adiponectin is protective against tumorigenesis, and its serum levels are usually decreased. Cancer patients show higher baseline levels of C-reactive protein and soluble TNFα receptor 2. Lipocalin 2 was associated with tumor invasiveness. Resistin, another proinflammatory adipokine, was increased in colon, breast, and prostate cancer. To date, many adipokines have been associated with cancer, contributing to enhanced inflammation, angiogenesis, cellular proliferation, and tumorigenesis [23].

3.1.1. Leptin

One of the main adipokines is leptin, a small protein (16 kDa), which is secreted by white, brown adipose tissue and cancer cells [24]. Leptin binding to its receptor, Ob-R, in the hypothalamus controls food intake and energy expenditure. Leptin also influences the reproductive function and is a long-term regulator of body weight. Leptin is also expressed in placenta, ovaries, skeletal muscle, stomach, and mammary epithelial cells. Leptin can inhibit bone formation. It regulates the ovulatory cycle and plays an important role in embryo implantation [25]. Obese and overweight individuals have high levels of leptin in blood but exhibit leptin resistance, failing to control food intake. Leptin blood levels in obese patients are 10 times higher (40 ng/ml) than in normal individuals (4 ng/ml). The underlying mechanism of leptin resistance in obese individuals is multifactorial that includes impairment of Ob-Rb signaling, hypothalamic neuronal wiring, leptin transport into the brain and Ob-R trafficking, endoplasmic reticulum (ER) stress, and inflammation [26]. High-leptin levels can induce cancer cell proliferation and thus can provide a link between obesity and cancer progression.

Several cancer cell types express leptin [25, 27, 28]. Both in vitro preclinical studies and patient, data suggest that leptin signaling is linked to the development of PC, breast, endometrial, colon, esophagus, stomach, thyroid gland, prostatic, hepatic, skin, brain, ovarian, lung and colon cancers, and leukemia [28–32]. Leptin can induce the development of nonalcoholic fatty liver disease, one of the major causes of hepatocellular carcinoma [33]. Leptin increases the proliferation of human myeloid leukemia cell lines and prostate cancer [34, 35]. In breast cancer, leptin increases the cancer cell proliferation and the expression of antiapoptosis-related proteins like Bcl-2 [36, 37]. Moreover, leptin induces the tumor angiogenesis, by promoting the expression of angiogenic factors, such as vascular endothelial-growth factor (VEGF) and fibroblast-growth factor 2 (FGF-2) [38]. Leptin has a direct effect on the proliferation of endothelial cells that were similar to VEGF [39]. Overall, leptin induces the production of inflammatory cytokines (IL-1, IL-6, and TNF-α), which can promote tumor invasion and metastasis [40].

There is a correlation between increased leptin levels and PC. Overexpression of leptin promotes the growth of human PC xenografts and lymph node metastasis in mice [41]. Ob-R is expressed by pancreatic cells, but its expression is increased in PC cells. Leptin binding to Ob-R induces proliferation, migration, angiogenesis and reduces PC cell apoptosis. The receptor

long isoform, Ob-Rb, is found more often in cancer cells and has full signaling capabilities, in contrast to the short isoform. Leptin and Ob-R have absolute affinity for binding. Leptin binding to Ob-R activates canonical (JAK2/STAT3, MAPK, PI-3 K/AKT1) and noncanonical signaling pathways (p38MAK, JNK, AMPK). The first leptin signaling event is the activation of JAK2, which phosphorylates Ob-R intracytoplasmic tail, leading to the phosphorylation of a tyrosine residue of STAT3 (pSTAT3). pSTAT3 forms a dimer that is translocated to the nucleus, inducing the transcription of specific genes, such as SOCS3, which acts as a potent negative feedback regulator of the JAK/STAT pathway [26]. Recently, it was reported that the central or peripheral administration of an Ob-R antagonist induced comparable changes in food intake, body weight, and hypothalamic SOCS3 expression in lean and diet-induced obesity (DIO) mice. These results suggest that endogenous Ob-R signaling may not be reduced in the context of DIO, thus challenging the established concept of leptin resistance under dietary-induced conditions [42].

4. Mechanisms of chemoresistance in PC

Cancer chemoresistance is a current PC challenge. Intrinsic chemoresistance occurs when chemotherapy is ineffective from the start of treatment, whereas acquired chemoresistance develops only after exposure to anticancer drugs. Although PC cells are more susceptible to Gemcitabine when compared with other anticancer agents, most patients develop resistance within weeks of treatment initiation, leading to poor survival [2]. Mechanisms of cancer chemoresistance include drug modification, reduction or inhibition of drug-induced apoptosis, overexpression of drug efflux proteins, increased expression of survival factors and deregulation of pathways, such as Notch, and expansion of cancer stem cells (CSCs), among others [43].

4.1. Pancreatic cancer stem cells (PCSCs)

The hierarchical model of cancer states that tumors arise from CSC or cancer-initiating cells that can reproduce all tumor cell types. CSCs have common characteristics associated to normal stem cells. CSCs are tumorigenic, show self-renewal capabilities, and can be differentiated into multiple cancer cell types. CSCs hide in the tumor niche causing relapse and metastasis. The tumor niche is composed of stromal and inflammatory cells, cytokines, ECM, and vasculature. It provides signals helping CSCs to maintain their undifferentiated state. The accumulation of ECM destroys the normal PC architecture and enhances the expression of PCSC markers [44].

PCSCs express various markers, including CD24+CD44+, CD133+, CD24+CD44+ESA+, ALDH+, or c-Met+. Metastatic PCSCs express CXCR4+CD133+. PCSC markers CD133 and CD44 correlated to CXCR1 expression. PCSC could be identified using Hoechst 33342 dye by flow cytometry. Hoechst-negative cells were called "side population" and were linked to chemoresistance [45]. ALDHs are a class of enzymes that oxidize aldehydes. ALDH + PCSC show clonogenic and metastatic potential that affects survival in PC. Positive PC cells for PCSC markers form tumors in mice, in contrast to negative PC cells. ALDH1 mediates resistance to Cyclophosphamide and

Gemcitabine in PC. TGF-β negatively regulates ALDH1 in PC in a SMAD-dependent manner. That can be disrupted by SMAD4 mutations and deletions. Therefore, targeting PCSC could induce sensitization of PC to chemotherapeutic treatment [46].

Chemotherapeutic agents target the bulk of the tumor but unfortunately allow the proliferation of CSC that exhibits chemoresistance. Gemcitabine kills tumor cells but increases PCSC (CD24+ and CD133+) that expresses stemness-associated genes, such as Bmi1, Sox2, and Nanog. PCSC expansion increased cell migration, chemoresistance, and tumorigenesis [47]. Drug resistant cells showed activated c-Met and increased expression of CD24, CD44, and ESA. The use of a c-Met+ cell inhibitor (Cabozantinib) abrogated Gemcitabine resistance in PC patients [48]. Administration of anti-CD44 monoclonal antibody to a human PC xenograft mouse model increased Gemcitabine sensitivity [49]. Similarly, Metformin enhanced the antiproliferation effects of Gemcitabine by inhibiting the proliferation of CD133+ cells in PC [50].

Another PCSC marker, Dclk1, was found in PanIN lesions, and PC at invasive stages [51], suggesting that PCSC may be used as diagnosis biomarkers. PCSCs show transcription factors found on embryonic stem cells (Oct-4, Sox-2, and Nanog). Increased levels of Oct-4 and Nanog correlate with early stages of carcinogenesis and worse prognosis. Oct-4 contributes to metastasis and cancer multidrug resistance. Sox-2 expression alone in PC could induce self-renewal and differentiation [24].

PCSC marker expression correlates with lymph node metastasis and poor survival. There are several factors that could affect PCSC maintenance and proliferation. For example, PCSC maintenance and survival are affected by miRNA34. In addition, stem cell factor (SCF) binding to its receptor, c-Kit, induces an increase in HIF-1α synthesis, which is involved in PC progression and chemoresistance [26].

Our data suggest that 5-FU (a common chemotherapeutic used in PC treatment) decreased PC tumorsphere formation. PC cells that expressed CD24 + CD44+, CD24 + CD44 + ESA+, and pluripotency (Oct-4, Sox-2, Nanog) markers were spared by the 5-FU treatment [30]. Therefore, the development of specific treatments against PCSC remains a challenge.

4.2. ATP-binding cassette proteins

Overexpression of drug efflux proteins (ATP-binding cassette proteins and ABC family of proteins) increases the elimination of anticancer drugs and decreases their accumulation inside the cancer cells. ABC proteins (ABCB1, ABCC1, and ABCG2) are found in PCSC and contribute to their resistance to Gemcitabine [52]. Indeed, ABCB1 was significantly increased in CD44+ PC cells during the acquisition of resistance to Gemcitabine [53]. PC chemoresistance correlated with increased expression of CXCR4, CD133, and ABCB1 by PCSC [54]. Interestingly, ABCG2 localization and activity were not confined only to the plasma membrane, as intracellular vesicles containing ABCG2 were detected within CSC in PC, colorectal, and hepatocellular cancers. Moreover, a direct relationship between the presence of these vesicles in CSCs and the maintenance of their stem-like properties, including chemoresistance, was found. Furthermore, the vesicles accumulated ABCG2-dependent substrates, such as the fluorescent vitamin riboflavin (vitamin B2). In addition, the vesicles could accumulate

ABCG2-depedent therapeutics, such as Mitoxantrone, to avoid apoptotic cell death [55]. Our data showed that PC tumorspheres treated with 5-FU were enriched in cells that overexpressed ABCC5 and ABCC11 efflux proteins [30].

4.3. Epithelial to mesenchymal transition (EMT) and PC metastasis

To gain invasive and migratory capacity, and resistance to apoptosis, cancer epithelial cells undergo EMT. The expression of transcription factors, including Snail, Slug, zinc finger E-box-binding homeobox 1 (ZEB1), and Twist, among others, induces EMT. ZEB1 deletion had a negative effect on tumor progression, invasiveness, and metastasis, reaffirming EMT's role in PC metastasis [55]. Gemcitabine-resistant PC cells had increased Vimentin and decreased E-cadherin expression. These alterations are hallmarks of EMT.

Our data showed that the use of 5-FU rendered different outcomes on EMT markers in tumorspheres derived from different PC cell lines. In BxPC-3 tumorspheres, 5-FU did not change the levels of expression of EMT markers (Vimentin and N-cadherin), while in MiaPaCa-2 tumorspheres, it slightly increased the expression of N-cadherin. Moreover, 5-FU spared PC cells that were N-cadherin+ [30]. Recently, the EMT concept was challenged by studies demonstrating the existence of a hybrid epithelial/mesenchymal phenotype in cells transitioning from EMT to mesenchymal to epithelial transition (MET). Because MET has been considered crucial for metastasis seeding in distant organs, this hybrid phenotype seems to be linked to drug resistance and tumor-initiating potential. Moreover, MET could allow tumor cells to collectively migrate in clusters to form metastases in a more effective way than pure EMT single cells [55].

4.4. Tumor microenvironment

PC desmoplasia results from proliferation of cancer-associated fibroblasts and increased deposit of ECM. This process reduces elasticity of tumor tissue and increases interstitial pressure, leading to decreased perfusion of chemotherapeutic agents [56]. The proliferative pancreatic stellate cells are the primary source of many of the ECM components in PC. These cells show increased proliferation and sensitivity to mitogenic factors. Fibrous proteins (e.g., collagen) and polysaccharide chain glycosaminoglycans (e.g., hyaluronan) are ECM factors that constitute the noncellular components of PC desmoplastic tissue. A significant overproduction of ECM components can be described as the failed resolution of a healing wound, which leads to fibrosis in PC. Immune cells (macrophages, neutrophils, and regulatory T cells [Treg]) contribute to PC desmoplasia. Therapeutics reducing the contribution of the desmoplastic reaction to chemoresistance are being actively pursued as a potential therapeutic approach [57].

4.5. Changes in signaling pathways

From the early lesions, PC cells harbor alterations in signaling pathways that remain throughout carcinogenesis. These changes not only impact tumor cells but also the surrounding stromal cells. Components of the Hedgehog (Hh) signaling pathway have essential roles in PC pathogenesis. In a global genomic analysis of PC, all tumors tested had alterations in at least one of the Hedgehog signaling genes. Hh signaling induced desmoplasia, playing a key role in chemoresistance [56]. Wnt signaling pathway is mainly involved in PC cell growth. The Wnt pathway is activated when ligands bind to the cell membrane Wnt receptor, resulting in the release of

β-catenin into the cytoplasm. Increased β-catenin levels and activity have been found in PC but not in the normal pancreas [58]. Wnt pathway induces PC formation by actions not only on the tumor cells but also on the stromal compartment through increases in ECM formation [59].

There are other dysregulated pathways in PC. The nuclear factor-κB (NF-κB) proteins constitute a family of transcription factors associated with mediating inflammatory responses. However, these transcription factors also control diverse genes involved in development, apoptosis, and cell proliferation. NF-κB has an important role in PC. Additionally, Notch and IL-1 induce NF-κB in PC [60]. NF-κB signaling crosstalks with other signaling pathways, oncogenic or cancer-related proteins, such as STAT3, p53, ALDH1, PI-3 K, and MAPK. A recent study that evaluated a large number of human PC samples along with a few PanIN lesions found amplification of c-Myc in 30% of the tumors [61]. c-Myc deregulation, in cooperation with other oncogenic pathways, such as Kras, is sufficient to promote tumorigenesis [62]. The complexity of the PC altered signaling pathways affects pathogenesis and could explain why there is no successful PC treatment. Relationships among tumor cells, stroma, and signaling pathway crosstalks demonstrate the importance of developing combined therapies targeting both compartments and altered signaling in PC.

4.6. Inhibition of apoptosis

Apoptosis or programmed cell death regulates the tissue homeostasis. Chemoresistance is in part due to impairment of apoptosis in cancer cells. Antiapoptotic protein Bcl-2 is not frequently overexpressed in PC, which differs from other cancer types. In contrast, an imbalance between antiapoptotic Bcl-XL and proapoptotic Bax was found in the TGF-α murine model of PC [63]. Moreover, inhibitors of apoptosis, such as survivin, are overexpressed in PC when compared with normal pancreatic tissue. Resistant PC cells can be sensitized to death receptor–mediated apoptosis by inhibiting the NF-κB prosurvival pathway or by decreasing the expression of antiapoptotic proteins. The p53 pathway plays an important role in cancer cells avoiding the apoptosis, with mutations in p53 gene leading to increased drug resistance in PC cell lines and poor survival in PC patients [63]. Our data showed that 5-FU treatment of PC tumorspheres reduced RIP and Bcl-XL levels and increased Bax. Moreover, 5-FU increased caspase-3 activation and decreased uncleaved PARP in PC [30]. These data indicate that 5-FU actions on PC induce apoptosis through several components of the pathway. Numerous chemotherapeutic drugs target DNA synthesis in cancer cells, leading to increased apoptosis.

4.7. Leptin and chemoresistance mechanisms in pancreatic cancer

Leptin induces a wide range of prooncogenic effects. We have shown, for the first time, that leptin could be secreted by PC cells and derived tumorspheres. Moreover, leptin induced PCSC in tumorspheres [28]. In line with these data, a study of a pool analysis from PC patients showed that leptin levels and elevated Ob-R expression correlated to Oct-4 [64]. Our data demonstrated that leptin increased PC cell proliferation, tumorsphere formation, and xenograft growth in an immunocompromised mouse model. Moreover, leptin induced cell cycle progression, PCSC markers (CD24 + CD44 + ESA+, ALDH+), and ATP-binding cassette protein expression (ABCB1) in PC cells [28]. Leptin has been shown to increase the expression of miR21, while the tumor suppressors (miR200a, miR200b, and miR200c) decrease the expression of Ob-R. Furthermore, these tumor suppressors could also interact with some of the

PCSC markers (c-Met, ABCB1, and CD44), which decrease their expression. Oncogenic miR21 increases the expression of ABCB1, ALDH, and CD44.

Leptin can directly regulate the expression of HDAC4 and HDAC5 and indirectly affect the expression of other HDAC via microRNA or PCSC markers. We have suggested that leptin can increase the expression of miR21, which in turn can increase the expression of HDAC3. Analysis of data from PC biopsies (TCGA databank) suggested that HDAC, miRNA21/200, and leptin could have complex signaling crosstalk that could be a novel therapeutic target for obese PC patients. We further determined the effects of leptin on HDAC expression in PC tumorspheres. HDAC3 and HDAC8 expression was increased by leptin. Furthermore, the Gemcitabine-induced decreased expression of HDAC2, HDAC3, and HDAC8 was reversed by leptin. Thus, we have shown that leptin through its effects on PCSC, ABCB1, and HDAC could be involved in PC chemoresistance [65]. Moreover, using another chemotherapeutic agent commonly used in PC treatment, 5-FU, we demonstrated that leptin impaired 5-FU cytotoxicity by increasing the expression and number of PCSC+, pluripotency+, and EMT+ PC cells. ABCC5 and ABCC11 expression as well as the number of positive cells for these ATP-binding cassette proteins were increased by leptin in PC tumorspheres. These leptin's effects protected the survival of PC tumorspheres treated with 5-FU and reduced its cytotoxicity. The survival of PC tumorspheres treated with 5-FU and leptin was linked to reduced apoptosis. Leptin increased the levels of PARP, Bcl-XL, and RIP and decreased Bax. 5-FU increased caspase-3 activation, which was reduced by leptin. These data could help to unravel the multiple mechanisms through which leptin signaling contributes to drug resistance in PC [30].

4.7.1. Leptin-Notch crosstalk in pancreatic cancer

Notch signaling controls the cell proliferation, PCSC maintenance and differentiation, apoptosis, invasion, and metastasis in cancer. Overexpression of Notch receptors (Notch1 and Notch2) was found in PCSC when compared with nonmalignant pancreatic stem cells [66]. DLL4 increase in PC cells stimulated the expression of Oct-4, Nanog, and stem cells [67]. PCSCs that express Oct-4, Sox-2, and Nanog show an increased aggressivity and chemoresistance. Notch4 overexpression was linked to PC chemoresistance to Docetaxel [68]. Expression of Notch3 and Hey1 was associated with reduced survival in PC [69]. Resistance to Gemcitabine correlated with Notch2, Notch4, and JAG1 overexpression [70]. The inhibition of Notch1 by siRNA suppressed proliferation, induced apoptosis, and reduced migration and invasion of PC cells [71].

Notch signaling induced EMT phenotype in Gemcitabine-resistant PC cells overexpressing Notch2, Notch4, and JAG1. Furthermore, the inhibition of Notch signaling decreased EMT markers, including Vimentin, Snail, Slug, and ZEB1, in human PC cell lines [72]. MiR200 members increased Notch activation by ZEB1 that regulates the expression of JAG1 and the mastermind-like coactivators (Maml2 and Maml3). In PC cells, miR200 expression showed an inverse correlation with JAG1 and ZEB1 levels [73]. Therefore, miR200 inhibits EMT by interacting with ZEB1/2 and the Notch pathway and represses self-renewal and differentiation in CSC. MiR200 is also involved in apoptosis [72].

Our data showed that leptin induced the expression of Notch family components in PC (Notch1–4, DLL4, JAG1, survivin, and Hey2), PCSC markers (CD24CD44ESA, ALDH, CD133,

and Oct-4), ABCB1 (MDR1), tumorsphere formation, cell cycle progression, proliferation, and tumorigenesis. These effects were reduced by GSI [28]. Moreover, mouse and human PC and cell lines treated with adiponectin, or an adiponectin receptor agonist, AdipoRon, suppressed leptin-induced STAT3 signaling in vitro and reduced PC growth in vivo [74]. The addition of leptin to 5-FU treated tumorspheres decreased 5-FU-induced cytotoxicity and increased colony forming ability, number of cells expressing pluripotency and EMT markers, drug efflux proteins (ABCC5 and ABCC11), and Notch. Leptin also reduced the 5-FU effects on apoptosis by decreasing proapoptotic (Bax, caspase-3 activation, and PARP degradation) and increasing antiapoptotic factors (RIP and Bcl-XL). Leptin's effects on PC tumorspheres were mainly Notch signaling dependent [30]. Therefore, the leptin-Notch axis could be a target to develop novel strategies for PC treatment.

5. Pancreatic cancer treatment

5.1. Chemotherapy

To decrease the risk of local and distant metastasis, adjuvant therapy is usually started 1–2 months after PC surgery. Although no regimen has been proven significantly more effective than others, a regimen based on 5-FU or Gemcitabine for 6 months is usually the option used to reduce PC patients' mortality [75]. The activity of 5-FU/Leucovorin has been compared to Gemcitabine as an adjuvant therapy in the European Study Group for PC (ESPAC)-3 trial [76]. However, the study showed that median overall survival for patients treated with 5-FU/Leucovorin was 23 months when compared with 23.6 months for patients treated with Gemcitabine. The ESPAC-4 study measured the efficacy of a combination treatment with Gemcitabine plus Capecitabine when compared with monotherapy with Gemcitabine alone. The results showed a survival of 28 months in the combined therapy when compared with 25.5 months in the monotherapy group. Because the dual therapy was well tolerated, the combination of Gemcitabine and Capecitabine has been used as a standard in the clinical setting [77]. Currently, regimens with Gemcitabine plus nanoparticle albumin-bound Paclitaxel (nab-Paclitaxel) and a combination of 5-FU, Irinotecan, and Oxaliplatin (FOLFIRINOX) are evaluated in the clinical setting [78]. Gemcitabine has usually some efficacy as an adjuvant therapy, but often patients develop chemoresistance. Nab-Paclitaxel, a water-soluble compound, has enhanced distribution properties within the tumor microenvironment when compared with Paclitaxel. However, studies have shown that nab-Paclitaxel treatment neither decreased tumor stroma nor increased tumor vascular perfusion in a mouse patient-derived xenograft (PDX) tumor model [79]. The infiltration of neoplastic lesions by CD8+ T lymphocytes is associated with improved prognosis. However, a CD40 monoclonal antibody that activated CD8+ T cells in Phase I clinical trial had only a partial response [80]. FOLFIRINOX and nab-Paclitaxel plus Gemcitabine have the potential to downstage local advanced disease and to improve tumor resection rates. The use of chemoradiation therapy as an adjuvant is controversial and with minimal effects on survival in clinical trials so far [81]. New studies that incorporate modern radiation techniques and current chemotherapy regimens are still needed to determine if radiation is beneficial in PC treatment.

5.2. Targeted therapy

A comprehensive genetic analysis of PC showed that these tumors contain an average of 63 genetic alterations in 12 cellular signaling pathways, including Notch pathway [82]. A Phase Ib trial for PC using a combination of Demcizumab (OMP-21 M18), a monoclonal antibody against Notch ligand, DLL4, with Gemcitabine and Abraxane, showed some clinical benefits [60]. An antibody against Notch2 and Notch3, Tarextumab, was tested in Phase 2 clinical trials in combination with Gemcitabine and nab-Paclitaxel in patients with metastatic PC. For these patients, the median progression-free and overall survival were 5.6 and 11.6 months, respectively. Gamma secretase inhibitors (GSIs) have been used in clinical trials in PC. For example, a GSI called RO4929097 was safely tolerated in combination with Gemcitabine and achieved clinical antitumor activity and more than 4 months of stable disease. However, the use of GSI has limitations and still represents a challenge because of the increased drug toxicity and lack of high specificity to Notch besides other substrates of γ-secretase [83].

Desmoplasia is a target in PC treatment. Hyaluronan, a component of the ECM of PC, is a naturally occurring nonsulfated glycosaminoglycan that was targeted using pegylated hyaluronidase (PEGPH20). In a Phase II study combining Gemcitabine, nab-Paclitaxel, and PEGPH20, there was no difference seen in the survival of PC patients that had this addition to their treatment. Also, due to the ubiquitous nature of hyaluronan, there were unexpected side effects, such as thrombosis. For the Gemcitabine, nab-Paclitaxel, and PEGPH20 study, a subset analysis was performed on the high-hyaluronan patients. In the arm receiving PEGPH20, the response rate was 45% when compared with 31% in controls, which was encouraging, and led to a Phase III clinical trial (HALO301) for patients that had high hyaluronan. In these studies, Lovenox was included for anticoagulation [84].

STAT3 inhibition has been shown to decreased PC growth in mouse models. Napabucasin decreased STAT3 transcription and tumorsphere formation and showed some efficacy in PC. Napabucasin induced a median progression-free survival of >7.1 months and a median overall survival of >10.4 months in PC patients. Based on these encouraging results, it is now being evaluated in a PC Phase III study in combination with Gemcitabine and nab-Paclitaxel (NCT02993731) [85].

The expression of leptin in gastroesophageal adenocarcinomas was associated with chemoresistance. Therefore, the addition of leptin antagonists to current chemotherapeutic treatment could represent a new strategy to overcome drug resistance and to improve survival of PC patients. SHLA, a leptin antagonist, increased the sensitivity of resistant gastric cancer cell line, AGS Cis5, and the esophageal adenocarcinoma, OE33, to cisplatin [86].

LPrA2 was designed and tested in vitro and in vivo in PC xenograft mouse models in our laboratory. LPrA2 is composed by a leptin sequence corresponding to its binding Site III of the leptin molecule. LPrA2 was conjugated to iron-oxide nanoparticles (IONP-LPrA2) to increase its bioavailability and effectiveness to block leptin signaling in cancer cells [28]. IONP-LPrA2 showed no toxicity and did not affect energy balance (body weight or food intake) or general health when it was administered to mice. IONP-LPrA2 reduced the expression of Ob-R, Notch, and PCSC markers. Furthermore, specific inhibition of leptin signaling by IONP-LPrA2 delayed tumor onset and decreased tumor growth in a PC xenograft mouse model. Our data also showed that IONP-LPrA2 could be used as an adjuvant therapy to 5-FU. In PC cells treated

	Human pancreatic cancer cells							
	BxPC-3 low aggressive				MiaPaCa-2 highly aggressive			
	B	CT	CT+L	CT+L+LI	B	CT	CT+L	CT+L+LI
Proliferation (%)	100	70	88	68[a]	100	32	91	58[a]
Tumorsphere formation (%)	100	96	161	97[a]	100	88	201	70[a]
Notch receptors								
Notch1+	100	263	234	167[a]	100	144	149	125[a]
Notch3+	100	91	113	111	100	240	325	210[a]
Notch4+	100	143	200	149[a]	100	146	219	186[a]
PCSC								
CD34+CD44+ESA+	100	122	144	91[a]	100	653	1088	403[a]
c-Met+	100	212	228	168[a]	100	81	142	69[a]
Pluripotency markers								
Oct-4+	100	141	144	89[a]	100	67	95	44[a]
Sox-2+	100	87	95	69[a]	100	84	116	62[a]
Nanog+	100	148	172	134[a]	100	84	78	57[a]
EMT								
N-cadherin	100	34	39	19[a]	100	159	276	137[a]
Vimentin	100	92	88	86	100	84	83	54[a]
ABC proteins								
ABCC5	100	93	123	110	100	55	76	34[a]
ABCC11	100	51	80	50	100	50	83	42[a]
Survival	100	60	104	67[a]	100	61	113	89[a]
Apoptosis								
Caspase-3 activity	100	980	300	441[a]	100	187	159	159
PARP	100	21	39	28	100	71	92	65[a]
RIP	100	26	38	20[a]	100	82	82	40[a]
Bcl-XL	100	87	109	80[a]	100	72	89	70[a]
Bax	100	136	103	130[a]	100	119	72	70

Table 1. Inhibition of leptin signaling using IONP-LPrA2 resensitizes PC cell lines to chemotherapy.

with 5-FU and leptin, IONP-LPrA2 reduced tumorsphere formation and cell proliferation, the number of Notch+, ABCC5/11+, and PCSC+ cells, and increased apoptosis. Thus, IONP-LPrA2 resensitized PC cells to 5-FU actions [28, 30]. In view of leptin multiple effects on PC and the involvement of Notch signaling in leptin's effects, targeting leptin-Notch crosstalk in PC patients might be a new treatment strategy for this deadly disease (**Table 1**). The addition of leptin antagonists to current chemotherapeutic treatment could represent a new strategy to overcome drug resistance and to improve survival of PC patients.

6. Conclusions

PC is a lethal systemic disease that is difficult to detect and treat. This is mainly due to the fact that even patients diagnosed with early stages eventually develop metastasis. The deep abdominal position of the pancreas is an additional factor that delays the onset of specific PC symptoms. Early PC diagnosis and potential cure remain important challenges due to the lack in screening methods and specific biomarkers. PC risk factors, such as high-fat diet, obesity, tobacco, and alcohol consumption, can be modified, leading to prevention of disease occurrence and

increased survival. PC desmoplastic stroma, which decreases chemotherapeutic drug delivery to the tumor, is an another current challenge to improve PC survival. Currently, combined chemotherapy strategies are used in selected patients with PC metastatic disease. The identification of novel PC targets is the key for the development of new individualized strategy for prevention and treatment. An emerging and promising area is the relationship between obesity and leptin-induced prooncogenic effects in PC, which could also affect chemoresistance and metastasis. In this respect, the use of leptin signaling antagonists as a novel sensitization adjuvant for current chemotherapeutic drugs appears as a potential new strategy to improve treatment effectiveness and patients' survival. The use of leptin signaling antagonists could also make possible the reduction of drug dosage and the improvement of patient quality of life.

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Conflict of interest

The authors declare that there are no conflicts of interest in writing this chapter.

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Chapter 3

Sporadic Pancreatic Cancer: Glucose Homeostasis and Pancreatogenic Type 3 Diabetes

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Additional information is available at the end of the chapter

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Abstract

Sporadic pancreatic cancer (SPC) has been frequently associated with impaired glucose homeostasis manifested by prediabetes, Type 2 diabetes or predominantly by T3c diabetes which develops as the first symptom of cancer. Pathogenic mechanisms in the development of T3c diabetes have not been fully elucidated although specific substances originating in the tumor cells are supposed to be the cause of β-cell dysfunction and insulin resistance. New biomarkers evaluated in patients with recent-onset diabetes are necessary for the early diagnosis of this tumor. Actual data characterizing risk factors, early symptoms, pathogenic mechanisms, biomarkers and structured programs in detection of SPC are described. A multidisciplinary team of primary care physicians, gastroenterologists, endoscopists, radiologists and pathologists should improve the prognosis of this malignant disease.

Keywords: sporadic pancreatic cancer, risk factors, early symptoms, T3c diabetes mellitus, β-cell dysfunction, insulin resistance, biomarkers, multidisciplinary team approach

1. Introduction

Pancreatic adenocarcinoma is a highly malignant cancer which occurs in three different forms: (1) sporadic pancreatic cancer (SPC) accounting for 90% of all pancreatic cancers, (2) familial pancreatic cancer accounting for 7%, and (3) pancreatic cancer as a part of genetic cancer syndromes, which account for the remaining 3%. A detailed program of long-term tertiary prevention (surveillance) is available for the two smaller groups. In contrast, there has been, up to now, no preventive program for the much larger SPC group.

The clinical diagnostics of SPC starts now much as it did in the middle of the past century, that is, after the appearance of local and/or systemic symptoms. They include abdominal and back pain, fatigue, loss of body weight, painless jaundice, anemia, peripheral phlebitis, and cachexia. These symptoms are nevertheless also harbingers of advanced disease.

High-resolution imaging methods (HRIMs: CT, MRI, MRCP, EUS) and histomorphology provide information suitable for diagnostics; nevertheless, their impact on patient prognosis is limited as they are typically ordered at an advanced disease stage. Radical surgery may only be suitable for 15–20% of patients. The relapses are frequent as well as early, and chemotherapy is basically palliative. This confluence of factors results in very low 5-year survival rates of only 3–6% of patients [1].

We recently recommended a screening program for early SPC detection based on cooperation of primary care physicians with gastroenterologists and other specialists [2].

2. Sporadic pancreatic cancer development

Pancreatic carcinogenesis begins with the transformation of pancreatic cells and evolution of the precancerous lesions (precursors). At present, six precursors with different morphologies and malignant potential are distinguished [serous microcystic adenoma (SMA); intraductal papillary mucinous neoplasm (IPMN); intraductal tubulopapillary neoplasm (ITPN); mucinous cystic neoplasm (MCN); pancreatic intraepithelial neoplasm (PanIN); and solid pseudopapillary neoplasm (SPN)] [3, 4]. The development of SPC based on the gradual accumulation of genetic and epigenetic alterations consists of three stages: (1) time prior to the invasive lesion, (2) time to the development of the metastatic subclone, and (3) time period of metastatic dissemination that leads to patient death. The average duration of the first two time periods is estimated to be about 18 years. Early detection must be concentrated during these two periods, when patients are often without any symptoms [5].

3. Sporadic pancreatic cancer and diabetes mellitus

The association between diabetes mellitus and pancreatic cancer has been repeatedly observed, and several case–control and cohort studies have been analyzed in meta-analyses [6]. The relationship between diabetes and SPC is reciprocal. While long-term diabetes is considered an etiologic/risk factor of SPC, new-onset diabetes may be the first manifestation of SPC [7] as recently summarized by D.K. Andersen [8].

3.1. Type 2 diabetes and obesity: important risk factors

Long-term Type 2 diabetes is a risk factor of SPC with a latency of more than 5 years, and an incidence that is approximately doubled [9, 10]. However, Type 2 diabetes develops from prediabetes and is frequently symptom-free for several years without clinical manifestations, which allows it to go undiagnosed. Exposure to the protumorgenic effects of Type 2

diabetes is in reality often longer than would be expected based on the time point at which the diagnosis was established. Hyperglycemia is the main factor inducing a cluster of events like higher oxidative stress, formation of advanced glycation end products, and inflammation. Such changes increase proliferation, invasiveness, and metastatic potential of pancreatic cancer [11]. Stimulation of receptors for advanced glycation end products (RAGE) promotes pancreatic cancer development, whereas their inhibition was reported to have opposite effects [12, 13]. Hyperinsulinemia exists in prediabetes and in the initial phase of Type 2 diabetes as a consequence of obesity and insulin resistance. Higher intrapancreatic insulin concentrations may stimulate proliferation of pancreatic tumor cells by activating insulin-like growth factor receptors (IGF-1R) and the downstream PI3K/Akt/mTOR signaling pathway [14].

Long-term Type 2 diabetes is frequently associated with ***obesity***, which by itself is another independent factor increasing the risk of pancreatic cancer development. Fat tissue as an endocrine organ produces and secretes hormones (adipokines) including leptin and adiponectin, which have been linked to cancer development. The key signaling pathway linking obesity and cancer is the PI3K/Akt/mTOR cascade which regulates cell proliferation and survival [15]. Leptin is positively correlated with adipose stores and nutritional status. It induces cancer progression by activating the PI3K, MAPK, and STAT3 signaling pathways [16]. In contrast to leptin, adiponectin is inversely associated with adiposity, hyperinsulinemia, and inflammation. It exhibits anticancer effects by decreasing insulin/insulin-like growth factor (IGF-1) and mTOR signaling via activation of 5′AMP-activated protein kinase (AMPK) and exerting anti-inflammatory actions via the inhibition of the nuclear kappa-light-chain enhancer of activated B-cells (NF-κB) [17]. Activation of NF-κB complex by stimulated RAGE is a possible mechanism through which inflammation may stimulate pancreatic cancer development [18]. In addition, obesity is frequently associated with hyperinsulinemia and may therefore through complex mechanism increase the risk of pancreatic cancer.

3.2. New-onset T3c diabetes: an early symptom of sporadic pancreatic cancer

Newly developed impairment of glucose homeostasis represented either by prediabetes (impaired fasting glucose or impaired glucose tolerance) or diabetes develops as the sole early symptom of SPC and is called pancreatogenic diabetes Type 3c (T3cDM), which appears up to 24 months (or even 36 months according to some investigators) before the clinical manifestation of SPC [19–21]. The relative probability of an already existing undiagnosed SPC is the highest in patients who were diagnosed with impairment of glucose homeostasis within the last 12 months (RR 5.4: 95% CI 3.5–8.3) [22]. A causal relationship between SPC and T3c diabetes is supported by the observation that diabetes resolves after surgical removal of the tumor in more than 50% of patients [23]. However, improvement of glucose homeostasis may be linked to the surgical procedure itself since it has also been demonstrated that subtotal pancreatoduodenectomy similarly improved diabetes in patients with or without pancreatic cancer [24].

T3c diabetes, which represents up to 8% of the total number of patients with diabetes mellitus, can occur secondary to other pancreatic disorders like chronic pancreatitis, hemochromatosis, or cystic fibrosis; however, in these cases, clinical manifestation of exocrine insufficiency usually precedes the development of pancreatic endocrine dysfunction [25]. Pancreatic cancers occur in about 9% of patients with T3cDM [26]. Therefore, one case of SPC per roughly 140

patients with new-onset diabetes can be expected. Patients with new-onset diabetes are associated with a 4- to 7-fold increase in risk of pancreatic cancer, such that 1–2% of patients with recent-onset diabetes were suggested to develop pancreatic cancer within 3 years [27].

4. Pathophysiology of T3cDM associated with sporadic pancreatic cancer

The pathophysiological relationship between T3cDM and SPC remains largely unknown. The high proportion of patients who develop T3cDM as the first clinical symptom of SPC (about 74% patients developing diabetes up to 24 months prior to SPC diagnosis) suggests that the tumor is the cause of the diabetes [28]. In addition, the prevalence of diabetes in patients with SPC is much higher (68%) compared to diabetes that develops in association with other cancers (up to 24%) [29].

4.1. β-cell dysfunction and insulin resistance

New-onset diabetes associated with SPC is a paraneoplastic phenomenon that is characterized by impaired insulin secretion and insulin resistance [30]. Impaired glucoregulation develops gradually. Approximately 15–20% SPC patients are normoglycemic with normal β-cell function but increased insulin resistance. Subjects with impaired glucose tolerance have disturbed β-cell function, but the insulin resistance is not significantly different from the preceding group. The changes in β-cells associated with SPC are initially functional as previously supposed in experimental study [31]. In contrast, morphological changes or a decrease of their counts are associated with other diseases of the exocrine pancreas, that is, chronic pancreatitis, cystic fibrosis, tropical pancreatitis, and hemochromatosis [32].

Several findings support the hypothesis that β-cell dysfunction is caused by substances overproduced by the cancer cells [21], which may impair glucose-stimulated insulin release and contribute to glucose dysregulation. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine which affects both inflammation and glucose homeostasis. Its overproduction by pancreatic cancer cells has been observed, and its effect on the inhibition of glucose-stimulated insulin release from β-cells as well as from isolated islets, through regulation of Ca^{2+} channels, has also been demonstrated [33]. In addition, increased serum levels of MIF have been found in new-onset diabetic patients with pancreatic cancer while no such increase has been seen in patients with pancreatic cancer without diabetes or in non-cancer new-onset Type 2 diabetic patients [33]. Cancer cells have also been shown to upregulate adrenomedullin, a potent inhibitor of insulin secretion (see below) [34, 35].

In addition to β-cell dysfunction, a significant increase in insulin resistance develops in SPC patients with diabetes [36]. Peripheral insulin resistance was confirmed by hyperinsulinemic clamps in patients with pancreatic cancer and was found to be higher in those with diabetes than in nondiabetic subjects [37]. Improved insulin sensitivity was observed after surgical removal of the pancreatic cancer [37]. Insulin resistance was found to be associated with reduced glycogen synthesis in muscles, which was also confirmed *in vitro* [37]. Impaired glycogen synthesis and glycogen storage in muscles were caused by defects at the post-receptor

level [38]. No changes in receptor tyrosine kinase activity, insulin-receptor substrate (IRS-1), or glucose transporter GLUT-4 were found in skeletal muscle biopsies of pancreatic cancer patients as compared to healthy controls [38]. Muscle insulin resistance was also unrelated to weight loss, plasma free fatty acids, or the energy status of cells and medium conditioned by pancreatic cancer cells did not induce insulin resistance in muscle cells in vitro [39]. Hepatic insulin resistance as determined by HOMA-IR indexes was observed in patients with pancreatic cancer [36]. Hepatic insulin resistance seems to be caused by pancreatic polypeptide deficiency and administration of pancreatic polypeptide has the potential to improve insulin sensitivity in the liver [40, 41]. In addition, adrenomedullin and tumor-derived exosomes may significantly contribute to the development of insulin resistance in SPC patients (see below).

4.1.1. Adrenomedullin

Adrenomedullin secreted by pancreatic cancer cells was found to be an important factor influencing β-cell function. It was first identified in 1993 in a pheochromocytoma as a hypotensive peptide [42]. It binds with three types of specific receptors (ADMR), which belong to the 7-transmembrane superfamily of G-protein-coupled receptors. One of them, the calcitonin receptor-like receptor (CRLR), is modulated by the receptor activity modifying protein (RAMP) [43]. Adrenomedullin is released by pancreatic cancer cells in **exosomes.** These membrane-bound vesicles contain proteins, miRNAs, and other molecules and traffic molecular cargo from the cell-of-origin to target sites in the body. After endocytosis or macro-pinocytosis of adrenomedullin-containing exosomes, adrenomedullin binds to its receptors, initiates endoplasmic reticulum (ER) stress and consequently the intracellular increase of reactive oxygen/nitrogen species (ROS/RNS) that can lead to β-cell dysfunction and death [30]. These observations provide new insights into the relationship between pancreatic cancer and new-onset diabetes. The SPC-associated diabetes was therefore proposed to be an example of an "exosomopathy," a novel exosome-based disease mechanism [44].

Body weight loss is another symptom frequently accompanying new-onset diabetes associated with SPC. It usually starts shortly after the onset of diabetes, precedes the development of other symptoms, and progresses up to the diagnosis of SPC. Weight loss varies extensively among individual patients with an average loss of between 4 and 5 kg. Weight loss may have a similar paraneoplastic origin as T3cDM. The adrenomedullin-containing exosomes secreted from pancreatic cancer cells interact with adipose cells and are internalized by endocytosis. Adrenomedullin via its receptors activates p38 and ERK1/2 MAPKs and promotes lipolysis through phosphorylation of hormone sensitive lipase [45]; thus, the loss of subcutaneous fat observed in SPC may be a paraneoplastic symptom mediated by exosomal adrenomedullin. Exosome induced β-cell dysfunction and lipolysis could be inhibited by adrenomedullin receptor blockade [30, 45], which underscores the role of adrenomedullin in the development of new-onset diabetes and weight loss in SPC. Nevertheless, exosomes are involved in several other aspects of cancer development including angiogenesis, stromal remodeling, chemo-resistance, and genetic intercellular exchange [46]. Cancer-derived exosomes can also enter muscle cells and inhibit insulin and PI3K/Akt signaling, leading to impaired GLUT 4 trafficking [47]. This effect leading to skeletal muscle insulin resistance may be mediated by microRNAs carried by exosomes [47]. This interaction between pancreatic cancer cells and normal cells represents another example of a "metabolic crosstalk" in

malignant tumors [47]. In additional to the peripheral insulin resistance expressed in skeletal muscles, impaired insulin action has been found in the liver where similar pathogenic mechanisms may be present [32].

4.1.2. Dipeptidyl peptidase 4 and fibroblast activation protein alpha

The membrane-bound proteases dipeptidyl peptidase 4 (DPP4, EC 3.4.14.5, CD26) and fibroblast activation protein alpha (FAP alpha, EC 3.4.21.B28, seprase) may represent other factors contributing to impaired glucoregulation in SPC [48]. DPP4 is a membrane glycoprotein expressed on the surface of many cell types including endothelial and epithelial cells, fibroblasts, and activated lymphocytes. Its soluble form is also present in the serum and other body fluids. FAP alpha is a close structural homolog of DPP4 with 52% amino acid sequence identity. Under physiological conditions, the expression of FAP alpha is restricted to alpha cells of pancreatic islets and stromal cells in the uterus. During carcinogenesis, FAP alpha is upregulated in the stromal fibroblasts of various malignancies [49]. FAP alpha positive fibroblasts have been found in primary and secondary cancerous lesions, whereas benign epithelial lesions rarely contain FAP alpha positive stromal cells.

DPP4 and FAP alpha are multifunctional proteins that exhibit both enzyme activity dependent and enzyme activity independent biological functions. The catalytic activity of DPP4 and FAP alpha cleaves off the N-terminal dipeptide from peptides and proteins containing proline or alanine in the penultimate position. In addition, FAP alpha also possesses endopeptidase enzymatic activity, with the potential to cleave among others FGF21 [49]. A number of DPP4 and FAP alpha substrates are related to the regulation of glucose metabolism and energy homeostasis (**Table 1**). The proteolytic cleavage significantly modifies the biological activity of the targets leading to inactivation, modified receptor preference, or increased susceptibility to cleavage by other proteases [50].

Biopeptide	Main physiological functions	References
GIP*	Stimulation of insulin and glucagon secretion	[78]
GLP-1*	Stimulation of glucose-stimulated insulin secretion, inhibition of glucagon secretion	[78]
PYY*,** NPY*,**	Regulation of food intake, adipogenesis, energy homeostasis, glucose-stimulated insulin secretion, lipolysis and blood pressure. Involved in stress reaction and pain perception	[78–81]
Glucagon*	Increase of glycemia and ketogenesis	[79, 82, 83]
FGF21*,**	Stimulation of glucose uptake in adipocytes, increase of energy expenditure	[84–86]
VIP*, PACAP*	Regulation of insulin and glucagon secretion, regulation of body weight, energy and lipid metabolism. Gastrointestinal motility. Immunomodulation	[87, 88]

GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1; PYY – peptide YY; NPY, neuropeptide Y; FGF21, fibroblast growth factor 21; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating peptide.

Table 1. Biopeptides involved in glucose and energy homeostasis that are cleaved by DPP4* and/or FAP**.

The role of DPP4 and FAP alpha has been studied in the context of various malignancies, including pancreatic cancer. Expression of both proteases is increased in SPC tissues and SPC patients with recent onset diabetes or prediabetes have increased plasma DPP4 enzymatic activity [51]. Increased expression and activity of these proteases may thus lead to decreased bioavailability of their substrates and thus contribute to impaired glucose homeostasis in SPC.

In summary, pancreatic cancer cells dysregulate the production of various substances with hormonal or enzymatic activities, which lead to impaired functioning of both the endocrine pancreas and other organs. New-onset T3cDM is therefore a consequence of impaired glucose homeostasis caused by the cancer cells.

5. Diagnosis of T3cDM

Early diagnosis of impaired glucose homeostasis is the first important step in the proper diagnosis of T3cDM associated with SPC. At this stage, the patient is usually without any clinical symptoms and a small decrease in body weight is frequently overlooked or considered unrelated. Determination of blood glucose every 2 years in patients over 50 years is highly recommended as a part of regular preventive examinations by general practitioners. A finding of impaired fasting glucose (IFG) or increased random blood glucose should initiate the next level of examination (i.e., oral glucose tolerance test or HbA1c), which can confirm a diagnosis of prediabetes or diabetes.

The main task for physicians is to distinguish T3c diabetes from the more common Type 2 or Type 1 diabetes, since in general practice only the latter two types are usually considered without any suspicion of T3c. Several indicators can be used for a better evaluation. Firstly, changes in body weight differ in subjects with T2DM vs. T3cDM after the appearance of diabetes. A decrease in body weight at the diagnosis of prediabetes or diabetes is significantly more frequent in patients with T3cDM than with T2DM, likely due to the tumor induced loss of subcutaneous fat tissue [45]. In SPC, the decrease in body weight usually precedes other systemic and local symptoms. T2DM frequently begins with increased body weight associated with insulin resistance and hyperinsulinemia and BMI is often higher compared to T3cDM [8]. A family history of diabetes is common in T2DM but not in T3cDM associated with SPC. The absence of markers of autoimmune disease may help exclude Type 1 diabetes. Therefore, an association of newly diagnosed prediabetes or diabetes with progressive weight loss should lead to the suspicion of T3cDM. Basic laboratory and clinical data that differentiates T2DM and T3cDM are presented in **Table 2**.

The plasma pancreatic polypeptide (PP) concentration in the fasting state and after meal-stimulation may also help discriminate between T2DM and T3cDM [8, 52]. The test is based on increased PP secretion after 30 min of nutritional stimulation in healthy controls and T2DM patients (usually by more than 100% of the baseline value); this increase is missing in T3cDM patients. The discriminative value of this test was found to be higher in cancer of the pancreatic head than in the other regions of the gland [53], since PP-cells are predominantly located within the head of the pancreas.

Indicator	Type 2 DM	Type 3c DM
Body weight	Increase	Decrease
Family history of DM	Positive	Frequently negative
Fasting plasma concentration		
Insulin	High or normal	Low or normal
PP	High or normal	Low or normal
GIP	Normal	Low or normal
Poststimulation levels		
Insulin	High or normal	Low
PP	High or normal	Low
GIP	Normal	Low

PP, pancreatic polypeptide; GIP, glucose-dependent insulinotropic peptide.

Table 2. Clinical and laboratory characteristics differentiating new-onset Type 2 from Type 3c diabetes associated with SPC.

6. Diagnosis of sporadic pancreatic cancer

Failure to diagnose SPC at an early stage is the main impediment to improving the prognosis of patients with this malignant disease. Currently, more than 80% of cases are diagnosed in advanced stages (T3 and T4), which generally excludes radical surgery, the only possibly curative treatment. The prerequisite for early diagnosis of SPC is the timely use of high-resolution imaging methods (HRIMs), which will lead to the identification of patients with early stage, effectively curable disease. The specificity and sensitivity of the classical tumor biomarkers currently used in the clinical practice is low. Therefore, novel biomarkers are critically needed to identify patients in whom HRIMs should be used. Recently, we have proposed a structured diagnostic strategy for individuals with newly diagnosed diabetes, who represent a significant risk group for SPC, involving primary care physicians (both general practitioners and diabetologists) [2].

6.1. Biomarkers

T3cDM with weight loss are alarming signs of a paraneoplastic origin and patients presenting with these signs require further examination. Recent reviews have summarized the present knowledge of biomarkers for the diagnosis of SPC [54–56]. A widely used biomarker, **carbohydrate antigen CA 19–9,** is neither sufficiently specific (68–91%) nor sensitive (70–90%) in patients with SPC and, as such, it is not a reliable marker for screening and early detection [57]. While a more sensitive assay for CA 19–9 has been developed, which also demonstrated higher specificity [58], a combination of different markers in multiplex detection appears to be more promising. A biomarker panel consisting of three proteins: (1) plasma tissue factor pathway inhibitor (TFPI), (2) Tenascin-C (TNC-FN III-C), and (3) CA 19–9, was better than CA 19–9 alone in early-stage cohorts (stage I and IIA/IIB), including the ability to discriminate

stage IA/IB/IIA from healthy controls [59]. This panel had the predictive power to detect early-stage pancreatic cancer and may have clinical utility for early detection of surgically resectable pancreatic ductal adenocarcinoma. In another study, a surface enhanced Raman spectroscopy (SERS) based immunoassay of CA 19–9 in combination with matrix metalloproteinase (MMP7) and mucin (MUC4) in serum had significantly enhanced sensitivity and could be a promising tool for liquid biopsy diagnostics [60].

MicroRNAs, small non-coding molecules circulating in blood, have been tested in patients with pancreatic cancer and healthy controls. They play roles in regulation of cell physiology, tumorigenesis, apoptosis, proliferation, invasion, metastasis, and chemoresistance. Many miRNAs found in serum have been suggested as reliable biomarkers of early SPC detection [61]. Combining several miRNAs with CA19–9 in a composite panel could improve diagnosis compared to a single biomarker. This was documented with six miRNAs (including miR-20a, miR-21, miR-25, miR-155, miR-196a, and miR-210), and CA19–9 [62]. The panels had a high specificity for pancreatic cancer compared to other gastrointestinal cancers and they showed better sensitivity and specificity than CA19–9 alone. A panel of miRNAs could be used to differentiate patients with new-onset diabetes with SPC, healthy controls, and new-onset Type 2 diabetes without SPC [63, 64]. MiRNAs were also analyzed using weighted gene co-expression network analysis (WGCNA). This method better discriminates between healthy and cancer patients and demonstrates that miRNAs can serve as prognostic biomarkers [65]. On the other hand, a set of 15 selected miRNAs was able to discriminate SPC patients from controls at the time of diagnosis but could not be used in earlier stages because their alterations only appeared in the later stages of the disease [66].

Another area of investigation provides new data from **metabolomic** studies that are based on metabolic differences between new-onset diabetes with and without pancreatic cancer as well as in comparison with Type 2 diabetes [67]. Sixty-two metabolites, from several hundred, were analyzed using liquid chromatography/mass spectrometry. The results were able to discriminate between the three abovementioned groups, although the procedure is not yet suitable for routine use. In another study, using a metabolomic profile of 206 metabolites, most significant changes were found in oleanolic acid, palmitic acid, taurochenodeoxycholate, and d-sphingosine, discriminating between healthy controls and pancreatic cancer patients [68].

T3cDM caused by pancreatic cancer is characterized by **abnormal concentrations of several hormones** which participate in glucose homeostasis. In cases where basal plasma concentrations of the hormone are within normal limits, the impairment may be disclosed after mixed-nutrient stimulation [52]. The determination of insulin, pancreatic polypeptide (PP), or glucose-dependent insulinotropic peptide (GIP) during the "meal test" may confirm their decreased levels, which would demonstrate their altered dynamics [19].

Exosomes bring new possibilities to the detection of SPC [69]. The proteins, miRNAs, and mRNAs transferred by these vesicles originating in cancer cells can be used as biomarkers. Several body fluids like serum, urine, and saliva were demonstrated to contain pancreatic cancer-derived exosomes [70]. Exosomes may improve early diagnosis of pancreatic cancer in stage I and IIA when the tumor is still localized [71]. Two miRNAs, miR-196a and miR-1246, were found to be highly enriched in pancreatic cancer exosomes and elevated in plasma exosomes of patients with localized pancreatic cancer. Exosomes can be examined

in pancreatic juice when new-onset diabetes is suspected as a paraneoplastic symptom of SPC [72]. Exosomes trafficking within pancreatic juice may facilitate the development of a pre-metastatic niche well before any symptomology that might support an early diagnosis of pancreatic cancer [72].

It appears that an early diagnosis is increasingly dependent on a combination of biomarkers with sufficient sensitivity to disclose localized tumors or, better still, their precursors.

6.2. Imaging methods

Diagnosis based on visualization of the tumor and classification of its stage is necessary for clinical decisions regarding treatment and the use of high-resolution imaging methods (HRIMs) is therefore immediately recommended in patients suspected of having SPC. The results of different methods were compared using a large database [73]. Effective screening procedures for early detection of pancreatic cancer were described by Hanada et al. [74, 75]. A review of the advances in various imaging methods, as well as their proper selection is beyond the scope of this review.

7. Risk groups of diabetic patients suggested for screening of sporadic pancreatic cancer

Early diagnosis and subsequent successful treatment of SPC associated with diabetes depends on proper evaluation of the **risk groups** of patients >50 years of age:

1. Patients with new-onset prediabetes or diabetes:
    - **a.** With decreasing body weight (>2 kg) and anorexia as the only clinical symptom
    - **b.** With failure of introductory antidiabetic drug therapy during the first 3 months and stagnation or a decrease in body weight (>2 kg)
    - **c.** With persistent impairment of glucose homeostasis despite the additional of a second antidiabetic drug during the next 3 months or a decrease in body weight (>2 kg)
2. Patients with long-term diabetes and obesity when there is a failure of antidiabetic drug therapy that developed during the preceding 6 months combined with a decreasing body weight (>2 kg).

In patients from the first group, the new-onset diabetes and the loss of body weight may be **early symptoms** of SPC. In the second group, long-term diabetes and obesity are **risk factors** for SPC [76]. A decline in diabetes control, as measured by glycated hemoglobin HbA1c, may precede clinical detection of pancreatic cancer by several months up to 5 years [77]. The failure of the antidiabetic drug treatment characterized by either poor or worsening diabetes control is a common feature of both T3c and T2 diabetic patients with pancreatic cancer [21].

Sometimes the fluctuations of blood glucose confirm unstable diabetes regardless of intensified insulin treatment. The findings of (1) worsening diabetes control and (2) failure of antidiabetic drug treatment indicate the need for SPC screening. Patients in both risk groups (i.e., new-onset and long-term diabetes) should be examined according to the structured protocol we described earlier [2].

8. Protocol for early sporadic pancreatic cancer detection

The program of early SPC detection has three steps [2]:

a. A clinical suspicion of SPC in the risk groups evaluated by general practitioners (GPs) or diabetologists,

b. A determination of biomarkers (oncomarkers, microRNAs, etc.) and hormones (GIP, PP, GLP-1) after nutritional stimulation as prescribed by a gastroenterologist,

c. An endoscopic examination of the patient and use of high-resolution imaging methods (HRIMs) as prescribed by an endoscopist/radiologist in collaboration with a pathologist.

A multidisciplinary team approach should improve the prognosis of this malignant disease. The early symptoms (new-onset T3cDM and weight loss), the effect of the initial antidiabetic drug therapy, as well as the failure of antidiabetic therapy in long-term diabetes control, with newly developing weight loss, should be properly evaluated by a GP or a diabetologist.

We suggested an algorithm for the examination of patients with new-onset diabetes (**Figure 1**) [2]. Regular screening of blood glucose in the general population above 50 years of age may disclose abnormalities in glucose homeostasis. Additionally, the evaluation of body weight and any changes during the months prior to the visit is critical. A decrease in body weight > 2 kg in a patient with newly confirmed prediabetes or diabetes should arouse suspicion of its paraneoplastic origin. In this case, a gastroenterologist should be consulted.

A patient with new-onset diabetes should be treated with the first line antidiabetic drug according to the guidelines for Type 2 diabetes. If the diabetes control is not satisfactory during the first 3 months and body weight remains stable or increases, then a second antidiabetic drug should be added. An inadequate response to intensified treatment or unintentional weight loss should lead to a suspicion of T3cDM. In this situation, the collaboration with a gastroenterologist, preferably in a tertiary center, is necessary. The patient should be tested for PP and GIP secretion after nutritional stimulation. A response by PP and GIP that is diminished or absent confirms the pancreatogenic origin of the diabetes (T3cDM). A gastroenterologist should arrange the next steps involving an endoscopic examination and HRIMs.

A patient with long-term diabetes with failing antidiabetic drug treatment combined with decreasing body weight should be included in the same multistep screening program as described for T3cDM patients.

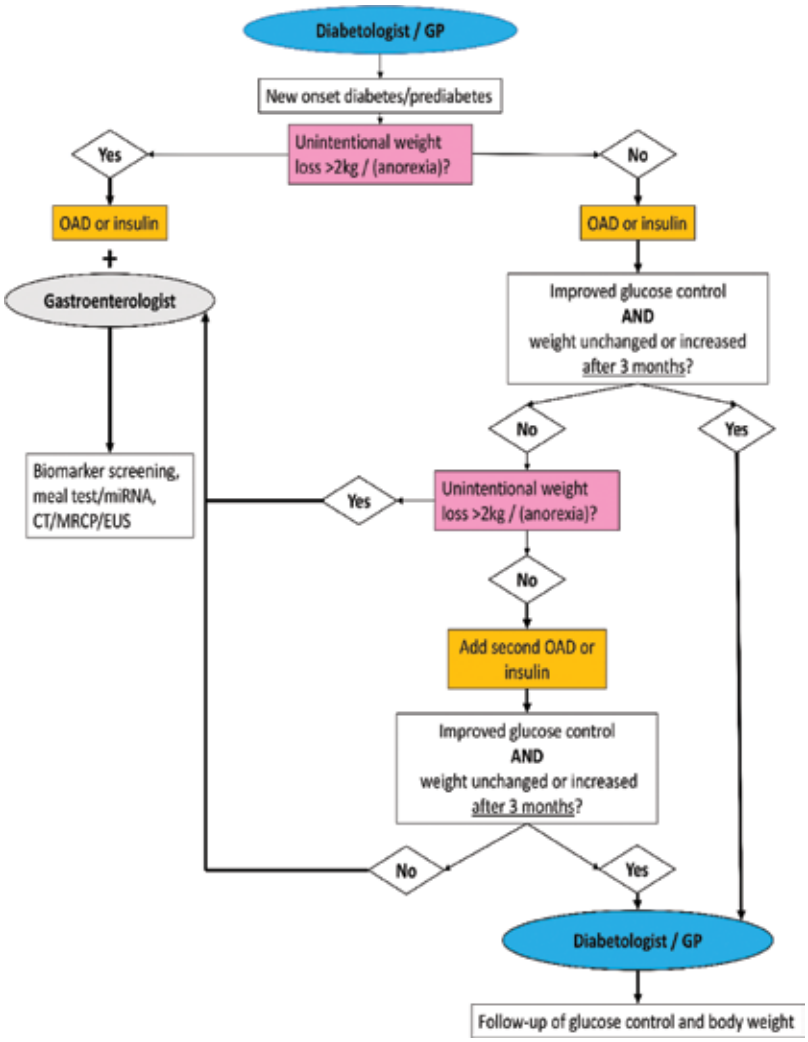

Figure 1. Differential approach to a patient with new onset diabetes/prediabetes. Unintentional weight loss, anorexia or no improvement in glucose control with appropriate treatment should prompt an evaluation by a gastroenterologist. OAD, oral antidiabetics; EUS, endoscopic ultrasonography; GP, general practitioner.

9. Conclusion

The association of SPC with diabetes mellitus offers an opportunity for early detection of this malignant disease. While long-term Type 2 diabetes is an important risk factor of SPC, new-onset T3cDM represents an early symptom as well as a pathogenetic feature of SPC. Thus, proper assessment of new-onset diabetes with a focus on the analysis of early symptoms, that is, failure of antidiabetic drug treatment including unstable diabetes requiring insulin administration, represents a promising step in shifting the diagnosis of SPC to an earlier stage. New biomarkers and high-resolution imaging methods may help discriminate between different pathologies with better accuracy, including identification

of the earlier stages of pancreatic cancer. A multistep and multidisciplinary preventive program based on collaboration between GPs, diabetologists and gastroenterologists offers an opportunity for timely SPC diagnosis. This approach may improve the prognosis for these patients.

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Conflict of interest

The authors have no conflict of interest.

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Chapter 4

Endoscopic Ultrasound in Pancreatic Cancer

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Additional information is available at the end of the chapter

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Abstract

Endoscopic ultrasound (EUS) has been developed over the course of the last 50 years. This technique has been shown to improve diagnosis, provide more accurate local information with regards to staging and enhance prediction of surgical resectability. Further to this, minimally-invasive local techniques have been developed, and continue to be developed, to provide both active and palliative management within the treatment schema for pancreatic cancer (PC).

Keywords: diagnosis, staging, therapeutics, gastroenterology

1. Introduction

Endoscopic ultrasonography (EUS) refers to the use of an ultrasound probe on a flexible endoscope to provide ultrasound images from within the GI tract and has applications for use in transoesophageal echocardiography (TOE), endobronchial ultrasound (EBUS), transrectal ultrasound-guided (TRUS) prostate biopsies, and evaluation of suspicious lesions in the upper GI tract, including the stomach and pancreas, as well as local lymph nodes. This chapter focuses on the utility of EUS in the assessment of pancreatic lesions. EUS is performed by experienced endoscopists and provides information regarding the sonographic characteristics of lesions of interest, as well as provides opportunity, through instrument channels in the endoscope, to take biopsies and perform minimally-invasive procedures for therapeutic or palliative benefit.

EUS has a vital role in the diagnosis, staging, and provision of local therapeutics in the management of PC. Emerging applications and future directions of EUS in PC are also discussed.

2. History

Endoscopy in its modern form began in 1806 with the invention of the Lichleiter, or 'light conductor', by Philipp Bozzini. This device consisted of two parts: the light container and viewing device, and the mechanical part (various speculae) that facilitated access to the subject's body. The fibre-optic endoscope was originally invented by the then medical student, Heinrich Lamm in 1930 [1]. Poor image quality limited the utility of this endoscope until scientific advances made by Harold Hopkins and Narinder Singh Kapany in 1954 [2] were adapted by Dr. Basil Hirschowitz to create the flexible fiberscope [3].

Ultrasound as an investigational modality was also being developed at this time, with Neurologist Dr. Karl Dussik publishing the first use of diagnostic ultrasound in 1941 [4]. The addition of radial ultrasound technology to endoscopy is credited to Dr. DiMagno in 1980, who felt that by internalising the ultrasound probe, problems with interfering gas patterns and nearby organs could be avoided, and the accuracy of ultrasound would be improved [4]. Although the intent at the time was to use this technique to image the pancreas, the coupling of endoscopy and ultrasonography also led to the development of transoesophageal echocardiography, endoscopic bronchial ultrasound, and trans-rectal ultrasound.

In 1991, Dr. Peter Vilmann and Søren Hancke utilised the curved linear array endoscope to facilitate minimally-invasive diagnostic and therapeutic interventions during endoscopic ultrasound [5]. The use of the linear array ultrasound probe enabled the use of instrument channels. These channels have facilitated the current utility of endoscopic ultrasound to perform fine needle aspirations (EUS-FNA) for diagnostic purposes, and for minimally-invasive therapeutic alternatives to radiologically-guided, or surgical drainage of collections, for biliary drainage (EUS-BD), and to perform celiac plexus neurolysis (EUS-CPN) [6, 7].

3. Diagnosis

Early PC is often detected incidentally, with identification of a non-specific pancreatic lesion. The gold-standard treatment of early PC is with pancreaticoduodenectomy ('Whipple's' procedure); a major surgical undertaking with significant morbidity. Ensuring an accurate diagnosis of malignancy is crucial to preventing unnecessary surgeries and the complications thereof.

Diagnosing early PC noninvasively has been historically a difficult undertaking. Clinical suspicion of PC is often based on either non-specific clinical features (asthenia, weight loss, abdominal pain, anorexia, etc.), or features that are associated with advanced disease (jaundice, hepatomegaly, abdominal distension, signs of pancreatic insufficiency, etc.), but specific to pancreatic malignancy. Contributory evidence of malignancy has historically involved clinical history, including presence of risk factors for PC (discussed previously), serum level of cancer antigen 19-9 (CA19-9), and radiographical appearance on transabdominal ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI). The advent of EUS and EUS-FNA has allowed for more accurate radiographical assessment of pancreatic lesions, as well as direct sampling to allow histological assessment of the lesion.

3.1. Tumour markers

CA19-9 is a useful biomarker for monitoring response to treatment, or disease progression or recurrence in patients with an established histological diagnosis of PC [8]. However, the specificity of CA19-9 (68–92%) and positive-predictive value (0.9% for serum concentrations >37 units/mL) negates the utility of CA19-9 in the diagnosis of PC [9].

3.2. Imaging

3.2.1. Transabdominal ultrasound (US)

US can be used to assess pancreatic masses ≥3 cm in size with up to 95% sensitivity [10]. Specificity of US is reported between 94 and 98%, however sensitivity decreases substantially when assessing smaller lesions, and is highly operator-dependent [11]. In order to improve detection of PCs at a size where curative resection is achievable, more sensitive investigations are necessary.

3.2.2. Computed tomography (CT)

Abdominal CT scan (multidetector CT, MDCT) has a sensitivity nearing 100% for pancreatic lesions >2 cm, which reduces to 77% for tumours ≤2 cm [12]. Its utility in assessing local extension is demonstrated by an accuracy for predicting surgical resectability of 80–90% [13], however is limited by its ability to detect liver metastases and early lymph node metastases [11].

3.3. Percutaneous biopsy

Percutaneous, image-guided pancreatic mass biopsies using ultrasound or CT, are safe and effective at obtaining the diagnosis of PC. Due to the direct sampling nature of the procedure, specificity is close to 100%, with varying sensitivity between 80 and 90% [14]. Theoretic concerns with regards to percutaneous biopsies include the risk of tumour seeding along the biopsy tract, or the increased risk of peritoneal carcinomatosis in patients having undergone percutaneous biopsy, and is contraindicated in potentially-resectable cases [15].

3.4. EUS-guided biopsy

EUS-guided fine-needle aspiration (EUS-FNA) uses the instrument channel of the endoscopy to pass a biopsy needle in front of the linear-array ultrasound probe to obtain tissue from lesions under direct ultrasound visualisation. The angle of the needle can be modified to target more cellular-appearing aspects of the target lesion. Two to 10 passes are made into the lesion with the needle and the use of an on-site cytopathologist, or specialist nurse trained in assessment of samples for cellularity is recommended. EUS-FNA allows for tissue acquisition for diagnostic purposes with a low rate of morbidity and mortality, and allows for early genetic and molecular analysis for research and therapeutic decisions [16].

Eloubeidi et al. conducted a review of 100 patients who underwent EUS-FNA, and found 95% sensitivity, 95% specificity, 100% positive predictive value, and 85.2% negative predictive value [17]. These results have been replicated and shown to hold in multiple studies, including

a meta-analysis and systematic review by Puli et al. , who identified 41 studies of EUS-FNA and found a pooled sensitivity of determining the correct nature of pancreatic masses of 86.8% (95% CI 85.5–87.9), a specificity of 95.8% (95% CI 94.6–96.7), a positive likelihood ratio of 15.2 (95% CI 8.5–27.3), and a negative likelihood ratio of 0.17 (95% CI 0.13–0.21) [18].

Chen et al. conducted a systematic review to determine the accuracy of EUS-FNA. They identified 15 studies, totalling 1860 patients and found 92% sensitivity (95% CI 91–93%, $p < 0.001$, $I^2 = 69.6\%$), 96% specificity (95% CI 93–98%, $p = 0.006$, $I^2 = 54.9\%$) [19]. From a practical point of view, the additional benefit of EUS in the assessment of pancreatic lesions is that radiological characterisation of the lesion, local extension and nodal involvement, and histological sampling can all occur in the one procedure, as opposed to US assessment followed by a separate imaging-guided biopsy.

However, a more recent Cochrane review highlighted the lack of quality studies in the area of comparative diagnostics with regards to PC; conclusions were unable to be drawn from the data as only three articles were identified that met the pre-defined quality parameters [20]. There is a paucity of good-quality head-to-head prospective, randomised controlled trials that compare the investigative modalities and heterogeneity in the inclusion criteria of many of the current studies within the literature. Coupled with variability in access and quality of EUS-FNA, interpreting the comparative efficacy and developing a standardised pathway for the investigation of pancreatic lesions remains open to debate.

Horwhat et al. reported an interesting randomised crossover trial comparing EUS-FNA with percutaneous biopsy. Patients with non-diagnostic first-line investigations were allowed to cross over to be investigated with the alternate modality. Fewer patients who received upfront EUS-FNA went on to have percutaneous biopsy (8/36 (22%) versus 16/36 (44%)). The comparative sensitivity of percutaneous biopsy and EUS-FNA was 62% (95% CI 0.41–0.80) and 84% (95% CI 0.64–0.95), respectively ($p = 0.1164$) [21]. In such a lethal disease, in a population where clinical deterioration often happens suddenly, accuracy in diagnosis is vital to facilitating early treatment. This study lends support to EUS-FNA over percutaneous biopsy for obtaining an early and accurate diagnosis.

Okasha et al. conducted a multicentre, prospective, controlled trial in a non-randomised population of EUS-FNA versus ultrasound-guided percutaneous biopsy (US-FNA) in the investigation of pancreatic head tumours. The investigative modality was dictated by accessibility and feasibility. One hundred and ninety seven patients underwent investigation and comparable accuracy (88.9% for EUS-FNA; 87.2% for US-FNA), sensitivity (84% EUS-FNA; 85.5% US-FNA), specificity (100% EUS-FNA; 90.4% US-FNA), positive predictive value (100% EUS-FNA; 94.7% US-FNA), and negative predictive value (73.3% EUS-FNA; 76% US-FNA) were found. Complications occurred in 1/72 patients (1.38%) in the EUS-FNA group (abdominal pain secondary to pancreatitis), compared with 7/125 (5.6%) in the US-FNA group (three cases of severe post-procedure epigastric pain, three cases of peritoneal seeding, and one case of pancreatic abscess requiring surgical debridement and drainage) [22].

It is important to recognise that peritoneal seeding after EUS-FNA has been reported [23], and is therefore not a delineating factor between choosing between percutaneous and EUS-guided biopsy. Of the 15 cases of needle tract seeding reported in this review of case studies of needle-tract seeding after EUS biopsy, 11 occurred during evaluation of pancreatic adenocarcinoma,

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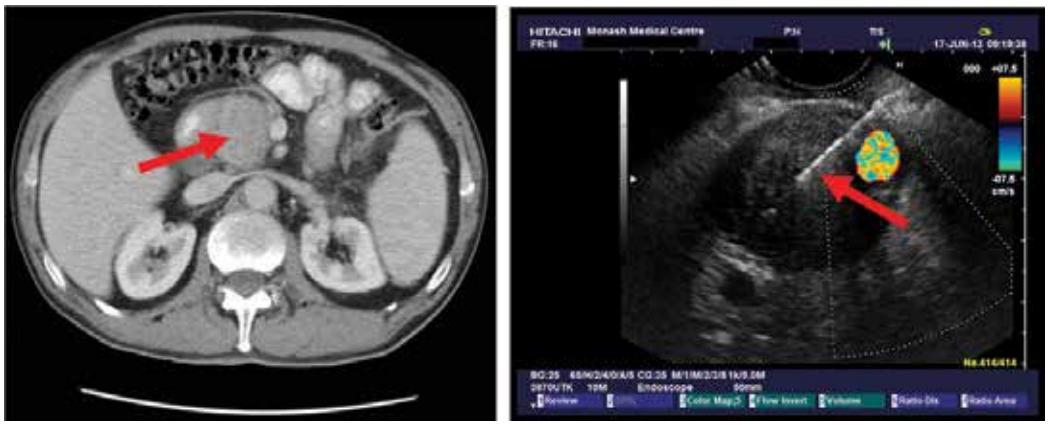

Figure 1. CT and corresponding EUS image of a pancreatic mass that proved to be autoimmune pancreatitis.

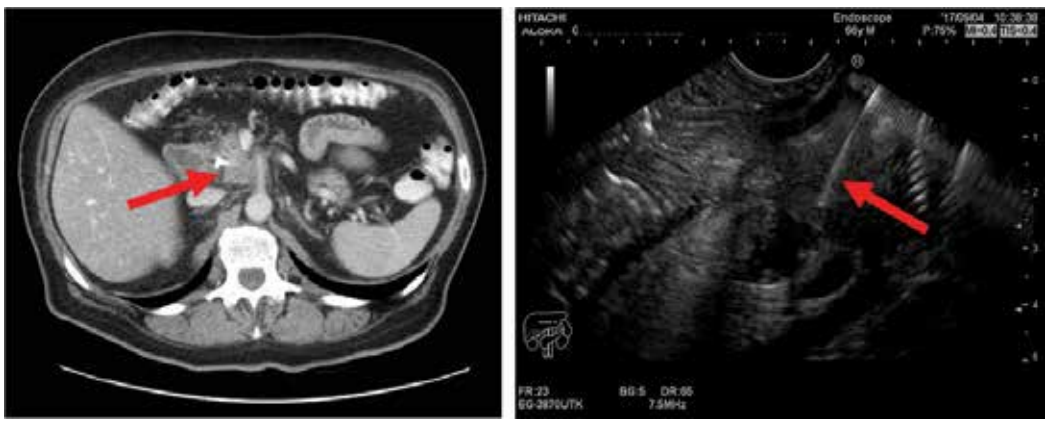

Figure 2. CT and corresponding EUS image of a pancreatic mass that proved to be pancreatic cancer.

with 1 case each of intraductal papillary mucinous neoplasia (IPMN), gastric cancer, malignant melanoma, and squamous cell cancer of unknown origin. All cases of needle tract seeding with relation to investigation of PC occurred with a transgastric approach and did not appear to be related to needle size (mostly 22G) or number of passes (range 1–5).

EUS-FNA of solid masses is generally a safe procedure, with a reported overall complication rate of 0.5–2.54% [24, 25]. Complications include infection, bleeding, and acute pancreatitis. The mortality rate of the procedure has been quoted at 0.04% [25]. Several studies have not found significant benefit in diagnostic yield or complication rate relative to needle size used [26–28]. The use of core (trucut) biopsy (EUS-TCB) instead of, or in combination with FNA has not been investigated to an extent to definitively support its use [29]. EUS-TCB has the potential to provide information about tissue architecture, as well as allow for retrieval of a larger volume of tissue, which in an era of expanding availability of histological and molecular analyses, may become a more desirable methodology, however more information regarding the comparative efficacy and safety is required.

Figures 1 and **2** below show the abdominal CT scan and EUS images of two patients referred to our institution for investigation of painless jaundice and a pancreatic mass. The red arrows in the CT images indicate the pancreatic lesion; the red arrows in the ultrasound image indicted the EUS-FNA needle within the pancreatic mass. In the first case (**Figure 1**), the CT scan and US findings were suspicious for autoimmune pancreatitis. The patient was commenced on high-dose steroids and the lesion resolved and liver function tests returned to normal. In the second case (**Figure 2**) the EUS FNA confirmed the clinical and radiological suspicion of pancreatic cancer.

4. Staging

Surgery is currently the only possibility for cure in PC. 15–20% of PC cases at time of diagnosis are eligible for resection. For those who undergo a successful surgical resection, the morbidity of the procedure is significant, and the 5-year survival rate (5YSR) remains low at 10–25% [30]. Surgery is indicated in the treatment of localised, or minimally-locally-advanced (Stages I-II) PC. Better cure rates are found with node-negative disease (5YSR ~30%); approximately 10% of patients who undergo complete (R0) resection with limited nodal disease progress to long-term survival [31].

The role of preoperative staging is to accurately assess the above features to guide the surgeon as to the likelihood of obtaining an R0 resection. This can be thought of in terms of assessing the extent of local invasion, as well as the presence of distal disease. Standard abdominal CT scanning is the investigation of-choice for assessing distant disease, but has a low sensitivity for assessing local invasion and peritoneal spread. In one study, 61% of cases deemed resectable by CT assessment were found to be unresectable at laparotomy [32]. This modality should not be used alone in assessing appropriateness for surgical intervention.

Standard abdominal CT scans are performed around 60–120 s after injection of intravenous contrast. The optimal timing for imaging of contrast within the pancreas is around 35 s. By using a pancreatic protocol CT, where images are captured at this time point, and then during the washout phase, both local configuration of pancreatic lesions and evidence of local hepatic metastases are elucidated. Pancreatic-protocol CTs are considered the standard imaging investigation for local staging of pancreatic cancer.

The accurate appraisal of the extent of local spread is crucial not only for identifying unresectable disease, but for avoiding false hope and subjecting a patient to an 'open-and-close' laparotomy for no therapeutic benefit.

Local surgical expertise often determines the definition of resectable disease on a pragmatic level, however the National Comprehensive Cancer Network (NCCN) guidelines [33] refer to the following factors when determining resectability:

- Relation to the superior mesenteric artery (SMA), celiac axis, superior mesenteric vein (SMV), and inferior vena cava (IVC)
- Unreconstructable SMV or portal vein

- Aortic involvement
- Distant metastases
- Presence of disease in lymph nodes beyond the field of resection

EUS provides high-resolution images of the primary mass, its relationship to local structures, and the appearance of regional lymph nodes. Conversely to CT, although EUS can detect some liver metastases, it provides insufficient information on distant disease. There have been few studies directly comparing the two modalities, however the combination of both modalities for their relative strengths seems to be the way forward. One study has shown an equivalent PPV of surgical resectability with regards to T-staging of either modality (63%), with a significant increase to 86% when used in combination [34]. While most studies have shown equivalence of EUS and CT with regards to N-staging, EUS has shown greater accuracy in assessing mesenteric vessel involvement, which often has a significant impact on determining surgical resectability [35].

EUS has previously been thought to be superior to CT scanning for the detection and assessment of smaller pancreatic lesions, however comment has been made that the technological advances in radiology continually improving the resolution of CT images that contemporary CT scans may show more accurate results. EUS has however, been shown to lead to less overstaging than multidetector CT (MDCT) and MRI [35]. This is crucial so that resectable cases are not appreciated as unresectable.

5. Screening

The use of EUS in screening patients at increased risk (high-risk individuals [HRIs]) has been suggested due to the lethality of the disease, and the often late-onset of clinical features leading to a very low rate of patients diagnosed at a sufficiently-early stage to undergo curative-intent treatment (15–20%) [30]. In line with the Wilson and Jungner criteria for screening, PC is an important health problem with an acceptable treatment, with a 'latent' phase wherein curative treatment can be undertaken. EUS is a suitable test for early-stage disease that would be likely acceptable to an at-risk population. The questions remain as to whether EUS is yet an accessible test from a resource-availability perspective, and accurately defining HRIs to whom screening could be offered. Subsequent to this, EUS screening of HRIs is yet to be proven to be efficacious, let alone cost-effective to offer as a screening tool.

Identifying HRIs should be based on risk factors for PC. Risk factors such as family history, presence of germline mutations (BRCA1, ATM, PALB2, CDKN2A, and MLH1), Peutz-Jeghers syndrome (PJS), cystic fibrosis, race, ABO blood group, chronic pancreatitis, diabetes mellitus, smoking history, and obesity, are all factors that could be combined to develop a pancreatic risk score. Wang et al. have developed PancPRO, a predictive model for PC using Bayesian modelling to provide risk stratification for developing PC based on family history. It was validated prospectively using the National Familial Pancreas Tumour Registry with an observed to predicted PC ratio of 0.83 (95% CI 0.52–1.20) [36]. The combination of risk stratification algorithms

that may include presence of germline mutations may prove to be a more accurate way of identifying HRIs – more research is needed in this area to more-accurately define an at-risk population in which a screening population can be shown to be efficacious and cost effective.

The use of EUS in HRIs has been explored in a review by Bhutani et al. [37]. They identified 10 studies utilising screening EUS in families with identified familial PC, PJS, familial atypical multiple mole melanoma syndrome, and several other mutations incurring increased risk. A total of 512 screening EUSs were performed across the 10 studies. The rate of abnormal EUS results (pancreatic duct dilatation or ectasia, observable solid or cystic masses, or parenchymal changes) in this study population was 212/512 (41%). Clinical outcome measures (rate of curative resection for detected cases, overall survival (OS), etc.) were not reported overall. Several studies have demonstrated the ability of EUS in HRI to identify pancreatic dysplasia and IPMN, with no reported false-positives when these cases with abnormal EUS progressed to surgical resection [38, 39].

The largest of these studies was performed in 216 individuals with one of the following risk factors:

- Relatives with known familial PC and two affected first-degree relatives (n = 195)
- Individuals with PJS (n = 2), or
- Known familial breast-ovarian cancer patients with at least one first-degree relative affected by PC (n = 19).

Screening was performed on all of these cases with MRI, CT, and EUS. Ninety-two (42%) of participants had an abnormal EUS (at least one pancreatic mass [cystic n = 84, solid n = 3], or pancreatic duct dilatation [n = 5]). Eighty-two of the abnormal EUS cases were IPMNs, and three were neuroendocrine tumours. Five participants went on to have surgical resection, returning three cases of pancreatic dysplasia in <3 cm IPMNs, multiple intraepithelial neoplasms. No cases were identified by CT or MRI that were undetected by EUS. This study lends support to the potential for pancreatic screening in HRIs and supports the choice of EUS as the screening modality over CT and EUS. Further investigation to properly define the characteristics of the at-risk sub-population is needed. The optimal timing and frequency of screening also requires further exploration. The potential merits of screening will need to be balanced against the resource-cost, access, and scalability considerations before routine EUS screening can be supported.

6. Therapeutics

6.1. Celiac plexus neurolysis

The first reported use of EUS-guided celiac plexus neurolysis (EUS-CPN) was published by Wiersema in 1996. EUS-CPN was performed on 30 patients with celiac plexus neuropathy; 25 with PC, and 5 with other intraabdominal malignancies. This single-arm study demonstrated efficacy in a mild to moderate reduction in pain scores at 2, 4, 8, and 12 weeks post-procedure (1–10 pain scale 6.1 +/− 3.1 versus 4.8 +/− 2.0, p = 0.004) [40]. Complications were minor and transient (diarrhoea in four patients).

Although no randomised clinical trial has been performed to compare the relative efficacy and safety of CPN via percutaneous versus endoscopic approach, a Cochrane Review of 102 studies concluded that CPN by any modality was associated with reduced pain at 4 weeks (mean difference in visual analogue scale (VAS) −0.42, 95% CI −0.70 to −0.13, p = 0.004). This less than one point improvement of VAS score begs the question of whether this is clinically-significant; coupling this data with quality of life would perhaps be more informative. This improvement was maintained at 8 weeks overall, the review noted significant heterogeneity of results at 8 weeks at this time point. Collective data on opioid consumption in these studies also showed a significant benefit in the CPN group [41]. A retrospective cohort study by Kambhampati et al. compared outcomes of patients who underwent either percutaneous or EUS-CPN between 2008 and 2015 at Johns Hopkins University School of Medicine. This study suggested a non-statistically significant reduction in procedural complications for EUS-CPN (7% EUS vs. 11% percutaneous, p = 0.51), as well as a non-significant higher immediate response rate in percutaneous CPN (87% versus 72% in EUS-CPN, p = 0.08). Response was defined as a decrease in numeric pain score by ≥3 points. There was no significant difference in quality of life measures, opiate usage, or pain response at 1 month between groups [42].

An interesting study of note by Wyse et al. looked at early EUS-CPN at the time of diagnosis by EUS of unresectable disease [43]. Patients with pain and suspected PC underwent a diagnostic and staging EUS. If diagnosis of unresectable adenocarcinoma was made, patients were randomised to either early EUS-CPN or conventional pain management. The early EUS-CPN group was found to have non-significant improvements in pain response (measured by the Likert scale) and morphine consumption at 3 months compared to standard analgesia (pain response −28.9 [95% CI −67.0 to 2.8], p = 0.09, morphine consumption −49.5 [95% CI −127.5 to 7.0], p = 0.10). Although not statistically significant, these data do suggest that early EUS-CPN at the time of diagnosis could be considered to assist with the often difficult-to-manage analgesic requirements in late-stage PC.

6.2. Biliary duct drainage

EUS-guided biliary duct drainage (EUS-BD) can be performed via several methods, but all involve the direct visualisation via EUS of the pre-obstructed biliary tract and puncture of the pre-obstructive system and confirmation with cholangiography. A guidewire is then inserted and the tract is dilated to create a fistula. These techniques rely on accurate EUS images to target the pancreatic duct, common bile duct, or intrahepatic bile ducts (IHBDs) to create a pancreaticogastrostomy, choledocoduodenostomy, or hepaticogastrostomy, respectively.

EUS-BD can be performed using several techniques:

- Transluminally, where the bile duct or common bile duct is accessed via the stomach or duodenum, respectively.
- Rendezvous, where the ampulla is accessed and the biliary duct is targeted with EUS to fistulise a guidewire to facilitate secondary endoscopic retrograde cholangiopancreatography (ERCP) and stenting over the guidewire
- Antegrade, where an IHBD is accessed from the upper intestine to bypass the anatomic biliary system altogether.

Method	Success rate, % (n)	Complication Rate
EUS-CDS	94.0 (282/300)	18.9 (53/280)
EUS-HGS	86.7 (137/158)	26.8 (41/153)
EUS-RV	80.5 (215/267)	11.1 (24/217)
Overall	87.4 (634/725)	18.2 (118/650)

EUS-CDS, EUS-choledochoduodenostomy; EUS-HGS, EUS-hepaticogastrostomy; EUS-RV, EUS-rendezvous.

Table 1. Summary of EUS-BD approaches reported in Iwashita et al. [44].

These techniques allow for bypass drainage of bile around the level of obstruction and have been shown to be efficacious with a low rate of serious complications. Iwashita et al. [44] conducted a literature review of EUS-BD and stenting. Results are summarised in **Table 1**.

Complications of EUS-BD were generally limited to peritonitis, pneumoperitoneum, abdominal pain, and perforation. No deaths or need for surgery were reported to have been required for complications arising from EUS-CDS or EUS-HGS. Two deaths were recorded in the 217 cases of EUS-RV; one of these was due to cirrhosis, the other was related to sepsis [44]. No comment on prophylactic antibiotic use was made in this review.

A more recent systematic review by Wang et al. of 1192 patients across 42 studies showed similar success rates, with an overall complication rate of 23.3%. The complications encountered were bleeding (4.0%), bile leakage (4.0%), pneumoperitoneum (3.0%), stent migration (2.7%), cholangitis (2.4%), abdominal pain (1.5%), and peritonitis (1.3%), with no differences in complication rate between transduodenal and transgastric approaches [45]. Grade of complications was not reported. It is important to recognise that EUS-BD has historically been utilised in the setting of failed ERCP for biliary drainage and that this may introduce some selection bias towards more difficult cases, or those who have had recent ERCP, which may be attributable to some of the complications documented in the follow-up period of the studies included in these reviews. A randomised controlled multicentre trial (BILPAL) is currently recruiting to compare EUS-BD with standard ERCP in the first-line setting for palliation of malignant obstructive jaundice [46].

7. The future

7.1. Local administration of anticancer therapies

The use of EUS as a delivery system for anticancer therapies is an attractive prospect. The poor vascularity and desmoplastic stroma displayed within a malignant pancreatic tumour is likely a significant factor contributing to the relatively poor efficacy of haematogenously-administered systemic therapies. EUS may circumvent this limitation by offering locally administered anticancer therapies directly into the tumour.

7.1.1. Intratumoural injections

EUS-fine needle injection (EUS-FNI) has the potential to improve the delivery of active cytotoxic agents such as chemotherapy or viral therapy to the target cancer more effectively, whilst

reducing systemic exposure and toxicity. Encouraging early phase data of several investigative approaches are emerging, although larger and randomised studies are lacking.

The use of EUS-FNI of ethanol was investigated in 19 patients with unresectable PC by Yang et al. (2009). At follow-up (between 2 and 7 months), a > 70% reduction in size of pancreatic lesions was identified in 12/19 patients (63%), and a 50–70% reduction in size was found in a further 6/19 patients (32%). Seven patients survived beyond 24 months. No major complications were encountered [47].

Levy et al. (2017) performed a prospective study on first-line EUS-FNI with gemcitabine in 36 patients with stage II-IV PCs. Conventional therapies were allowed in all cases at the discretion of the treating Oncologist, but not described in the results. 95 mg (2.5 mL of 38 mg/mL) of gemcitabine was administered via EUS-FNI. OS at 6- and 12-months was 78 and 44%, respectively. Four (20%) patients with stage III disease who underwent EUS-FNI were down-staged and were able to undergo R0 resection [48].

Immunogenic approaches have included EUS-FNI of allogenic mixed lymphocytic culture, immature dendritic cells, tumour necrosis factor alpha (TNF-α), and gene-deleted replication-selective viruses such as ONYX-015. These agents are still under investigation and have been shown to be feasible and safe, however early clinical data has not been overwhelmingly positive [49].

7.1.2. Brachytherapy

Brachytherapy induces cell death through the delivery of short-wave beta radiation-emitting particles being placed within the tumour. The local delivery allows for a larger total dose to be delivered to the tumour when compared to external beam radiotherapy (EBRT), with relative sparing of surrounding tissue. Endoscopic brachytherapy (EUS-BT) is being investigated in the management of PC, particularly in locally-advanced unresectable PC, currently treated with either combined chemoradiotherapy with EBRT or palliative chemotherapy alone. Although, the efficacy of EUS-BT has not yet been established, trials in this area including at our institution are ongoing. **Figure 3** below shows the placement of brachytherapy seeds under direction visualisation into an unresectable pancreatic cancer through EUS. **Figure 4** shows a Bremm study taken 1 week after implantation of brachytherapy seeds showing the radiation field created by the implanted seed. **Figures 5** and **6** taken from the same patient shows the radiological response achieved by this technique in this case. More investigation is required to optimise patient selection and delivery techniques.

Sun et al. utilised EUS-BT in 15 patients with stage III (n = 8), and stage IV (n = 7) pancreatic adenocarcinoma [50]. 27% of cases experienced a partial response, with a mean duration of response of 4.5 months. Rate of disease control was notable at 80% (partial 27%, mild 20%, stable 33%), and 30% of patients showed a clinical benefit (defined by an improvement in Karnofsky Performance Score and pain response to treatment), particularly with regards to pain reduction. Local complications occurred in three patients (pancreatitis and pseudocyst formation), and grade III haematologic toxicity was encountered in three patients without clinical impact [50].

Brachytherapy with several radiation-emitting sources has been trialled (Ra^{226}, Rn^{222}, Au^{198}, Ir^{192}) with significant complications and post-treatment mortality. More recently, I^{125} has been

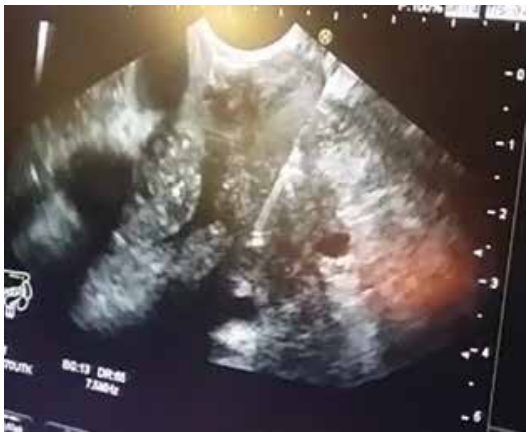

Figure 3. Brachytherapy seed implantation under direct EUS visualisation.

investigated, with much improved mortality rates, but showed no benefit to cancer-related mortality [51]. Current phase III studies are under way with P^{32}; phase II safety studies have shown a moderate increase rate of serious adverse events per patient when used with 5-fluorouracil (5FU) chemotherapy followed by gemcitabine, compared to EBRT with 5FU chemotherapy, followed by gemcitabine [52]. The varying complication rates reported across studies may also be due to interoperator variability or the low numbers of cases treated. More studies with larger numbers are needed and are currently underway.

There are also some efforts to improve the planning and delivery of brachytherapy to the intended area. Sun et al. (2017) developed a computer-based treatment planning system that was studied in 42 patients with unresectable PC. In this study, EUS-BT using this software

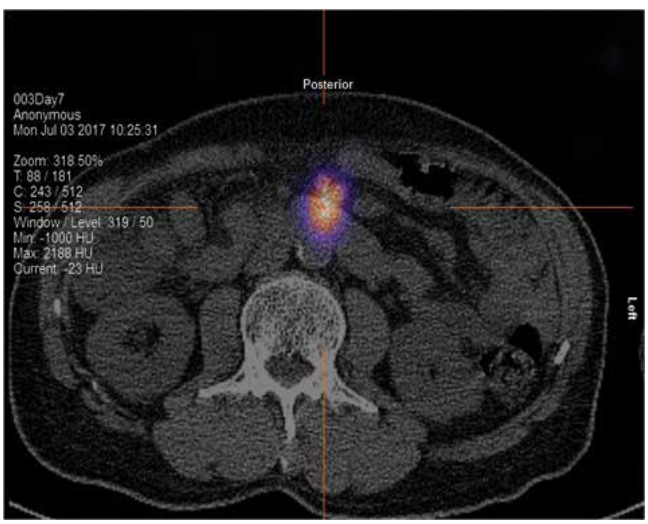

Figure 4. Bremm study one week after brachytherapy.

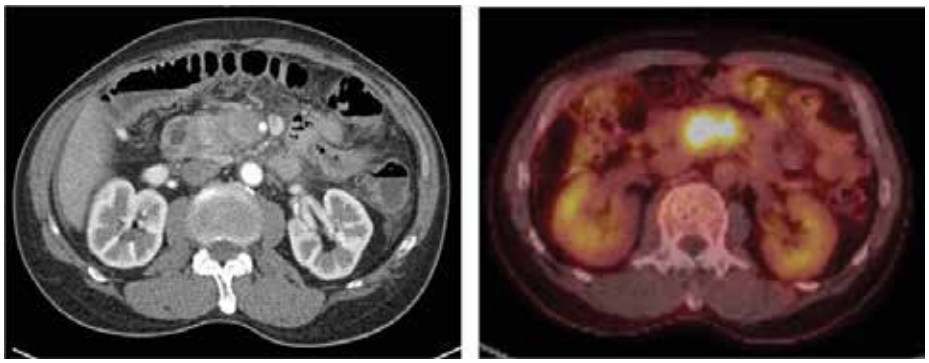

Figure 5. CT and PET scan of pancreatic cancer pre-brachytherapy implantation.

was performed and showed an OS for stage III patients of >12 months with an overall median survival time of 9.0 months (95% CI 7.6–10.4 months) [53]. Interestingly, the use of this treatment planning system resulted in no serious adverse events in the study population, which has been a significant criticism of this treatment modality previously.

7.1.3. Radiofrequency ablation

Radiofrequency ablation (RFA) induces coagulative necrosis through the application of heat induced by a medium-frequency alternating current [54]. RFA as an anticancer technique is currently utilised in the management of several other malignancies (hepatocellular, renal, etc.), but is also employed in the disruption of aberrant electrical pathways in the heart, as well as in pain medicine, for the ablation of nerve in certain conditions. Until EUS, the utility of external application of RFA has been limited by the sensitivity of pancreatic tissue and nearby gastrointestinal tissues to RFA, leading to significant complications.

EUS-radiofrequency ablation (EUS-RFA) has been studied in several small case series. There have been two recent systematic reviews published on EUS-RFA in pancreatic malignancies [55, 56]. Rustagi and Chhoda (2017) reported on four clinical studies performed in locally-advanced,

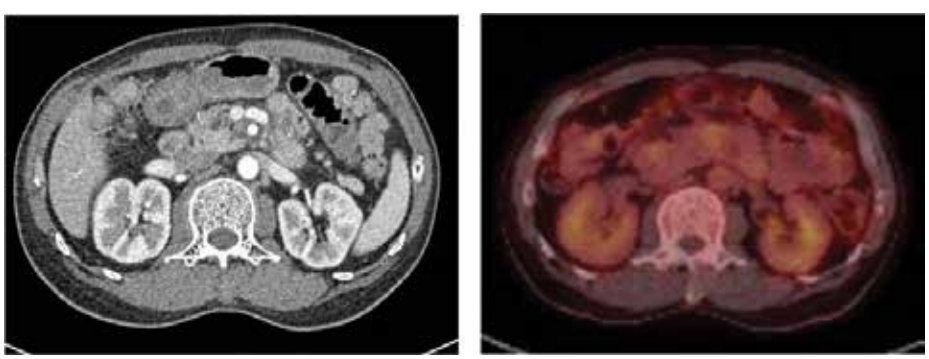

Figure 6. CT and PET scan of pancreatic cancer post-brachytherapy.

unresectable adenocarcinoma, pNETs, and pancreatic cystic neoplasms (PCNs). The endpoint of most of the reported studies was complication rate, rather than efficacy or survival. The follow-up period for the articles addressing better-prognosis pancreatic lesions (PCNs / pNETs) was also likely too short to draw conclusions from. Of the 37 cases included across the four studies, adverse events included mild abdominal pain in seven cases, minor duodenal bleeding in one case, jaundice in two cases, duodenal structuring in one case, and cystic fluid collection in one case. The authors concluded that EUS-RFA is feasible and safe in the management of pancreatic lesions, and that more studies are needed with larger sample sizes and longer follow-up periods to investigate EUS-RFA as a treatment modality for PC [56].

7.1.4. High-intensity focused ultrasound

A high-intensity focused ultrasound (HIFU) transducer has been developed for use with endoscopy. HIFU induces cell death by thermogenic coagulative necrosis, similar to RFA, but by emitting ultrasound waves, rather than radiofrequency waves. Tong et al. (2015) have successfully used this probe to induce lesions in normal porcine pancreatic models in vivo [57] to show proof of concept in inducing targeted areas of cell necrosis in pancreatic tissue. HIFU's use in inducing cell death in malignant pancreatic lesions has yet to be elucidated.

7.2. Artificial intelligence

EUS images can be digitised for analysis by artificial neural networks (ANNs) to quantitatively analyse EUS images as to their likelihood of there being a malignant lesion within them. The use of ANN analysis in pancreatic EUS image analysis was reported by Norton (2016). In a study of 21 patients with PC and 14 patients with focal pancreatitis, ANN analysis was able to differentiate between PC and focal pancreatitis with an accuracy of 89%. This was similar to the endosonographer's impression at time of EUS (accuracy 85%) [58].

Saftoiu et al. performed a similar study among 68 patients; 22 with a normal pancreas, 11 with chronic pancreatitis, 32 with pancreatic adenocarcinoma, and 3 with pancreatic neuroendocrine tumours (pNETs). Reported sensitivity, specificity and accuracy were 91.4, 87.9, and 89.7% respectively and the study concluded that larger, prospective randomised controlled trials were needed to further investigate the use of this adjunct diagnostic tool [59].

With constant improvements in image quality, and further development of ANN models, this may prove a useful adjunct to EUS-based diagnosis, particularly if used by inexperienced endosonographers, and may help to broaden the accessibility of this imaging modality.

7.3. Elastography

The act of vibrating tissues and measuring the elasticity of their resultant movement is being used in analysis of pancreatic lesions. In general, firmer lesions tend to be malignant; soft lesions are more likely benign. By qualitatively or quantitatively assessing their rebound potential, inferences can be made on the composition of pancreatic lesions.

Due to the differing relative consistency of benign and malignant lesions, quantitative strain elastography results can assist in differentiating subtypes of pancreatic mass. The use of EUS-Elastography has been assessed to have excellent sensitivity (95–99%) for differentiating benign

from malignant lesions, however due to the fibrotic nature of many of the benign pancreatic lesions (tumour-forming pancreatitis, and benign pancreatitis with fibrosis), specificity is inadequate (67–76%) to replace direct tissue sampling by way of EUS-FNA [60]. Moreover, there are currently several guidelines on the strain ratio cut-off value for differentiation between tissue subtypes, thus harmonisation and standardisation are required between techniques.

Elastography can be measured by either strain elastography by measuring propagated external pressure in the axis of the direction of the applied force, or by shear wave elastography [61]. The latter utilises acoustic radiation force impulses to generate perpendicular 'shear' waves, the velocity of which can be measured in the field of the ultrasound, and are not affected by structures posterior to the target organ in question. Currently, only strain elastography is available via endoscopic approach. Due to the pulsations of the nearby aorta, the future use of shear wave elastography may be advantageous over current strain elastography.

7.4. Contrast-enhanced harmonic ultrasound

Contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS) uses intravenously administered hyperechoic microparticles at the time of ultrasound to provide further information regarding the vascularity of the lesion in-question. In the presence of contrast, PC generally appears hypoenhanced and heterogenous, pancreatitis appears isoenhanced, and pNETs cancers appear hyperenhanced [62]. Sensitivity has been reported to be above 90% in multiple studies [63, 64]. However, as some PCs have been reported as being isoenhanced, the specificity of this modality (68%) is also insufficient for replacing EUS-FNA.

CEH-EUS has been combined with EUS-FNA to improve the accuracy of diagnosis of EUS-FNA. Due to the highly desmoplastic stroma in and around PCs, targeting hypoechoic or isoechoic appearance on CEH-EUS for FNA has been shown to improve diagnostic yield when compared to EUS-FNA alone. Sugimoto et al. (2015) have also shown that CEH-EUS-FNA has the potential to reduce the number of needle passes required for diagnosis [65]. In their conclusion, the authors make the valid point that in all reported cases of needle-tract seeding in EUS-FNA, multiple needle passes were performed. Although in need of validation, CEH-EUS-FNA has the potential to reduce the risk of needle-tract seeding by reducing the required needle passes.

8. Confocal laser endomicroscopy

Confocal laser endomicroscopy (CLE) allows for in vivo histological analysis of tissues in real time. The technique is being developed for the assessment of early pancreatic masses and the surveillance of precancerous lesions. A laser is used to illuminate the target tissue, which is then reflected back through a pinhole to the user. Local or systemic use of fluorescence agents, such as fluorescein can also be used to enhance the image. In endoscopy, CLE can either be done through an integrated endoscope tip, which has been useful for assessing and targeting biopsies of the luminal wall (e.g. oesophagus or stomach), or through needle-based CLE (nCLE), which uses a microfiber that can pass through a 19-gauge needle to assess tissue at the site of the needle tip.

nCLE has been studied mostly in investigation of pancreatic cystic lesions and shown to have an accuracy of 46–95% for diagnosing PC, with a low sensitivity 46–59%. Overall complication rate ranges from 0 to 2.5%; complications include bleeding, infection, pancreatitis and perforation [66]. Nakai et al. used a combination of EUS-guided cystoscopy (direct visualisation of the internal wall of a cystic lesion) and nCLE in the assessment of 30 patients with cystic pancreatic lesions. The sensitivity of cystoscopy was 90% for determining PCN vs. BPC, nCLE was 80% sensitive, and the combination of the two modalities was 100% sensitive [67].

Kongkam et al. conducted a study to validate the CLE diagnostic criteria and found a 90.9% accuracy of EUS-nCLE among 22 patients [68]. They found malignant lesions displayed dark clumping with or without dilated vessels (<40 μm), while benign lesions were more likely to display white fibrous bands and normal acini. They also found good inter-observer agreement between the three blinded endoscopists ($\kappa = 0.82$) [68]. These results contrast that of Karstensen JG et al. (2018), who conducted a prospective, dual-centre study on 28 patients with pancreatic masses referred for EUS-FNA and found limited benefit above EUS-FNA alone by using the current proposed nCLE criteria. This study also found significant interobserver and intraobserver analysis of the proposed CLE criteria, suggesting the reproducibility of the procedure is currently suboptimal. They concluded that further development of the technology is needed to permit better delineation between benign and malignant disease [69]. More studies are required in this area before EUS-CLE can be recommended as an adjunct to EUS-FNA for routine analysis of solid pancreatic lesions.

The use of EUS-nCLE to enable direct visualisation of molecular expression with pancreatic cancers has also been explored. Nakai et al. have shown proof-of-concept in the ability to directly image EGFR and survivin expression in porcine models in vivo. This study utilised the direct injection of fluorescein isothiocyanate-labelled antibodies against EGFR and survivin into the pancreas 30 min before EUS-nCLE to highlight the expression of EGFR and survivin, and showed good correlation between the EUS-nCLE images and histological analysis of the porcine pancreas ex vivo [70]. The use of similarly labelled antibodies to KRAS could assist in the stratification of precancerous lesions and direct early-stage treatment.

What about a section on molecular diagnosis personalised therapy. You cannot tell me that this will not be important in the future. I know self-citation is frowned upon but we have published 3 articles in this space recently?

9. Conclusion

EUS now has an integral and indeed indispensable role in the diagnosis, staging, and treatment of pancreatic cancer and its complications. It is likely that this technique will become increasingly important in the management of patients with this condition.

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Types of Treatment

Chapter 5

Irreversible Electroporation in Pancreatic Cancer

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Additional information is available at the end of the chapter

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Abstract

Pancreatic cancer is the deadliest of the gastrointestinal tract with 5-year survival rates of less than 5%. Given common asymptomatic early disease course, most patients (50%) present with an already metastatic disease, while only 20% can undergo potentially curative resection. The remaining 30% present with locally advanced disease, defined as extended vascular encasement, where the risk of surgical therapy often outweighs its benefits. Traditional thermal local ablative modalities (RFA, MWA, or cryotherapy) have the disadvantage that they are not applicable in proximity to vital vascular structures, which are abundant in the peripancreatic region. Irreversible electroporation (IRE) is an emerging non-thermal alternative that induces apoptosis of tumor cells by the delivery of short repetitive impulses of high-voltage electric current. Given its mostly non-thermal modality, IRE is not hampered by a heat-sink effect and is applicable with little risk around vascular structures, bile and pancreatic ducts. Recent research suggests that local tumor destruction through IRE improves overall survival, progression-free survival and quality of life in patients with locally advanced pancreatic cancer.

Keywords: locally advanced pancreatic cancer, borderline resectable pancreatic cancer, irreversible electroporation, local tumor destruction, apoptosis, overall survival

1. Introduction

Pancreatic adenocarcinoma is a prevalent disease with 53,760 newly diagnosed patients in the United States in 2017 [1]. Despite the rapidly growing medical progress in the twenty-first century and extensive efforts in cancer research, pancreatic adenocarcinoma remains a highly aggressive malignancy with 5-year survival rate still not exceeding 5% [1, 2]. By extrapolating annual incidence rates, pancreatic adenocarcinoma is estimated to rise to the second leading

cause of cancer-related death in the United States by 2020 [3]. Unfortunately, only a minority of patients presented early during the disease course, and screening programs have been crowned by little success so far [4]. It is globally accepted that early detection of the tumor provides the only chance for cure, given that treatment modalities other than surgical resection are inherently palliative. Several clinical staging systems for patients with pancreatic adenocarcinoma exist, while all of them comply with the only potentially curative treatment option, that is, surgical resection. Thanks to more elaborate imaging techniques now widely available (high-resolution CT, MRI, endoscopic ultrasound), the tumor-vessel relationships can be determined with high precision rendering pretreatment staging increasingly accurate. Among others, one of the largely used and accepted staging definitions has been established by the National Comprehensive Cancer Network (NCCN) [5]. This classification groups pancreatic adenocarcinoma into four categories such as resectable, borderline resectable, locally advanced and metastatic disease [6].

Definition according to the National Comprehensive Cancer Network (NCCN) [6]:

Resectable tumor: no distant metastases, no radiographic evidence of superior mesenteric vein (SMV) and/or portal vein (PV) abutment, distortion, tumor thrombus, or venous encasement. Clear fat planes around the celiac axis, hepatic artery and superior mesenteric artery (SMA).

Borderline resectable: no distant metastases, encasement of the SMV/PV but without encasement of the nearby arteries or short segment venous occlusion resulting from either tumor thrombus or encasement but with suitable vessel proximal and distal to the area of vessel involvement, allowing for safe resection and reconstruction. Gastroduodenal artery encasement up to the hepatic artery, without extension to the celiac axis. Tumor abutment of the SMA not to exceed >180° of the circumference of the vessel wall (**Figure 1**).

Locally advanced (unresectable): tumor involvement or occlusion of the SMV or PV, which precludes reconstruction of vessels or greater than 180° tumor contact with either SMA, celiac artery or any involvement of first jejunal branch of SMA or Aorta (**Figure 2**).

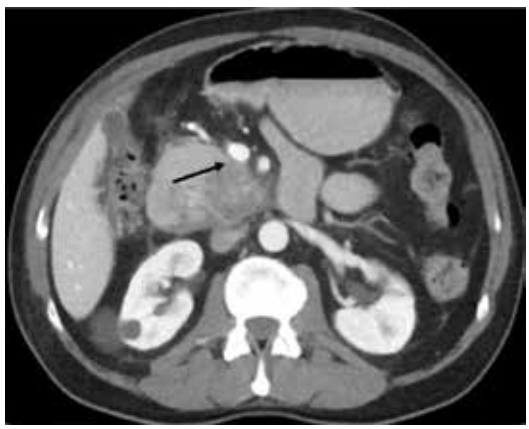

Figure 1. Borderline resectable pancreatic cancer. Tumor with contact to the SMV (black arrow).

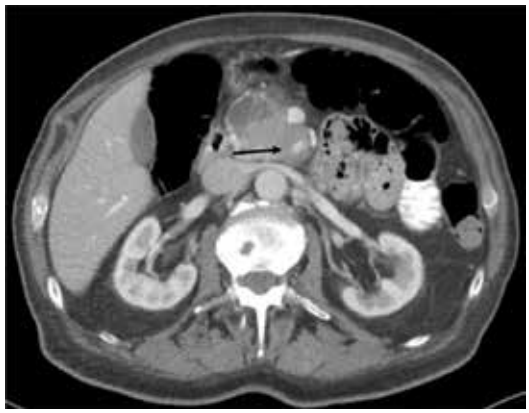

Figure 2. Locally advanced pancreatic cancer. Encasement of the superior mesenteric artery (black arrow).

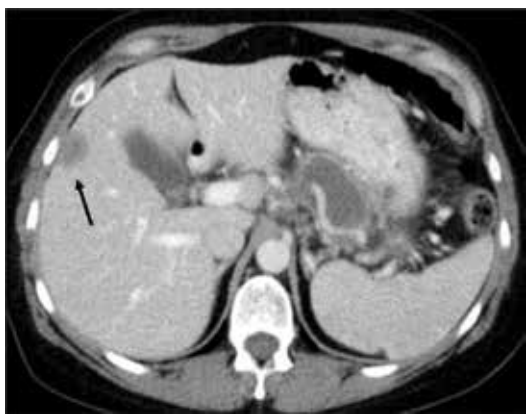

Figure 3. Metastatic pancreatic cancer. Liver metastasis (black arrow).

Metastatic disease: presence of distant metastases (e.g. hepatic, peritoneal, or other) (**Figure 3**).

At the time of diagnosis, only approximately 20% of patients present with a resectable or borderline resectable disease. If treated by surgical resection with negative histological margins (R0) followed by adjuvant chemotherapy, this patient group can achieve a 5-year survival rate of about 20% [2, 5]. While 50% of patients initially present with metastatic disease, the remnant 30% show a locally advanced stage with involvement of vital adjacent structures as defined earlier. While the diagnosis of resectable and metastatic disease usually is simple, the distinction between borderline resectable and locally advanced disease can be challenging. In patients with borderline resectable disease, the involvement of major vessels is less extensive, and a macroscopic negative resection margin (e.g., with segmental/partial resection of venous structures) is potentially achievable without adding major morbidity [7]. In contrast, patients with locally advanced pancreatic cancer are often characterized by arterial vessel involvement. A resection with negative margins in these cases is technically rarely feasible

and often implies arterial resection associated with high perioperative morbidity and mortality [8–11]. A timely meta-analysis by Mollberg et al. assessed the impact of arterial resection on perioperative outcomes among patients undergoing pancreatic resection. They showed that perioperative morbidity and mortality was, with 53 and 12%, significantly higher for patients with arterial resection compared to around 25–30 and 6% reported for pancreatoduodenectomies, not requiring arterial reconstruction, respectively [12–14]. In summary, given the high perioperative risk and its limited impact on survival, arterial resection can at present not be justified as a standard procedure in the treatment of pancreatic cancer [2, 10]. Accordingly, the current treatment recommendations for patients with locally advanced pancreatic cancer are chemo- and/or radiotherapy, which achieve a median overall survival of 9–13 months on average [2]. There is notably a difference in the standard treatment regimens according to the geographic location; most patients in the USA with locally advanced pancreatic cancer are currently undergoing combined chemoradiotherapy, whereas patients in the Europe are usually treated with chemotherapy alone. Recent advances in chemotherapy allow to downstage certain patients with locally advanced disease, making a surgical resection of some tumors possible [15, 16]. Patients treated with neoadjuvant chemotherapy followed by surgical resection can achieve similar survival rates like patients diagnosed in a resectable or borderline resectable state. It is estimated that in select patient cohorts, up to one-third of patients initially judged as non-resectable can be converted into a resectable state by neoadjuvant chemotherapy. However, the neoadjuvant regimen is still debated and is not internationally accepted yet as standard of care [17, 18].

Given that patients with locally advanced disease have a poor prognosis despite multimodal therapy, additional treatment alternatives are desperately needed. In the group of locally advanced pancreatic cancer, the tumor is confined to the location of origin without evidence of distant spread—rendering local therapy an attractive additional treatment option. As such, loco-regional therapies including radiofrequency ablation (RFA), microwave ablation (MWA) and irreversible electroporation (IRE) have gained increased attention in the treatment of locally advanced pancreatic adenocarcinoma over the last years [19, 20].

2. Local ablative strategies

2.1. Thermal local ablative strategies

Radiofrequency (RFA) and microwave ablation (MWA) have been used in an attempt to achieve local control among patients with locally advanced pancreatic cancer. Both treatments generate thermal energy by a high-frequency alternating current, which is delivered to the cancerous tissue by one or more needle electrodes. The created high local temperature at the tip of the electrodes leads to cell death by coagulative necrosis and protein denaturation [21, 22]. RFA has been used with success in the setting of unresectable tumors in multiple solid organs (liver, lung, kidney, brain, breast, prostate, bone, adrenal glands, spleen) [22]. Over the last years, it has also been deployed in the palliative setting of locally advanced pancreatic cancer. However, RFA in patients with pancreatic adenocarcinoma has not been widely accepted because of considerable morbidity and mortality rates [19]. The high complication rate was thought to be due to thermal injury to the multiple delicate structures (bile duct, pancreatic duct, duodenum, vital vessels)

surrounding the pancreas. By adjusting the administered temperature from 105 to 90°C for 5 min' length, complications in recent patient cohorts treated by RFA were substantially reduced [20]. Still, gastrointestinal hemorrhages, acute pancreatitis, pancreatic/biliary fistulas, duodenal injury and portal vein thrombosis are regularly reported in the literature [19]. A systematic review from 2014 cited an RFA-related morbidity ranging from 10 to 37% and an RFA-related mortality from 0 to 19% [23]. Another important downside of thermal ablative therapies is the so-called "heat-sink effect." During the ablation process, adjacent blood vessels are "cooling the tissue down" leading to an insufficient temperature in the immediate proximity of the vessels, where therefore efficient cell death cannot be induced [24]. Given the anatomical complexity of the pancreatic region and bearing the abovementioned aspects in mind, it is self-evident that the application of thermal ablative therapy in locally advanced pancreatic cancer is delicate. It is at this state unknown whether RFA should be combined with chemo- and/or radiotherapy as a standard treatment. A retrospective analysis of patients with locally advanced pancreatic cancer with short induction chemotherapy and RFA compared to a patient group with RFA did not show a difference in early disease progression or overall survival [25]. While there is no evidence from randomized controlled trials regarding the oncological outcome of RFA in locally advanced disease, several case series show a significantly increased median overall survival in patients where RFA was part of the treatment concept [26, 27].

MWA is less prone to the heat-sink effect compared to RFA and is therefore more suitable for application closer to large vessels. Similar to RFA, no randomized data using MWA in locally advanced pancreatic cancer are available [28]. Given the heterogeneous reports of MWA and RFA, direct comparisons between the two techniques in regard of long-term survival are currently not available. However, based on published evidence, MWA seems to lead to less postoperative pain and decreased ablation time with similar results in morbidity and mortality compared to RFA [29]. However, at present, MWA is still studied less extensively than RFA [30, 31].

2.2. Irreversible electroporation

2.2.1. Introduction to IRE

Irreversible electroporation (IRE) is an emerging ablative modality that gained enormous interest over the last years. In contrast to the abovementioned thermal ablative strategies, IRE leads to cell death mainly through a nonthermal technique. In IRE, high voltage (maximum of 3000 V) electrical pulses of 70–90 μs duration are applied through a minimum of two electrodes positioned next to or into the target neoplastic tissue. The thus created electrical field leads to a disruption of the cell membrane's lipophilic bilayer by formation of nanoscale micropores. This damage to the cell membrane eventually leads to a collapse of intracellular homeostasis and an activation of apoptotic pathways, finally resulting in cell death. The distinct advantage of this technique compared to thermal ablative strategies is the preservation of structural components like collagen and elastin as thermal damage does only occur in the very close proximity to the ablation needles depending on pulse length, exposure of the needle tips, delivered energy, distance between the electrodes and underlying tissue. Another advantage of IRE compared to thermal ablative modalities is its nonexisting "heat sink effect," which means that the efficiency of IRE will not be reduced in proximity to large

vessels [21, 32]. For the above-cited reasons, IRE is a very attractive local ablation method in pancreatic cancer, given the inherent proximity of the pancreas to vital vascular structures as well as the bile and pancreatic duct.

However, IRE cannot be applied under any circumstances given that several contraindications for its use exist. The presence of metallic material in close proximity to the placed IRE needles (e.g., metallic biliary stent that is not removable) is a relative contraindication for IRE, given that the conductivity of the metal could potentiate the minimal thermal effect of it. Even more importantly, the presence of metal can distract, respectively, and derivate the electrical current used in IRE, rendering prediction of the ablation zone impossible. Hence the effect of IRE is potentially dangerous [33, 34]. Also, a tumor size >5 cm is generally seen as a contraindication, given that the volume of the ablation zone of a tumor exceeding this size is technically difficult to achieve [21]. Additionally, IRE is contraindicated in patients with certain cardiac arrhythmias, and patients with pacemakers should be evaluated by a cardiologist prior to IRE, as the high-voltage electric current applied can itself provocate potentially serious arrhythmias [32]. To avoid such complications in ablations at the level of the pancreas, the electrical pulses are applied during the complete refractory phase of the heart (50 ms after the R wave). To achieve the coordination of the IRE pulses and the heart rhythm of the patient, the IRE device is synchronized with the patient's ECG. Furthermore, application of IRE is not recommended in patients having a history of epilepsy or recent myocardial infarction. No data exist about the use of IRE in pregnancy.

2.2.2. IRE in locally advanced pancreatic cancer

IRE has first been established as a complementary local therapy in conjunction with chemotherapy for patients with locally advanced pancreatic cancer, which is not amenable to surgical resection [30]. In situations where surgical resection seems too risky (e.g., a tumor encapsulating the superior mesenteric artery), IRE has shown to be a safe and valuable treatment alternative. Standalone IRE without surgical resection of the primary tumor is called "in situ" IRE. Its primary aim is to achieve maximal local tumor control. As in thermal ablative strategies, there is currently no randomized data available that look at oncological outcomes of (radio-) chemotherapy and IRE compared to (radio-) chemotherapy alone. However, there is encouraging evidence that suggests a relevant improvement of overall survival in patients with in situ IRE after induction chemotherapy/(radio-) chemotherapy [2, 35]. A propensity-matched score analysis by Martin et al. showed a survival benefit of induction chemotherapy and/or radiation followed by IRE compared to (radio-) chemotherapy alone. The additional treatment with IRE showed a prolongation of local progression free survival from 6 to 14 months, distant progression free survival from 9 to 15 months and overall survival from 13 to 20 months [35]. Another study analyzing 200 patients with locally advanced pancreatic cancer, either undergoing in situ IRE or margin accentuation IRE after an induction chemotherapy/(radio-)chemotherapy showed an encouraging median overall survival of 24.9 months and local recurrence rates of only 3% [36]. These results indicate that local tumor control with IRE is achievable and has a significant positive effect on patients with locally advanced pancreatic cancer. However, the interpretation of data on long-term oncological outcomes after IRE is still difficult, given that the studies available are of substantial heterogeneity and mostly lacking direct control groups. Additionally, most studies were not primarily designed to demonstrate oncological efficacy of the procedure but rather aimed to demonstrate safety and efficacy of the IRE procedure itself [2]. Some authors emphasize

the possible impact of neoadjuvant chemotherapy over the direct effect of IRE given that the specific impact of IRE has not yet been demonstrated. However, Gillen et al. found a median overall survival of 22 months in patients with locally advanced pancreatic cancer treated with neoadjuvant chemotherapy and if possible subsequent pancreatic resection [17]. These survival outcomes are still slightly worse than the ones of the 200 patients documented by Martin et al. in his cohort undergoing in situ IRE/margin accentuation IRE with pancreatic resection. IRE thus seems to add some additional benefit that systemic chemotherapy cannot provide, most probably by its local field of action. This observation has led to the hypothesis that the resection margin in pancreatic cancer deserves further investigation, as IRE might contribute to better overall survival by achieving more "true" R0 resections (see Chapter 4) [37]. Additional studies focusing on overall survival are certainly needed to further investigate the potential of IRE in improving the outcomes of patients with locally advanced pancreatic cancer.

2.3. Induction therapy in locally advanced pancreatic cancer before in situ IRE

At present, in situ IRE is mainly recommended in combination with upfront chemotherapy or (radio-) chemotherapy for at least 3 months. This does not only allow a "test for time" to get familiar with the biology of the patients underlying tumor, but also avoids local treatment with in situ IRE among patients with metastatic disease. Several induction treatment regimens have been suggested while so far no specific data are available, which favor one regimen over the other. Gemcitabine-based chemotherapy and/or radiotherapy is an option; however, more recent studies show beneficial results with the more aggressive FOLFIRINOX regimen as initial therapy [38–40]. Given the significant toxicity of FOLFIRINOX, a modified regimen has been suggested, where the 5-FU bolus is left out [15]. An alternative chemotherapy regimen often used in advanced pancreatic cancer and also in the setting of induction therapy before IRE is the combination of gemcitabine and Nab-paclitaxel [41]. Further studies assessing the best inductive treatment before IRE are needed before any general recommendation can be given.

Whatever induction therapy is used, it must be followed by restaging investigations. While there is no standard algorithm recommended, we perform a 3-phase contrast enhanced pancreas protocol computer tomography including the chest, to exclude pulmonary metastases and to plan the IRE procedure in detail. High quality CT-scans allow for sound judgment of tumor vessel relationships. In addition, given that diffusion MRI of the liver has shown to outperform CT-scans in regard of detection of liver metastases, all patients will undergo this imaging tool prior to surgical exploration [42].

3. Technique of IRE

3.1. General considerations

As mentioned earlier, all eligible patients for in situ IRE with locally advanced pancreatic cancer have to complete at least 3 months of neoadjuvant (radio-) chemotherapy, mainly to avoid local IRE treatment in patients with metastatic disease. This said, restaging after finishing induction treatment is crucial and should be performed with major diligence. Noteworthy is the usually absent "radiographic response" in pancreatic imaging after neoadjuvant

therapy—consensus is therefore to proceed to in situ IRE unless imaging shows local disease progression or newly observed distant metastases [2, 35, 36].

In case restaging confirms the presence of locally advanced pancreatic adenocarcinoma, every patient should be discussed at an interdisciplinary tumor board including medical oncologists, radiation oncologists, radiologists, pathologists and surgical oncologists. If tumor response is achieved and the lesion can be downstaged to borderline resectable disease, patients should be considered for surgical resection with margin-accentuation IRE (see part 4). In cases of stable disease without development of distant metastases and a maximal tumor diameter of <5 cm, patients can be planned for in situ IRE.

3.2. Practice of IRE

3.2.1. Open approach

Technically, there are different ways to apply IRE to a target lesion. Practice in our institution is at the moment the "classical" open abdominal approach. An upper midline or transverse incision is performed followed by a meticulous abdominal exploration looking for occult metastatic disease. IRE needles are then placed under ultrasound guidance covering the suspected tumor area. At least two unipolar probes are needed to deliver the high-voltage current. Parallel orientation of the needles is of utmost importance, with ideally a distance of about 2 cm between each needle pair (**Figures 4** and **5**).

A maximum of six probes can be inserted at the same time [43]. During the IRE procedure itself, full neuromuscular relaxation has to be guaranteed as the high voltages transmitted by the electrodes can produce significant muscular contractions [44]. If successful ablation was performed at one site, needle pull-back can be repeated as many times as needed with performance of the treatment at another level in order to cover the full desired ablation volume. Early imaging documentation of the success of IRE treatment is not possible, given the unspecific postoperative changes. As such, control imaging by CT-scan is not recommended before 3 months after IRE, as the images can be altered by ongoing edema following electroporation [45].

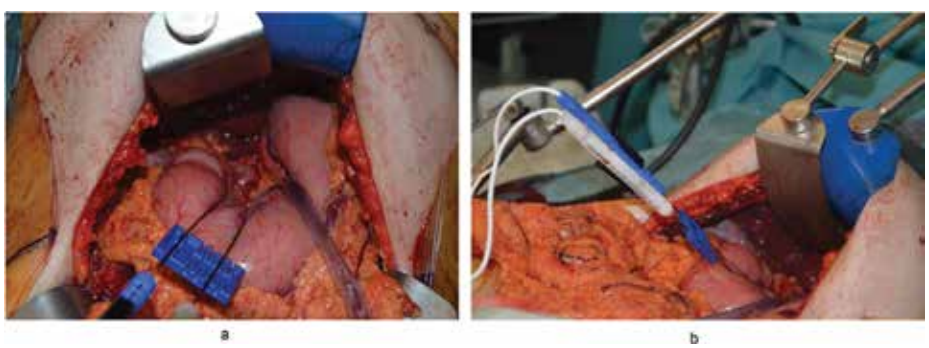

Figure 4. (a and b) Parallel placement of two needles at the distance of 2 cm for an IRE treatment around the common hepatic artery.

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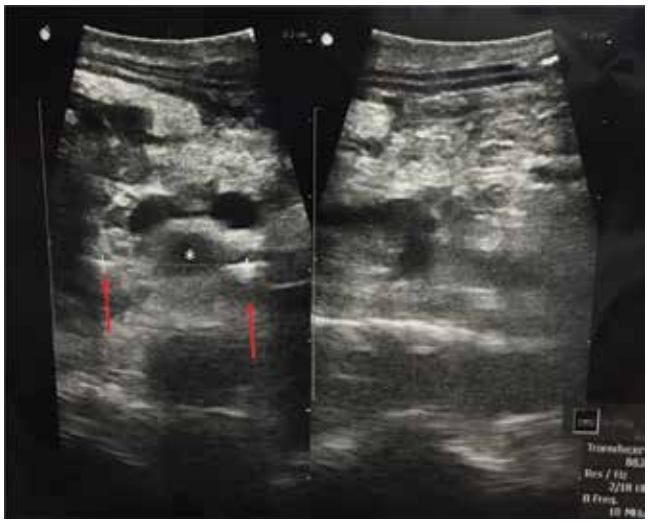

Figure 5. Intraoperative needle positioning under ultrasound guidance. In this example the two needle tips (red arrows) are placed to the left and right of the superior mesenteric artery (*).

3.2.2. Minimal-invasive approach

IRE may also be applied in the setting of minimally invasive surgery under laparoscopic ultrasound guidance [46].

Additionally, surgical interventions like hepaticojejunostomy or gastroenterostomy, which have the potential to improve the quality of life in patients suffering from locally advanced pancreatic adenocarcinoma, can be performed during the same intervention in patients receiving IRE by either an open or a laparoscopic approach.

3.2.3. Percutaneous approach

Several groups have gained experience in the percutaneous application of IRE supported by different imaging modalities. Narayanan et al. reported a series of 50 patients with CT-guided percutaneous IRE. The procedure was technically feasible in all patients. A median overall survival of 27 months from the time of diagnosis and 14 months from the time of IRE was reported, which is comparable to the oncological outcomes observed in open IRE [47]. Another group around Mansson performed IRE under ultrasound guidance. Of the 24 patients, all treatments were completed using ultrasound guidance only [48]. The case series presented are small, but the data suggest that the percutaneous approach is technically feasible and generally safe [47, 48]. A potential drawback of the percutaneous approach is the lack of visual assessment of the peritoneal cavity. Small liver/peritoneal lesions can be missed and patients with potential metastatic disease might be "locally overtreated," given that at present no data for application of IRE in metastatic settings exist.

3.3. Potential complications of IRE

Despite its nonthermal technique, also IRE is associated with potential complications. In the so far largest population from Martin et al. consisting of 200 patients treated with IRE, a total rate of 37% adverse events were recorded along with a mortality rate of 2%. The most common adverse events reported were pancreatic leak, pancreatitis and duodenal ulcer formation. Also, less frequently vascular complications (such as hepatic arterial thrombosis or mesenteric/portal vein thrombosis) and liver dysfunction/failure have been observed [36]. A group from Scandinavia analyzed the so-far gained IRE experience in a recent review including 10 studies comprising 446 patients in total (304 patients treated with open IRE and 142 patients treated percutaneously). A total of nine fatalities (2%) were recorded, while overall complication rate was summarized to be 35% [37]. It has to be kept in mind that complications after open IRE are challenging to interpret, as in many cases, patients had major gastrointestinal surgery in addition to their IRE treatment. However, whereas most complications seemed self-limited, there have been several reports on severe complications in open IRE such as portal vein thrombosis, pancreatic fistula and pancreatitis. Overall complications following percutaneous IRE vary from 0 to 20% in the different study groups. In the abovementioned population of 50 patients from Narayanan et al., most patients described postinterventional abdominal pain, 10 patients (20%) were reported to have a severe complication, but no IRE-related deaths occurred [47].

4. Navigated IRE

An additional, novel technology is the so-called navigated IRE. One of the most critical and difficult steps of IRE is the correct positioning of the needles in accurate depth and perfect parallelism. If IRE is performed in an open fashion, the placement of the needles is normally controlled under ultrasound guidance. However, given the complex anatomical situation around the pancreas, 3D reconstructions based on preoperative imaging can provide the surgeon with a better topologic understanding of the patient's specific anatomy. Those reconstructions can nowadays be transferred to planning tools and even be used intraoperatively as navigational help. It has been shown that the ability to plan procedures on these image data and visualize them during the surgery holds significant value as different surgical strategies can be evaluated on the 3D models preoperatively and can be used as additional patient-specific information throughout the surgery (**Figure 6**) [49, 50].

In 2005, Grenacher et al. discussed the role of computer-assisted surgery (CAS) in the field of liver and pancreas surgery [51]. Up to then, CAS was well established in surgical procedures related to orthopedics and neurosurgery, but the advantage of transferring the knowledge to soft tissue applications was insufficiently studied. However, advances in computer science nowadays enable intraoperative navigation in hepato-pancreato-biliary surgery. Using the CAScination® system, the real world can be linked to tThe virtual scene, either by using landmarks on the surface of the organs or by using ultrasound to mark internal structures like

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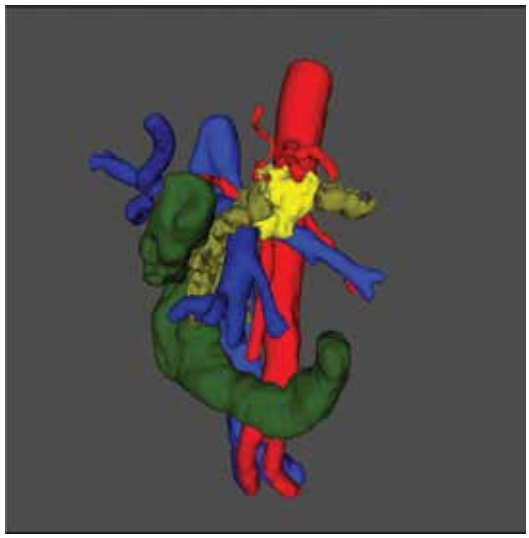

Figure 6. 3D reconstruction showing the arteries (red), veins (blue), tumor of the pancreatic body (yellow), duodenum (green) and the pancreas (light green).

Figure 7. Demonstration of equipment for navigated IRE: touch screen (red arrow); infra red detection device (black arrow); metal spheres required for real-time instrument tracking consisting of instrument and ultrasound (white arrow).

tumors or bifurcations of vessels [52]. The surgical instruments are then equipped with markers, which can be detected by an optical tracking system in real time (**Figure 7**).

A specific solution for IRE treatment of the pancreas has been implemented, which provides the surgeon with the possibility to preoperatively verify the needle placement based on given constraints like parallelism and spacing between the needles [53]. The aim of this novel technique in IRE would be the placement of the needles under live CAS-guidance according to the preoperatively defined plan. Nevertheless, further improvements of the intraoperative navigation

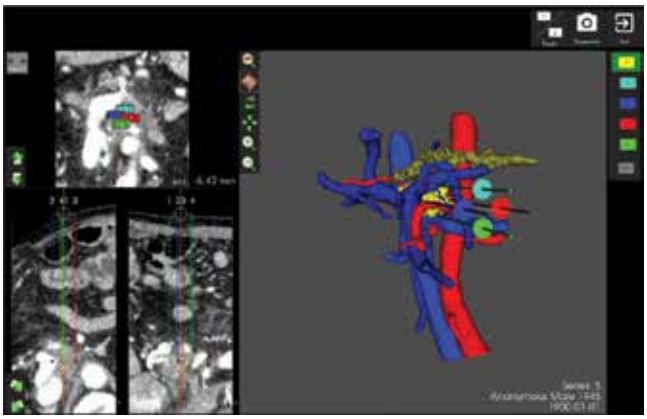

Figure 8. Preoperative 3D planning of an in situ IRE with 4 needles in a patient with locally advanced pancreatic cancer.

tools are required before they can be implemented to clinical routine and will be tackled by our team in the near future (**Figure 8**).

5. Additional indications: margin accentuation IRE

In recent years, the indications of IRE have been expanded to the so-called "margin accentuation" IRE, typically in patients with borderline resectable pancreatic cancer. In this patient group, IRE is used as an adjunct to surgery intraoperatively, aiming to achieve a higher percentage of negative margin resections [32]. It is well known that margin-negative resection is a strong indicator for better overall survival in pancreatic cancer. However, isolated local recurrences are observed in 35–80% of patients after intended R0 resection, raising the hypothesis that R1 resections are underestimated [54, 55]. A comprehensive work-up done by Esposito et al. confirmed that almost 80% of the patients had a true R1 resection, if a thorough examination is performed by the pathologist [56]. It is commonly accepted that R1 resections are associated with worse outcome as compared to R0 resections. In addition, there are different R0 definitions used in the current literature: no microscopic tumor at the or within 1 mm of the resection margin [57]. Hence, the role of R1 resections is not yet entirely clear—some advocate that R1 margins have a negative impact on the overall survival, whereas others state that R1 margins do influence local recurrence rates, without having a significant effect on survival [58–61]. Margin-accentuation IRE has been implemented in multiple pancreatic centers aiming to achieve a higher "true" R0 percentage and to therefore potentially increase overall survival and decrease local recurrences. At the present time, there are no clear recommendations of when margin accentuation IRE should be performed, because there are no clear radiological signs of when a microscopic positive resection margin has to be expected. Given the true R1-resection rate of up to 80%, one might argue that every patient with suspected or proven pancreatic cancer should have a margin accentuation IRE, if the additional procedure risk is limited and operation time is not significantly prolonged. However, as long as no data are published on the benefit of margin accentuation IRE over surgical resection only, the indication remains somewhat arbitrary. Further

research is definitely needed to assess the independent effect of margin accentuation IRE on local recurrence rates and overall survival.

6. Conclusions

Pancreatic cancer remains a highly lethal disease. Especially patients with locally advanced pancreatic cancer usually face a discouraging prognosis with limited treatment options. The local ablative therapy with IRE is a valuable additional treatment modality, which, looking at present evidence, seems to have the potential to improve disease-specific and overall survival among patients with this dreadful disease. IRE is technically feasible and generally safe in its open and minimal-invasive access. It can either be applied as in situ IRE in unresectable cases or as a complementary treatment to surgery in borderline resectable patients in order to improve the percentage of true R0 resections. Despite being now an accepted and increasingly applied therapy, there are still a lot of open questions regarding the use of IRE. Future efforts should aim toward the establishment of standard treatment protocols for IRE, in order to make its potential benefit available to more patients suffering from pancreatic adenocarcinoma.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

None.

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Chapter 6

Enhanced Electric Pulse Technology for the Ablation of Pancreatic Cancer

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Additional information is available at the end of the chapter

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Abstract

Electric pulse based technology has been developed and studied as a non-thermal ablation method for local control of pancreatic cancer. Irreversible electroporation (IRE) has shown a significant survival benefit for local advanced pancreatic cancer in clinical trials. However, incomplete ablation with local recurrence and major complications limit the potential of this new technology. We have developed an integrated moderate heating electric pulse delivery system which consists of controllable tumor heating, multi-parameter monitoring and electric pulse delivery. The impedance of tumor is greatly decreased after moderate heating at 42°C for 1–2 min, which does not cause any cell death. Moderate heating significantly enlarges the ablation zone of tumor treated with IRE. In contrast to IRE alone, moderate heating assisted IRE results in a high rate of complete tumor regression and a significant longer median survival. Another electric pulse technology, nanosecond electric pulses, has been assessed for the treatment of pancreatic cancer as well. Nanosecond electric pulse treatment achieves more survival benefit in animals with partial tumor ablation than those treated with IRE and leads to a vaccine-like protective effect in animals with complete local ablation. More studies are needed to demonstrate the advantages and translational feasibility of the enhanced electric pulse technologies.

Keywords: electric pulses, tumor ablation, pancreatic cancer, irreversible electroporation, moderate heating, nanosecond electric pulses

1. Introduction

The incidence of pancreatic cancer is relatively low. It only counts for 2% of all cancers [1, 2]. However, pancreatic cancer is a serious global health issue due to its extremely high mortality

and poor prognosis. The overall 5-year survival rate is 5–8% [2–4]. In contrast to other major cancers with decreasing mortality rates, the mortality rate of pancreatic cancer has been gradually increasing in the past 50 years [2]. It is predicted to be the second leading cause of cancer-related deaths in the United States and Europe by 2030 [5]. There are several reasons for the dismal outcome of pancreatic cancer. There are no early signs and symptoms for pancreatic cancer. There is no screening tests or early diagnostic methods. Pancreatic cancer is often diagnosed at a late stage with a large tumor burden. It is notoriously resistant to chemotherapy and radiotherapy [6–8]. Surgery is the only way to potentially achieve complete cure of early stage pancreatic cancer, which counts for approximately 15–20% of the patients [9]. Nevertheless, the 5-year survival rates of surgical resection are only 13.6–17.5% [10]. The poor prognosis after surgery is due to a high incidence of local recurrence and distant metastases [11, 12].

In the last decade, much effort has been made to develop ablative technologies for local pancreatic tumor control and the improvement of quality of life and survival. An electric pulse technology, irreversible electroporation (IRE) as a non-thermal ablation method has been investigated in animal models for tumor ablation [13, 14]. Recently IRE has been studied in clinical trials for liver [15], renal [16], prostate [17] and pancreatic cancers [18–21]. Martin et al. reported that overall survival increased 6–8 months in patients with local advanced pancreatic adenocarcinoma treated with IRE [22]. A systematic review demonstrated significant survival benefits with reducing the risk of injury to vessels and ducts after treating advanced pancreatic cancer with IRE [23]. IRE has been demonstrated some advantages in contrast to thermal ablation technologies, which are associated with high morbidity and mortality due to thermal damage to adjacent structures. However, local recurrence [22, 24–26] and various rates of major complications [18, 22, 25] are two major issues that restrict the benefit of IRE treatment. Thus, the enhancement technologies for IRE or novel non-thermal electric pulse technologies, which can increase complete tumor ablation and/or decrease adverse effects, are needed to further improve the quality of life and long term survival.

Here we introduce two promising electric pulse technologies, moderate heating (MH) enhanced IRE and nanosecond electric pulses (nsEPs) for the treatment of pancreatic cancer, and present our preclinical findings demonstrating their potential advantages in contrast to current IRE technology.

2. Moderate heating enhances the therapeutic efficacy of irreversible electroporation for pancreatic cancer

2.1. Background

The impedance change of biological tissues at various temperatures has been investigated for over three decades [27, 28]. A decrease of impedance means the increase of tissue conductivity, which is equal to an elevated current and a large electrical energy delivery to the tissue or tumor under a certain electric field. We found that tumor ablation zone could be significantly

enlarged when preheating with moderate temperature increase was applied. This result led to our hypothesis that a moderate increase in the temperature of the target tumor could decrease tumor impedance, thereby sensitizing the target tumor for IRE tumor ablation. To test this hypothesis, we first developed a controllable tumor heating unit and an impedance monitoring unit, then integrated these two units into an electric pulse supplier. In additional to treating tumor with IRE, this integrated electric pulse delivery system has the capacity to heat the targeting tumor, maintain at a set temperature and monitor impedance changes of the treated tissue in real time.

2.2. Experimental design

Ex vivo IRE tumor ablation was assessed in a 3D agarose cell culture model, which was described in the literature [29]. Pan02 mouse pancreatic cancer cells were used to make the 3D tumor model. The IRE parameters were pulse duration 100 μs, frequency 1 Hz, 80 pulses, and electric fields 750 V/cm. The four-needle electrode was utilized to deliver this electric pulse protocol. A thermopile was integrated into the electrode for a real-time temperature monitoring and a fiber optic laser located at the center of the electrode was used for tumor heating. After IRE treatment, tumor was stained with propidium iodide (PI, 4 μg/ml) for 30 minutes. Images were taken using a Leica MZFLIII fluorescence stereomicroscope equipped with a Leica DFC420 C CCD camera. Cell death or ablation zone was quantified with ImageJ software (imagej.nih.gov/ij/).

A syngeneic mouse Pan02 pancreatic cancer model was established for the evaluation of this moderate heating enhanced IRE (MHIRE) system. Female C57BL/6 mice (6–8 weeks of age) were injected with 1×10^6 Pan02 cells in 50 μL Dulbecco's phosphate buffered saline on the left flank. The size of primary tumor was assessed by digital calipers twice a week. Tumor volume was determined as described in the literature [30]. Tumors were treated when it reached 8–10 mm in diameter with an average tumor volume of 250–300 mm^3. The IRE parameters were pulse duration 100 μs, frequency 1 Hz, 90–120 pulses, and electric fields 1500–2500 V/cm. A four-needle electrode arranged in an array of 7×5 mm spacing was used to treat pancreatic tumor.

MH was defined at 42°C within 2 min, which is below the threshold of pain sensation [31] and does not cause cell death [32]. A calibration of MH protocol was done prior to the MHIRE treatment. A thin thermocouple was inserted in the bottom part of tumor and the time it took the reading to reach 42°C was recorded. It took 20–60 s for the internal tumor temperature to reach 42°C when the surface target temperature was set to 45°C by laser heating. So, based on the calibration results, we decided that pre-heating tumor for 60 s would allow the inside of the tumor to be at the correct internal temperature before IRE was performed.

2.3. Results and discussion

2.3.1. Moderate heating decreased the impedance of tumor

The change of impedance during the IRE or MHIRE treatment is shown is **Figure 1**. The baseline impedance of each tumor was different; however, preheating tumor with a moderate

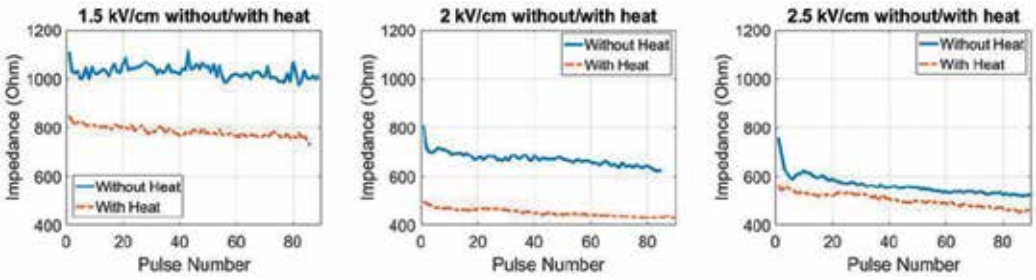

Figure 1. Tumor impedance change during IRE or MHIRE treatment. Each data point represents an average impedance reading of 4–5 tumors. IRE parameters: 100 μs pulse width, 90 pulses, frequency of 1 Hz and applied electric fields of 1.5 kV/cm (left), 2 kV/cm (middle) and 2.5 kV/cm (right).

temperature increase could reduce all of them by 200–300 Ω or 15–38% of the baseline impedance, which occurred prior to the IRE treatment. Consistent to the other group's report [33], IRE could result in the decease of tumor impedance as well. It appears that the reduction of the impedance was associated with the strength of the electric field. The higher electric field, the more the impedance was reduced by the end of the treatment. Additionally, MH also reduced the fluctuation of impedance changes, which may indicate that MH improves homogeneity of the tumor physical property. The average drop of impedance was 39.1 to 46.6% for MHIRE with 2000 to 2500 V/cm and 22.4 to 30.5% for IRE with the same electric field.

The impedance decrease of tumor was likely correlated to the complete tumor ablation of IRE [33]. Given an approximate 40% decrease of tumor impedance, theoretically IRE at 2500 V/cm should be equivalent to MHIRE at 1500 V/cm. It means MHIRE could reduce the electric field of IRE and achieve the same level of efficacy for tumor ablation. Interestingly, MH was observed to decrease the impedance fluctuation of the tumor as well. Tumor is not a homogenous structure but with multiple types of cells and extracellular matrix [34, 35]. A heterogeneous impedance map of tumor tissue [36] is expected and may contribute to the incomplete ablation of IRE. This feature of MH might also contribute to more complete tumor ablation.

2.3.2. Controllable MH enlarged ex vivo *tumor ablation and enhanced the therapeutic efficacy of IRE for pancreatic cancer*

More cell death was observed after Pan02 tumor cells in a 3D agarose gel were treated with MHIRE with a four-needle electrode, while tumor cells treated with MH alone did not result in any cell death (**Figure 2**). The ablation zone or total cell death increased 1.4-fold with MHIRE at an electric field of 750 V/cm ($p < 0.05$) comparing to those treated with IRE at the same electric field.

Tumor bearing animals were treated with either IRE or MHIRE at 1500 V/cm. The IRE treatments alone had no significant influence on the tumor growth. However, a synergistic effect was seen in the IRE treatment when the tumor was preheated to 42°C (**Figure 3**). Tumors treated with MHIRE were all significantly smaller than those in the control group or those in IRE-alone group on post-treatment days 4, 7, 11, 13 and 14. However, no long-term complete tumor regression was obtained under either IRE or MHIRE protocols. In order to obtain

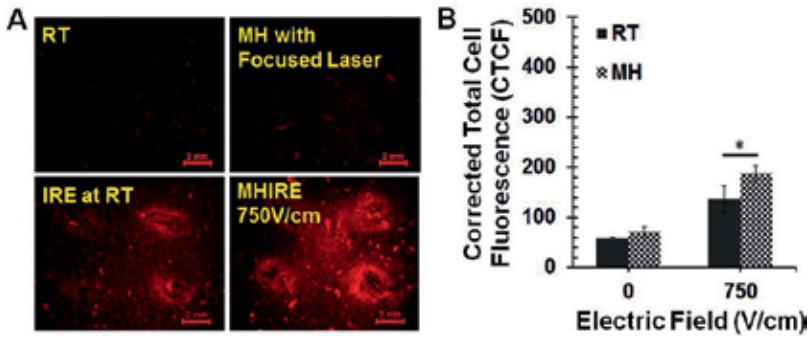

Figure 2. Enlargement of *ex vivo* tumor ablation zone with MHIRE. A 3D agarose gel Pan02 tumor model was treated by IRE or MHIRE. Area with red color was zone of dead cells indicated by propidium iodide (PI) staining. RT: room temperature; MH: samples preheated with laser. Corrected total cell fluorescence (CTCF) was analyzed by ImageJ software. n = 3–4. *: $p < 0.05$ (t-test).

complete tumor regression, IRE or MHIRE with elevated electric fields were adopted to treat tumor. At the electric field of 2000–2500 V/cm, MHIRE significantly prolonged median survival by roughly two times with 84 days in contrast to the control mice ($p < 0.001$) (**Figure 4**). Despite the higher electric fields, IRE treatment alone could not achieve long-term complete tumor regression. It only extended medial survival for 3 days. Median survival was 43 days for the control tumor animals and 46 days for the IRE treated animals. More importantly, 55.6% (5/9) of the tumor-bearing animals treated with MHIRE were long term tumor-free.

It was noticed that IRE alone was unable to achieve complete tumor ablation in this mouse Pan02 tumor model though the IRE protocol was similar to those used in other animal studies and clinical trials. Jose et al. reported that IRE treatment resulted in 25% of complete tumor ablation [37]. In that study, a comparable IRE protocol (100 μs, 1 Hz, 2500 V/cm and pulse number 90) was used to treat xenograft human BxPC-3-luc pancreatic tumors in athymic nude mice. Local recurrence was reported relatively low in clinical trials, 11% by Kluger's group [25] or 27.8% by Martin's group [22]. Though multiple factors including the tissue type of tumor, its size, the IRE protocol and electrode configuration could contribute to the incomplete ablation, the physical properties of the target tumors especially to the impedance likely play a critical role. The 750–800 Ω impedance of mouse Pan02 tumor (**Figure 1**) is much higher than the 100–120 Ω impedance of human pancreatic cancer reported by Dr. Martin's group [33]. Such a big difference of impedance may explain why the mouse Pan02 tumor is difficult to be successfully ablated by the IRE treatment.

Though MH alone did not result in any cell death (**Figure 2**) and had no impact on tumor regression and animal survival (**Figure 3**), it was demonstrated to synergize with IRE on tumor ablation zone *in vitro* (**Figure 2**), to diminish tumor growth (**Figure 3**) and to improve long-term survival *in vivo* (**Figure 4**). Together with the impedance changes of tumors (**Figure 1**), we have validated a novel technology and concept that the therapeutic efficacy of IRE can be enhanced by MH with a consequent decrease of tumor impedance. Is it feasible to translate this technology into an effective therapy for pancreatic cancer? The MHIRE system developed in this study has been utilized to successfully treat tumor with relative small size (less than 1 cm).

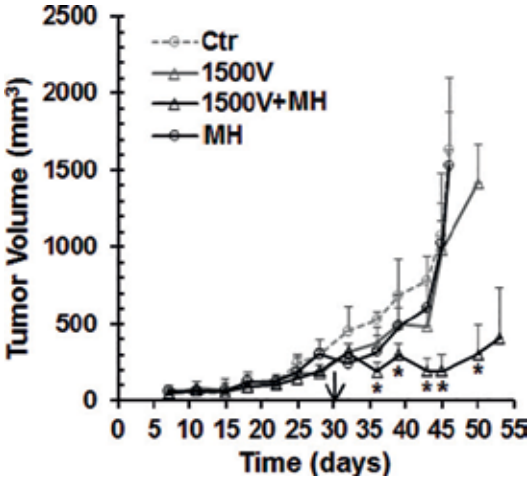

Figure 3. Pancreatic tumor growth after IRE or MHIRE treatment. Pan02 pancreatic tumors with the size of 8–10 mm were treated with IRE or MHIRE at day 31 indicated by black arrow. IRE parameters: pulse duration 100 μs, frequency 1 Hz, pulse number 90 and applied electric fields 1500 V/cm. Ctr: no treatment (n = 4); MH: tumor heated with laser at 42°C for 2 min; 1500 V: IRE at 1500 V/cm (n = 4 mice); 1500 V + MH: Tumor preheated with laser at 42°C with IRE at 1500 V/cm (n = 8). *: $p < 0.05$, or $p < 0.01$ or $p < 0.001$ for MHIRE vs. IRE or Ctr (one way ANOVA).

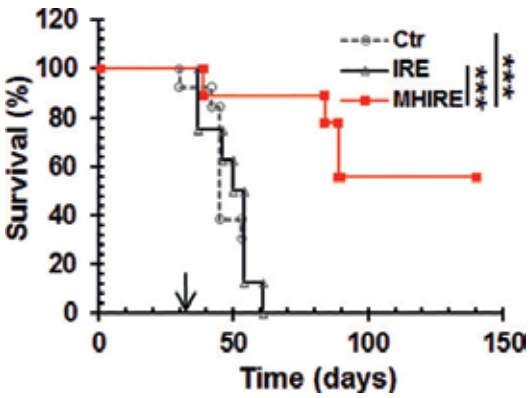

Figure 4. Kaplan-Meier survival curves of mice treated with IRE or MHIRE. Pan02 pancreatic tumors with the size of 8–10 mm were treated with IRE or MHIRE at day 31 indicated by arrow. IRE parameters: pulse duration 100 μs, frequency 1 Hz, pulse number 90 and applied electric fields 2000-2500 V/cm. Ctr: No treatment (n = 8 mice per treatment group); IRE: Treated with IRE (n = 8); MHIRE: Tumor preheated with laser at 42°C with IRE (n = 9). ***: $p < 0.001$ (LogRank test).

However, as is known, most tumors in patients are larger than 1 cm, especially for later stage cancers, which are the targets of the IRE treatment. This limitation of treatable size can be addressed with the adjustment of the electrode configuration, to cover a larger area. Meanwhile, the depth of laser heating and its thermal distribution needs to be profiled, and the refined MHIRE system will be calibrated/reprogrammed and optimized in an *in vivo* pancreatic cancer

model with tumor size relevant to the clinical settings. The extension of heating area can be resolved by the integration of multiple infrared laser beams and/or additional optic lens, increase of needle gap and length. To heat large and deep tumors, different laser sources [38] can be adopted. Moreover, other heating methods, including focused ultrasound [39], microwave [40] or radiofrequency [41], could be employed for the purpose of MH.

3. Nanosecond electric pulses for the treatment of pancreatic cancer

3.1. Background

An electrical engineering technology, nanosecond electric pulses (nsEPs), has been developed and studied by Dr. Schoenbach's [42] and other groups [43]. NsEPs are assumed non-thermal if the appropriate parameters especially the low frequencies are selected. Similar to IRE, nsEPs have been utilized to treat cancer in animal models for local tumor ablation [44–46]. Beyond the local tumor ablation, a vaccine-like protective effect has been observed by two groups [44, 47]. The vaccine like-protection effect has been demonstrated by our group [48] in a poorly immunogenic breast cancer model as well. We have demonstrated that local nsEP tumor ablation elicits an anti-tumor immunity to prevent distant metastases, reject established distant tumors and protect animals from secondary tumor challenge. Thus, nsEP therapy shows additional advantages, in addition to local tumor eradication.

NsEPs have been reported for the treatment of pancreatic cancer in two studies [36, 49]. However, whether immune protection is induced by the nsEP treatment is unknown because xenograft tumors in immune deficient animals have been used in both studies. To assess if nsEP ablation could induce antitumor immunity and achieve additional benefits beyond local ablation for pancreatic cancer, a syngeneic mouse Pan02 pancreatic cancer model was utilized in this study.

3.2. Experimental design

A syngeneic mouse Pan02 pancreatic cancer model was established as above mentioned. Tumors were treated when it reached 5–7 mm or 8–10 mm in diameter with an average tumor volume of 40–120 mm^3 (small) or 250–300 mm^3 (large). The nsEP parameters were pulse duration 100 or 200 ns, frequency 1–3 Hz, pulse number 600–1200, and electric fields 30–50 kV/cm. Pancreatic tumors were treated with either a four-needle electrode with gaps of 5 × 7 mm or a pitch electrode, which was selected from three configurations including 2 mm gap with 6 mm in diameter, 3 mm gap with 8 mm in diameter and 4 mm gap with 10 mm in diameter. In comparison, pancreatic tumors were also treated with IRE. The IRE parameters and the choice of electrode were described in the previous section.

To assess if a vaccine-like protection occurred after pancreatic cancer was treated with nsEPs, tumor free mice were challenged with 0.5 million live Pan02 tumor cells on the right flank. Tumor growth was monitored as above mentioned.

3.3. Results and discussion

3.3.1. NsEP treatment resulted in complete tumor regression or extension of survival for animals with incomplete tumor regression

As shown in **Figure 5**, a single nsEP treatment achieved 50–100% complete tumor regression dependent on the doses of nsEPs. In contrast to the IRE treatment, muscle contraction was greatly reduced with the nsEP treatment. Both pitch electrode and two-plate suction electrodes were safe and no mortality was found. A minor issue was that scab was formed after the nsEP treatment. It usually shed within 2–3 weeks and left a small scar or no visual changes on the skin.

Extension of survival was achieved even with partial tumor ablation regardless of whether pancreatic cancer with small size (5–7 mm) or big size (8–10 mm) was treated, and median survival was extended to 63 days (**Figure 6A**) if small tumors were treated, or to 68 days (**Figure 6B**) if large tumors were treated, in contrast to 45 days for the control animals. However, the survival benefit was only present in large tumors treated with IRE but not in small tumors if the tumors were partially ablated. Median survival was extended to 50 days if large tumor was treated, in contrast to 45 days for the control animals (**Figure 6B**). Actually, the median survival was shortened to 40 days if the small tumors were not completely ablated with IRE. Obviously, tumor growth was accelerated and lost heterogeneous pattern after partial IRE ablation (**Figure 7**). The same phenomenon was reported in literature and explained as cancer stem cell activation [50]. Nevertheless, this was not seen in the animals treated with nsEPs. It suggests different cell death mechanisms or possible inhibition of immune responses may occur.

3.3.2. A vaccine-like protective effect was resulted from the nsEP treatment

As shown in **Figure 8**, tumor free mice after the nsEP treatment were able to impede or prevent the growth of challenging tumors. Noticeably, there was a significant difference between the two nsEP protocols. Majority of tumor free mice (66.7%) after the 100 nsEP treatment were successfully protected from the second live tumor challenge whereas no protection but only growth inhibition of tumor was observed in animals treated with the 200 nsEPs. Nevertheless, neither protection nor growth inhibition was seen in the animals treated with IRE.

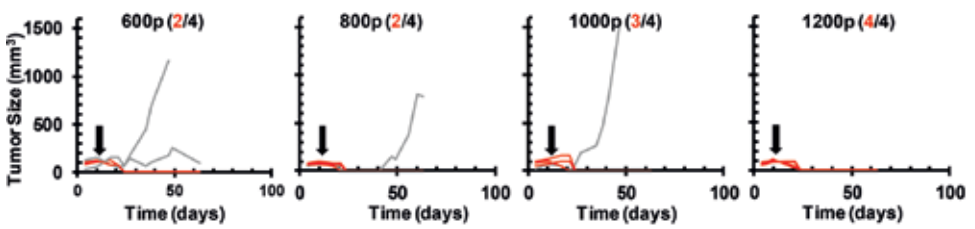

Figure 5. Pancreatic tumor growth after the nsEP treatment. Pan02 pancreatic tumors with the size of 5–7 mm were treated with nsEPs at day 11 indicated by black arrow. nsEP parameters: 200 ns, 2 Hz, 30 kV/cm, and pulse numbers 600, 800, 1000 or 1200, indicated by 600p, 800p, 1000p or 1200p, separately. Number of tumor free mice vs. total number of treated mice was indicated.

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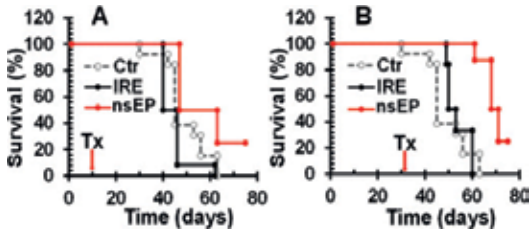

Figure 6. Survival extension in animals with incomplete tumor regression after the nsEP treatment. Pan02 pancreatic cancer was treated with IRE or nsEP. Only animals with incomplete tumor regression were included here. **A,** tumor size with 5–7 mm was treated (n = 13, 12 or 4 for Ctr, IRE or nsEP). Tx: Treatment at day 7 (IRE) or 11 (nsEP). **B,** tumor size with 8–10 mm was treated. Ctr: No treatment; IRE: Treatment with IRE; nsEP: Treatment with 200 ns, 2 Hz, 30 kV/cm and 600–1200 pulses (n = 13, 6 or 7 for Ctr, IRE or nsEP). Tx: treatment at day 31.

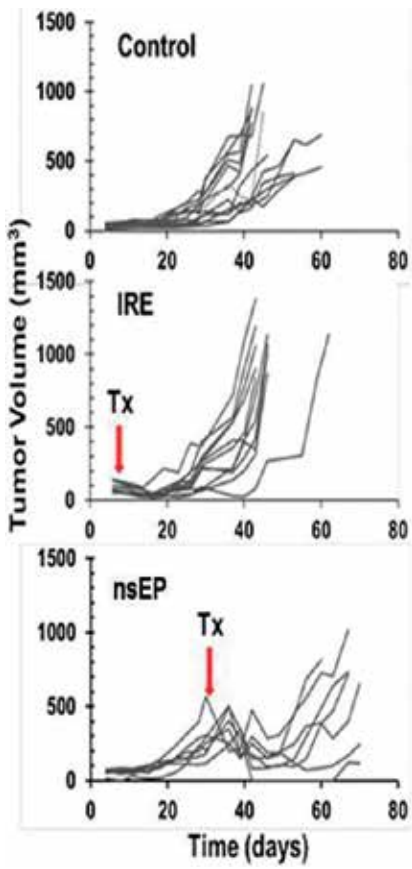

Figure 7. Pancreatic cancer growth after treatment with IRE or nsEP*. Control (n = 13): no treatment. IRE (n = 12): treated with IRE. Tx: treatment day 7. nsEP (n = 7): treated with nsEP (200 ns, 30 kV/cm, 2 Hz with 800–1000 pulses), Tx: treatment day 31. *: Only animals with partial tumor regression were included to assess the effect of treatment on tumor regrowth.

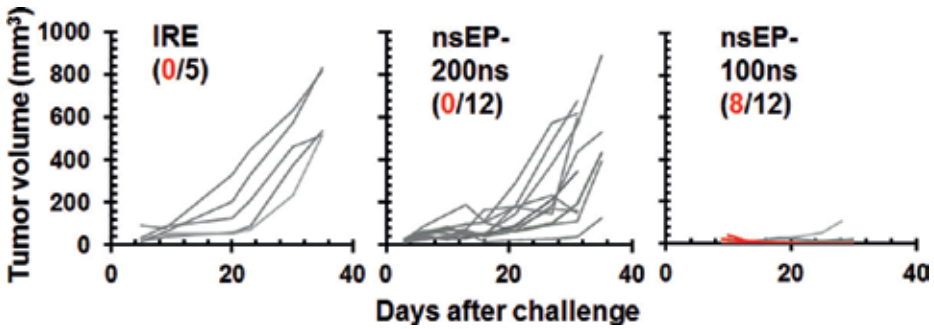

Figure 8. A vaccine-like protection effect after the nsEP treatment. Growth curves of second challenge pancreatic tumors in tumor-free animals after IRE or nsEPs. Primary pancreatic tumors were treated with IRE (**IRE**), nsEPs with 200 ns, 2 Hz, 30 kV/cm and 600 -1200 pulses (**nsEP-200 ns**), or nsEPs with 100 ns, 2 Hz, 50 kV/cm and 800 -1200 pulses (**nsEP-100 ns**). Number of protective mice vs. total number of challenged mice was indicated. $p < 0.05$ for nsEP-200 ns vs. IRE and $p = 0.001$ for nsEP-200 ns vs. nsEP-100 ns (Chi Square test).

Surprisingly, the protective rates between two sets of nsEP parameters are very different. A high rate of protection from the second live tumor challenge, 100%, has been observed in both mouse breast cancer [48] and rat hepatocellular cancer models [44] after the same 100 nsEP treatment. Does this mean 100 nsEPs are more favorable to induce immune protection than 200 nsEPs? The answer is not clear because 100 nsEPs has eradicated local mouse lung squamous cell cancer (KLN205) but has failed to result in any vaccine-like protection (0/19 protection in our unpublished data). It's very likely that cancer cell types and distinctive tumor microenvironments play a critical role on the induction of immunity following the nsEP tumor ablation.

The growth inhibition of local recurring tumors and the second challenging tumors suggests that underlying common immune responses are induced after the nsEP treatment. It's critical to understand the mechanisms causing the differential responses and outcomes between IRE and nsEPs or among various nsEP parameters, so it is possible for researchers to design more effective therapeutic strategies, such as further optimization of the system or a combination therapy with other immunomodulators. Currently, we are investigating cell death mechanisms, local and systemic immune responses, and the changes of tumor microenvironments following the nsEP tumor ablation.

4. Conclusion

Two electric pulse-based technologies have been studied to treat pancreatic cancer in a syngeneic mouse pancreatic cancer model. A novel MHIRE system has been developed. This MHIRE system has three functions including controllable tumor heating, impedance monitoring and electric pulse delivery. MH has been demonstrated to decrease the impedance of tumor, to enlarge the tumor ablation zone of IRE *ex vivo* and to enhance the complete tumor ablation of the IRE treatment *in vivo*. The MHIRE treatment significantly improves the therapeutic efficacy of the IRE treatment. In contrast to the IRE treatment, nsEP tumor ablation showed distinctive outcomes and potential advantages. If partial ablation occurred after either the IRE or the nsEP treatment, animals treated with nsEPs received survival benefit. If complete local ablation was achieved, animals treated with nsEPs but not with IRE were able to reject secondary tumor

challenge or to diminish its growth. An induction of antitumor immunity following the nsEP treatment is highly suggested to account for this vaccine-like protective effect. For both MHIRE and nsEPs for the treatment of pancreatic cancer, our data are preliminary and more studies are needed to further optimize these technologies, elucidate the underlying mechanisms and evaluate their translational feasibility.

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Conflict of interest

R. Heller and S.J. Beebe own stock in Pulse Biosciences, Inc.

All other authors declared no potential conflicts of interest.

Notes

The data present in the second section have been published in or modified from the journal of Scientific Report (see [27]).

Author contributions

S.G. conceived, designed, and supervised the studies. S.G., K.S., R.H, and C.J. designed and developed the MHIRE system. N.B., C.E., J.H. S.B., and S.G. conducted the experiments. S.G. analyzed and interpreted the data. All authors contributed to writing, editing, and review of the manuscript.

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Chapter 7

Pancreatic Resections for Metastatic Disease

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Additional information is available at the end of the chapter

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Abstract

Although the incidence of metastases to the pancreas from various primaries is very low, these lesions are usually being described as part of the systemic recurrence of different malignancies, in certain cases isolated pancreatic metastases might be encountered. When it comes to the malignancies, which might lead to the apparition of pancreatic metastases, the most common origins have been reported to be renal cell carcinoma, colon cancer, ovarian cancer and melanomas. In certain cases, patients with pancreatic metastases might be submitted to surgery with curative intent. However, it should not be omitted that pancreatic resections can be associated with higher rates of perioperative morbidity; therefore, a precise selection of the cases that are considered suitable for such procedures is mandatory. It seems that the best results in regard with long-term survival are expected in cases with isolated pancreatic metastases as well as in cases with limited extrapancreatic lesions, amenable to complete cytoreductive surgery. This chapter reviews the most important studies conducted on the theme of pancreatic resections for metastatic disease from various primaries.

Keywords: pancreatic metastases, resection, cytoreductive surgery, morbidity

1. Introduction

Pancreatic metastases from other primaries are rare eventualities, the reported incidence being less than 2% from all pancreatic lesions [1, 2]. When it comes to the most frequent tumors that might induce the apparition of pancreatic metastases, they consist of renal cell carcinomas, melanomas, breast, lung and gynecologic primaries [3]. Most times these patients are asymptomatic and are detected during the follow-up examinations with pancreatic lesions with various localizations. When symptomatic, the most commonly reported symptoms and signs

consist of vomiting, abdominal pain, jaundice, upper digestive hemorrhage or weight loss, the apparition of such symptoms being usually associated with poor prognostic.

Once the diagnostic of malignant tumor of the pancreas is established, most of the times it is very difficult to distinguish between primary malignant lesions of the pancreas and metastatic ones [4]. However, patients presenting metastatic disease are usually associated with better outcomes when compared to pancreatic primary tumors [5].

2. Imagistic studies for metastatic pancreatic lesions diagnostic

When it comes to the most efficient imagistic study in order to determine the existence of such lesions, computed tomography seems to play an important role, most commonly such lesions being diagnosed as singular, multinodular lesions or as a diffuse infiltration with hypervascular aspect [1]. However, in up to 10% of cases, metastatic lesions of the pancreas might not be seen at standard computed tomography (CT), a positron emission tomography/computed tomography (PET-CT) is being needed. In this case, a diffuse uptake revealed by PET-CT studies might be associated with benign conditions (such as Graves' disease or autoimmune thyroiditis), while focal uptake is rather significant for the presence of a malignant lesion. In such eventualities, a percutaneous biopsy is recommended in order to have a positive diagnostic of malignancy [6].

3. Therapeutic strategies in pancreatic metastases from various primaries

Unfortunately, pancreatic metastases usually develop as part of the systemic recurrence and are associated with other disseminated lesions; therefore, the patient will become a candidate for palliative oncological treatment, in order, to alleviate the symptoms. In certain cases, the pancreatic lesions will develop as oligometastatic disease and by this way, the patient will become the perfect candidate for surgical treatment with curative intent. In such cases, an important benefit in terms of survival might be obtained. However, even in cases presenting metastatic lesions, surgery has been lately implemented as standard of care due to the high morbidity rates of this kind of surgical procedures. For a long period of time, it has been considered that performing a gesture of pancreatic surgery in such cases is associated with unacceptable rate of postoperative complications including the risk of pancreatic leaks or acute pancreatitis. In the last decades, improvement in hepatobiliopancreatic techniques in association with the improvement of the postoperative care lead to a successful association of such resections as part of cytoreductive surgery for various primaries [7–10]. However, the prognostic in such cases is strongly related to the origin of the pancreatic lesion.

An interesting study conducted on the subject of pancreatic resection for metastatic disease was published by Sweeney et al. in 2009. The study had as departure point a case series of three patients with pancreatic metastases from various primaries submitted to different therapeutic

strategies; the first reported case was the one of a 51-year-old patient known with lobular breast cancer, 5 year earlier treated with lumpectomy, axillary node dissection and adjuvant hormonal therapy and renal cell cancer, submitted to left nephrectomy and adjuvant therapy consisting of interferon and thalidomide. The patient was further diagnosed with pulmonary metastases and was submitted to atypical lung resection and with an isolated pancreatic lesion measuring 4 × 3.1 cm at the level of the distal pancreatic tail. The pancreatic lesion was successfully resected, the histopathological studies confirming the metastatic origin from the renal cell carcinoma. However, the patient was diagnosed with disseminated liver metastases and died of disease 20 months after pancreatic surgery. The second case was the one of a 56-year-old man submitted to surgery 4 years earlier, a left pneumonectomy being performed at that moment. Four years later, he was diagnosed with an isolated lesion in the pancreatic neck, the biopsy demonstrating the metastatic origin of the lesion. He was submitted to an exploratory laparoscopy but due to the local invasion of the hepatic artery, resection was not feasible. In consequence, the patient was submitted to palliative chemotherapy and remained alive 3 years after the diagnostic of pancreatic metastases. The third case had been previously diagnosed with breast cancer for which she had been submitted to radical mastectomy followed by six cycles of chemotherapy; 5 years later, she was diagnosed with a pancreatic tumor at the junction between head and body of the pancreas, so she was submitted to a biopsy which confirmed the metastatic character of the lesion. Therefore, she was successfully submitted to surgery, an oncologic resection of the pancreatic metastasis being performed. However, the surgical procedure was followed by adjuvant chemotherapy with good long-term outcomes [11].

The authors went further and reviewed the literature regarding pancreatic metastases of various origins published until that moment. They reported a total number of 220 patients with this pathology with a median age of 59.2 years. Among cases which reported the symptomatology at the time of presentation, the authors underlined that up to 27.6% of cases were asymptomatic, among symptomatic cases the most commonly reported signs and symptoms are abdominal pain, upper digestive bleeding, weight loss and pancreatitis. When it comes to their localization, the most common pancreatic sites of metastases included pancreatic head (in 41.8% of cases) followed by body and tail (in 34.9% of cases), periampullary region (in 8.9% of cases) and uncinate process (in 1.1% of cases); when reported, the tumor size ranged between 1 and 11.5 cm, the average size being of 3.9 ± 2.1 cm. As the originating tumors had leaded to the apparition of pancreatic metastases, the most common primary tumor was the kidney (in 70.5% of cases) followed by the colorectal tumors (in 6.5% of cases), melanomas (in 2.7% of cases) and malignant fibrous histiocytomas (in 1.8% of cases). Among the 220 patients initially introduced in this study, surgery was performed in only 177 cases, the other 43 patients being diagnosed with unresectable lesions. The most commonly performed surgical procedures consisted of distal pancreatectomies in 25.9% of cases and pancreatoduodenectomy in 49.7% of cases, while total pancreatectomy was needed in 18.6% of cases submitted to surgery. When it comes to the short-term outcomes, the authors underlined that the reported incidence of complications was similar to the one reported in patients submitted to pancreatic resections for pancreatic primaries and consisted most often of pancreatic fistulas (in 6.5% of cases). As for the long-term outcomes, the authors calculated the median survival as well as the 2 year and 5 year survival rates only for pancreatic metastases from renal cell carcinoma

(this subtype being the most frequently reported in the present study); therefore, among 177 patients submitted to surgery for pancreatic metastases from renal cell cancer, the median survival was 70 months, while the 2 year and 5 year overall survival rates were of 78 and 65%, respectively. These data suggest the potential benefit of pancreatic resections for metastatic lesions; it seems that the short-term outcomes are not significantly influenced by the metastatic character of the lesion, while the long-term outcomes seem to reveal significant long-term survival especially in oligometastatic lesions from renal primaries [11].

In a study conducted by Reddy et al. on 49 patients with metastatic pancreatic lesions, the main included primaries were renal cell carcinoma (in 21 cases), gallbladder cancer (in 6 cases), pulmonary cancer (in 4 cases), ovarian cancer (in 4 cases), sarcomas (in 4 cases), melanomas (in 3 cases), colorectal cancer (in 2 cases), breast cancer (in 1 case), hepatocellular carcinomas (in 1 case), seminomas (in 1 case), Langerhans cell histiocytosis (in 1 case) and nonpancreatic endocrine cancers (in one case). The study was conducted for a 38 year time period and reviewed data from 3830 patients submitted to pancreatic surgery; among these cases, the metastatic origin of the tumor was demonstrated in 1.6% of cases. The median age at the time of resection of pancreatic lesions was 60 years, while the most commonly encountered symptoms were abdominal pain (in 48% of cases), followed by jaundice (in 31% of cases). When it comes to the most commonly performed surgical procedures, they consisted of pancreatoduodenectomy in 31 cases, distal pancreatectomies in 14 cases and total pancreatectomy in 4 cases; among the 14 cases submitted to distal pancreatic resections, splenectomy was associated in 13 patients. When it comes to the short-term outcomes, the reported morbidity rates were 52% after pancreaticoduodenectomy, 46% after distal pancreatectomy and 25% after total pancreatectomy; however, the overall mortality rate was 0. The most often reported complications were wound infections, followed by delayed gastric emptying or pulmonary complications. When it comes to the histopathological findings, the most often reported lymph node metastases originated from renal cell carcinomas, gallbladder carcinomas, lung, colorectal carcinomas, melanomas, seminomas, sarcomas and nonpancreatic endocrine tumors, while perineural and vascular invasion were reported in gallbladder, lung, renal cell cancers and melanomas. As for the long-term outcomes, the authors reported a median overall survival rate after pancreatic resection of 3.7 years. Among long-term survivors (defined as a longer than 10 year survival after pancreatic resection), the most commonly reported origins were renal cell carcinomas, followed by Langerhans histiocytosis and seminomas. When performing an univariate analysis, the most important prognostic factors affecting the long-term survival were represented by the presence of perineural invasion and vascular invasion; surprisingly, the diameter of the metastatic tumor or lymph node metastases did not significantly influence survival. Moreover, patients who experienced any type of surgical complication as well as male patients trended to report a poorer outcome. When it comes to the influence of the cancer type on the overall prognostic, a significant influence was reported. The poorest outcomes were reported in patients submitted to surgery for pancreatic metastases originating from melanomas, followed by cases with breast cancer. No patient diagnosed with pancreatic metastases from colorectal, lung cancer or sarcoma did experience an overall survival longer than 5 years. The best outcome was reported by the patient diagnosed with metastatic Langerhans cell histiocytosis and by the patient diagnosed with seminoma, both cases being alive more than 11 years after pancreatic surgery.

Another interesting conclusion of the study was the one regarding the time of diagnostic of the pancreatic lesions; in three cases pancreatic resection was performed synchronously with the resection of the primary tumor, the origin of the pancreatic metastases being represented by renal cell carcinoma, gallbladder cancer and ovarian cancer. In the remaining cases, pancreatic resection was performed for metachronous lesions; however, there was no difference in terms of survival between the two groups [12].

4. Pancreatic metastases from renal cell carcinoma

Renal cell carcinomas represent almost 2% of all malignant tumors in adults, being the third most common genitourinary tract cancer [13]. Although renal cell carcinoma is associated with an overall good prognostic, with 5-year survival rate of up to 95%, patients presenting distant metastases report a significant poorer outcome, with 5-year survival rates lower than 10%.

Pancreatic metastases with renal cell carcinoma origin can be diagnosed at the time of the diagnostic of the primary tumor (as synchronous lesions) or after a disease free interval (as metachronous lesions). In the second case, it seems that the prognostic is significantly influenced by the disease free survival interval, a longer period of time between the initial diagnostic and the diagnostic of metastatic lesions being associated with a lower biological aggressivity and better chances of long-term survival [14, 15]. However, it should not be omitted that pancreatic metastases from renal cell carcinoma can occur even at 10–32 years from the diagnostic of the primary tumor, so that differential diagnosis should be kept in mind any moment in which a patient known with previous history of renal cell carcinoma is diagnosed with a metachronous pancreatic tumor [16].

Patients with pancreatic metastases from renal cell carcinomas can remain asymptomatic for a long period of time or can develop signs and symptoms such as weight loss, abdominal pain, jaundice or even pancreatitis due to the Wirsung duct obstruction caused by tumor growth; in certain cases lesions located in the pancreatic head will lead to the apparition of upper gastrointestinal bleeding [17, 18].

Once a pancreatic metastasis with renal cell carcinoma origin is suspected at the imagistic studies, a fine needle biopsy might be needed in order to confirm the origin of the lesion and to decide which should be the therapeutic protocol. However, pancreatic metastases from renal cell carcinoma are the most common situation in which pancreatic resection for metastatic disease has been proposed.

When it comes to the most important prognostic factors after pancreatic resections for pancreatic metastases with renal cell carcinomas, it seems that the disease free survival plays a central role; patients diagnosed with pancreatic metastases with a disease free survival longer than 2 years seem to have an improved outcome. Other factors which seem to influence the long-term outcomes are represented by the diameter of the tumor (tumors larger than 5 cm being associated with poorer outcomes), stage at the initial diagnostic and the tumoral degree of differentiation [19, 20].

The largest series of cases submitted to pancreatic resections for metastatic renal cell carcinomas was conducted by Schwarz et al. and was published in 2014 in *Annals of Surgical Oncology* [21]. The study was conducted between May 1987 and June 2003 in 12 Franco-Belgian surgical centers and involved 62 patients submitted to surgery for pancreatic metastases from renal cell carcinomas. The median age at diagnostic was 54 years (range 31–75 years) while the most common reported symptoms were abdominal pain (in 24% of cases), anemia and gastrointestinal bleeding (in 13% of cases) and jaundice (in 10% of cases). The mean interval from the diagnostic of the primary tumor to the diagnostic of the pancreatic lesion was 9.8 years (range 0–25 years—two patients presenting with synchronous pancreatic lesions). When it comes to the most commonly performed surgical procedures, they consisted of pancreatoduodenectomy in 31% of cases, distal pancreatectomies in 40% of cases, total pancreatectomies in 23% of cases and enucleation in 6% of cases. In order to achieve negative resection margins, in six cases major vascular resections with reconstruction were performed, while *en bloc* visceral resections were needed in other four cases (consisting of colonic resections in three cases and omentectomy in one case); in other six patients the presence of other distant metastases imposed performing other visceral resections such as liver resection in three cases, contralateral adrenalectomy in three cases and contralateral nephrectomy in other two cases. The histopathological studies confirmed an unique pancreatic lesion in 39 cases, while in the other 23 cases, 2 or more metastatic lesions were described. Moreover lymph node involvement was reported in 27% of cases submitted to lymph node dissection. During the early postoperative period, the authors reported an overall mortality rate of 6.4%; after a median follow-up of 91 months, 32 patients were dead of disease, 11 cases died of other non-malignant causes and 15 patients were still alive (5 cases being alive with disease while the remaining 10 cases were alive with no signs of recurrent lesions). The authors reported a 3 year, a 5 and a 10 year survival rate of 72, 63 and 32%, respectively. Among the 37 patients who experienced recurrences, 9 cases presented pancreatic relapse, the median time to recurrence being of 44 months. Pancreatic relapsed presented as isolated metastases in five cases and as part of systemic relapse—in association with lung and liver metastases in other four patients; therefore four cases were submitted to pancreatic re-resection, the median survival time after re-resection being of 52.6 months (significantly higher compared to the one reported after conservative therapy—11.2 months, $p = 0.019$). When it comes to the most important prognostic factors influencing survival, it seems that the presence of extrapancreatic disease as well as the presence of lymph node metastases significantly decreased survival. Surprisingly, the study failed to demonstrate a significant influence of the disease free survival interval on the overall survival rate, this fact being explained by the authors by the limited number of patients introduced in the current study [21].

In a similar study conducted by Ruckert et al. and published in 2016 in the *International Journal of Surgery,* the authors reviewed data from 40 patients submitted to surgery for pancreatic metastases from renal cell carcinomas in 2 German centers between January 1993 and October 2014. These cases were submitted to surgery for pancreatic disease after a median period of 125.4 months, the most commonly performed surgical procedures consisting of pancreatoduodenectomy (in 37.5% of cases), total pancreatectomies (in 22.5% of cases), distal pancreatectomies (in 30% of cases), segmental resections (in 7.5 cases) and papillary resections (in 2.5% of cases). The most commonly encountered complication was pancreatic leak and it was

reported in 12 cases while the postoperative overall mortality was 7.5%. When it comes to the long-term outcomes, the authors reported a mean overall survival of 147.9 months; however, none of the studied factors (including body mass index, sex, time of resection, synchronous/metachronous lesions, symptomatic/asymptomatic lesions, resection status, existence of other extrapancreatic lesions, tumor dimension or lymph node status) did not significantly influenced survival. This fact was explained by the authors by the relative small number of cases introduced in the current study [22]. Another important aspect pointed out of these authors is the one regarding the lymph node status: among the 21 patients who benefited from lymph node resection 5 patients were diagnosed with lymph node metastases; therefore the authors underlined the necessity of association of lymph node dissection in such cases [22].

The outcomes after pancreatic resection for pancreatic metastases with renal cell origin are shown in **Table 1**.

Name, year of the study	Period of the study	No. of patients	Disease free interval	Type of pancreatic resection	Early postoperative outcomes	Long-term outcomes
Schwarz, 2014 [21]	1987–2003	27 patients	9.8 years	PD: 19 cases DP: 25 cases TP: 14 cases Enucleation: 4 cases	Postoperative mortality: 4 cases	Overall survival after pancreatic resections: 52.6 months
Ruckert, 2016 [22]	1993–2014	40 patients	125.4 months	PD: 15 cases DP: 12 cases Enucleation: 3 cases Broad papillary resection: 1 case	Postoperative mortality: 3 cases	Overall survival after pancreatic resections: 147.9 months
Markinez, 2013 [23]	2000–2011	8 patients	12.42 years	TP: 6 cases DP: 1 case Atypical resection: 1 case	Postoperative mortality: 1 case	Survival between 6 months and 95 months
Benhaim, 2015 [24]	1997–2012	20 patients	130 ± 59 months	PD: 6 cases DP: 5 cases TP: 3 cases Metastasectomy: 6 cases	Postoperative mortality: 1 case	Overall survival rate at 4 years: 72%
Zerbi, 2008 [25]	1998–2006	23 patients	8 years	PD: 4 cases DP: 11 cases TP: 2 cases Metastasectomy: 5 cases MP: 1 case	Postoperative mortality: 0	Overall survival after pancreatic resections: 44 months
Yuasa, 2015 [26]	1999–2013	15 patients	13.4 years	TP: 2 cases Metastasectomy: 13 cases	Postoperative mortality: 0	Overall survival after pancreatic resections: not reached at 3.5 year follow-up

Name, year of the study	Period of the study	No. of patients	Disease free interval	Type of pancreatic resection	Early postoperative outcomes	Long-term outcomes
Sohn, 2001 [27]	1989–1999	10 patients	9.8 years	PD: 7 cases DP: 2 cases TP: 1 cases	Postoperative mortality: 0	Overall survival after pancreatic resections at 5 year follow-up: 75%

Abbreviations: DP, distal pancreatectomy; PD, pancreatoduodenectomy; TP, total pancreatectomy; MP, middle pancreatectomy.

Table 1. Outcomes after pancreatic resections for pancreatic metastases from renal cell carcinomas.

5. Pancreatic metastases from colorectal cancer

Colorectal cancer represents one of the most common reported malignancies worldwide, being the third cause of death following breast and lung cancer and the second cause of death among non-smokers [28]. When it comes to the most common patterns of spread in colorectal cancers, they are represented by the peritoneal, lymphatic and hematogenous spread; the hematogenous route is usually related to the apparition of parenchymatous lesions located in liver, lung or brain. In certain cases, pancreatic metastases from colorectal malignancies can occur, the estimated incidence being of 2%; these types of lesions are usually associated with peritoneal carcinomatosis and less often as single lesions [29]. When it comes to the most appropriate imagistic study in order to confirm the presence of such lesions, CT has been proposed, followed by PET-CT (in cases in which although the clinical symptoms are highly suggestive for a pancreatic lesion but standard CT failed to diagnose it). It has been reported that PET-CT is a highly sensitive method of diagnostic in such cases (with an estimated sensitivity of 90–95%) while the specificity ranges between 65 and 85%; therefore performing a PET-CT in such cases seems to be responsible for the change of the therapeutic approach in up to half per cent of cases [6].

Isolated pancreatic metastases from colorectal cancer suitable for resections are scarce eventualities, only few cases being reported so far. Therefore is difficult to establish whether surgical resections of such lesions is superior to the conservative treatment such chemotherapy, due to the small number of cases submitted to surgery. However, it seems that surgery is especially useful in patients with symptomatic lesions, a satisfactory symptom relief being reported [10].

6. Pancreatic metastases from melanomas

Pancreatic metastases from melanomas are rare situations, only few such cases being presented so far; moreover, most often the cases are presented as case reports or small case series involving less than 10 cases, a standard therapeutic protocol being hard to be established. Up to half of patients presenting with pancreatic metastases from melanomas present in fact disseminated metastatic lesions in the context of the systemic recurrence, being associated

with an extremely poor prognostic. Less than 2% of patients with pancreatic metastases from melanomas will be diagnosed with oligometastatic disease, most often the primary tumor being an ocular melanoma [30, 31].

As for the long-term outcomes, patients presenting pancreatic metastases originating from melanomas with various locations seem to have a poorer prognostic when compared to other primaries due to the aggressive biological behavior of melanomas [19]. However, compared to patients treated in a conservative manner, it seems that patients submitted to surgery might benefit from a better outcome especially in cases in which complete resection of the pancreatic lesion is feasible [32, 33]. When it comes to the most important prognostic factors which might influence the long-term outcomes, it seems that a long disease free survival interval is usually associated with a lower biological aggressivity of the primary tumor, and, therefore, with a better outcome [34].

In a case series of two patients diagnosed with pancreatic metastases from melanomas at *Melbourne University*, *Austin Hospital*, *Victoria*, *Australia* published in 2003, the authors reported encouraging results; the first case was the one of a 45-year-old woman with personal history of ocular melanoma treated by transscleral resection 12 years earlier, the histopathological studies revealing at that moment a 10 mm mixed spindle and epithelioid cell melanoma; however, she experienced an early local recurrence 1 year postoperatively, laser therapy being performed at the time of relapse. About 11 years later (after the first local relapse), the patient was diagnosed with a pancreatic head tumor in association with three to four well defined hepatic nodules measuring 5–10 mm, while the fine needle biopsy confirmed the metastatic character of the tumor originating from the melanoma. The patient was submitted to a pylorus preserving pancreatoduodenectomy and segmental liver resection, the histopathological studies confirming the metastatic character of all the resected tumors. The patient was free of disease at 6 months follow-up. The second reported case from the same authors was the one of a 55-year-old patient known with a previous history of ocular melanoma enucleated 13 years earlier who complained of epigastric pain and was diagnosed with a tumoral mass at the level of the pancreatic head; intraoperatively multiple pigmented lesions were seen on the whole surface of the pancreas, so the patient was submitted to total pancreatectomy and remained free of disease 7 months later [34].

Wood's series conducted on a group of six patients with pancreatic metastases forming melanomas, complete surgical resection of the pancreatic lesion was associated with a median overall survival rate of 24 months, significantly higher than the survival rates after palliative chemotherapy (where the median overall survival rate does not surpass 12 months) [35, 36]. However, in Woods' study one of the most important prognostic factors was related to the resection margins, patients presenting with positive resection margins or incomplete resection being associated with a significantly poorer outcome (in fact in these cases the median survival rate was 8 months, similar to the one reported after palliative chemotherapy) [35]. In conclusion incomplete resection has no benefit in terms of survival.

7. Pancreatic metastases from breast cancer

Intra-abdominal breast cancer metastases are usually diagnosed at the level of the liver, spleen or axial skeleton [37]. When reported, pancreatic metastases from breast cancer are

usually associated with other disseminated lesions. Oligometastatic disease has been rarely diagnosed; however, these kinds of lesions might be seen after a long disease free survival interval; therefore, the diagnostic of metastatic disease should be kept in mind whenever a pancreatic tumor is diagnosed in a patient with previous history of breast cancer [38, 39]. In such cases, the clinical signs and symptoms can range from totally asymptomatic lesions to diffuse upper abdominal pain, jaundice or acute pancreatitis due to the concomitant obstruction of the common bile duct or of the Wirsung duct [38, 39].

Pancreatic metastases from breast cancer have been reported with an incidence of 13% in autopsy studies and are usually associated with other disseminated lesions, transforming the patient into a candidate for a palliative oncologic treatment [40]. However, in cases presenting as oligometastatic lesions, surgery has been proposed, this therapeutic approach being encouraged by the success reported by hepatobiliary surgeons who performed surgery for isolated hepatic metastatic with mammary origin [41].

Bednar et al. reported a case series of two patients diagnosed with pancreatic metastases from breast cancer origin. The first one was the case of a 75-year-old patient diagnosed with an invasive lobular breast carcinoma at 58 years of age for which she was submitted to a radical mastectomy at that moment, followed by adjuvant hormonal therapy based on tamoxifen. About 18 years later, the patient was investigated for weight loss, jaundice and she was diagnosed with a pancreatic head tumor; she was resubmitted to surgery, a pancreatoduodenectomy being performed. The histopathological studies confirmed the presence of a metastatic lesion originating from the primary breast cancer; postoperatively, she was resubmitted to hormonal treatment. At 4 year follow-up, the patient was alive with disease, disseminated metastatic lesions in the contralateral axilla being found. The second case was the one of a 57-year-old patient initially diagnosed with stage IIA mixed cellularity Hodgkin's lymphoma initially treated by radiotherapy, which developed 19 years after breast tumor. At that moment the patient was submitted to surgery, the histopathological studies demonstrating the presence of a high grade phyllodes tumor. About 4 years later, the patient was diagnosed with a pancreatic head tumor, in association with lung nodules which were biopsied, the histopathological studies confirming the metastatic character originating from the phyllodes tumor. Therefore the patient was submitted to palliative chemotherapy and died 15 months later [42].

8. Pancreatic metastases from ovarian cancer

Ovarian cancer remains one of the most aggressive gynecological malignancies due to the fact that most often patients are diagnosed in advanced stages of disease, when disseminated lesions are already present. In such cases, the principles of debulking surgery were successfully applied especially for pelvic confined disease. However, patients presenting extended upper abdominal lesions were considered to a have a poorer outcome due to a more aggressive surgical biology. This myth was destroyed by the first studies which incorporated extended upper abdominal resections as part of debulking surgery; in Eisenhauer's study conducted between 1998 and 2003, 262 patients with advanced stage ovarian cancer

were included. These patients were divided in 3 groups according to the time when the surgical procedure was performed: there were 57 patients submitted to surgery after the date of May 2000 when extensive upper abdominal resections were performed as part of debulking surgery. Groups 2 and 3 were submitted to surgery before that date and included 122 patients submitted to cytoreductive surgery for pelvic confined disease (group 2) and 83 patients, respectively, submitted to debulking surgery for extensive lesions (group 3); therefore, most patients in the third group were suboptimally cytoreduced due to the extension of the tumoral process in the upper abdomen. The authors demonstrated a median progression free survival of 24, 23 and 11 months, respectively, for groups 1, 2 and 3. Moreover the author reported a median overall survival of 84 months for group number 2, 28 months for group number 3 and was not reached by the end of the study for group number 1. In conclusion, the authors underlined that the long-term outcomes were not influenced by the extension of the upper abdominal resections as part of debulking surgery, the only factor which strongly shortened survival being the presence of residual disease [43]. Therefore the upper abdominal resections were successfully included as part of debulking surgery for both advanced and recurrent ovarian cancer.

Pancreatic metastases from ovarian cancer usually develop as part of systemic dissemination of the malignant process, the main patterns of spread including peritoneal, hematogenous or lymphatic spread. When it comes to the pancreatic involvement due to ovarian cancer, the most commonly involved mechanisms include peritoneal and hematogenous spread.

When it comes to the association of pancreatic surgery as part of debulking surgery for advanced stage or relapsed ovarian cancer, it has been initially considered that association of such procedures will lead to the apparition of an unacceptable risk of perioperative complications. However, a study conducted by Kehoe et al. demonstrated that these surgical procedures can be successfully associated as part of debulking surgery. The authors reported a series of 17 patients submitted to distal pancreatectomies for pancreatic metastases with ovarian origin, the median age of patients being of 63 years. When it comes to the surgical outcomes, nine patients were submitted to debulking surgery to no residual disease, seven cases were submitted to optimal cytoreductive surgery while in one case a suboptimal cytoreductive surgery was performed; however, in this last case the presence of tumoral residual lesions was described at the level of the diaphragm and in the liver. When it comes to the short-term outcomes, the authors reported a morbidity rate of 24%, all patients being diagnosed with pancreatic fistulas. However, the presence of pancreatic fistulas did not impede the administration of the adjuvant therapy. Moreover, the rate of pancreatic leaks was similar to the one reported by surgeons performing pancreatic resections for pancreatic primaries, demonstrating in this way that pancreatic surgery can be safely associated as part of debulking surgery for advanced stage or recurrent ovarian cancer [44].

When it comes to the long-term outcomes after pancreatic resections for advanced stage or recurrent ovarian cancer, an interesting study was conducted in *Fundeni Clinical Hospital, Bucharest, Romania,* and was conducted by Bacalbasa et al. The study included one patient submitted to pancreatic resections as part of primary cytoreduction, four cases submitted to surgery as part of secondary cytoreduction and one case submitted to pancreatic resection

as part of tertiary cytoreduction. The patient submitted to primary cytoreduction benefitted from a distal pancreatectomy in association with splenectomy which was associated to a total hysterectomy with bilateral adnexectomy, pelvic and para-aortic lymph node dissection and parcelar gastrectomy; the long-term outcome was a favorable one, the patient being diagnosed with relapse at 54 months follow-up. Patients submitted to pancreatic resections as part of secondary cytoreduction experienced a median disease free interval of 32 months and necessitated in all cases a distal pancreatectomy. Postoperatively, two patients developed pancreatic leaks which were treated conservatively in one case and through reoperation in the second case. When it comes to the long-term outcomes, the median overall survival was 36.38 months, all cases being dead of disease at the end of the study. At the time of tertiary cytoreduction, pancreatic resection was performed in a single case, 52 months after the initial diagnostic. Although the early postoperative outcome was favorable, the patient died of disease 10 months later. The authors demonstrated in this way the effectiveness of pancreatic resections as part of cytoreductive surgery in the setting of advanced stage disease as well as for patients diagnosed with recurrent lesions [45].

Name, year of the study	Period of the study	No. of patients	Type of pancreatic resection	Early postoperative outcomes	Long-term outcomes
Chi, 2004 [47]	2001–2002	70 cases, 3 patients necessitating pancreatic resections	DP: 3 cases	0	Not reached at the end of the study (for the entire group)
Eisenhauer, 2000 [43]	2000–2003	57 cases, 6 patients necessitating pancreatic resections	DP: 6 cases	NR	Not reached at 36 months follow-up (for the entire group)
Chi, 2006 [48]	1989–2003	465 cases, 2 patients necessitating pancreatic resections	DP: 2 cases	0.6%	106 months—in cases submitted to complete cytoreductive surgery
Chi, 2009 [49]	2001–2004	210 cases, 9 patients necessitating pancreatic resections	DP: 9 cases	1%	Overall survival after pancreatic resections: 54 months
Hoffman, 2007 [50]	2002–2004	6 cases, 2 patients necessitating pancreatic resections	DP: 2 cases	0	Overall survival after pancreatic resections: not reported
Chi, 2010 [51]	2001–2006	141 cases, 17 patients necessitating pancreatic resections	DP: 17 cases	1.4%	Overall survival after pancreatic resections: 57 months
Rodriguez, 2013 [52]	2001–2004	482 cases, 12 patients necessitating pancreatic resections	DP: 12 cases	Not reported	Overall survival after pancreatic resections: 54.6 months
Heitz, 2016 [53]	2005–2010	578 cases, 13 patients necessitating pancreatic resections	DP: 13 cases	2%	Overall survival after pancreatic resections: 49 months

Abbreviations: DP, distal pancreatectomy.

Table 2. Outcomes after pancreatic resections for pancreatic metastases from ovarian carcinomas.

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An interesting such case was reported by Rania Abadeer in 2010 and referred at a 43-year-old patient who was initially submitted to surgery for an adult granulosa cell tumor for which a salpingo-oophorectomy was performed. About 7 years later, the patient was diagnosed with disseminated lesions infiltrating the pelvic wall, so she was resubmitted to surgery, a total hysterectomy with left adnexectomy and bilateral pelvic lymph node dissection being performed; at that moment debulking surgery to no residual disease was achieved, the histopathological findings confirming the metastatic origin from the adult cell granulosa tumor; therefore the patient was submitted to adjuvant taxol and platinum-based chemotherapy. However, 3 years later, the patient was diagnosed with a 4.2 × 4.1 cm pancreatic cyst located at the cephalic level so a fine needle aspiration was performed, the cystic fluid presenting no signs of malignant cells. Due to the fact that the cyst continued experiencing a fast growth process, the patient was submitted to surgery, the frozen section of the cystic wall being suggestive for malignancy; due to this aspect, the surgical procedure was completed by performing a pancreatoduodenectomy. The immunohistochemical studies confirmed the metastatic origin of the lesion originating from the initial adult granulosa cell tumor. The long-term outcome was favorable, at 30 months follow-up the patient being free of any recurrent disease [46].

The outcomes after pancreatic resection for pancreatic metastases with ovarian cancer origin are shown in **Table 2**.

9. Pancreatic metastases from uterine body or cervix cancer

Pancreatic metastases from uterine primaries are other rare eventualities, only few cases being described so far [1, 54–56]. The main pattern of spread responsible for the apparition of pancreatic metastases with endometrial origin consists of hematogenous disseminations and it is usually responsible for the apparition of other distant lesions such as hepatic, pulmonary or splenic metastases [57]. Due to this aspect, pancreatic metastases from uterine carcinomas can be rarely treated with curative intent. The first authors who reported performing a surgical procedure for a pancreatic metastasis originating from an endometrial carcinoma came from the USA, in 1998; it was the case of a patient known with previous history of endometrial cancer who presented for upper digestive stenosis 3 years later. At this time, a 4 cm tumor located at the level of the uncinate process of the pancreas was found, so the patient was successfully submitted to surgery; unfortunately the authors did not report the performed surgical procedure or the outcome of this patient [2]. The first successful pancreatic resection for pancreatic metastases from endometrial cancer came from Dan Blazer, at *M.D. Cancer Center, Houston, Texas, United States of America,* in 2008. It was the case of a 56-year-old patient who had been previously submitted to surgery for endometrial cancer, at that moment a total hysterectomy with bilateral adnexectomy, pelvic and para-aortic lymph node dissection being performed; postoperatively, the patient was submitted to adjuvant radiotherapy. However, 31 months later, she was diagnosed with a pancreatic lesion measuring 3 × 3 cm in the pancreatic tail. The fine needle biopsy confirmed the metastatic origin, so the patient was resubmitted to surgery, a distal pancreatectomy being performed. The histopathological studies confirmed the metastatic origin of the lesion; however, 6 months later, the patient remained free of recurrent disease [55].

Pancreatic oligometastases with uterine cervix origin is another rare situation, a successful resection of such a lesion being reported for the first time by Wastell et al. in Westminster, London. The authors reported the case of a patient who had been initially treated by radiotherapy with curative intent for a squamous cell carcinoma; however, 5 years later, the patient was diagnosed with a pancreatic head tumor. At that moment a pancreatoduodenectomy was performed, the histopathological studies confirming the metastatic origin of this lesion; unfortunately the postoperative course was complicated by the apparition of a bronchopneumonia, the patient being dead 16 days later [58].

Another interesting case was reported by the Japanese authors in 2013 [59]. The authors reported the case of a 44-year-old patient who had been initially diagnosed with a stage IB uterine cervix cancer, the histopathological studies reporting a mixed adeno-neuroendocrine carcinoma; 8 years later, the patient was diagnosed with an isolated pancreatic tumor which was biopsied, a metastatic neuroendocrine tumor being revealed. At that moment a central pancreatectomy was performed, the histopathological studies confirming the presence of a metastatic lesion; however, only the neuroendocrine component seems to be responsible for the apparition of the recurrent disease. The long-term outcomes were favorable, the patient being free of disease at a 7 month follow-up [59].

10. Pancreatic metastases from lung cancer

Lung cancer is associated with the highest mortality rates, being associated most often with liver, brain, bone or lymph node metastases [60].

Pancreatic metastases from lung cancer are not a usual condition; in a review of 333 cases diagnosed with pancreatic metastases, the most common origin of the pancreatic lesions was represented by the renal cell carcinomas, being responsive for 45% of cases; among the remaining cases, the lung was reported as the origin of the pancreatic metastases in 14.7% of cases [61].

When it comes to the most common histopathological subtype of lung cancer which might induce the apparition of pancreatic lesions, small cell lung cancer has been most often reported; other incriminated histopathological subtypes included large cell carcinomas, squamous cell carcinomas and anaplastic bronchial carcinomas [62].

When diagnosed, pancreatic metastases from lung cancer are usually encountered as part of the systemic recurrence, with metachronous character; therefore the patient will be a candidate for palliative oncologic treatment, with low rates of long-term survival. Oligometastatic pancreatic disease with lung cancer origin appears in rare situations and it seem to be best treated through surgery with curative intent; however, the long-term outcomes failed to demonstrate good survival rates, the median overall survival ranging from a few months to a few years [10] due to biological aggressiveness of the primary tumor.

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11. Pancreatic cancer from sarcomas

Metastatic lesions with sarcomatous origin are usually associated with an extremely poor outcome due to the biological aggressiveness of such primaries. When encountered, pancreatic metastases with sarcomatous origin are reported as part of the systemic disease so most often surgery is no longer a valid therapeutic option. In cases presenting oligometastatic disease, surgery might be proposed whenever the biological status of the patient will permit it. A particular problem in such cases is related to the multifocality of such lesions and to the feasibility of resection with curative intent [7].

Successful resection of pancreatic metastases from soft tissue sarcomas has been reported by the Japanese authors in two cases. The first patient had been initially diagnosed with a mesenchymal chondrosarcoma of the left thigh in 1986; 3 years later, the patient was diagnosed with isolated pancreatic lesions, the patient being submitted to surgery with curative intent; the patient remained alive for the next 10 years. The second case was initially diagnosed with a synovial sarcoma followed by pulmonary resection for metastatic disease; the case was further diagnosed with a solitary pancreatic lesion for which she was submitted to pylorus preserving pancreatoduodenectomy with good results, the patient remaining alive for more than 6 years after pancreatic resection [63].

Another extremely interesting situation was reported by another Japanese team in 2016. The authors reported the case of a 44-year-old woman who had been previously submitted to surgery for a right fibular head osteosarcoma; 3 years later, the patient was diagnosed with a metastasis in the distal pancreas, so a laparoscopic distal pancreatectomy with spleen preservation was successfully performed; the histopathological studies confirmed the metastatic origin from the initial osteosarcoma. Although the patient also reported the apparition of lung metastases, 1 year later, she was resubmitted to surgery with curative intent, the patient being still alive at the time of publishing the case [64].

A particular situation is represented by patients diagnosed with pancreatic metastases from uterine sarcomas, in cases presenting oligometastatic disease, surgery being considered as a valid option. Most often these lesions occur in patients who had been previously diagnosed with uterine leiomiosarcomas and might experience good long-term outcomes whenever a curative resection is performed [65, 66].

12. Conclusion

Isolated pancreatic metastases suitable for resection are rare eventualities; the renal cell carcinoma origin being the most frequently reported situations. When diagnosed as metachronous isolated lesions, such metastases can be submitted to surgery with curative intent, long-term survival rates being reported. Another primary with good outcomes after pancreatic resections for metastatic disease is represented by ovarian cancer, debulking surgery to no residual disease

including pancreatic resections being associated with long-term survival. When it comes to the other origins, the reported results are inconstant and no standard therapeutic protocol can be established due to the paucity of cases. However, it seems that the best outcomes should be expected in cases diagnosed with isolated metachronous lesions with long disease free survival intervals and in the absence of extrapancreatic disease. In such cases, association of surgery as part of multidisciplinary approach might improve the long-term outcomes.

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Chapter 8

Drug Discovery from Natural Products for Pancreatic Cancer

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Additional information is available at the end of the chapter

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Abstract

Since ancient times, natural products (NPs) have been used as anti-infectives, anti-inflammatories, antioxidants, analgesics and antitumorals and many compounds derived from NPs are in clinical use. The use of plants in traditional medicine for multiple purposes is well known, and throughout recent history, metabolites of microbial origin have had an extraordinary impact on the welfare of humanity. There is an outstanding diversity of chemical structures that nature, and especially microorganisms, are able to produce, due to millenniums of evolution. Since only a small amount of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery, the challenge being how to access this natural chemical diversity. However, the validation and selection of primary screening assays, both phenotypic and target-based, are vital to guaranteeing a selection of extracts or molecules with relevant pharmacological action. The screening of antitumor agents against pancreatic cancer (PC) involves the use of established cell lines, cancer stem cells and spheroids that mimic the patient's tumor. Improvements in the discovery of natural products along with the emergence of new technologies in cancer screening assays, promise the discovery of new and valuable drugs to tackle pancreatic cancer in the coming years.

Keywords: natural products, pancreatic cancer, fungi, bacteria, plant, dereplication, phenotypic-based screening, target-based screening, high-throughput screening, spheroids, organoids

1. Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer mortality in the United States and accounts for about 3% of all newly diagnosed cancers each year. The American Cancer

Society estimated that 43,090 people would die from this disease in 2017 [1]. PC is a multifactorial disease, making it difficult to treat with conventional antitumoral therapy. The poor survival rate is likely due to the lack of early diagnosis, rapid disease progression, high metastasis rate and unsuccessful outcome of treatment. Although there are different types of treatment available for PC, most patients have no recognizable symptoms, making early diagnosis difficult [2], and chemotherapies or radiation therapies are often ineffective. While surgery is generally considered the best treatment option, only 10–20% of patients with PC are surgical candidates. Radiation therapy and chemotherapy are two other common methods for treating PC. However, the biochemical and physiological characteristics of PC appear to limit the effectiveness of these standard forms of therapy, in part due to genetic alterations.

Furthermore, recent studies have shown that PC is highly enriched with cancer stem cell (CSC) subpopulations that are resistant to current chemotherapeutic drugs and therefore promote tumor recurrence [3]. CSCs, also called tumor-initiating cells, share many characteristics with normal stem cells, such as asymmetric cell division, where each CSC generates one daughter cell with self-renewal capacity and another cell destined to differentiate. The self-renewal capacity helps to maintain the number of CSCs within the tumor, and its descendent progeny generate the mass of the tumor [4]. CSCs also exhibit unique features, such as their metastasis ability and the ability to remain in a quiescent state, which protect them from the chemotherapeutic drugs developed to target actively dividing cells. Another phenomenon frequently seen in PC is the cancer cell epithelial to mesenchymal transition (EMT), associated with metastasis, CSC generation and treatment resistance [5].

Although many efforts have been made to find a cure, there is much work left to be done because PC is still an unmet medical need affecting an increasing number of patients every year. Current research categorizes anticancer agents into two major groups based on their mechanisms of action and origins: cytotoxic anticancer agents and molecular targeted therapeutic agents. But the failure of current drugs and treatments can be attributed, in part, to our limited understanding of the targets of the disease and to a lack of reliable disease-relevant screening methods that mimic the key pathophysiological features of cancer. Therefore, efforts now should be addressed to developing new models and assay formats, innovative screening technologies that better summarize *in vivo* physiology [6], and new, effective and safe compounds or combinations.

NPs have been, and still are, one of the main sources of drug discovery. According to the data from Newman and Cragg [7], most new FDA-approved drugs between 1981 and 2014 were derived from NP structures. Natural constituents are widely distributed in various natural sources, including plants, microorganisms and invertebrates. Plant-derived molecules continue to make up a large portion of the pharmaceuticals in the clinic, and the production of antibiotics by microorganisms was one of the biggest breakthroughs in the history of drug discovery in the twentieth century. Bacteria and fungi represent two of the most important sources for novel therapeutic agents exhibiting the most diverse biological actions. Since only a small amount of the world's biodiversity has been evaluated for potential biological activity, many more useful natural lead compounds await discovery, the challenge being how to access this natural chemical diversity.

In the last decades, despite the difficulty of finding novel scaffolds, an increasing number of research groups have dedicated numerous efforts to exploring alternative sources, such as the marine environment, which has become an extraordinarily rich source of new drugs. At present, plants, microorganisms and marine invertebrates are major sources of NPs for discovering novel drugs.

To better understand the huge impact of NPs on cancer pharmaceuticals, it is worth mentioning that out of 155 small molecules used as chemotherapeutics, 73 are directly NPs and another 40 are derivatives or synthetic NP mimetics [8]. Furthermore, current research trends in the field suggest an optimistic future for NPs in cancer prevention and new therapeutics drug discovery. Because of the complex chemistry generated by centuries of evolution of NPs, more success is expected in drug discovery with NPs than with synthetic molecules. However, that complexity of the natural molecules requires a coordinated effort from the interaction of multidisciplinary research areas with new and more sophisticated analytical and technical expertise in order to extract, isolate, identify and turn them into promising leads.

This chapter provides insights into the advances in cancer drug discovery from NPs using high-throughput screening (HTS) technologies, with a special emphasis on the biological tools and cell-based assay platforms implemented to untap new NP scaffolds with novelty in their mode of actions, and the most promising natural molecules under development today.

2. Natural products in drug discovery

The use of plants in traditional medicine is well known for multiple purposes. Over the millennia, plants have been used as anti-infectives, anti-inflammatories, antioxidants, analgesics and antitumorals, and there are many compounds derived from plants used in the clinic. The most famous example to date is probably the synthesis of the anti-inflammatory agent acetylsalicylic acid (aspirin), derived from salicin and isolated from the bark of the willow tree *Salix alba* L [9]. Other examples are morphine, codeine, digitoxin, quinine and the antitumorals paclitaxel, vincristine and vinblastine, and a long list of other drugs. Metabolites of plants still constitute a major area of research but the microbial secondary metabolism of bacteria and fungi has been intensely explored in industrial screening programs in the last decades.

The biosynthesis and breakdown of proteins, fats, nucleic acids and carbohydrates, which are essential to all living organisms, is known as primary metabolism, while the mechanism by which an organism biosynthesizes other compounds is known as secondary metabolism. These secondary metabolites are known as NPs and are often found to be unique to an organism or species [10]. Generally, secondary metabolites are not essential for the growth of an organism and are produced either as a result of the organism adapting to its surroundings or to act as a possible defense mechanism [11]. The biosynthesis of secondary metabolites derived from the fundamental processes of photosynthesis, glycolysis and the Krebs cycle which generate limited building blocks, but the formation of novel secondary metabolites is infinite. The most important building blocks employed in the biosynthesis of secondary metabolites are those derived from the intermediates:

acetyl coenzyme A, shikimic acid, mevalonic acid and 1-deoxyxylulose-5-phosphate, which are involved in innumerable biosynthetic pathways. The catabolic systems using these secondary metabolites are directed by the polyketide synthase (PKS) and the non-ribosomal peptide synthetase (NRPS), which catalyze the elongation of polyketides and synthesis of oligopeptides and also the biosynthetic pathways of terpenoids and alkaloids; these systems are really responsible for ensuring the diversity of the NPs. The evolution in the biosynthetic pathways may be due to natural causes (e.g., viruses, horizontal transfer or environmental changes) or unnatural causes (e.g., chemicals or radiation), in an effort by the microorganism to adapt to the environment. These modifications and alterations have resulted in a huge library of chemical structures optimized by natural selection that possess a broad array of biological activities. The challenge is how to access this chemical diversity and find the appropriate assays to test these biological activities.

In the 2000s, the traditional natural products screening was gradually abandoned because of frequent re-discovery of previously isolated compounds, the inherent technical difficulties associated to the isolation of active constituents of extracts, the incompatibility of NP extracts with HTS campaigns, and the structural complexity and low titer production of NPs, which required total synthesis and derivatization, sometimes economically and synthetically challenging. NP discovery was therefore replaced by molecular target-based drug discovery using large synthetic combinatorial libraries. However, the success of these combinatorial libraries in cancer have been brought into question since only one compound from this origin has been approved by the FDA, in 2005, for treatment of renal cell carcinoma, and in 2013, for treating thyroid cancer. This antitumor compound is sorafenib, co-developed and co-marketed by Bayer and Onyx Pharmaceuticals. Combinatorial libraries lack the structural diversity and complexity given by nature to NPs. In a further step, the diversity-oriented synthesis (DOS) approach was developed to mimic NPs and the resulting compounds are currently being tested in a large number and variety of biological screens in order to determine their role as a promising *hit* [12].

Nonetheless, recent advances in technology and sensitive instrumentation for the rapid identification of novel bioactive NPs and structure elucidation have opened up a new era and greatly improved the NP discovery process [13]. NPs, their semi-synthetic derivatives and natural product-inspired compounds still represent one of the most important sources of chemical diversity and bioactive novel structures ever described [8, 14].

The extremely prolific production of novel molecules by some groups of microorganisms, especially some taxa of actinomycetes (a phylum of Gram-positive bacteria) and fungi, did not require the use of an unlimited number of cultivation conditions to ensure that novel molecules were produced. In fact, for decades researchers have agreed on the application of a maximum of three to four production media at a time are sufficient to exploit the production of new bioactive molecules [15]. Microbial extracts have been largely exploited in antibiotic discovery, but new applications of these secondary metabolites are emerging, such as their relevant activity as antitumor agents. In recent years, increasing numbers of complete annotated genomes have been confirming the presence of a huge biosynthetic potential in bacteria and fungi, in many cases only detected as cryptic pathways from genome mining of biosynthetic pathways [16]. We still do not know the most important factors conditioning the nutritional requirements and secondary metabolism regulatory factors of most of the species screened which might be producing molecules quite below the detection threshold. Further

studies are in progress to modulate and heterologously express the secondary metabolism of microorganisms, opens up an emerging vast field of research in synthetic biology.

Furthermore, the difficulty of discovering novel molecules and the recurrent re-discovery problem of old, well-known molecules has required in parallel moving away from traditional approaches challenging the secondary metabolism of these species from quite different culture-based perspectives. The challenge of finding new molecule classes from libraries of secondary metabolites produced by microorganisms has required a change of paradigm with a shift in the number of new extracts tested and an improvement in the strain selection conditions and the nutritional conditions required for the production of novel molecules.

Access to the microbial diversity in the environment has traditionally been focused not only on intensive sampling from widely diverse geographical locations and habitats, but also other novel approaches were introduced in the 2000s. One of these approaches for drug discovery from microbial strains involves the application of the One Strain Many Compounds (OSMAC) method, which attempts to induce silent biosynthetic clusters leading to the accumulation of compounds by a combination of cultivation and nutritional conditions. OSMAC helps to determine the modulating effect that altered culture conditions (i.e., media composition, temperature, osmolarity and pH) may have on the secondary metabolite production of microorganisms [17]. Examples of such culture variations include the use of different liquid or solid media, such as solid beans or rice medium or the mimicry of extreme habitats by cultivating at colder temperatures or using highly saline media [18]. The OSMAC approach has been routinely used at Fundación MEDINA, a reference center for natural products drug discovery, to exploit our microbial strain collection of 190,000 strains (in part inherited from the Merck & Co. Inc. and Cubist Collections) to generate a NP Library of more than 180,000 extracts. MEDINA has introduced small-scale bacterial and fungal fermentations in tubes and deep well microplates that can be readily adapted to automated liquid handling equipment for further extraction and processing. Thus, the reduction of the fermentation volumes opens up the possibility of testing multiple nutritional conditions while offering the possibility of exploring minor groups of isolates and understanding their requirements up to as many as 20 different media. An average of the best eight fermentation media covering the largest metabolic space of the producing strains was shown to ensure an increase in the numbers and diversity of the strains tested [19]. All crude extracts are obtained from these cultures, using organic solvents to collect most of the secondary metabolites generated both, inside the microbial cells and excreted to the culture media. In the case of extracts from plants or invertebrates, different parts of the plant or animal are crushed and then extracted, also with organic solvents.

Screening of NPs, just as synthetic compounds, is performed following different approaches depending on the paradigm chosen for each application. In the case of antitumor screening, both target- and phenotypic-based assays are normally used. The screening methods are discussed below in Section 2.1 of this chapter, and the process of identifying the new molecules is summarized in **Figure 1**.

Once the active extracts in screening are detected, chemical identification of the novel compounds is necessary. Although diverse strategies are followed for isolating the active compounds from microorganisms, here we describe one of the most exhaustive approaches (**Figure 1**). The process of identifying known compounds responsible for the activity of an

extract prior to bioactivity-guided isolation is referred to as dereplication, and must be done as soon as possible in drug discovery process [20]. An early dereplication of known molecules may be performed through different systematic analysis techniques. One of the most successful methods combines liquid chromatography with high resolution mass spectrometry (LC-HRMS). Identification of known NPs with no chemical or pharmacological interest is inevitable and should be detected early through the comparison of analytical data against proprietary LC-MS libraries of microbial metabolites in research groups with long experience, or against public and commercial libraries of NPs, such as Chemspider or the Chapman & Hall Dictionary of NPs.

Active extracts containing novel components are of great interest and the molecular identification and isolation of these novel compounds are required. To do this, first, the microbial strain is regrown in the same conditions on a medium scale (150 mL), and bioassay-guided extract fractionation is carried out. Enriched fractions are generated through semipreparative HPLC method using proper separation columns and solvent gradient. The fractions are then tested for activity following the screening paradigm. In some cases, LC/HRMS and NMR dereplication allow identify the bioactive components at this stage, but in most cases, regrowth on

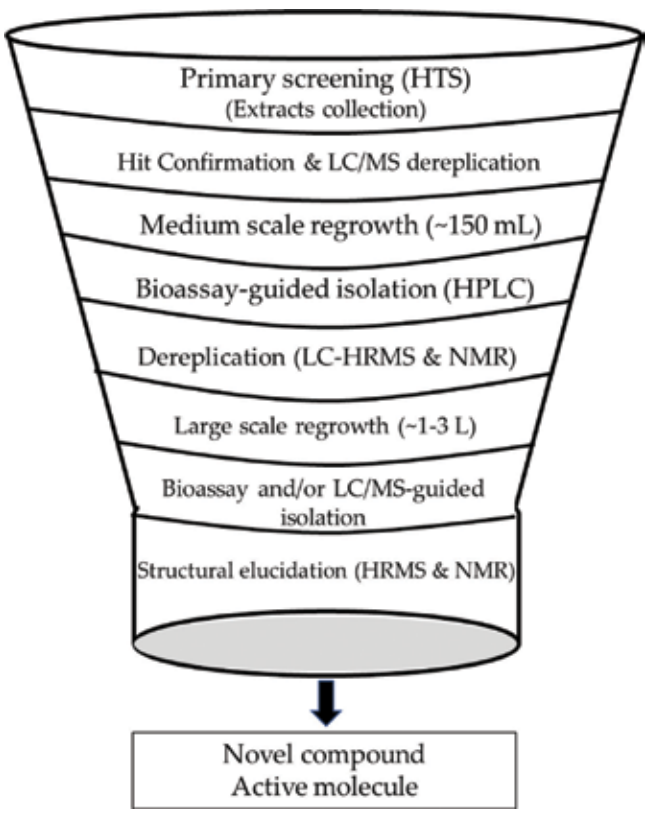

Figure 1. Schematic representation of the high-throughput screening and integrated dereplicating chemistry platform used to discover molecules from microbial extracts.

a large scale (1–3 L) is needed to have enough quantity of the bioactive compounds, whose structure elucidation is eventually performed using LC-HRMS and nuclear magnetic resonance (NMR) [21]. In the case of NPs from plants or invertebrates, similar bioassay-guided fractionation, dereplication, elucidation and chromatographic purification steps are followed [22, 23].

Undoubtedly, the discovery of drugs from natural products requires a multidisciplinary team: a group of experienced microbiologists or biologists working on plants or invertebrates to generate a collection of samples with the greatest possible biodiversity, researchers with experience in screening and with skills to work with HTS and robotic equipment, and lastly, a group of chemists with extensive knowledge about the chemistry of natural products.

2.1. Screening in pancreatic cancer

Screening has played a critical role in the discovery of leads that are further optimized for their properties, eventually leading to clinical candidates and drugs. The tremendous progress made in life sciences has resulted in the definition of many pathological processes and mechanisms of drug action. Drug discovery for cancer is carried out using both, target-based and phenotypic-based approaches. Target-based approaches to drug discovery are extensively used in the pharmaceutical industry but there are very few fully validated drug targets in cancers that are dependent on the tumor microenvironment, such as pancreatic cancer [24]. Due to this lack of avowed target in PC and the advances in cell culture, the most widely used approaches are phenotypic.

The latest advancements have led to the establishment of various molecular and cellular bioassays in conjunction with HTS methods. HTS decreases the amount of testing compound required so that only microgram quantities are needed. This is advantageous for certain NPs that are difficult to isolate and purify, and permits assaying compounds that are difficult to synthesize. Fluorescent methods are probably the classic choice for HTS, as they allow the best discrimination of the signal of interest from the background, though luminescent methods or other new technologies like AlphaScreen™, fluorescence resonance energy transfer (FRET) technology, time-resolved fluorescence (TRF) and fluorescence polarization are becoming more relevant. The current move is away from the traditional 96-well plate to 384- or 1536-well plates, where reagent costs are typically 100 times lower, and assay volumes decrease from 200 to 5–10 μl, and the quantity of compound or crude extract assayed drops to nanoliters. The use of such a low volume in assays leads to the need for liquid handling equipment, robotic platforms and new technology advancements such as Acoustic Droplet Ejection Technology [25].

Furthermore, image acquisition using robotic fluorescent microscopy and automated image analysis, generally referred to as high-content screening (HCS), has become an essential tool in early drug discovery programs, especially in cancer research. High-content cellular imaging is increasingly meeting the challenges of high-throughput needs and facilitating the integration of disease-relevant screens in cancer models such as three-dimensional (3D) cultures. NP screening has been adapted to HTS technologies, and a huge effort has been made to adapt the classical NP research laboratories to centralized HTS facilities.

The increase of chemodiversity, together with HTS methods and novel assay models in cancer research, make the use of NPs a promising source of anticancer drugs. The two main approaches to drug discovery for PC, target- and phenotypic-based screening, are described.

2.1.1. Target-based screening in pancreatic cancer

A target for a disorder is only fully validated when there is a registered drug for which it can be shown that the principle mode of action is by modulation of the target. For decades, PC was commonly treated with 5-fluorouracil (5-FU), also used in other types of cancer. The suicide inhibitor 5-FU works through irreversible inhibition of thymidylate synthase [26]. The cytidine analog Gemcitabine (2′,2′-difluoro 2′-deoxycytidine), which replaced 5-FU, was approved by the Food and Drug Administration (FDA) in 1996 [27], becoming the standard first-line treatment for PC. In 2005, the FDA approved the combination of gemcitabine and erlotinib [28]. Erlotinib is a receptor tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR). Other combination clinical trials have been conducted since then.

A combination of oxaliplatin, irinotecan, 5-FU and leucovorin, called FOLFIRINOX [29], is chemotherapy regimen confined to patients with good performance status because of its high toxicity and severe side effects. Oxaliplatin is a platinum-based antineoplastic agent, irinotecan prevents DNA from unwinding by inhibition of topoisomerase 1 and leucovorin (5-formyltetrahydrofolate) is an adjuvant in cancer chemotherapy.

Nab-paclitaxel, a nanoparticle albumin-bound paclitaxel, which is natural product from extract of *Taxus brevifolia* (Pacific yew) [30], targets tubulin and destroys cancer cells by preventing the normal breakdown of microtubules during cell division. In September 2013, the FDA-approved nab-paclitaxel for use in treating advanced PC. The last therapy approved for PC, in 2015, was the combination of Onivyde (irinotecan liposome injection), 5-FU and leucovorin. Despite great efforts, many years of research and numerous studies, these chemotherapeutic options for treating PC are far from satisfactory at present [31].

In recent years, further attempts have been made to discover new chemotherapeutics directed to new targets which may provide an approach for PC prevention and treatment. Pathways with relevant novel targets are: K-ras (Raf [32], MAPK, Erk, PI3Ks, PDK-1 [33]; p53 [34]; growth factor (EGF, EGFR [35], FGF, FGFR [36], VEGF [37], IGF [38]) and the pathway of epithelial to mesenchymal transition (Wnt/β-catenin [39], TNFα [40], Notch [41], Snail-1, Slug, E-cadherin [42]). Clinical trials have been developed to evaluate some of these targets but the results have been disappointing. Studies testing the antibody bevacizumab, which blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A) [43] and cetuximab, which blocks ligand-binding domain of EGFR [44], have been negative.

Although these experimental targets are widely used for target-based screening in PC, there is an urgent need to identify innovative therapeutic targets. Current approaches to the discovery of new biomarkers and targets range from the use of microarrays for gene expression profile of PC patients versus healthy controls [45], to proteomic [46] or secretome profiles [47].

Few examples of NPs have been reported for PC with different target-directed mechanisms. Some of the more representative ones are described below and summarized in **Table 1**.

NP compound or derivate	Ref.	Source	Target
Nab-paclitaxel (derivated from paclitaxel)	[30, 48]	*Taxus brevifolia* (tree)	Tubulin (microtubules)
Hispidulin	[49]	Artemisia and salvia (plants)	VEGF receptor 2-mediated PI3K/Akt/mTOR signaling pathway
Betulinic acid	[51]	*Betula pubescens* (tree)	Lamin B1
Ixabepilone	[52]	*Sorangium cellulosum* (Gram-negative bacterium)	Microtubule stabilizer
Apigenin	[54, 55]	Widely distributed in plants	COX-2, IKK-β-mediated NF-κB activation
Baicalein	[56]	*Scutellaria baicalensis* and *S. lateriflora* (plant)	Lipoxygenases (LOXs)
Ellagic acid	[57]	Fruits, nuts and vegetables	Arachidonic acid (AA) pathway
[6]-Gingerol	[58]	*Zingiber officinale* (plant)	Arachidonic acid (AA) pathway
Thymoquinone	[59]	*Nigella sativa* (plant)	Arachidonic acid (AA) pathway
Triptolide	[60]	*Tripterygium wilfordii* (plant)	Arachidonic acid (AA) pathway

Table 1. Summary of experimental NPs and derivatives for PC treatment with known target.

The most advanced, which has been approved by FDA, is nab-paclitaxel, derived from natural paclitaxel [48], mentioned above in the chapter. Another example, reported by He et al. [49] is hispidulin, a flavone found in some plants including artemisia and salvia, which targets the VEGF receptor 2-mediated PI3K/Akt/mTOR signaling pathway in endothelial cells, leading to the suppression of pancreatic tumor growth and angiogenesis.

Other class of secondary metabolites of plants with effectiveness in PC is terpenoids. The mechanism of the terpene antitumor effects is the inhibition of posttranslational isoprenylation of proteins regulating cell growth [50]. For instance, betulinic acid, a triterpenoid obtained from the bark of *Betula pubescens*, exhibits potent antitumor activities and can downregulate lamin B1; knockdown of lamin B1 significantly attenuates the proliferation, invasion and tumorigenicity of PC cells [51]. Moreover, the epothilone B lactam ixabepilone, a microtubule stabilizer produced by a Gram-negative bacterium *Sorangium cellulosum*, which was approved by the FDA for breast cancer treatment in 2007, was assayed for PC in a Phase II trial in patients with advanced pancreas cancer [52], showing encouraging activity in the patients.

Additionally, the arachidonic acid (AA) pathway plays a key role in carcinogenesis. The AA pathway metabolic enzymes phospholipase A2s (PLA2s), cyclooxygenases (COXs) and lipoxygenases (LOXs), and their metabolic products, such as prostaglandins and leukotrienes, have been considered novel preventive and therapeutic targets in cancer [53]. AA pathway inhibitory NPs have been developed as chemopreventive and therapeutic agents against several cancers. For example, apigenin, a flavonoid widely found in plants, suppresses inducible COX-2 expression and inhibits the growth of PC cells *in vitro* and *in vivo* by IKK-β-mediated NF-κB activation [54]. Epidemiologic studies suggested that apigenin is related to a decreased risk of certain cancers, including PC [55]. Another is baicalein (5,6,7-trihydroxyflavone), found

in the roots of *Scutellaria baicalensis* and *S lateriflora*, which inhibits LOXs and in turn down-regulates Bcl-2, increases Bax, increases cytosolic cytochrome c, and activates caspase-9, promoting apoptosis and exhibiting anticancer activity against PC cells [56]. Ellagic acid, a hydrolyzed metabolite of ellagitannin found in certain fruits, nuts and vegetables, has been reported to possess anti-pancreatic cancer properties, targeting AA pathway. Zhao et al. [57] described that ellagic acid inhibited PC growth in PANC-1 xenografted mice, by suppressing various pro-tumorigenic mediators. Other natural compounds with described activity for this pathway are [6]-gingerol, a phenol constituent of the plant *Zingiber officinale* Roscoe (ginger) [58]; thymoquinone [59], derived from black cumin *Nigella sativa* and triptolide [60], a diterpenoid isolated from *Tripterygium wilfordii*. Many more NPs that modulate AA pathway are under research for other cancer types and could also be active against PC.

2.1.2. Phenotypic-target screening in pancreatic cancer

While all of the early drugs were discovered by phenotypic screening, the past three decades have given rise to new technologies for performing HTS that have since dominated the pharmaceutical industry. Development of patient-derived cell cultures, three-dimensional (3D) culture, organotypic systems, advances in cell imaging, microfluidics and nanotechnologies are the future trends in drug discovery and development.

Patient-derived cell cultures offer a more clinically relevant model for testing novel gene and cell-based therapies. These models are decidedly valuable in cancer research, where highly selective drugs targeted at genetically defined clinical subtypes are needed to support a more patient-centric approach to drug development [61]. Potential drugs have been tested against patient-derived primary cancer subtypes for various cancers, with promising results in glioblastoma [62] and leukemia [63]. The problem in PC research is that there are few *in vitro* models of exocrine pancreas development and primary human pancreatic adenocarcinoma (PDAC), and the models are just starting to be established. The use of surgically resected pancreatic cancer tissue is difficult because of the limited amount of residual pancreatic cancer tissues remaining after the large amount of cancer tissue has been used in the histopathologic examination for diagnosis. To overcome this limitation, researchers have tried to establish cancer cell lines from patient-derived cancer tissues, but this approach has not been very successful due to specific histopathologic characteristics of PC, such as low cancer cellularity and the extensive desmoplastic reactions by the associated fibroblasts. Consequently, the number of established patient-derived PC cell lines is currently much lower than that of other cancers [64–66], there currently being only 11 cell lines from the American Type Culture Collection and another 4 from the European Collection of Authenticated Cell Cultures (ECACC). These are widely used despite the limitations, such as the *in vitro* cell culture conditions which modify cells over time, losing the expression of markers or enriching specific cell populations. Patient-derived tumor xenograft mice models (PDXs) have been used recently for predicting patient responses for patient-selection strategies [67], but this animal model cannot be used for massive drug discovery for obvious reasons.

Recent evidence suggests the existence of small populations of CSC, which are believed to be responsible for tumor initiation and progression as well as resistance to chemotherapy and

radiation. Identification of the regulatory mechanisms and signaling pathways involved in CSC are expected to help researchers identify and design novel agents that target this resistant cell population in PC. Pancreatic CSC can be allowed to divide and grow in ultra-low binding tissue culture dishes to form multicellular spheroids that will favor the formation of multicellular tissues with the appropriate cell-cell and cell-matrix (ECM) interactions necessary for full functionality. Natural and synthetic biomaterials and nanomaterials are used to build these 3D cultures, providing a robust architecture in 96- and 384-well formats. This technology is being successfully applied in cancer models [68], although the culture medium and materials used still need to be improved.

Coupled with 3D cultures, HCS imaging systems have been developed, with huge advances in microscopy and image-informatics solutions [69]. Image acquisition using robotic fluorescent microscopy and automated image analysis has become an essential tool in early drug discovery programs. HCS cellular imaging has increasingly met the challenges of high-throughput needs and facilitates the integration of disease-relevant models and screens at early stages of the drug discovery process [70]. Although various PC cell lines can grow as spheroids in 3D cultures, it is unclear how well they reflect the properties of the original human tumor [71, 72].

There are some examples of promising NPs against pancreatic cancer obtained and described through phenotypic-based screening and nutritional studies, which have been reported in scientific publications and patents and are summarized in **Table 2**.

One of the main currents for treating cancer using natural plants is traditional Chinese medicine. Chinese medicine is an old form of medicine built on a foundation of more than 2500 years of Chinese medical practice. In recent times, new studies have been made and new patents have been registered in relation to this ancient science with the aim of modernizing traditional Chinese medicine [73]. Some patents of PC treatment have been registered on a combination of tens of herbal and other NPs, based on a secret prescription handed down from ancestors and on traditional Chinese medical theory [74, 75]. These patents report a very high efficacy in clinical studies and have shown a total recovery of up to 90% in 2–5 years. This percentage is incredibly high so a confirmation in other populations would be necessary to validate the results, but there is no record of the patents being tested in other countries. Additionally, the principal components of the flower *Paeonia lactiflora*, albiflorin and paeoniflorin, which are a functional food ingredient widely used for more than 2000 years in traditional Chinese medicine, have been patented for pancreatic cancer prevention and treatment [76]. *In vitro* and *in vivo* experiments show that albiflorin has antitumoral activity and may provide a new option for the clinical treatment of tumors, although these trials are only in the first stage of drug development.

Furthermore, dietary proanthocyanidins mostly present in apples, pears and pulses, has been suggested to reduce the risk of pancreatic cancer by 25% [77]. Another example is *Chelidonium majus* L. (Papaveraceae), a medicinal herb with antitumoral activity that is widely found in Europe. High cytotoxic activity against pancreatic cancer has been reported by an extract of *C. majus* [78]. Also, Sarcaboside B was isolated from the whole plant of *Sarcandra glabra* and shows *in vitro* results of antineoplastic activity in PC cells according to a patent posted by Ding Shengyu [79]. Usnic acid is extracted from lichen and has been used for its antimicrobial activities. Its effect against cancer cells was first reported over 30 years ago, and specifically its effect has been observed in PC cells [80].

NP compound or derivate	Ref.	Source	Approach
Albiflorin and paeoniflorin	[76]	*Paeonia lactiflora* (flower)	*In vitro* and *in vivo*
Proanthocyanidins	[77]	Apples, pears and pulses (fruit)	Epidemiological study
Extract from *Chelidonium majus L.*	[78]	*Chelidonium majus L.* (plant)	*In vitro* and *in vivo*
Sarcaboside B	[79]	*Sarcandra glabra* (plant)	*In vitro*
Usnic acid	[80]	Lichen	*In vitro*
Extract from *Spirulina platensis*	[81]	*Spirulina platensis* (blue-green alga)	*In vitro* and *in vivo*
Aplidine	[82]	*Aplidium albicans* (ascidian, invertebrate marine)	*In vitro* and *in vivo*
Manzamenone O	[83]	*Plakortis sp.* (marine sponge)	*In vitro*
Polysaccharide-K	[84, 85]	*Trametes versicolor* (mushroom)	*In vitro*
Antroquinonol	[86]	*Antrodia camphorate* (mushroom)	*In vitro*
MMH01	[87]	*Antrodia cinnamomea* (fungi)	*In vitro*
Beauvericin	[88]	*Fusarium oxysporum* (fungi)	*In vitro*
Globosumones	[89]	*Chaetomium globosum* (fungi)	*In vitro*
MDN-0090	[90]	*Onychola* sp. (fungi)	*In vitro*

Table 2. Summary of experimental NPs and derivatives for PC treatment from phenotypic-based screening and epidemiological studies.

Marine samples, from marine microorganisms, algae or invertebrates, are increasingly more relevant as sources for cancer chemoprotectives. The lead compound, Ecteinascidin-743 (Yondelis®), is the first marine anticancer agent approved in the European Union for patients with Soft Tissue Sarcoma (STS) and for the treatment of Relapsed Ovarian Cancer. In the case of PC, marine samples still have a long way to go before they can be used in treatment but there is a whole universe of compounds waiting to be discovered. One example is *Spirulina platensis*, which is a blue-green alga used as a dietary supplement because of its hypocholesterolemic properties. Among other bioactive substances, it is also rich in tetrapyrrolic compounds closely related to bilirubin molecule, a potent anti-proliferative agent. The anti-proliferative effects of *S. platensis* were observed against PC cells and were also shown *in vivo* where inhibition of PC growth was evidenced from the third day of treatment in a mice model [81]. Another compound of marine origin, Aplidine (Dehydrodidemnin B), extracted from the invertebrate ascidian *Aplidium albicans*, shows dose-dependent cytotoxic activity against PC cells as well as significant activity against mice bearing human cancer xenografts. Aplidine's mechanisms of action seem to be mediated by the AKT pathway and the reduction in ERK activation [82]. Also, Manzamenone O was isolated from a marine sponge and has been patented against PC [83].

From the fungi kingdom, polysaccharide-K (PSK, krestin) is one of the most commonly used medicinal mushroom extracts, with a long history in cancer therapy in Asia, especially in Japan [84]. Zhang et al. [85] have reported that PSK decreases the invasiveness of a human PC cell line. Also, antroquinonol, a ubiquinone derivative isolated from the mushroom *Antrodia camphorata*, induced a concentration-dependent inhibition of cell proliferation in PC [86].

Among the microbial NPs, very few are described in bibliographies. One of those published is the compound MMH01, which is isolated from the parasitic fungus *Antrodia cinnamomea* on aromatic tree *Cinnamomum kaneirai Hay.* (Lauraceae), which markedly inhibited growth of a PC cell line [87]. Other examples are the compound beauvericin, extracted from the fungal strain *Fusarium oxysporum*, which inhibits migration of the metastatic PC cell [88], and globosumones, from the Sonoran Desert endophytic fungus *Chaetomium globosum*, with inhibitory activity of cell proliferation in PC cells [89]. Also noteworthy is MDN-0090, a compound patented by our research group at Fundacion MEDINA, from a fungus identified as *Onychola* sp. [90], with *in vitro* activity against PC. This compound was obtained through a phenotypic-based screening of more than 90,000 microbial extracts from Medina's NP Library following a cytotoxicity screening in 2D cancer cell culture and a dereplication and identification of the novel compound. Promising results with this novel compound have been obtained from an *in vivo* mice model of disease (unpublished data).

2.2. Future trends in screening in pancreatic cancer

Although many efforts have been made to improve the technology, research models and sources for finding a cure, there is much work left to be done in pancreatic cancer research. Here, we summarize the future trends in this research.

One solution that more closely mimics tumor properties is the use of an alternative 3D model for tissues, termed organoids, which go one step further. 3D organotypic models have potential for bridging the gap between cell-based discovery and complex animal models. Adult stem cells are prepared from human adult tissues and embedded in a three-dimensional matrix, where they self-organize into epithelial structures that resemble the original organ. One example for PC was developed by Huang et al. [91], who differentiated human pluripotent stem cells (PSCs) into exocrine progenitor organoids that formed ductal and acinar structures in culture, expressing the mutations frequently found in patients. These organoids would recapitulate the properties of the original tumor, maintaining the differentiation status, histoarchitecture, phenotypic heterogeneity and retaining patient-specific physiologic changes. The publication of Boj et al. [92] describes the obtaining of PC tissues from patients undergoing surgical resection. The tissue is minced, digested with enzymes, embedded in matrigel and culture with propagation until 20 passages (~6 months) or is cryopreserved, which makes it a very useful model. Related to this is the new creation of living organoid biobanks [93], which consist of a collection of cryopreserved organoids from patients. The ability to create organoids from individual tumors and the enormous clinical diversity of these specimens can be extremely useful for drug discovery. One large collection of these cultures is the Hubrecht Organoid Technology (HUB) "living" biobank [94]. The HUB is part of the Human Cancer Models Initiative in collaboration with The National Cancer Institute, Cancer Research UK, and the Wellcome Trust Sanger Institute. This "living" biobank stores approximately 1000 cancer cell models that best represents the hallmarks and diversity of human breast, colorectal, lung, pancreatic and prostate cancer. The organoids generated have been assayed to analyze drug sensitivity to a vast array of anticancer drugs. This well-characterized library of cultures, with genome sequenced and clinical data from patients, has been created to aid in basic research and explore novel therapeutic strategies and drugs, being accessible to industry and academia [95].

Therefore, tumor organoids can be used in PC models and for drug screening to identify precision therapy strategies, but nowadays only a few laboratories have enough equipment, facilities and access to patient samples to develop them. One step further is the organ-on-a-chip, still under development. This technology is essentially miniaturized microfluidic perfusion systems which allow long-term *in vitro* growth of primary cells and tissues in a format that is viable for scaling up for high-throughput discovery campaigns. These systems model the complex tissue microenvironment and communication, reproducing *in vivo* tissue and organ functionality. One example reported by Maschmeyer et al. is a four-organ-chip system that mimics human liver, skin, intestine and kidney [96]. Furthermore, the use of microfluidic perfusion chambers in these systems permits the homeostatic function of the organ as if it were the blood flow, supplying nutrients and discharging catabolic metabolites [97]. We look forward to seeing how these promising advances develop in the new era of drug discovery in PC research.

3. Conclusion

In this scenario, PC is still an unmet medical need and new therapies need to be discovered. The ancestral use of natural products in medicine continues in force, but with novel approaches. The increase of chemodiversity together with HTS methods and novel assay models in cancer research make the use of NPs a promising source of novel anticancer drugs. Many more useful natural lead compounds await discovery and the challenge is how to access this natural chemical diversity. More and more multidisciplinary teams are needed to access the world's biodiversity, identify novel compounds and evaluate their potential biological activity.

Promising compounds are currently being tested and many more are expected to be found from diverse natural product sources, with the help of the new trends in cancer research.

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Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject-matter or materials discussed in the manuscript apart from those disclosed.

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Neoadjuvant Treatment

Chapter 9

Neoadjuvant Treatment for Nonmetastatic Pancreatic Cancer

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Abstract

Pancreatic adenocarcinoma is one of the most lethal malignancies among solid tumors. Unfortunately, several patients are diagnosed at metastatic stage or with unresectable disease due to vascular compromise involving the pancreas without any chance of curative treatment. There are also two other groups of patients: resectable patients at upfront diagnosis and "borderline resectable" pancreatic cancer patients. This last group represents those patients where surgery is not always possible without a preoperative treatment allowing surgeons to perform an R0 resection. Achieving an R0 resection is the only curative option for pancreatic cancer patients; nevertheless, many R0-resected patients will relapse within 2 years from surgery. Despite adjuvant treatment, reported median overall survival is only 28 months for patients with resectable pancreatic adenocarcinoma; thus, neoadjuvant treatment has been explored in order to improve survival. We aim to describe the controversial reported data and to show the recommendations that are suggested for these patients; however, we need to remark that there is no strong data that support neoadjuvant treatment. Currently, clinical trials are ongoing, and probably soon this approach will become a standard of care among borderline resectable patients and probably in selected resectable patients too.

Keywords: neoadjuvant treatment, pancreas cancer, borderline resectable pancreatic cancer

1. Introduction

Pancreatic cancer is one of the most lethal malignancies among all types of solid tumors. Most of the patients are diagnosed at clinical and radiological late stages when curative

treatments are no feasible to perform. To date, surgical resection is still the only potential curative treatment for the adenocarcinoma of the pancreas; however, only 15–20% of all the newly diagnosed patients will be candidates for curative pancreatectomy as an upfront treatment.

A complete radiological evaluation defines three subtypes of patients: metastatic and/or unresectable patients, resectable patients, and borderline resectable patients. This last group includes patients with vascular tumor compromise that could become resectable after an adequate neoadjuvant treatment.

The prognosis of the pancreatic cancer is poor, even in those patients with resectable disease who underwent oncological surgery and adjuvant treatments if they were recommended, but also for those patients with borderline resectable disease who achieved oncological resection after neoadjuvant treatment that may include chemotherapy, radiotherapy, or a combination of both. Despite an optimal treatment, many of the resected patients will relapse within the 24 months after completing adjuvant treatment or after surgery. The 5-year survival following pancreaticoduodenectomy is only 25–30% for node-negative and 10% for node-positive tumors. The need to improve these results has led us to the development of new treatment strategies that will be discussed ahead in this chapter.

2. Epidemiology of the adenocarcinoma of the pancreas

As mentioned earlier, pancreatic adenocarcinoma is one of the malignancies with worse prognosis among all solid tumors, with a small number of patients who will achieve cure after an optimal treatment, only if they have access to a good quality of cancer therapies based on specialized oncological surgeons who usually perform pancreas cancer surgery. In the United States, pancreatic cancer is the second most common malignant tumor of the gastrointestinal tract and the fourth malignancy related to cancer death in adults [1], with an estimated incidence of 48,960 new cases by 2015 and 40,560 deaths during the same year. Reported incidence and mortality are slightly higher in men than in women [2]. According to the reports of "Surveillance, Epidemiology and End Results Program" (SEER), the incidence of pancreatic adenocarcinoma is greater in males than in females (male-to-female ratio 1.3:1) and in Afro American population than in white population (14.8 per 100,000 in Afro American males compared with 8.8 per 100,000 in the general population) [3]. Worldwide, pancreatic cancer is the eighth leading cause of death related to cancer in men (138,100 deaths per year) and the ninth cause of death related to cancer among female population (per year) [4].

In some developing Latin American countries, for reasons associated with industrialization and with the increasing life expectancy, pancreatic cancer and biliary tract malignancies are becoming more frequent diseases in adult population regardless of the educational and socioeconomical level. As an example, in Chile, one of the most developed countries in South America, with an estimated population of almost 17 million inhabitants, the reported annual

mortality rate for pancreatic adenocarcinoma was 5.8 for 100,000 in men and 5.6 for 100,000 in women by 2012. Curiously, due to problems with the cancer registries in Chile as in other countries of the region, the reported mortality may be higher than the reported incidence for this malignancy during the same period [5]. Despite the lack of better cancer registries, it is well known that the incidence and mortality rates are similar among patients with pancreatic cancer, and both curves get closer in low- and middle-income countries; nevertheless, in developed countries, the chance of surviving a pancreatic cancer is still low and the incidence rate is just a little higher than the mortality rate.

Assuming a correct diagnosis and a complete staging, there are different rates of mortality among pancreatic cancer patients according to the extension and probability of resection of the whole tumor. The 5-year survival for all the patients is 7.2%. The highest survival is found in 27.1% with very localized disease, but this rate dramatically decreases up to 10.7% for regional disease, and for metastatic disease, the 5-year survival is almost anecdotic with less than 2.5% of survival patients in that space of time [3].

3. Molecular biology and genetics

Several attempts looking for driver mutations and for trying to find target therapies to control pancreatic cancer spread have been made. Unfortunately, despite all the efforts, researchers have not conducted positive results in the clinical field, or at least their impact has not been relevant. Driver mutations such as KRAS, CDKN2A, TP53, and SMAD 4 have been involved in pancreatic cancer tumorigenesis [6], but without any impact on patients' selection of treatment yet. In other side, current immunotherapies that have achieved a great impact in the treatment of malignancies such as melanoma, lung cancer, bladder cancer, and others were not able to show benefit when tested in pancreatic cancer patients [7].

It is estimated that only 4–16% of pancreatic adenocarcinoma has a family history of this disease [8], while the rest of the cases may be considered as sporadic. To have a first-degree relative with an apparently sporadic pancreatic cancer has a moderate effect on the risk to develop this disease (odds ratio (OR), 1.76; 95% confidence interval (CI), 1.19–2.61) [9]. Selective mutations that have a recognized role in ovarian and breast cancer such as BRCA2 and, in a lesser degree, BRCA1 have been associated with familial pancreas cancer [10]. As previously mentioned, there are other selected genes that may have been associated with pancreatic cancer, for example, PALB2 [11], CDKN2A [12], and SMAD4 [13]. There are also genetic syndromes linked to pancreas cancer (e.g., hereditary pancreatitis, HNPCC, familial breast cancer with BRCA2 mutations, p16 mutations, Peutz-Jeghers syndrome, ataxia telangiectasia) [14]. Routine genetic testing for patients with newly diagnosed pancreatic cancer is controversial but it could give some clinical benefit by reducing the risk of associated cancers and by identifying family members of the index case who might benefit from screening for the cancer-predisposing mutation. Nevertheless, this is not considered a standard practice by current guidelines [15].

4. Resectability

For patients with tumors that appear resectable during the baseline staging, based on tomography of the abdomen with pancreatic phase, which together represent probably only the 20% of all pancreatic cancers, surgery remains the only potentially curative treatment option [16, 17]. The conventional surgical procedure for pancreatic cancer of the head and or the uncinate process is the pancreaticoduodenectomy. Conventional pancreaticoduodenectomy (i.e., Whipple procedure) involves removal of the pancreatic head, duodenum, the first 15 cm of the jejunum, common bile duct, gallbladder, and a partial gastrectomy [18]. Many times, despite a good quality of the surgery and adequate adjuvant treatments, pancreatic cancer has recurrences that will not be able to be treated with a curative intention. Complete R0 resections have a high incidence of recurrence before 2 years after surgery [17], R1 and R2 resections will have a higher and faster incidence of recurrence and in general should not be considered as patients who underwent a curative surgery. Among patients who underwent an R0 surgery, 75% of them will have a recurrence due to microscopic metastatic disease that was undetectable at diagnosis, or due to resistance of locoregional residual tumor cells to adjuvant treatments that include adjuvant chemotherapy, adjuvant radiotherapy, or chemoradiation. Most of the patients who did not achieve a complete resection will relapse with a recurrence rate very close to 100% [19, 20].

At the time of taking decisions to define resectability of pancreatic tumors, a multidisciplinary approach, including surgeons who have expertise in pancreatic tumor resection, medical oncologists, radio- oncologists, and well-qualified radiologists should be mandatory. With the support of specialized radiologists and the rest of the team as well, surgeons will be able to define if the patients may undergo a surgery as an upfront treatment or if they are definitely unresectable (including locally advanced unresectable disease and metastatic disease).

A third group will be considered as "borderline" resectable patients. "Borderline resectable" definition is variable and somehow imprecise. As a global conception of this definition, we might consider that borderline pancreatic cancer involves those patients who, based on images and on oncological surgery team expertise, are not considered as unresectable but at the same time are not clearly resectable as an upfront treatment but could became resectable after a neoadjuvant treatment.

Some reserve the term "borderline resectable" for cases where there is focal (less than one-half of the circumference) tumor abutment of the visceral arteries or short-segment occlusion of the superior mesenteric vein or portal vein confluence. Others suggest that venous narrowing without occlusion should be included in the definition of borderline resectable disease [21]. Due to that, the aim of surgery in pancreatic cancer is to achieve an RO resection to give the chance of a curative treatment; borderline resectable patients are the best candidates to be treated with neoadjuvant therapies, and most of the time they should not undergo surgery as a first treatment due to a higher risk of not achieving a complete resection resulting in a potential negative impact in survival.

5. Adjuvant therapy in pancreatic adenocarcinoma

Until recently, gemcitabine chemotherapy was the standard of care as adjuvant treatment in complete resected pancreatic cancer patients [22]. The use of radiation therapy or chemoradiation has been controversial, without clear data to support its use among complete resected patients [23, 24]; however, there are groups that considered its use [25] mainly in the group of R1-resected patients and or among node-positive patients. It is important to remark that most of the recurrences will be distant metastasis and only a small percentage of patients will die due to local recurrence or due to local progression after resection; therefore, systemic treatments should always be considered unless a clear justification for local regional treatment has been made.

Since 2017, the standard of care for early stage, resectable pancreatic adenocarcinoma patients is surgery followed by adjuvant chemotherapy combination of gemcitabine plus capecitabine according to ESPAC-4 trial. The median overall survival for these patients in the gemcitabine plus capecitabine group was 28.0 months (95% CI, 23.5–31.5) compared with 25.5 months (22.7–27.9) in the gemcitabine group (hazard ratio (HR) 0.82 (95% CI, 0.68–0.98), $p = 0.032$). Reported results showed a positive impact for the adjuvant therapy in most of the clinical subgroups, including patients with R1 resection margins [26].

S-1 is an oral 5 FU prodrug that has been tested in several malignancies with good results but with a limited efficacy among Asian population. In the phase III JASPAC 01 trial, adjuvant chemotherapy with S-1 showed a 5-year overall survival rate of 43.6 versus 24.2% for gemcitabine (HR 0.60; $P < 0.0001$) and was relatively well tolerated [27]. These data support the use of S-1 as a new standard of care for adjuvant treatment among Japanese population that underwent surgery for pancreatic adenocarcinoma, but it should not be considered in non-Asiatic population due to the lack of existing data.

The use of adjuvant chemotherapy can be delayed or affected by postoperative complications but also by the appearance of early recurrences that can be found before systemic treatment starts or during early image control during adjuvant treatment. Prospective observational trials have shown that up to 38% of resected pancreatic cancer patients did not receive chemotherapy due to those reasons [28, 29]. Considering the bad prognosis of this disease, despite a complete resection when feasible, neoadjuvant treatments have been explored, which focused on improving those outcomes.

6. Locally advanced and unresectable disease

Locally advanced unresectable and metastatic pancreatic cancer patients have a similar dismal prognosis. In case of patients with a good performance status (ECOG 0–1), they should be strongly considered for treatment with high-intensity palliative chemotherapy, with the aim of improving quality of life and overall survival. Conroy et al. showed in the PRODIGE trial that FOLFIRINOX regimen when compared with gemcitabine was associated with a significative

better survival, with a reported median overall survival of 11.1 months versus 6.8 months, respectively (HR for death 0.57 (95% CI, 0.45–0.73), p < 0.001) [30]. This trial was basically conducted among French population and did not include patients of 76 years or older. In patients with ECOG 2 and those with ECOG 0–2 older than 75 years, a lower intensity chemotherapy regimen like gemcitabine with or without nab-paclitaxel should be considered. Von Hoff et al. published in 2013 that the combination of gemcitabine and nab-paclitaxel improves overall survival compared with gemcitabine alone (median OS 8.5 vs. 6.7 months, HR 0.72, CI 0.62–0.83, P < 0.001) [31]. Patients older than 75 years were also included in this study. Chemotherapy should not be recommended in patients with a poor performance status (ECOG 3–4) due to lack of benefit and because a higher risk of toxicity with worsening of quality of life.

7. Neoadjuvant treatment

Regardless of the relative poor prognosis of the disease and considering that an adequate treatment is the only option for surviving a pancreatic cancer, resectable and borderline resectable pancreatic cancer patients should be considered for curative intention treatments [32].

Theoretically, treating patients with neoadjuvant chemotherapy might favor the eradication of microscopic metastatic disease to obtain better results in terms of survival. It may also help in making a "selection" of patients to undergo surgery: if the patient presents disease progression during treatment, an unnecessary surgery could be avoided in patients that otherwise would have had a rapid disease progression after surgery, considering also that oncological surgery for pancreatic cancer is not free of morbidity and mortality [33].

A decision analysis model to assess what was the best treatment strategy for resectable pancreatic cancer supported the use of neoadjuvant chemotherapy showing that it provided longer survival in comparison to surgery followed by adjuvant chemotherapy [34].

Geus et al. [35] reviewed 12,857 non-metastatic pancreatic adenocarcinoma patients who underwent pancreatectomy and initiated adjuvant chemotherapy. Across propensity score-matched analysis, comparing the clinical outcomes of neoadjuvant therapy versus upfront surgery for pancreatic cancer by stage, neoadjuvant therapy was associated with a significant survival benefit after matching (median survival 22.9 vs. 17.3 months; log-rank P < 0.0001) compared with conventional upfront surgery followed by adjuvant therapy, in stage III patients.

Mokdad et al. [36] reviewed the data from a cohort of 15,237 patients (National Cancer Database 2006–2012) with stage I–II adenocarcinoma of the head of the pancreas that were treated with curative intention, comparing neoadjuvant treatment (chemotherapy or chemoradiotherapy combination) with patients who underwent upfront resection with or without adjuvant treatments (chemotherapy or chemoradiotherapy combination) to evaluate the overall survival impact of those modalities. The authors of this manuscript showed that patients who had received neoadjuvant treatment had better results in terms of survival when compared with patients who underwent surgery as an upfront treatment. The median survival

was 26 months for the neoadjuvant group and 21 months for the group who underwent surgery as an upfront treatment, but also a higher pathological tumor stage, a higher incidence of lymph node compromise, and a lesser R0 resection in the group that did not receive neoadjuvant treatment were seen. A two-arms Markov model showed that the median overall survival was longer for the neoadjuvant cohort (22 months) in comparison with the adjuvant group (20 months) [37]. Despite this information that shows better outcomes in terms of survival when neoadjuvant treatments have been done, to date, there are no phase 3 randomized clinical trials that support the use of neoadjuvant treatments in pancreatic cancer patients. Most of the available data are limited to retrospective evidence or to one-arm design-prospective clinical trials [38].

In recent years, the use of systemic preoperative chemotherapy alone or in combination with radiation therapy has been offered to an increasing number of patients with the main intention of reducing the size of the tumor, increasing the likelihood of negative resection margins, and testing the effects of cytotoxic medications in vivo [39].

Phase 2 clinical trials have evaluated the use of neoadjuvant therapy for resectable and borderline resectable pancreatic cancer patients, either with chemotherapy or with chemoradiotherapy combination (**Table 1**).

One quarter of the patients who underwent neoadjuvant chemoradiotherapy had disease progression, and surgery was not performed. Disparities on reported results among patients who underwent surgery showed an R0 resection rate between 12.5 and 96% of the total resected patients. Patients who had progression after treatment did it mainly with distance metastasis (59–73%), most of them located at the liver. Local recurrence rate was seen between 0 and 25% according to different reports. Reported overall survivals show also differences; patients who only received neoadjuvant treatment with chemoradiation had reported survival between 8 and 34 months; patients who underwent only neoadjuvant chemotherapy achieved survivals up to 19 months.

Chemotherapy without radiation has been explored as an option for neoadjuvant treatment in pancreatic cancer. A phase 2 clinical trial in the neoadjuvant setting using gemcitabine with or without cisplatin showed a resection rate of 54% and a median overall survival of 28 months in resected patients [40]. Unfortunately, similar trials using gemcitabine plus cisplatin doublet showed inferior results [41]. Due to the heterogeneity of these studies that included different types of patients such as resectable, borderline resectable, and unresectable patients at diagnosis, but also that have used different modalities of radiotherapy and different schedules and schemes of chemotherapy, no conclusion can be drawn regarding the overall impact on survival and what are the most effective chemotherapy agents or the best combination of chemotherapy agents for resectable pancreatic cancer.

Since 2011, after the results of PRODIGE trial, many case series with neoadjuvant FOLFIRINOX for locally advanced pancreatic cancer have been published, but sample sizes of most studies have been too small to draw definitive conclusions about the efficacy and safety of this treatment approach; however, its use followed by chemoradiation as a multimodality treatment has shown promising results (**Table 2**).

Author / Year	No. Patients	Clinical Stage / Duration Neoad. therapy	Chemotherapy / Radiation	Radiological Response (%)	R0 resection (%)	Median Overall survival (mo)
Jeamy/ 1998	16	Unresectable / NA	Tuboroucil (IC 225 mg/m2) 7 days per week / 45Gy	Partial: 20 Progression: 62	12.5	8 protocol vs 12.2 unprotocol
Hoffman/ 1998	53	Resectables / 2.8 mo	Mitomycin 10 mg/m2 day 2 and thereafter 1 000 mg/m2/d continuous infusion / 50 Gy	Partial: 8 Progression: 16	67	15 surgery with resection vs 8.3 surgery without resection
Talamonti/ 2006	20	Resectables / 3.8 mo	Gemcitabine (1000 mg/m2 weekly) / 36Gy	Partial: 15 Progression: 0	84	26 with surgery vs NA
Pahnec/ 2007	26	Resectables / 4 mo	Gemcitabine (1000 mg/m2 weekly) + Cisplatin (35 mg/m2)	Partial: 0 Progression: 1	75	29.4 with surgery vs 8.6 Dyspnea
Le Scodan/ 2009	41	Resectable / 3 mo	5-FU (300 mg/m2 daily) Cisplatin (20 mg/m2) / RT (50 Gy)	Partial: 10 Progression: 25	81	11.7 with surgery vs 5.7 unresectable
Heinrich/ 2008	28	Resectable / 2mo	Gemcitabine (1000 mg/m2 twice weekly) Cisplatin (50 mg/m2)	Partial: 4 Progression: 13	80	19.1 with surgery
Evans/ 2008	80	Resectable / 3 mo	Gemcitabine (100 mg/m2 weekly) / 50 Gy	NA	82	34 with surgery vs 7 without
VauxLaclaire/ 2008	90	Resectable / 4.8 mo	Gemcitabine (350 mg/m2 weekly) + Cisplatin (30 mg/m2) every 2 wk / 36Gy	NA	86	32 with surgery vs 10.5 without
Landy/ 2010	21	Resectable / 2 mo	Arm A: Gemcitabine (500 mg/m2) weekly / RT (30 Gy) Arm B. Gemcitabine (175 mg/m2) + Cisplatin (70 mg/m2) 5-FU (600 mg/m2) / RT (30 Gy)	Arm A Partial: 10, Progression: 80. Arm B Partial: 18, Progression: 36	NA	26.8 with surgery vs NA
O'Reilly/ 2011	38	Resectable / 2mo	Gemcitabine (1000 mg/m2) Oxaliplatin (80 mg/m2) every 2 wk for 4 cycles	Partial: 10.5 Progression: 7.9	71	27.2 with surgery vs range of 5 to 32 for unresected
Gokhe/ 2015	33	Resectable / 1 mo	Arm B 800 mg/m2 gemcitabine and 30 mg/m2 cisplatin on days 1, 8, 22, and 29 of radiotherapy / 50Gy	NA	Arm B. 48	25 Arm B vs 18.9 Arm A (surgery first)
Van Buren/ 2013	99	Resectable / 3 mo	Gemcitabine (1000 mg/m2) over 2 wk + Bevacizumab (10 mg/kg) – RT (30 Gy)	Partial: 8.4 Progression: 7.9	88	19.7 with surgery vs NA

Neoad: Neoadyuvant, IC: Infusea continuous, NA: Not Available, Mo: Month

Table 1. Phase II trials of patients treated with neoadjuvant therapies.

In general, according to data mainly obtained from retrospective studies with a small number of patients with disease in borderline and locally advanced stages, between 13 and 68% of patients could undergo surgery after neoadjuvant treatment, achieving R0 resection in a range of 24–100%, with a median survival that usually exceeds 20 months.

Published results of a meta-analysis that included 13 different publications, with different methodologies including 325 patients with locally advanced pancreatic cancer treated with FOLFORINOX regimen, with some of the patients that after this treatment underwent radiotherapy or chemoradiation, showed that 28% of the patients (91 of 325 included in this analysis) underwent surgery with a pooled proportion of patients who achieved R0 surgery of 78% [42]. In this same meta-analysis, which included a total of 689 patients with different stages, as mentioned before, all received FOLFIRINOX; some of them underwent other therapies such as radiation or radio-chemotherapy, and not all the patients treated with FOLFIRINOX as neoadjuvant treatment could undergo surgery, the reported median overall survival across all the studies was 10–32.7 months and the reported progression-free survival ranged from 3 to 20.4 months.

In a small multicenter prospective single-arm trial that included 22 borderline resectable pancreatic cancer patients, Katz et al. assessed the use of four cycles of neoadjuvant-modified

Author/year/type	No. patients with Noady FOLFIRINOX/ total	Clinical stage	Radiation	Resection	R0 resection (%)	Median overall survival range (mo)
Boone/2013/ Retrospective	25/ 25	BR: 12 (48%), LA: 13 (52%)	SBRT 36 Gy	11 (44%)	7 (63%)	NA
Faris/2013/ Retrospective	22/ 22	LA: 22 (100%)	IMRT 50.4 Gy	12 (54.5%)	5 (42)	24.7 (19.0–30.3)
Ferrone/2015/ Retrospective	40/127	BR:15 (12%), LA 25(20%)	CHRT: 50.4 Gy and 5-FU*	40 (31.4%)	35(92)	34
Hosein/2012/ Retrospective	18/18	BR 4 (22%), LA 14 (78%)	CHRT: 50.4 Gy and GEM**	10 (55.5%)	8 (80%)	32.7 (23.1-42.3)
Marthey/2015/Cohort	77/77	LA: 77 (100%)	54 Gy***	28 (36.3%)	25 (89)	22 (12.3-29.9)
Mellon/2015/ Retrospective	21/159	BR 110 {69%), A: 49 (31%)	SBRT 30-40 Gy#	21 (13.2%)	5 (24)	15
Sadot/2015/ Retrospective	101/101	BR: 31 (30.6%), LA: 70 (69.3%)	CHRT··	31 (30.6%)	16 (52)	26 (18-33)
Katz/2016/ Prospective single-arm	22/22^	BR 22 (100%)	CHRT 50.4 Gy^	15 (68%)	15 (100)	21.7

Noady: neoadjuvant, BR: borderline resectable, LA: locally advanced, SBRT: stereotactic body radiosurgery, IMRT: intensity-modulated radiation therapy, HIGRT: hipofractionated radiation therapy, NC: not correspond, CHRT: chemoradiation, 5-FU: fluorouracil, GEM: gemcitabine.*After FOLFIRINOX and before surgical exploration.

**For unresectable patients post FOLFIRINOX, radiation sensitization patients received concurrent gemcitabine plus IMRT.

***External radiotherapy for consolidation.

#After neoadjuvant chemotherapy.

··Patients who appeared to convert to resectable disease underwent surgical exploration, and patients with stable disease were typically initiated with chemoradiotherapy with 5-FU or GEM.

^Modified FOLFIRINOX treatment: (85 mg/m^2 of oxaliplatin, 180 mg/m^2 of irinotecan hydrochloride, 400 mg/m^2 of leucovorin calcium, and then 2400 mg/m^2 of 5-fluorouracil for 4 cycles) followed by 5.5 weeks of external-beam radiation (50.4 Gy delivered in 28 daily fractions) with capecitabine (825 mg/m^2 orally twice daily) prior to pancreatectomy. 10 patient initiated adjuvant therapy with GEM.

Table 2. FOLFIRINOX studies only with BR and LA pancreatic cancer.

FOLFIRINOX followed by 5.5 weeks of radiation therapy with a total dose of 50.4 Gy in 28 fractions with concurrent capecitabine twice daily during radiation [43]. Grade 3 or higher toxicity was reported in 64% of patients. Fifteen patients underwent pancreatectomy, 80% of them required vascular resection, and R0 resection was achieved in 93% of the resected patients. The reached median overall survival was 21.7 months. Using another regimen of neoadjuvant chemotherapy, a single patient case report showed efficacy achieving R0 resection in this patient who had unresectable locally advanced diseased and was treated with gemcitabine plus nab paclitaxel combination followed by FOLFIRINOX before surgery [44].

To address the question if neoadjuvant treatments or adjuvant treatments will result with better outcomes, different prospective trials are currently ongoing, and their aim is to find out the real impact of the neoadjuvant treatments in resectable and borderline resectable pancreatic cancer patients. Those trials include neoadjuvant chemotherapy, neoadjuvant versus

adjuvant chemotherapy, neoadjuvant chemoradiation, neoadjuvant chemoradiation versus upfront surgery, and other modalities as well [45–49].

Concerning toxicity among patients treated with neoadjuvant treatments that have been reported, mostly as local experiences or as small institutional trials, there are no clear data concerning side effects of neoadjuvant therapies, nor there are data on perioperative morbidity and mortality, comparing patients who underwent upfront surgery and patients who received neoadjuvant treatment and then underwent surgery. The biggest data on quality of life come from reports in the metastatic setting. The quality of life report of the PRODIGE-4 trial (mentioned earlier), FOLFIRINOX chemotherapy reduced the quality of life impairment compared with gemcitabine, but also it has benefit in the quality of life that can be a surrogate for survival, as physical functioning and some symptoms severity were prognostic factors for survival [50]. In a meta-analysis of FOLFIRINOX in locally advanced pancreatic cancer, 60% of the patients presented G3 or higher side effects, neutropenia and diarrhea being the most frequent events among treated patients. There were no related deaths attributable to FOLFIRINOX [42]. In a retrospective analysis of patients undergoing FOLFIRINOX as neoadjuvant treatment followed by surgery, Marchegiani et al. concluded that among patients who underwent neoadjuvant chemotherapy, there were less postoperative pancreatic fistula and less postoperative pancreatic hemorrhage but delayed in gastric emptying [51].

8. Current guidelines

Due to a lack of strong data based on phase 3 clinical trials, it is not possible to talk about a gold-standard treatment in the neoadjuvant setting for pancreatic cancer patients. Most of the groups support the idea to perform surgery as an upfront treatment in resectable patients followed by adjuvant chemotherapy.

Current ESMO guidelines support the use of FOLFIRINOX followed by chemoradiotherapy in borderline resectable patients as a main option in pancreas cancer [52]. Contrarily, ASCO guidelines indicate that there is no clear evidence to support one regimen over another, and physicians may offer therapy based on extrapolation from data derived from studies in the metastatic setting [53].

Pancreatic cancer patients with resectable or borderline resectable disease should always be discussed in a multidisciplinary team. Neoadjuvant treatment should always be considered to attempt an R0 resection; otherwise, the chance of cure in non-R0-resected patients and also due to the meaning of the diagnosis itself will be similar to metastatic patients. Multidisciplinary team should at least include a digestive oncological surgeon with expertise in pancreatic surgery, a medical oncologist, a radiologist with expertise in pancreas, a radiation oncologist, and a pathologist, given the disparity of opinions and the importance of treatment agreement looking forward the best chance to those patients.

At SLAGO 2015 (Latin American Gastro-Enterology Cancer Symposium) congress [54], a meeting held every 2 years in Latin America that focuses on digestive malignancies, specialists from different Latin American countries met to discuss about pancreatic cancer. Concerning

borderline resectable pancreatic cancer patients, SLAGO's main recommendation is to consider FOLFIRINOX schedule as the best choice for neoadjuvant treatment; then after selected patients that do not have disease progression after chemotherapy could be considered for radiotherapy with capecitabine as radio-sensibilizer before surgery. For patients who have contraindication to receive FOLFIRINOX and in older than 76 years, neoadjuvant treatment with gemcitabine plus nab paclitaxel combination can be an option [55].

9. Conclusions

Pancreatic cancer is one of the most lethal malignancies among all types of solid tumors. Most of the patients are diagnosed at unresectable or at advanced stages with no chances of cure. Early diagnosis is critical to give the patient the chance of cure; however, most of the patients are diagnosed where the tumor is not amenable to be resected. Even more, many of the patients who will undergo an R0 resection will relapse before 2 years after surgery.

We would like to remark that there is no strong evidence to make final conclusions in order to define the best upfront treatment in non-metastatic resectable and borderline resectable pancreatic cancer patients. For resectable patients at diagnosis, upfront surgery is still the standard of care followed by adjuvant chemotherapy. In this subgroup of patients, radiotherapy and chemoradiotherapy do not seem to be the best choice. On the other hand, neoadjuvant chemotherapy has not been explored yet in well-designed clinical trials, and its use has been just limited to small experiences. Borderline resectable pancreatic cancer patients are a subgroup where upfront surgery has a low chance of achieving an R0 resection; therefore, these patients must be considered to receive neoadjuvant treatments in order to improve complete tumor resection and as a consequence to improve survival. As in the resectable subset of patients, radiotherapy or chemoradiotherapy has not shown a real impact in this group. FOLFIRINOX followed or not by chemoradiotherapy seems to be the best option to improve resectability, for achieving complete resection and pathological downstaging and for improving overall survival in resected patients. Final reports from clinical trials will set the key whether or not neoadjuvant treatment, in resectable and borderline resectable pancreatic cancer patients, should be mandatory or recommended.

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Chapter 10

The Role of Neoadjuvant Therapy in Surgical Treatment of Pancreatic Cancer

Dealing with Borderline Resectable Pancreatic Cancer, What Comes First?

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Additional information is available at the end of the chapter

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Abstract

Pancreatic cancer is a leading cause of cancer-related death worldwide, and its burden is destined to increase. Multimodal treatment is crucial to achieve a cure, but standardization is far to come. Borderline resectable disease is the most challenging situation to face. An anatomically resectable disease may hide a biologically aggressive or undiagnosed systemic disease. Whether the patient has to undergo surgery first or after locoregional or systemic therapy is still unknown. Decision-making stands on low-quality evidences since RCTs are lacking. Neoadjuvant treatment may downstage the tumor and treat an early systemic disease, selecting patients for surgery in order to achieve a margin-free resection and avoid early recurrences and useless pancreatectomies. Resectable patients without other worrisome features may benefit from a surgery-first approach, while all other nonmetastatic patients should be enrolled in trials to rule out the outcomes of neoadjuvant treatments.

Keywords: pancreatic cancer, neoadjuvant treatment, pancreatic surgery, borderline resectable, locally advanced

1. Introduction

By 2030 pancreatic cancer (PaC) is expected to be the second cancer-related cause of death [1]. Its 5-year survival in nonmetastatic stages currently ranges between 3 and 14% [2] regardless of treatment. Surgery remains the only chance for cure since the 5-year survival in T1 N0 resected patients reaches 55.2% [3]; therefore, the standard of care advocates a surgery-first approach in case of resectable disease followed by adjuvant treatment (ADT), but neoadjuvant approaches are spreading either in resectable or borderline resectable (BLR) and locally advanced (LA) patients. The National Comprehensive Cancer Network (NCCN) states that there is limited evidence to recommend specific neoadjuvant regimens off-study [4]. While the only choice in LA PaC is a locoregional chemoradiation (CRT) or systemic chemotherapy (CHT) and subsequent revaluation, for resectable and BLR, we must choose between a surgery-first approach and a neoadjuvant treatment (NADT). Over 40% of patients who have clinically a resectable disease are found unresectable at surgery, even though this percentage drops to 20% if a diagnostic laparoscopy is added to the preoperative diagnostic panel [5]; one out of five patients are eventually misdiagnosed as resectable or BLR while having a LA disease. Even in a high-volume referral hospital, the percentage of successfully resected patients at surgical exploration is as low as 51% [6]. Results of first-line pancreatectomy may be very poor with only 20% of patients receiving radical surgery and 80% presenting tumor within 1 mm from margin or direct microscopic margin infiltration [7]. In a Korean series, 9.1% of patients presenting with PaC diagnosis were clinically staged as BLR [8], about 27% of whom required a vascular resection (VR) in order to achieve their pancreatectomy [9], but histological invasion of resected vessels is confirmed only in 56.7% of specimens [10]. Finally, up to 28% of successfully resected patients will not undergo ADT because of surgical morbidity, poor performance status, refusal, or early recurrence [10]. As Buchler said, unfortunately available evidences supporting NADT come from retrospective studies in which treatment protocols vary greatly and patient cohorts are often mixed with resectable, BLR, and LA [11].

Whereas features of a metastatic disease are evident, dealing with PaC and NADT, a foreword has to be spent to clarify the terminology "resectable," "borderline resectable," and "locally advanced." To that end, we will first focus on the definition of borderline resectable disease and then analyze the outcomes of NADT from a surgical point of view.

2. Definition of borderline resectable pancreatic cancer

In origin the term "marginally resectable" pancreatic cancer was used for tumors without a 180° free fat plane around SMA, SMV, or PV for at least 1 cm [12]; this outlined a tumor with a high probability of positive-margin surgery. In the following years, several revisions took place, and the term "borderline resectable" was adopted, but still there is no universal consensus on its definition.

2.1. Anatomic criteria

The pancreatic glands lay in the deepest abdomen in direct contact with several major vascular structures. It is encased between the mesenteric root and the two main splanchnic arteries.

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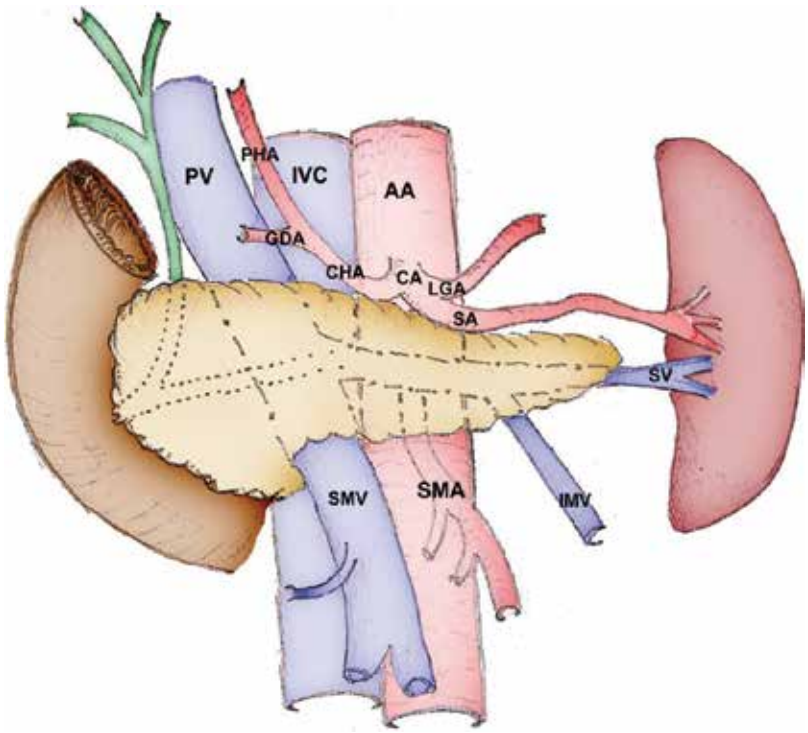

Figure 1. Schematic representation of pancreatic vascular relationships. AA, Aorta; IVC, inferior vena cava; PHA, proper hepatic artery; PV, portal vein; SMV, superior mesenteric vein; SMA, superior mesenteric artery; IMV, inferior mesenteric vein; SV, splenic vein; SA, splenic artery; LGA, left gastric artery; CA, celiac axis; CHA, common hepatic artery; GDA, gastro-duodenal artery.

With the PV/SMV-SMA plane being its bed and the celiac trunk being its roof, resectability and thus possibility of cure are played in few millimeters. As shown in **Figure 1**, PaC may arise in the head, body, or tail of the pancreas; therefore, respectability definition differs along with its location whether on the right of the left border of PV/SMV (head) or on the left of the left aortic border (tail) or in between (body). In surgery few things are technically impossible; this is heavily surgeon-dependent because it relies in its skills and will. That is why several institutions/associations have tried and classified PaC resectability depending on its involvement of nearby structures. In **Table 1** the anatomic criteria for definitions of borderline resectable disease from the classifications of five major institutions are shown: MD Anderson Cancer Center [9], American Hepato-Biliary-Pancreatic Association/Society of Surgery of the Alimentary Tract/Society of Surgical Oncology (AHBPA/SSAT/SSO) [13], Alliance A021101 [14], IAP [3], and NCCN [4]. Any situation with a more extensive vascular involvement will obviously be classified under the "locally advanced/unresectable" definition, whereas a less extensive one will define a resectable disease. Despite the effort to standardize definitions and make patients and features comparable among radiologist and surgeons, in some classifications, terms like "allowing for safe reconstruction" still appear increasing confusion among professionals and trials. It is interesting how some may consider a unique SMV/PV <180° involvement that implies a venous resection, as a resectable disease, whereas an arterial involvement is always considered at least borderline resectable; this is due to the surgical

International consensus IAP [3]	BR-PV (SMV/PV involvement alone): • SMV/PV: tumor contact 180° or greater or bilateral narrowing/occlusion, not exceeding the inferior border of the duodenum. • SMA, CA, CHA: no tumor contact/invasion. BR-A (arterial involvement): • SMA, CA: tumor contact of less than 180° without showing deformity/stenosis; • CHA: tumor contact without showing tumor contact of the PHA and/or CA.
NCCN Guidelines [4]	VENOUS • Solid tumor contact with the SMV or PV of >180°, contact of ≤180° with contour irregularity of the vein or thrombosis of the vein but allowing for resection and reconstruction • Solid tumor contact with the inferior vena cava (IVC). ARTERIAL If head/uncinate process: • Solid tumor contact with CHA without extension to CA or hepatic artery bifurcation allowing for resection and reconstruction. • Solid tumor contact with the SMA of ≤180° • Solid tumor contact with variant arterial anatomy If body/tail: • Solid tumor contact with the CA of ≤180° • Solid tumor contact with the CA of >180° without involvement of the AA and with intact and uninvolved GDA thereby permitting a modified Appleby procedure.
Intergroup criteria Alliance A021101 [14]	• a TVI with SMV or PV ≥180° of the circumference of either vein's wall or short-segment occlusion of either vein amenable to reconstruction; • any TVI with CHA amenable to reconstruction; • a TVI with SMA <180° of the circumference of the vessel wall.
AHBPA/SSO/SSAT consensus statement [13]	• Venous involvement of the SMV/PV demonstrating tumor abutment with or without impingement and narrowing of the lumen, encasement of the SMV/PV but without encasement of the nearby arteries, or short segment venous occlusion resulting from either tumor thrombus or encasement but allowing for resection and reconstruction. • GDA encasement up to the CHA with either short segment encasement or direct abutment of the CHA, without extension to the CA. • Tumor abutment of the SMA not to exceed >180° of the circumference of the vessel wall.
MD Anderson Cancer Center [9]	• Tumor abutment (<180° of the circumference of the vessel) of the SMA or CA; • Tumor abutment or encasement (>180° of the circumference of the vessel) of a short segment of the CHA; • Short-segment occlusion of the SMV, PV, or SMV-PV confluence amenable to vascular resection and reconstruction.

BR, borderline resectable; PV, portal vein; SMV, superior mesenteric vein; SMA, superior mesenteric artery; CA, celiac artery; CHA, common hepatic artery; PHA, proper hepatic artery; AA, aorta; GDA, gastroduodenal artery; IVC, inferior vena cava; TVI, tumor-vessel interface.

Table 1. Anatomic criteria for borderline resectable disease.

implications of venous and arterial resections, as the former does not increase either postoperative morbidity or mortality [15].

2.2. Biologic criteria

Besides anatomical features, PaC may have an intrinsic undiagnosed risk of early recurrent or micrometastatic disease linked to its biology; indeed, early recurrence occurs in 25% of resected patients [16], thus making surgery ineffective. Herein, some examples of biomarkers are predicting more aggressive disease. Preoperative CA19.9 > 300 U/ml and tumor size >3 cm [16], preoperative CEA >3 ng/ml and CA19.9 > 75 U/ml, N+ disease, and T3–T4 [17] are independent negative prognostic factors. Moreover, patients presenting with local disease have a 22% of SMAD-4 loss versus 78% of patients with metastatic disease [18]. In an era of tailored treatments, it should be reminded that every patient and every disease have their own characteristics that have to be taken into account in the therapeutic decision-making. Lately, circulating tumor cells have been investigated as prognostic biomarkers, three or more CTCs/4 ml were an independent prognostic factor for the overall survival, and it accurately predicted occult metastatic disease [19]. That is why during the 20th meeting of the International Association of Pancreatology held in Sendai in 2016 a consensus of borderline resectable definition has been drawn up that includes biological and conditional criteria. Biological criteria are a CA19.9 above 500 IU/ml and regional lymph node metastasis proven by biopsy or PET-CT. Conditional host-related criteria depends on the patient's performance status defined by a PS score of 2 or more [3]. Thus, patients satisfying at least either an anatomic, biologic, or conditional criteria are classified as borderline resectable.

3. Neoadjuvant treatment

3.1. Indications

A preoperative treatment has several theoretical advantages. First of all it delivers systemic therapy to all patients: at least 30% of patients do not receive adjuvant therapy after resection for a variety of reasons [20]. Then, it is shed in a highly perfused tumor bed that allows an in vivo testing of the tumor sensibility to the chemotherapeutic agent. Moreover, NADT should increase the probability of negative margin surgery (R0) and decrease the likelihood of nodal involvement and vascular resection (VR). Finally, it identifies tumors with an aggressive biology and picks out patients who would not benefit from surgery because of early progression, recurrence, or previously undiagnosed metastatic disease. Whether those presumed advantages translate into real world is still under investigation. The role of NADT for PaC, especially for primary resectable ones, still remains controversial among other reasons because a quote of those patients undergoing neoadjuvant treatment will experience severe side effects and complications [21]. A comprehensive meta-analysis provides marginal support to the assumed benefit of contemplating neoadjuvant therapies for patients whose tumor was judged resectable at preoperative staging [22]. Neoadjuvant treatment should always be offered to BLR and LA diseases; nevertheless, since preoperative staging in PaC is far from being accurate, with 22.5% of patients brought to the operating room with curative intent found to be metastatic [6], it is crucial to treat every patient within registered trials.

3.2. Outcomes

3.2.1. Toxicity

Unfortunately, every neoadjuvant regimen brings its own risk of toxicity, and a successful resection is more likely in case of completion of NADT [23]. A serious side effect might indefinitely postpone surgery; that is why resectable patients are exposed to a shift from being a surgical patient to never being proposed for cure. This has to be taken into account while proposing such treatment in selected patients. As shown in **Table 2**, it is reported that 29.4–36% of patients will experience grade 3 or 4 adverse effects during preoperative treatment [22, 24, 25], with 6% of patients giving up treatment because of its toxicity [26]. But up to 91% of patients initiated to NADT achieve the intended preoperative protocol [24].

3.2.2. Pathologic response

As seen in **Table 3**, up to 11.3% of BLR or LA PaC presented a complete pathologic response (ypT0) after NADT [27]. In this paper 83% of patients with ypT0 were dead or relapsed at a

Author, year	Article type	Grade 3/4 toxicity
Dhir, 2017 [24]	Metanalysis	36%
Marthey, 2015 [26]	Cohort study	26%
Andriulli, 2012 [22]	Metanalysis	31%
Gillen, 2010* [25]	Metanalysis	29,4%
Kapoor, 2014 [37]	Prospective	0%

*PaC AND periampullary tumors.

Table 2. Major toxicity.

Author, year	Article type	Complete pathological response	Partial pathological response	Stable disease	Progression
Dhir, 2017 [24]	Metanalysis	n.a.	20%	59%	16%
Hashemi-Sadraei, 2017 [27]	Retrospective	11.3% (of resected patients)	n.a.	n.a.	n.a.
Marthey, 2015 [26]	Cohort study	5.2%	28%	56%	16%
Addeo, 2015 [40]	Retrospective	8.8%	n.a.	n.a.	n.a.
Andriulli, 2012 [22]	Metanalysis	n.a.	22%*	50%	25%
Gillen, 2010† [25]	Metanalysis	3.9%	29.1%	43.9%	20.8%
Heinrich, 2008 [38]	Phase II trial	0%	n.a.	n.a.	n.a.
Kim, 2017 [8]	Retrospective	0%	65%	35%	0%

*Including complete path. resp.
†Pancreatic cancers AND periampullary tumors.

Table 3. Pathological response.

median follow-up of 21.3 months [27], suggesting a systemic undiagnosed or uncontrolled disease. A 2010 meta-analysis shows 3.9% of complete pathologic response, 29.1% partial response, 43.9% stable disease, and 20.8% of progression during NADT [25]. A more recent 2017 meta-analysis confirms those data with partial response or stable disease in 79% of treated patients (20 and 59%, respectively) while in progression in 16% of cases [24]. According to Gillen and coll. Pooled percentages of pathologic response did not vary much in the two groups of initially deemed resectable and non-resectable tumor patients [25]; this may be due to the fact that resectability is defined only by anatomical features, while probably a biological understanding of the disease would enhance clinical staging. Anyhow unfortunately, there are 16–32% of patients that will have to stop treatment because of progression [22, 26].

4. Surgery

Surgery is ideally recommended 4 to 8 weeks after neoadjuvant treatment [4] although it has been postulated that patients with a longer (>10 weeks) interval between RT and CHT and surgery could be more likely to have an improved pathological response, R0 resection, and OS [28]. According to a consensus statement drafted in 1999 [29], "standard" pancreatoduodenectomy includes regional lymphadenectomy around the duodenum and pancreas; "radical" pancreatoduodenectomy includes regional lymphadenectomy plus skeletonization of the proper hepatic artery (PHA), common hepatic artery (CHA), superior mesenteric artery (SMA) between the aorta (AA) and inferior pancreaticoduodenal artery, and the CA; dissection of the anterolateral aspect of the aorta and inferior vena cava (IVC) includes Gerota's fascia; and lastly "extended radical" pancreatoduodenectomy includes "radical" pancreatoduodenectomy and clearance of the anterior AA between the diaphragmatic hiatus (around the CA) and the origin of the common iliac arteries. Currently, extended lymphadenectomy is no more recommended as it increases costs [30], blood loss, and operative time without adding survival or staging advantages [31]. For what concerns vascular involvement, venous resection doesn't affect preoperative mortality even if it may slightly increase morbidity; instead, arterial resection is still under major debate since it seems to have acceptable outcomes only in single high-volume centers' reports [32]. In a French experience, patients that received a venous resection but whose tumor did not infiltrate the vessel at final histology, lived longer either than patients whose tumor eventually infiltrate the vein either than patients who did not require a vascular resection (42 months vs. 24 vs 22 respectively $p = .04$) [33]. This may even justify extreme positions such as calling upon routine VR during pancreatectomies. According to the latest staging system of the American Joint Committee on Cancer (8th edition), venous infiltration doesn't modify T stage: indeed T1 to 3 stages relies on tumor's dimension and T4 is defined only in case of arterial involvement [34]; therefore, venous resection should not hold back surgeons from performing a pancreatectomy with curative intent.

4.1. Resectability

In a 2010 meta-analysis, surgical exploration after NADT was attempted in 69.5% of patients, but only 50.7% of NADT patients were eventually successfully resected (that is 77.9% of explored patients) [25]. In a more recent meta-analysis, the rate of resected patients raised to

Author, year	Article type	ITT population	Explored/ITT	Resected/ITT	Vascular resection/ resected
Epelboym, 2014 [42]	Retrospective	Mixed	ITT=explored	82.2%	64.3%
Gillen, 2010* [25]	Metanalysis	Mixed	69.5%	50.7%	n.a.
Sherestha, 2017 [36]	Retrospective	BLR	54.9%	44%	n.a.
Kim, 2017 [8]	Retrospective	BLR	ITT = explored	85%	26.5%
D'Angelo, 2017 [35]	Metanalysis	Mixed	n.a.	65%	n.a.
Addeo, 2015 [40]	Retrospective	Mixed	ITT=explored	77.5%	97.7%
Marthey, 2015 [26]	Cohort study	LA	n.a.	66%	n.a.
Kapoor, 2014 [37]	Prospective	LA	n.a.	26.7%	n.a.
Andriulli, 2012 [22]	Metanalysis	Mixed	66%	74% (of explored)	n.a.
Heinrich, 2008 [38]	Phase II trial	Resectable	93%	89.28%	12.5%

ITT, intention to treat; PaC, pancreatic cancer; BLR, borderline resectable; LA, locally advanced.
*PaC AND periampullary tumors.

Table 4. Resectability.

65%, but reported percentages vary from 26.7% to 89.28% depending on variability of protocols and patients [35]. In fact resection was more likely in resectable patients (73.6%) than in non-resectable ones (33.2%) [25]. In John Hopkins Hospital's experience, recently published, resection in BLR patients after neoadjuvant treatment was achieved in 44% of cases [36], while it was possible only in 26.7% of LA patients in an Indian report [37] versus 89.28% of pancreatectomies in resectable patients of a Swiss trial [38]. Those are single experiences that cannot reflect general reality, and the few existing neoadjuvant RCTs report a protocol achievement range of 18.18–70% [39]. Anyway, after neoadjuvant treatment between 26.5% [8] and 97.7% [40] of patients successfully receiving a pancreatectomy will require a VR. **Table 4** reports resection's outcomes of selected experiences and meta-analysis.

4.2. Morbidity and mortality

According to the recently reported experience of an Italian group with more than 150 pancreatectomies per year, NADT exposes patients to a reduced incidence of postoperative fistula and hemorrhage; unfortunately, in spite of this, the average clinical burden is increased [41]. Back in 2010 a morbidity of 34.2% with a mortality of 5.3% in eventually resected patients was reported as a meta-analytical data after NADT [25]. Some claimed perioperative mortality to be much higher (6.7–7%) after NADT with FOLFIRINOX [26, 40, 42] compared to upfront

resected patients regardless of VR, while others reported mortality in PV/SMV resection to be as low as 3% [33]. In literature a great amount of data make it muddler to understand the picture of the actual situation.

4.3. Resection margins

The goal of multimodal treatment is to achieve a margin-free surgery, taking into account that additional resection to achieve negative neck margin after R1 frozen section is not associated to improve survival [43]. In pancreatectomies' specimens the most frequently involved margin is the retroperitoneal one (39%) [6]; that is why VR assumes a central role in academic discussions. In fact among patients requiring VR NADT reduced significantly R1 rate (from 34.9 to 19.6%) [40]. After NADT, intention-to-treat (ITT) R0 rates have been reported to be 23–63% depending on their preoperative assessed resectability [24]. In resected patients R0 rate was estimated by a meta-analysis to be as high as 94%; that is to say that this data comes from nonrandomized trials [35]. Indeed, clear resection margins were present in 40% and 75% of cases in Landry [44] and Palmer's [45] RCTs. Lastly, pathologists have to be aware that after a preoperative treatment what seems to be a tumor-free margin could be only the expression of a reduction of density of tumor cells [46].

5. Role of adjuvant treatment

In several trials a significant benefit of ADT after pancreatectomy has been demonstrated [39], but whether additional adjuvant treatment is necessary in preoperatively treated patients is not clear as it may not provide additional survival benefit [40, 47]. In a Korean series 5.9% of patients undergone NADT and pancreatectomy recurred before having the chance to begin ADT [8]. In a Japanese experience, NADT was found to be a negative factor in predicting failure to achieve ADT therapy along with preoperative prognostic nutritional index, intraoperative blood transfusion, organ/space surgical site infections, and advanced UICC stage; however, this association was not confirmed at multivariate analysis, and only poor prognostic nutritional index, intraoperative blood transfusions, and organ/space surgical site infections were confirmed to be significantly associated with ADT dropout [48]. What is the real weight of NADT in precluding the administration of ADT? An American group reported the administration of ADT to 90% of resected patients after a long-term NADT regimen [23]; thus, all that matters is probably only a correct patient selection.

6. Survival

Survival goes hand in hand with successful surgical resection with a wide clear (R0) margin (>1 mm) giving the chance for an OS of 35 months, while R0 < 1 mm of 16 months involved margin (R1) resections of 14 months and unresected patients only 11 months ($p < .001$) [6]. Even in case of complete pathologic response (ypT0) after NADT and pancreatectomy cure is not guaranteed; indeed, in a series of ypT0 patients, 83.3% were dead or relapsed after a median of 21.3 months [27]. In NADT patients, resection hangs the scales in survival: in a meta-analysis OS in eventually resected patients was 22.78 months versus 9.89 in non-resected patients with

Author, year	Type of article	ITT-OS (months)
Andriulli, 2012 [22]	Metanalysis	16.4
D'Angelo, 2017 [35]	Metanalysis	16.7
Sherestha, 2017 [36]	Retrospective	15.1

ITT-OS, intention-to-treat overall survival.

Table 5. Survival.

an ITT OS of 16.7 months (please see **Table 5**) [36]. Such results are in line with a high-volume center such as Johns Hopkins Hospital, in which after NADT median overall survival (mOS) of resected patients was 25.8 months versus 11.9 months in eventually non-resected patients [36]. Results in the setting of RCTs aren't equally encouraging with ITT OS ranging 9.9–19.4 months in NADT setting versus 12.5–29.8 months in ADT one [39], but it shouldn't be forgotten that in the former we are dealing with resectable patients, while in the latter with resected ones; therefore, we could make a comparison only including in the latter group also patients who undergone explorative laparotomies. In a retrospective series, thanks to less lymphovascular invasion, less perineural invasion, and lower T and N stages, NADT-treated and NADT-resected patients presented a better median overall survival than primarily resected ones (27.3 months vs. 19.7 months, $p < .05$) [42]. In their experience, concerning vascular resections, there was no difference among NADT patients between VR+ and VR- in terms of OS [42].

7. Discussion

Every surgery resident is raised with two warnings:

> *"Eat when you can, sleep when you can, and don't mess with the pancreas."*

and

> *"God put the pancreas in the retroperitoneum so the surgeon won't mess with it."*

Perioperative mortality in pancreatectomies has been as high as 15% in the 1950s–1970s and since then has dropped to 1.5% in selected centers [49]. Nevertheless, pancreatic surgery for PaC has still high in-hospital mortality rates, as highlighted by an analysis of the German national database; it ranges from 12.2% in very-low-volume hospitals (with a median of four resections per year) to 7.1% in high-volume ones (with a median of 105 resections per year) [50]. Surgery is the only chance for cure of patients affected by PaC—and besides it decreases costs compared to palliative treatments [51]—but multimodal treatment is crucial for long-term survival [52]. Therefore, patients' selection has to be accurate since in one hand patients sent to NADT may miss the window for resection and in the other surgical complications may indefinitely postpone systemic treatment. Currently, there are no reliable clinical predictors of resectability [36]: in order not to lose the chance for resection, all patients receiving NADT should be surgically explored unless evident metastatic disease as fibrosis and inflammation can mimic a LA unresectable disease. As Buanes said, "one of the major problems worldwide is the underutilization of surgery in resectable pancreatic cancer" [53], and, especially after NADT, clinical staging

is unreliable. Indeed, there may be relevant tumor regression during NADT around involved vessels despite the absence of radiographic signs of tumor downstaging [54]. Not even PET-CT has shown to be reliable in differentiating benign from malignant disease after NADT [55].

In Miura' study, while in the ITT analysis, clinically BLR disease was an independent poor prognostic indicator, among resected patients OS did not differ between preoperatively classified resectable and BLR patients [56]. Similarly, OS of previously resected patients (20.87 months) was not better than the overall resected ones (22.78 months) in a recent meta-analysis [35]; this reflects the inadequacy of current preoperative staging and confirms that once resected, preoperative staging doesn't influence patients' outcomes. Once more, our efforts have to be straight at bringing patients to a curative surgery.

Supporters claim NADT to increase patients' selection, but unfortunately despite NADT, there is still a proportion of patients early progressing after surgery [8], for those patients we need more accurate staging and prognostic biomarkers in order to avoid useless surgery.

Overall, only 57.7% of PaC patients will receive the intended ADT—of which 24.1% more than 70 days after surgery—and this is mainly due to surgical complications: a wound dehiscence may seem trivial, but it lowers the percentage of patients receiving systemic therapy to 43.6% versus 61.8% in patients without any postoperative morbidity [57]. NADT bypasses this dropout administrating treatment before surgery; the price to pay is that about one-third of patients experiencing major toxicity and about one-fifth progressing, but in resected ones, surgery seems not to be affected by the worst outcomes.

That NADT is safe and helpful in upfront technically unresectable patients and is self-evident, which other choices would they have? But how can we know if it is advisable in resectable patients regardless to vascular (whether venous or arterial) or en bloc multi-organ resections? A German group tried to design an RCT comparing NADT versus upfront surgery, in both cases followed by ADT, to rule out the question. Unfortunately, even if a slight increase in OS, R0, and N0 rates was seen in NADT arm, the trial had to be stopped due to slow recruiting; thus, sample size was not reached and results were not significant [58]. According to Mellon and colleagues, patients with BLR or LA PaC and sufficient response to neoadjuvant multi-agent chemotherapy and stereotactic body radiation therapy have similar or improved perioperative and long-term survival outcomes compared to upfront resected patients [59].

The problem dealing with NADT is that RCTs are lacking; the existing three trials conducted on resectable PaC report a protocol achievement of 18.18–70% and an ITT survival of 9.9–19.4 months [39]. Selected retrospective single-institution experiences over resectable BLR and LA PaC report OS up to 43.4 months in resected patients following chemotherapy or chemoradiation [60]. In a paper comparing NADT in BLR-LA to upfront resected patients, the ITT analysis showed worse survival for the former (17.0 vs. 22.1 mo, $p = 0.029$); such comparison has little significance because in the first group 61.6% of patients was eventually unresectable, while the upfront surgery group accounted only resected and adjuvant-treated patients [59]. Indeed, there was no significant difference and even a slight trend favoring NADT, in survival between the two groups among only resected patients (33.5 vs. 23.1 mo, $p = 0.057$) [59].

Histological confirmation of the disease is mandatory before administering NADT even though up to 16% of preoperatively cyto−/histologically diagnosed PaC eventually receive a

final pathological diagnosis other than PaC [38], thus receiving a useless neoadjuvant treatment. In Golcher's study pathological diagnosis of PaC at biopsy has been rejected in 4.5% of resected patients (because of the finding of a distal choledochal adenocarcinoma and a duodenal adenocarcinoma) [58].

8. Conclusions

The use of different resectability classifications, different NADT protocols, and selective reporting in the past years makes the comparison of literature extremely tricky. Outcomes tend to be better outside an RCT context; literature is influencing our conduct, but strong evidences come only from well-designed randomized trials. The unanimous adoption of the International Association of Pancreatology's classification [3] and standardized protocols and trials might clarify the impact of neoadjuvant treatments on the survival of those patients.

Assuming that patients are unresectable at diagnosis in the vast majority of cases; that even if they are suitable for NADT, more than 20% give up because of progression or toxicity; that barely an half is then resected; that, of those, up to 20% have positive margins; and that nor a negative resection margin nor a complete pathologic response shelters the patient from recurrence, we may say that nowadays PaC treatment desperately needs un upgrading.

Waiting for strong evidences, a reasonable behavior could be to resect all patients primarily resectable without any biologic worrisome feature (high CA19.9, high CEA, tumor >3 cm, positive nodes) and to offer all nonmetastatic patients neoadjuvant treatment in order to select those eligible for surgical exploration. Obviously, this has always to be done in the context of randomized controlled trials.

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Conflict of interest

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Abbreviations

PaC	Pancreatic cancer
ADT	Adjuvant treatment
BLR	Borderline resectable

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LA	Locally advanced
NCCN	National Comprehensive Cancer Network
CRT	Chemoradiation
CHT	Chemotherapy
NADT	Neoadjuvant treatment
VR	Vascular resection
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
PV	Portal vein
AA	Aorta
IVC	Inferior vena cava
GDA	Gastroduodenal artery
PHA	Proper hepatic artery
CHA	Common hepatic artery
AHBPA	American Heptad-Biliary-Pancreatic Association
SSAT	Society of Surgery of the Alimentary Tract
SSO	Society of Surgical Oncology
IAP	International Association of Pancreatology
CA19.9	Carbohydrate antigen 19.9
CEA	Carcinoembryonic antigen
CTCs	Circulating tumor cells
PS	Performance status
R0	No cancer cells seen microscopically at the resection margin
R1	Cancer cells present microscopically at the resection margin (microscopic positive margin)
ypT0	Complete pathological response after neoadjuvant treatment
RCT	Randomized controlled trial
ITT	Intention to treat
OS	Overall survival
mOS	Median overall survival

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Pancreatic cancer is a growing source of cancer-related death and has poor survival rates, which have not improved in the last few decades. Its high-mortality rate is attributed to pancreatic cancer biology, difficulty in early diagnosis, and lack of standardized international guidelines in assessing the pancreatic masses. This book aims to provide an update in the current state of play in pancreatic cancer diagnosis and to evaluate the benefits and limitations of the available diagnostic technology and therapy. The main modalities for diagnosis are imaging with HCT, MRI, USE, and PET. Some chapters review the improvements in the techniques used. Timely and accurate diagnosis of pancreatic cancer can lead to improve in the current poor outcome of this disease.

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