Pancreatic cancer: why is it so hard to treat?

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Abstract: No common malignancy is as rapidly and inevitably fatal as pancreatic ductal adenocarcinoma (PDA). This grim fact has driven substantial research efforts into this disease in recent decades. Unfortunately, the investment has yet to result in a meaningful increase in 5-year survival. This has prompted many pancreatic cancer researchers and advocates to redouble their efforts, but also requires one to step back and ask why the previous efforts were lacking and to consider why pancreatic cancer is so difficult to treat. The difficulties are legion. PDA is characterized by an insidious clinical syndrome, but is rarely diagnosed at a time when surgical resection is feasible. We lack markers of early detection and screening programs remain unproven even in high risk populations. The location of the tumor in the retroperitoneum, the advanced age of patients, and the systemic effects of disease limit the options for local therapy. Chemotherapy may provide a small benefit, but most efforts to improve on the current regimens consistently and stubbornly fail in advanced clinical trials. The molecular and cellular features of ductal pancreatic tumors are aggressive and underlay multiple levels of therapeutic resistance. Non-cell-autonomous features including stromal proliferation, reduced vascular density and immune suppression also contribute to therapeutic resistance. Growing awareness of these the fundamental features of PDA has begun to guide ongoing research efforts. Clinical trials are now specifically targeting these tumor properties and actively focusing on the therapeutic implications of tumor stroma. As reviewed here, reflecting on the fundamental question of why pancreatic cancer is so difficult to treat is a necessary and informative exercise that will aid our efforts to improve patient outcomes. These efforts will lead to improvements in clinical trial design, expand our focus to include the molecular and histologic implications of novel treatment paradigms, and ultimately change the lives of our patients.

Keywords: pancreatic cancer, chemotherapy resistance, tumor desmoplasia

Introduction

In the modern era of cancer research, pancreatic ductal adenocarcinoma (PDA) has proven to be among the most unyielding of adversaries. The oncology community has expended its entire arsenal at this disease with little effect: the 5-year survival rate has ticked up to 6% over the past 40 years, but nearly all diagnosed patients ultimately succumb to the disease. An estimated 37,390 people will die of pancreatic cancer in the US in 2012 [Siegel *et al.* 2012] with a similar pattern in the rest of the developed world [Jemal *et al.* 2011]. Over 80% of them will be found to have unresectable tumors at diagnosis [Stathis and Moore, 2010], giving them an expected overall survival of just 6 months. There are few therapeutic options for these patients and the most efficacious are also the most burdensome. Those who do undergo surgery improve their overall survival compared with patients of a similar stage by about 10 months [Bilimoria *et al.* 2007], but must tolerate significant morbidity and face almost inevitable recurrence. Given the slow progress against this disease, one must ask the question 'why is pancreatic cancer so hard to treat?'.

The particular problem of pancreatic cancer is multifactorial in its nature. The patient population in PDA is predominantly elderly and in poor overall health. There is no simple early detection method for pancreatic cancer and the earliest indications of disease are nonspecific. The tumor

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Ipsen Ex. 2072, Page 1 or 17

itself has its own peculiarities. For example, it has become apparent that PDA metastasizes microscopically early in the disease course, limiting the effectiveness of local therapies such as surgery and radiation. At the cellular level, the actual neoplastic epithelial cells at the heart of the disease harbor some of the most profoundly oncogenic alterations known to biology, and these are found at unusually high frequencies in PDA. In addition to driving growth and promoting cell survival, these alterations alter the metabolism of pancreatic cancer to one that can better support the manufacture of new cellular components. Layered on top of these high penetrance mutations is a host of rare alterations that are found in effectively unique combinations in each patient. The extent of genetic alterations in pancreatic tumors bears witness to a genomic instability phenotype that appears to play a significant role in the biology of PDA and implies an ability to rapidly develop acquired resistance to therapies that do manage to provoke an initial response. In addition to features of the tumor epithelium, PDA harbors a dense, desmoplastic stroma that can serve to limit the delivery of agents to tumors and foreshadows an incredibly complex interplay of intercellular signals that confound our ability to study the disease *in vitro*. Certain cell types within this stroma construct an immune-suppressed microenvironment that prevents the local immune system from clearing the tumor. Finally, PDA manifests as a syndrome, not just a mass, with systemic comorbidities that have a profoundly negative impact on quality of life.

Together, these raw observations paint a grim picture of the battle against pancreatic cancer that has at times led to a sense of nihilism. In reality, there are many signs that the research efforts of the past few decades have altered the momentum of this battle. Each of the challenges listed above has, in recent years, been the subject of intense research, leading to new ideas that are now being developed in the lab and in the clinic. For example, an understanding of the dynamics of drug delivery in PDA has led to a focus on targeted agents with desirable pharmacological properties. Another approach is to target the tumor stroma directly in order to facilitate the delivery of genotoxic agents or relieve local immune suppression. Other agents take advantage of the hypoxic microenvironment conferred by the desmoplastic stroma, or specific metabolic dependencies. Furthermore, decades of failed trials have led to improvements in clinical trial design and in the diagnostic and interventional techniques used in patients. By addressing the manifold difficulties that underpin the challenge of pancreatic cancer, a new sense of optimism is apparent. These barriers are surmountable and the nascent efforts to address them will ultimately be reflected in improved patient outcomes.

Patient population and diagnosis

Pancreatic ductal adenocarcinoma is largely a disease of old age, with an average age of diagnosis of 71 years. Yet the presenting symptoms are nonspecific such as weight loss and abdominal pain [Bakkevold *et al.* 1992]. This population of patients (and their general practitioners) is accustomed to aches and pains, and so in most cases, the earliest signs of malignancy go unnoticed; a high level of perception is required to avoid delays in diagnostic workup. Furthermore, in contrast to breast, prostate, melanoma and testicular cancers, there are no simple examinations that can elevate the level of suspicion: the pancreas is too deep to palpate and there is no specific blood test available for PDA. Other symptoms at diagnosis can include new onset of diabetes [Chari *et al.* 2005], unexplained jaundice [Porta *et al.* 2005] and unprovoked thrombosis [Khorana and Fine, 2004]; the most specific of these is unexplained painless jaundice, but many other explanations are possible. Thus, by the time that a patient seeks medical advice and their GP successfully navigates the diagnostic maze, often many months have passed and the patient's condition has further deteriorated.

PDA is associated with a syndrome of comorbidities that affect patients' overall health and in some cases can be life threatening. Symptoms related to pain [Porta *et al.* 2005] and depression [Kelsen *et al.* 1995] are components of this syndrome and are often present at the time of diagnosis, but become more severe with progression of the disease. PDA is intrinsically associated with biliary obstruction, infection, jaundice, ascites and pancreatic insufficiency, but beyond these factors PDA patients frequently experience the hypercatabolic state of cachexia and muscle wasting [Pausch *et al.* 2012]. In addition, PDA is classically associated with hypercoagulability and development of thromboembolic disease (Trousseau's syndrome) [Khorana and Fine, 2004]. Combined with the host of unrelated ailments typical of patients in their seventh, eighth and ninth decades of life, the average condition of PDA patients is poor, and many in this population may never be eligible or receive therapy.

Ipsen Ex. 2072, Page 2 of 17

From an epidemiological standpoint, efforts to change the long-term outcome of PDA patients through modification of risk factors have also been disappointing. Few behaviors reliably predict an increased risk for PDA [Raimondi *et al.* 2009]. Of those factors, cigarette use [Iodice *et al.* 2008; Heinen *et al.* 2010] should be discouraged but others, such as dietary habits, are less definitive [Thiebaut *et al.* 2009] and there is not sufficient evidence to recommend dietary changes to reduce the risk of PDA. The most promising chemopreventative agent is low-dose aspirin, which has been shown to significantly reduce the risk of pancreatic cancer in a dose-dependent manner [Tan *et al.* 2011]. There are familial clusters of PDA [Hruban *et al.* 1999; Bartsch *et al.* 2004; Shi *et al.* 2009] and first-degree relatives of affected patients are at increased risk [Klein *et al.* 2004; Hruban *et al.* 2010]. However, these comprise a minority of the overall population of PDA patients (5–10%) [Bartsch *et al.* 2012].

Furthermore, this knowledge is of limited benefit due to the lack of validated screening tests for early diagnosis of PDA. Due to its location in the retroperitoneum, the pancreas is difficult to access and sample with traditional endoscopic techniques. Endoscopic ultrasound techniques provide for higher yields but the morbidity associated with this procedure makes it unsuitable as a screening tool in an unselected population. Studies are ongoing in targeted populations of patients at high risk [Langer *et al.* 2009; Verna *et al.* 2010; Canto *et al.* 2012]. Cross-sectional imaging has the potential to identify small and even asymptomatic pancreatic lesions while they are still amenable to surgical resection [Canto *et al.* 2012]. However, due to poor innate contrast between PDA and the surrounding pancreas, specialized imaging protocols are required to optimally image pancreatic cancer by computerized tomography (CT) and magnetic resonance imaging (MRI) [Erkan *et al.* 2012]. As discussed later, PDA is characterized by hypovascularity and reduced perfusion compared with normal pancreatic tissue and this property may be utilized to obtain greater resolution in the detection of early lesions using techniques such as diffusion-weighted MRI [Holzapfel *et al.* 2011].

Serum sampling has not yet identified a suitable screening test for early detection of PDA. Many pancreatic lesions secrete CA19-9 (carbohydrate antigen 19-9) and this serum assay has a role in some patients in monitoring disease activity and response to therapy [Steinberg, 1990]. However CA19-9 has little use alone as a screening test due to high rates of false positivity in patients with non-malignant hepatobiliary disease [Frebourg *et al.* 1988]. There are ongoing efforts to identify molecular markers for early diagnosis of PDA [Goggins, 2005] but so far there are no validated agents and, as a consequence, diagnosis is often delayed.

The limited effect of local therapies

Currently, complete surgical resection provides the only potential for long-term cure of PDA but only a minority of patients have tumors that are amenable to surgery [Shaib *et al.* 2006]. This is due to the fact that, upon diagnosis, tumors have generally spread to involve critical abdominal vessels as well as adjacent organs. Significant advances have been made in the technical aspects of surgical resection with decreases in short-term morbidity and mortality at major centers [Winter *et al.* 2012]. Yet even in the most experienced centers, long-term survival after surgery is poor [Farnell *et al.* 2005; Ferrone *et al.* 2012], with tumors recurring in virtually all patients [Allison *et al.* 1998]. Due to the high rate of recurrence, local targeted therapy with radiation has been suggested following surgery. However, controlled studies of the long-term impact of adjuvant radiation therapy have proved inconclusive to date [Neoptolemos *et al.* 2004]. The cytotoxic effect of radiation therapy relies in part on the presence of oxygen [Harrison *et al.* 2002]. However, intraoperative oxygen measurements on human patients have found that these tumors are extremely hypoxic [Koong *et al.* 2000], which may contribute to the limited impact of this modality.

The limited long-term efficacy of surgery and adjuvant radiation therapy has led many to conclude that residual tumor tissue remains even in the case of complete surgical resection with no evidence of residual tumor. One possible explanation is that of 'field effect' mutations that may affect otherwise normal appearing cells present in the residual pancreatic tissue. Alternatively, PDA may simply metastasize at a microscopic level at a very early stage. Indeed, provocative data in a genetically engineered mouse model of PDA suggest that mutant cells may delaminate from the pancreatic epithelium and enter circulation in the very early stages of tumorigenesis even prior to the development of an overt carcinoma [Rhim *et al.* 2012]. If this is true, then PDA should be considered an inherently metastatic disease for which local therapy is simply a delaying action. This also highlights

Ipsen Ex. 2072, Page 3 of 17

the importance of identifying chemotherapeutic agents that effectively target microscopic metastases. It is notable that mutation evolution analysis based on deep sequencing of human pancreatic tumor samples has suggested a long latency period for PDA, estimating that it may take an average of 17 years for a tumor to evolve from a single common progenitor [Yachida *et al.* 2010]. However, a computational modeling study is consistent with the notion that PDA metastasizes early in disease [Haeno *et al.* 2012].

Clinical data of therapeutic efforts following resection of PDA are summarized in Table 1. These trials are consistent in demonstrating a small benefit when assessing recurrence but limited impact on long-term survival regardless of the intervention [Neoptolemos *et al.* 2004, 2010; Stocken *et al.* 2005; Oettle *et al.* 2007]. The modest achievements of adjuvant therapy compare poorly with the experience in other common cancers. Unfortunately PDA cells display broad and intractable resistance to chemotherapy, the subject of the remainder of this review.

Chemotherapy resistance in PDA

The track record of the clinical trials in advanced and metastatic pancreatic cancer is dismal (summarized in Table 2). Gemcitabine and erlotinib (Tarceva) remain the only two agents approved for use in advanced disease despite their modest benefits. Gemcitabine was approved on the basis of a study [Burris *et al.* 1997] showing that it was superior to 5-FU (5-fluorouracil) in providing a clinical benefit among advanced PDA patients with pain symptoms (clinical benefit rate = 23.8% *versus* 4.8%; $p = 0.0022$) and modestly prolonged median survival from 4.4 to 5.6 months ($p = 0.0025$). The incremental median survival benefit seen with the addition of erlotinib to gemcitabine is even smaller (5.9 to 6.2 months, $p = 0.038$), albeit statistically significant [Moore *et al.* 2007].

As summarized in Table 2, over 20 phase III trials have been conducted to improve on the modest efficacy of gemcitabine and these have been overwhelmingly disappointing. These trials covered traditional chemotherapeutic agents and combinations, targeted therapies such as the anti- vascular endothelial growth factor (anti-VEGF) monoclonal antibody, bevacizumab [Kindler *et al.* 2010] and the anti-epidermal growth factor receptor (anti-EGFR) antibody, cetuximab [Philip *et al.* 2010], as well as experimental targeted therapies including farnesyltransferase inhibitors [van Cutsem *et al.* 2004]. It is difficult to overstate the physical, financial and psychological costs of these unsuccessful attempts.

Two notable exceptions to this tale have been reported in the past 2 years. In 2011, a robust clinical benefit was found in a phase III randomized trial of FOLFIRINOX (a four-drug combination of 5-FU, leucovorin, oxaliplatin and irinotecan) compared with gemcitabine in metastatic PDA (median overall survival [OS] of 11.1 *versus* 6.8 months; $p \leq 0.001$) [Conroy *et al.* 2011]. However, this advantage comes at the cost of significant toxicity, making the regimen appropriate only for those patients with good performance status. As recently as November 2012, after exciting phase II results [von Hoff *et al.* 2011], a phase III trial of *nab*-paclitaxel (Abraxane) plus gemcitabine was reported to have met its primary overall survival endpoint. We look forward to learning the magnitude of this effect in the coming months and are excited at the prospect of having a range of chemotherapeutic tools to treat patients in different states of health.

This collective history has delivered a consistent overarching message: the response of PDA to chemotherapy is poor. Using standard criteria that define radiographic response as a decrease of 30% (or 50% in older studies) in tumor size, very few patients treated with chemotherapy experience an objective response (noted in Table 2). Thus, the initial resistance of these tumors is primary (innate), rather than the secondary (acquired) resistance that is classically observed in most cancers. This is an important clue to understanding the recalcitrant nature of pancreatic cancer.

Cell-autonomous mechanisms of resistance to chemotherapy

It is informative to think of the resistance of PDA to chemotherapy as occurring due to cell-autonomous and non-cell-autonomous pathways. Although ductal pancreatic tumors display clinical and pathologic heterogeneity, a striking characteristic of PDA is the consistent pattern of high penetrance genetic alterations that occur in four genetic loci: K-ras, p53, cdkn2a and smad4/DPC4. Over 90% of pancreatic tumors harbor activating mutations in K-ras, one of the most potent of all human oncogenes, far exceeding the rate of any other cancer [Almoguera *et al.* 1988; Pellegata *et al.* 1994; Hezel *et al.* 2006; Maitra and Hruban, 2008]. Mutant K-ras initiates a signal

Ipsen Ex. 2072, Page 4 of 17

Table 1. Phase III trials of adjuvant therapy following resection of PDA and comparison with other common tumors.

Study	Study period	Study population	Treatment arm(s)	Survival		
				Median OS (months)	*p* value	5-year survival %
EORTC 40891 [Klinkenbijl *et al.* 1999]	1987–1995	Stage 1–3 resected PDA (others excluded from this analysis)	Observation	12.6	NS	10
			Chemoradiotherapy (5-FU based)	17.1		20
ESPAC-1 [Neoptolemos *et al.* 2004]	1994–2000	Stage 1–3 resected PDA	Observation	16.9	0.009 (for chemo *versus* no chemo)	11
			Chemotherapy alone (5-FU)	21.6		29
			Chemoradiotherapy	13.9		7
			Chemoradiotherapy + chemotherapy	19.9		13
CONKO-001 [Oettle *et al.* 2007]	1998–2004	Stage 1–3, resected PDA	Observation	20.7	NS	11.5
			Chemo (gemcitabine)	22.1		22.5
RTOG 9704 [Regine *et al.* 2008, 2011]	1998–2002	Stage 1–3, resected PDA in head of pancreas.	5-FU + chemoradiotherapy	16.9	NS	22
			Gemcitabine + chemoradiotherapy	20.5		18
ESPAC-3 [Neoptolemos *et al.* 2010]	2000–2007	Stage 1–3, resected PDA	5-FU	23.0	NS	NR
			Gemcitabine	23.6		NR
Lung cancer				mOS		5 year survival
ANITA [Douillard *et al.* 2006]		Stage IB–IIIA resected, NSCLCa	Observation	43.7	0.017	43
			Chemotherapy	65.7		51
Colon cancer						6 year survival
MOSAIC [Andre *et al.* 2004, 2009]	1998–2001	Stage II–III, resected colon cancer	Chemotherapy (FOLFOX)	NR		78.5
Breast cancer						5 year survival
[Sparano *et al.* 2008]	1999–2002	Stage II–III breast cancer	Chemotherapy (most effective group)	NR		89.7

5-FU: fluorouracil; chemo: chemotherapy; NR: not reported; NS: not significant ($p > 0.05$); NSCLCa: non-small cell lung cancer. OS: overall survival; PDA: pancreatic ductal adenocarcinoma.

transduction cascade that provides a strong pro-growth signal, increases cell motility and invasion, and profoundly rearranges cell metabolism to a growth-promoting state. In pancreatic cancer, Ras mutation initiates paracrine signals that promote and maintain stromal desmoplasia, a key mediator of non-cell-autonomous resistance (see below). Unfortunately, K-ras is an extremely challenging therapeutic target for which no effective targeted inhibitors have been identified to date.

Overlaid on this oncogenic scaffold are mutations in four extremely potent tumor suppressor genes. The cdkn2a locus encodes two tumor suppressor genes, $p16^{Ink4a}$ and $p15^{ARF}$. These genes are inactivated through a variety of mechanisms in >90% of human pancreatic tumors [Caldas *et al.* 1994; Schutte *et al.* 1997]. The p53 tumor suppressor gene is another major tumor suppressor and is altered in 75–90% of pancreatic tumors [Pellegata *et al.* 1994; Redston *et al.* 1994], resulting in impaired DNA damage responses, impaired apoptosis, loss of cell cycle control, and promotion of genomic instability. p53 is typically altered through 'gain of function' missense mutations that may further promote cancer beyond the loss of classical p53 tumor suppressor functions [Olive *et al.* 2004; Morton *et al.* 2010]. Alterations in DPC4 [Hahn *et al.* 1996a, 1996b] are observed in more than half of cases and confer a prometastatic phenotype. The combined effect of these mutations

Ipsen Ex. 2072, Page 5 or 17

Table 2. Phase III randomized trials with gemcitabine comparison in advanced PDA.

Study*	Accrual period	Number of patients	Treatment groups	Median OS		Response rate	
				Months	*p*	%	*p*
[Burris *et al.* 1997]	1992-1994	126	5-FU	4.4	0.0025	0	NS
			Gemcitabine	5.6		5.4	
[Bramhall *et al.* 2001]	1997-1998	239	Gem	5.5	NS	11	NS
			G + marimistat	5.5		16	
[Moore *et al.* 2003]	1997-1999	277	Gem	6.6	<0.001	NR	
			G + BAY 12-9566 (MMP inhibitor)	3.7		NR	
[Heinemann *et al.* 2006]	1997-2002	195	Gem	6.0	NS	8.2	NS
			G + cisplatin	7.5		10.2	
[Berlin *et al.* 2002]	1998-1999	322	Gem	5.4	NS	5.6	NS
			G + 5-FU	6.7		6.9	
[Van Cutsem *et al.* 2004]	1999-2001	688	Gem	6.1	NS	8	NS
			G + tipifarnib	6.4		6	
[Rocha Lima *et al.* 2004]	2000-2001	360	Gem	6.6	NS	4.1	<0.001
			G + irinotecan	6.3		16.1	
[Louvet *et al.* 2005]	2001-2003	313	Gem	7.1	NS	17.3	0.044
			G + oxaliplatin	9.0		26.8	
[Herrmann *et al.* 2007]	2001-2004	319	Gem	7.2	NS	7.8	NS
			G + capecitabine	8.4		10	
[Abou-Alfa *et al.* 2006]	2001-2003	349	Gem	6.2	NS	5.2	NS
			G+ exatecan	6.7		6.8	
[Moore *et al.* 2007]	2001-2003	569	Gem	5.9	0.038	8.0	NS
			G + erlotinib	6.2		8.6	
[Oettle *et al.* 2005]	2001-2003	565	Gem	6.3	NS	7.1	0.004
			G + pemetrexed	6.2		14.8	
[Colucci *et al.* 2010]	2002-2007	400	Gem	8.3	NS	10.1	NS
			G + cisplatin	7.2		12.9	
[Cunningham *et al.* 2009]	2002-2005	533	Gem	6.2	NS	12.4	0.034
			G + capecitabine	7.1		19.1	
[Poplin *et al.* 2009]	2003-2005	832	Gem (standard rate)	4.9	NS	6	NS
			Gem-FDR	6.2		10	
			Gem-FDR + oxaliplatin	5.7		9	
[Philip *et al.* 2010]	2004-2006	745	Gem	5.9	NS	7	NS
			G + cetuximab	6.3		8	
[Kindler *et al.* 2010]	2004-2006	602	Gem	5.9	NS	10	NS
			G + bevacizumab	5.8		13	
[Van Cutsem *et al.* 2009]	2005-2006	607	Gem + erlotinib	6.0	NS	8.6	NS
			Gem + erlotinib + bevacizumab	7.1		13.5	
[Conroy *et al.* 2011]	2005-2009	342	Gem	6.8	<0.001	9.4	<0.001
			FOLFIRINOX	11.1		31.6	
[Heinemann *et al.* 2012]	2006-2008	281	Gem + erlotininb (capecitabine for second line)	6.2	NS	16	NR
			Capecitabine + erlotinib (gem for second line)	6.9		5	

(Continued)

Ipsen Ex. 2072, Page 6 of 17

Table 2. (Continued)

Study*	Accrual period	Number of patients	Treatment groups	Median OS		Response rate	
				Months	*p*	%	*p*
[Goncalves *et al.* 2012]	2006-2009	104	Gem G + sorafenib	9.2 8	NS	19 23	NS
[Kindler *et al.* 2011]	2007-2008	632	Gem G + axitinib	8.3 8.5	NS	2 5	0.018

5-FU: fluorouracil; FDR: fixed dose rate.G and Gem: gemcitabine; NR: not reported; NS: not statistically significant ($p > 0.05$);
*In addition to these trials, several phase III trials have been completed or terminated and remain unpublished. These include fluorouracil plus triacetyluridine [ClinicalTrials.gov identifier: NCT24427], aflibercept [ClinicalTrials.gov identifier: NCT574275], TS-1 [ClinicalTrials.gov identifier: NCT498225], GV1001 vaccine [ClinicalTrials.gov identifier: NCT358566], virulizin [ClinicalTrials.gov identifier: NCT40092] and AMG 479- ganitumab [ClinicalTrials.gov identifier: NCT1231347].

is formidable and likely explains a large portion of the difficulty in treating this disease. Indeed, patients with three or four of these alterations in their tumors have a much worse prognosis than those with one or two (median survival of 9 *versus* 23 months) [Yachida *et al.* 2012].

Besides these well-established 'driver' variations, many other genetic changes occur at lower frequencies [Hansel *et al.* 2003; Jones *et al.* 2008; Biankin *et al.* 2012; Perez-Mancera *et al.* 2012]. An effort to sequence the entire exome of 24 PDA samples revealed that the average PDA contains more than 60 genomic changes [Jones *et al.* 2008]. Some of these may contribute to the specific resistance to chemotherapy in as yet unidentified ways. This high degree of genomic changes seen in PDA is suggestive of significant genomic instability and may limit the effectiveness of therapy, especially targeted agents, by contributing to secondary or acquired chemoresistance.

Despite the survival benefits observed in clinical studies, only 5–10% of pancreatic tumors exhibit a radiographic response to gemcitabine therapy. Pharmacological investigations into the mechanisms of gemcitabine activity have led to some of the best characterized determinants of patient prognosis. Gemcitabine [2′,2′-difluorodeoxycytidine (dFdC)] is a nucleoside analog of cytidine that must be actively transported into cells and then sequentially phosphorylated to the active triphosphate [2′,2′-difluorodeoxycytidine triphosphate (dFdCTP)] [Heinemann *et al.* 1988, Mini *et al.* 2006]. Transport across the cell membrane is primarily mediated by human equilibrative nucleoside transporter (hENT1), though other transporters play a minor role [Mackey *et al.* 1998, Mini *et al.* 2006]. Cell lines that are resistant to gemcitabine are often hENT1 deficient [Achiwa *et al.* 2004] and hENT1 expression in human tissues can predict response to gemcitabine [Oguri *et al.* 2007]. In pancreatic cancer, patients with elevated hENT1 have improved survival when treated with gemcitabine but not among untreated patients [Marechal *et al.* 2012]. In a large clinical trial [Farrell *et al.* 2009], patients treated with gemcitabine who had no hENT1 staining had poorer survival than those with positive hENT1 staining (hazard ratio for survival = 0.51, 95% confidence interval [CI] = 0.29–0.91; $p = 0.02$).

Enzymes associated with the metabolic activation and inactivation of gemcitabine may also impact tumor sensitivity. The monophosphorylation of gemcitabine is a rate-limiting step in its activation and is mediated by deoxycytidine kinase (dCK). Reduced levels of dCK are associated with gemcitabine resistance in some tumor cell lines [Achiwa *et al.* 2004], while elevated dCK expression is associated with improved survival among those receiving adjuvant gemcitabine in PDA [Marechal *et al.* 2012]. Conversely, gemcitabine can be deaminated to its inactive metabolite [2′,2′-difluorodeoxyuridine (dFdU)] in a process catalyzed by the enzyme cytidine deaminase (CDA) [Eliopoulos *et al.* 1998], levels of which are a key determinant of gemcitabine activity. One frequent polymorphism 79A>C (Lys27Gln) is associated with decreased enzymatic activity, improved clinical outcomes, and increased toxicity in combination therapy with gemcitabine in lung cancer [Tibaldi *et al.* 2008]. In pancreatic cancer, the data are conflicting; one group failed to find an effect of this polymorphism on gemcitabine activity [Sugiyama *et al.* 2007], while another group saw increased toxicity but no change in

Ipsen Ex. 2072, Page 7 of 17

outcomes in patients with intact CDA treated with gemcitabine [Farrell *et al.* 2012]. Other polymorphisms have also been identified [Sugiyama *et al.* 2007; Tanaka *et al.* 2010] and may be clinically relevant. Other studies suggest that the most relevant measure of CDA is functional testing which can predict rate of severe toxicity to gemcitabine [Ciccolini *et al.* 2010], though the clinical implications of these findings await validation in prospective trials [Giovannetti *et al.* 2010]. It is the high levels of CDA in human plasma that leads to the short (~15 minute) half-life of gemcitabine. This short half-life is compounded by non-cell-autonomous features of pancreatic tumors that limit the delivery of drugs to pancreatic tissues.

Non-cell-autonomous barriers to drug efficacy

A defining characteristic of PDA is the presence of a dense fibrotic proliferation surrounding the epithelial cells that may form the majority of the tumor mass [Chu *et al.* 2007; Neesse *et al.* 2011]. This 'desmoplastic reaction' is composed of various leukocytes, fibroblasts, endothelial cells and neuronal cells, as well as extracellular matrix components such as collagen and hyaluronan. The desmoplastic reaction is driven by paracrine signals originating in the epithelial compartment. These signals are driven by the oncogenic signals such as those initiated by mutant K-ras. A pair of studies in genetically engineered mouse models that utilized 'switchable' alleles of mutant K-ras found that the loss of mutant K-ras expression led to rapid quiescence and involution of the stroma over the course of just a few days [Collins *et al.* 2012; Ying *et al.* 2012]. In recent years, significant effort has been invested in identifying the signals that mediate the relationships between the different cell types in pancreatic tumors. For example, early in pancreatic tumor development, the neoplastic epithelial cells begin to overexpress Sonic Hedgehog (SHH), a secreted ligand that normally plays a role during organ development [Berman *et al.* 2003]. This upregulation has no effect on hedgehog pathway activity in the epithelial compartment [Nolan-Stevaux *et al.* 2009]. Rather, SHH activates the pathway in nearby stromal fibroblasts, promoting their activation and proliferation [Bailey *et al.* 2008; Tian *et al.* 2009]. This pathway has served as a paradigm for how tumor cells influence the behavior of their neighboring stromal cells.

The desmoplastic stroma of pancreatic cancer has physiological effects on the tumor that have a direct impact on drug efficacy. In contrast to many tumors that are dependent on neo-angiogenesis, ductal pancreatic tumors are very poorly vascularized relative to normal tissues and consequently poorly perfused [Olive *et al.* 2009]. Indirect evidence suggests that this is mediated by an anti-angiogenic effect of the tumor stroma, though the precise mechanism is an area of active research. Regardless, the poor perfusion of pancreatic tumors has the unfortunate consequence of limiting the delivery of therapeutic agents into the tumor parenchyma. Indeed, studies in a genetically engineered mouse model found that the delivery of two different chemotherapeutic agents, gemcitabine and doxorubicin, was approximately one third that of surrounding normal tissues [Olive *et al.* 2009]. Furthermore, poor perfusion in pancreatic tumors has been correlated with poor prognosis in patients [Komar *et al.* 2009]. The drug delivery effect is visualized every time a contrast agent is used to image a patient with pancreatic cancer: the finding of a 'hypoenhancing mass' in the pancreas is diagnostic for pancreatic ductal adenocarcinoma, particularly compared with endocrine carcinomas of the pancreas, which are hyperperfused [Fusaroli *et al.* 2010; Matsubara *et al.* 2011; Saftoiu *et al.* 2012]. Another consequence of poor perfusion is a hypoxic microenvironment, which can have important effects on radiosensitivity, cell metabolism and cell invasion. Direct measurements of oxygen partial pressure in human pancreatic tumors found that pancreatic tumors are profoundly hypoxic [Koong *et al.* 2000].

Tumor stroma is also the site of interaction between cancer and the immune system. Pancreatic tumors establish a profoundly immunosuppressed microenvironment that is nearly devoid of T lymphocytes. Several stromal cell types harbor immunosuppressive properties, including tumor associated macrophages (TAMs), cancer associated fibroblasts (CAFs), regulatory T cells (T_{reg}) and myeloid derived suppressor cells. Recently, two groups identified a K-ras dependent signal that promotes immunosuppression. Following activation, the principle upregulated cytokine is granulocyte-macrophage colony-stimulating factor (GM-CSF), which was found to promote the recruitment and activation of immature myeloid progenitor cells to become myeloid derived suppressor cells [Bayne *et al.* 2012; Pylayeva-Gupta *et al.* 2012]. Undoubtedly, other such signals exist and should be explored in the coming years.

Ipsen Ex. 2072, Page 8 of 17

Future therapeutic options

The many complexities of pancreatic tumors have, to date, overwhelmed our best clinical efforts. However, the investments of the past 30 years in establishing a fundamental understanding of the disease uncovered a number of important and promising leads for new therapeutic approaches. At the most basic level, improved patient management and the advent of multidisciplinary centers specializing in the care of pancreatic cancer patients is improving the quality of life of our patients. High volume centers clearly have improved surgical outcomes [Lieberman *et al.* 1995; Birkmeyer *et al.* 2002] and similar expertise in the endoscopy suite is likely to also improve patient care. Indeed, a recent report described the diagnostic and financial advantages of direct histological processing of fine needle aspiration (FNA) samples rather than use of cytology [Brais *et al.* 2012]. The recent introduction of endoscopic core biopsy needles has also improved the ability to acquire samples for both diagnostic and experimental purposes.

Several promising techniques are under development that may improve diagnostic imaging. Advanced MRI sequences such as diffusion weighted imaging (DWI) and dynamic contrast enhanced (DCE) MRI capitalize on the altered perfusion of pancreatic tumor to provide functional contrast relative to normal or inflamed pancreatic tissue [Fattahi *et al.* 2009; Bali *et al.* 2011; Hur *et al.* 2012; Wiggermann *et al.* 2012; Yao *et al.* 2012]. Advanced endoscopic ultrasound techniques such as contrast ultrasound (which measure tissue perfusion) and ultrasound elastrography (which measures tissue stiffness) have shown initial promise as diagnostic and prognostic indicators [Sofuni *et al.* 2005; Sakamoto *et al.* 2008; D'Onofrio *et al.* 2009]. A number of efforts are under way to identify targeted contrast agents that discriminate early cancer or late-stage premalignancies. Among the most promising is a peptide that recognizes extracellular expression of Plectin-1, which was identified initially through a phage-display screen in genetically engineered mouse models of pancreatic cancer [Kelly *et al.* 2008; Bausch *et al.* 2009]. This probe appears to be upregulated in carcinomas *in situ* (PanIN 3) and is being developed for clinical trials as a single photon emission computed tomography (SPECT) probe.

For patients with advanced or metastatic PDA, a number of new therapeutic avenues are being explored. A variety of approaches have been taken to target components of the pancreatic tumor stroma. PEGPH20, a modified enzyme that breaks down hyaluronan crosslinks in the extracellular matrix, has been shown by two groups to facilitate the delivery of drugs to pancreatic tumors in genetically engineered mice and increase their overall survival [Jacobetz *et al.* 2013; Provenzano *et al.* 2012] A phase Ib/II clinical trial of PEGPH20 in combination with gemcitabine is now active at multiple sites. Two agents are in clinical trials that take advantage of the paucity of vasculature in pancreatic cancer. The gamma secretase inhibitor RO4929097 (Hoffman La Roche) is being evaluated in previously treated metastatic pancreatic cancer patients in a phase II study. Gamma-secretase is required for activation of Notch pathway signaling, which plays a role in pancreatic tumor angiogenesis. In a genetically engineered mouse model, inhibition of γ-secretase reduced vascularity to critically low levels within the tumor, resulting in cavitating necrosis and increased overall survival when combined with gemcitabine [Cook *et al.* 2012]. A second approach utilizes the presence of hypoxia to activate a chemotherapeutic prodrug. TH-302 is a potent DNA alkylator that is selectively activated in regions of hypoxia and is now undergoing clinical evaluation in combination with gemcitabine. Unfortunately, inhibition of the Hedgehog pathway, one of the earliest stroma targeting strategies, has so far failed to meet expectations. Two different targeted inhibitors of the Smoothened protein, IPI-926 (Saridegib, Infinity Pharmaceuticals) and GDC-0449 (Vismodegib, Genentech) were evaluated in phase II clinical trials, with negative results reported for IPI-926. Investigations are ongoing to understand this disconnect between preclinical and clinical results for these agents. On a positive note, a phase III study of gemcitabine plus Abraxane, a nanoparticle reformulation of taxol, was recently found to have met the primary endpoint, after an encouraging phase II study in which metastatic patients lived an average of 12.2 months [von Hoff *et al.* 2011]. One proposed mechanism of action is the targeting of SPARC, an extracellular matrix protein that is upregulated in the stroma of pancreatic tumors [Desai *et al.* 2009]. However, a recent analysis in genetically engineered mice found that Abraxane alters the sensitivity of pancreatic tumors to gemcitabine through downregulation of cytidine deaminase, leading to higher concentrations of dFdCTP in tumors [Frese *et al.* 2012]. In either case, with a toxicity profile that may be more reasonable than FOLFIRINOX, the regimen may prove to be a welcome new tool for the treatment of pancreatic cancer patients.

Ipsen Ex. 2072, Page 9 or 17

Finally, two novel immunotherapy approaches are under development in pancreatic cancer. The observation that GM-CSF promotes a paracrine circuit that helps maintain an immunosuppressive microenvironment has led to the proposal that anti-GM-CSF targeted antibodies may be useful in treating patients with pancreatic cancer [Bayne *et al.* 2012; Pylayeva-Gupta *et al.* 2012]. Another approach was reported in a phase I trial of a CD40 agonist in combination with gemcitabine in metastatic PDA patients. CD40 is an immunostimulant, and the combination therapy resulted in partial responses in 19% of patients and stable disease in 52% of patients. Contrary to initial expectations, the regimen relied on a macrophage-based mechanism of action, as revealed in a genetically engineered mouse model [Beatty *et al.* 2011].

Lessons learned

It is important to heed the hard-won lessons of a generation of clinical researchers [Tabernero and Macarulla, 2009]. As our molecular understanding of cancer in general and PDA specifically increases, it will become increasingly useful to obtain more information about the tumors we are treating. Currently many patients are diagnosed with PDA on the basis of imaging characteristics and a fine needle aspiration, which demonstrates adenocarcinoma but provides little additional tissue for further analysis. In many advanced clinical trials, the reason for failure of encouraging agents is never determined and this limits further directions in PDA research. Where therapies are developed and justified on the basis of tumor biology, it is critical to include pretreatment biopsies and, whenever feasible, to obtain additional post-treatment tumor samples to monitor the effect of targeting interventions on tumor histology and biology. Although this can increase trial costs and is an additional burden to patients, many patients believe in the importance of research efforts and are willing to undergo these procedures as a meaningful contribution to this effort. Moreover, in successful trials, correlative studies can provide valuable guidance for future development efforts. Treatment-related biopsies also facilitate the ability to prospectively test biomarkers, an important tool in identifying appropriate agents for advanced clinical trials [Philip *et al.* 2009].

It is also useful to bear in mind the many missed signals: situations where single arm phase I or II trials led to great hope, only to be disappointed by randomized phase III trials. In some cases this relates to the marked heterogeneity in outcomes among patients with advanced PDA based on other clinical characteristics such as age, pain status, function status and other comorbidities. These factors are complex and difficult to control. One way to address this limitation is to incorporate a control group in phase II trials to provide context to the reported results. Although this will require more patients and will still necessitate large phase III trials of positive agents [Rubinstein *et al.* 2011], it may reduce the number of agents that progress to expensive, large-scale, but ultimately futile phase III studies [Sharma *et al.* 2011].

Consideration must also be given to the caution necessary when utilizing surrogate markers in clinical trials. Particularly in pancreatic cancer, response rate and progression-free survival have often failed to correlate with increased overall survival. Although additional surrogate markers may prove to be beneficial, ultimately well-designed randomized trials with survival or clinical benefit outcomes will remain the gold standard of therapeutic effect.

In addition, it is important to remember that the majority of patients with PDA are elderly and many have poor functional status related to their tumors. These patients may ultimately benefit from different therapies than a younger, fitter population, and they should be represented in clinical trials. We therefore advocate for dedicated trials of less toxic regimens in the setting of performance status 2 patients. Perhaps most importantly, numerous advanced clinical trials have been terminated early and the data from many of these experiences are not publicly available. An effort must be made to make these data available to researchers in a timely fashion to inform future clinical trial design.

In conclusion, while the clinical outcomes for PDA have not improved sufficiently in the last decades, a large wealth of knowledge has been developed and is now being translated towards the ultimate goal of improving treatment outcomes for patients with PDA. We are filled with optimism that these efforts will be successful.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

Ipsen Ex. 2072, Page 10 of 17

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Ipsen Ex. 2072, Page 12 of 17

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Ipsen Ex. 2072, Page 13 or 17

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Ipsen Ex. 2072, Page 14 of 17

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Ipsen Ex. 2072, Page 15 or 17

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Ipsen Ex. 2072, Page 16 of 17

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Ipsen Ex. 2072, Page 17 or 17