

# Emerging concepts in pancreatic cancer medicine: targeting the tumor stroma

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**Abstract:** Pancreatic ductal adenocarcinoma is a stroma-rich and highly challenging cancer to treat. Over recent years, it has become increasingly evident that the complex network of soluble cytokines, growth factors, proteases, and components of the extracellular matrix collaboratively interact within the tumor microenvironment, sustaining and driving cancer cell proliferation, invasion, and early metastasis. More recently, the tumor microenvironment has also been appreciated to mediate therapeutic resistance in pancreatic ductal adenocarcinoma, thus opening numerous avenues for novel therapeutic explorations. Inert and soluble components of the tumor stroma have been targeted in order to break down the extracellular matrix scaffold, relieve vessel compression, and increase drug delivery to hypovascular tumors. Moreover, targeting of antiapoptotic, immunosuppressive, and pro-proliferative effects of the tumor stroma provides novel vantage points of attack. This review focuses on current and future developments in pancreatic cancer medicine, with a particular emphasis on biophysical and biochemical approaches that target the tumor microenvironment.

**Keywords:** pancreatic cancer, chemoresistance, tumor stroma, drug delivery

## Introduction to pancreatic cancer

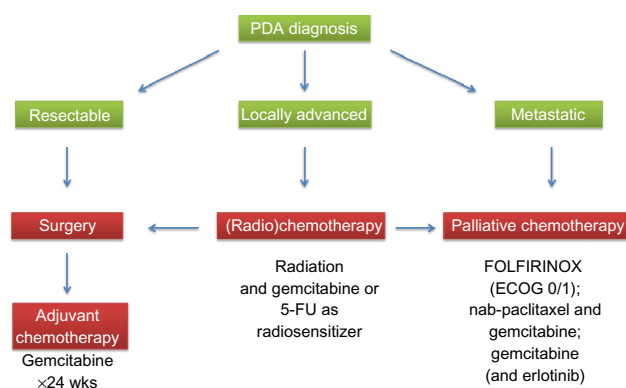
Pancreatic ductal adenocarcinoma (PDA) is an extremely stroma-rich and highly aggressive solid tumor within the exocrine compartment of the pancreatic gland, and its incidence rates are steadily increasing in the Western world.<sup>1</sup> In the US, an estimated 43,920 new cases are diagnosed each year, and many diagnosed patients succumb to the disease after only a few months.<sup>2</sup> The inability of clinicians to substantially improve the prognosis of PDA patients over the last few decades is reflected in a virtually unchanged 5-year survival rate of 5%–6% and a median survival of less than 12 months.<sup>2</sup> Potential reasons for such a poor clinical outcome reflect both the clinical and biological characteristics of pancreatic cancer. First, PDA is chiefly a disease of the elderly, and patients often initially note relatively nonspecific symptoms, including back pain and dyspepsia.<sup>3</sup> These symptoms often point towards degenerative and relatively harmless conditions that may not immediately raise concerns on the part of patients and general practitioners. More alarming symptoms include new onset of diabetes,<sup>4–6</sup> painless jaundice, weight loss, or spontaneous deep vein thrombosis.<sup>7,8</sup> As a result, a timely diagnostic work-up, including use of specialized imaging modalities such as abdominal ultrasound scans, computed tomography (CT), or magnetic resonance imaging is often delayed for several months after initial symptom onset.<sup>9</sup> Second, no specific blood or urine biomarkers are presently available that would help to identify subgroups of patients with increased risk of developing PDA. Due to these

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issues and the lack of early detection methods, 80% of PDA patients are diagnosed with unresectable locally advanced or metastatic disease.

Families having at least two first-degree relatives with confirmed PDA that does not meet the criteria of other inherited tumor syndromes, such as Peutz–Jeghers syndrome or hereditary chronic pancreatitis, comprise a small subset of the overall population of PDA patients (5%–10%).<sup>10</sup> Increased surveillance by high resolution imaging and various chemopreventive strategies are under investigation as potential modalities to detect PDA early or prevent its onset.<sup>11–16</sup> While the optimal treatment of locally advanced PDA without detectable distant metastases remains to be determined, patients with distant metastases are candidates for systemic palliative chemotherapy dependent on comorbidities and general performance status. A relative minority of patients (15%–20%) who qualify for pancreatic resection receive a 6-month course of adjuvant gemcitabine or 5-fluorouracil plus folinic acid chemotherapy (Figure 1).<sup>17</sup> Perioperative morbidity and mortality have improved in high-volume centers owing to improved surgical resection techniques, postoperative care, and multidisciplinary approaches;<sup>18–20</sup> however, tumor relapse is unfortunately common, and the median survival rate for patients with R0 resection is approximately 2 years, with a 5-year survival of 15%–20%.<sup>21</sup>

This review focuses on current and future developments in pancreatic cancer medicine, with a particular emphasis on novel treatment options that target the tumor microenvironment, in particular the tumor stroma.



**Figure 1** Schematic overview of current clinical treatment strategies for pancreatic cancer patients according to whether they have resectable, locally advanced, or metastatic pancreatic cancer, and ECOG performance status 0–5.

**Notes:** ECOG 0, fully active without restrictions; ECOG 1, restricted in hard physical work, but ambulatory and able to carry out light work. The FOLFIRINOX protocol includes folinic acid, fluorouracil, irinotecan, and oxaliplatin.

**Abbreviations:** 5-FU, 5-fluorouracil; ECOG, Eastern Cooperation Oncology Group; PDA, pancreatic ductal adenocarcinoma; nab-paclitaxel, albumin-bound paclitaxel.

## Patient outcomes and current treatment options

Despite intensive clinical research activities aiming to identify effective chemotherapies, PDA has remained virtually unresponsive to conventional and targeted therapies.<sup>22</sup> A large number of randomized clinical trials have been conducted in an attempt to improve overall survival in PDA patients. To this end, the nucleoside analogue gemcitabine was combined with a second cytotoxic agent, eg, 5-fluorouracil, capecitabine, cisplatin, oxaliplatin, irinotecan, pemetrexed, or exatecan, but all combinations failed to achieve significant improvement in overall survival.<sup>23–29</sup> Although much hope lay in novel targeted therapies such as the vascular endothelial growth factor inhibitor bevacizumab,<sup>30</sup> the matrix metalloproteinase inhibitor marimastat,<sup>31,32</sup> and the anti-epidermal growth factor receptor agent cetuximab,<sup>33,34</sup> none of these drugs alone or in combination with standard chemotherapies had a significant impact on patient outcome. Therefore, the relatively well tolerated nucleoside analogue gemcitabine remained the standard of care chemotherapy in most countries, mainly on the basis of modest patient benefit with only a marginal increase in median survival.<sup>35</sup>

The only targeted agent that has been approved for PDA patients is the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib (Tarceva®, Genentech, CA, USA), since the combination of gemcitabine and erlotinib conferred a marginal survival benefit over gemcitabine alone (6.24 months versus 5.91 months).<sup>36</sup> Although statistically significant, the clinical relevance remains questionable. More recently, the gemcitabine-free FOLFIRINOX protocol (folinic acid, fluorouracil, irinotecan, and oxaliplatin) was reported, and achieved a significant survival benefit for patients with metastatic PDA compared with gemcitabine monotherapy (11.1 months versus 6.8 months).<sup>37</sup> Although FOLFIRINOX significantly improves quality of life compared with gemcitabine,<sup>38</sup> severe side effects such as grade 3 and 4 neutropenia and dehydration limit the use of this aggressive combination chemotherapy to carefully selected patients with good performance status. More recently, results from the Phase III Metastatic Pancreatic Adenocarcinoma Trial (MPACT) in 861 patients comparing nanoformulated albumin-bound paclitaxel (nab-paclitaxel, Celgene, NY, USA) with gemcitabine and gemcitabine monotherapy were presented. The data show a significant survival benefit for the combination of nab-paclitaxel and gemcitabine (8.5 versus 6.7 months), with a reasonable toxicity profile.<sup>39</sup> Although the mechanism of action of nab-paclitaxel remains unclear,

its tolerability profile is superior to that of conventional paclitaxel dissolved in cremophor, and the MPACT regimen will likely become a new standard of care treatment and new backbone for novel targeted therapies in the future. Furthermore, the nab-paclitaxel and gemcitabine combination is undergoing investigation in localized resectable and unresectable PDA for its potential clinical benefit in these disease stages. Figure 1 shows the different clinical treatment algorithms for resectable, locally advanced, and metastasized PDA.

## Cellular and molecular evolution of pancreatic cancer

Pancreatic carcinogenesis occurs through an accumulation of genetic alterations that result in deregulation of tumor cell-autonomous and nontumor cell-autonomous pathways. These genetic changes are accompanied by typical morphologic and histologic alterations in epithelial, stromal, and inflammatory cells within the pancreas that eventually culminate in desmoplastic, highly invasive, and metastatic ductal adenocarcinoma. Activating mutations in the *K-ras* gene represent a signature event in almost all pancreatic cancers (>90%), followed by subsequent somatic mutations involving the tumor suppressor genes *p16*, *p53*, and *DPC4/SMAD4*.<sup>40–45</sup> Interestingly, the number of actual mutations of these key drivers of pancreatic carcinogenesis correlates positively with a poor prognosis and shortened survival for patients.<sup>46</sup> Mechanistically, oncogenic *K-ras* activation governs a multitude of mitogenic signals with profound cell-autonomous and noncell-autonomous effects that initiate epithelial transformation, dynamic rearrangement of a proinflammatory and immunosuppressive microenvironment, metabolic requirements, and finally drive frank malignancy.<sup>47–54</sup> Recent evidence from genetically engineered mouse models (GEMMs) with pancreas-specific and doxycycline-inducible expression of oncogenic *K-ras*<sup>G12D</sup> indicates that abrogation of mutant *K-ras* in established tumors led to dramatic tumor shrinkage and depletion of the surrounding stroma after only a few days,<sup>55,56</sup> further highlighting the key function of mutant *K-ras* in shaping tumor biology in PDA.

Global sequencing analysis and transposon-mediated insertional mutagenesis screens have also discovered genetic alterations at low frequency and provide multiple examples for the genomic instability and heterogeneity of PDA.<sup>57–59</sup> For the small subset of patients with inherited predisposition to PDA, several germline mutations, including *BRCA2*, *STK11/LKB1*, *p16/CDKN2A*, *ATM*,<sup>60</sup> and *PRSS1*, have been reported.<sup>10</sup>

Alongside these molecular discoveries, a unique histopathologic progression model similar to that of the adenoma-carcinoma sequence in the development of colon cancer<sup>61</sup> was proposed to describe the progression from a normal pancreas via preneoplastic lesions to invasive cancer.<sup>62,63</sup> Preneoplastic lesions are classified as pancreatic intraepithelial neoplasms (PanINs) 1a/b, 2, and 3, according to their stepwise accumulation of histopathologic and molecular alterations. The discovery that high-grade PanIN lesions increase the risk of developing PDA has sparked attempts to detect these lesions early by cross-sectional and endoscopic imaging techniques.<sup>16,64,65</sup> Further, early (partial) pancreatectomy in patients with high-grade PanIN lesions is considered in high-risk individuals (eg, familial PDA), but the ideal timing is still debated and data on overall survival from randomized clinical trials are currently not available.<sup>10</sup>

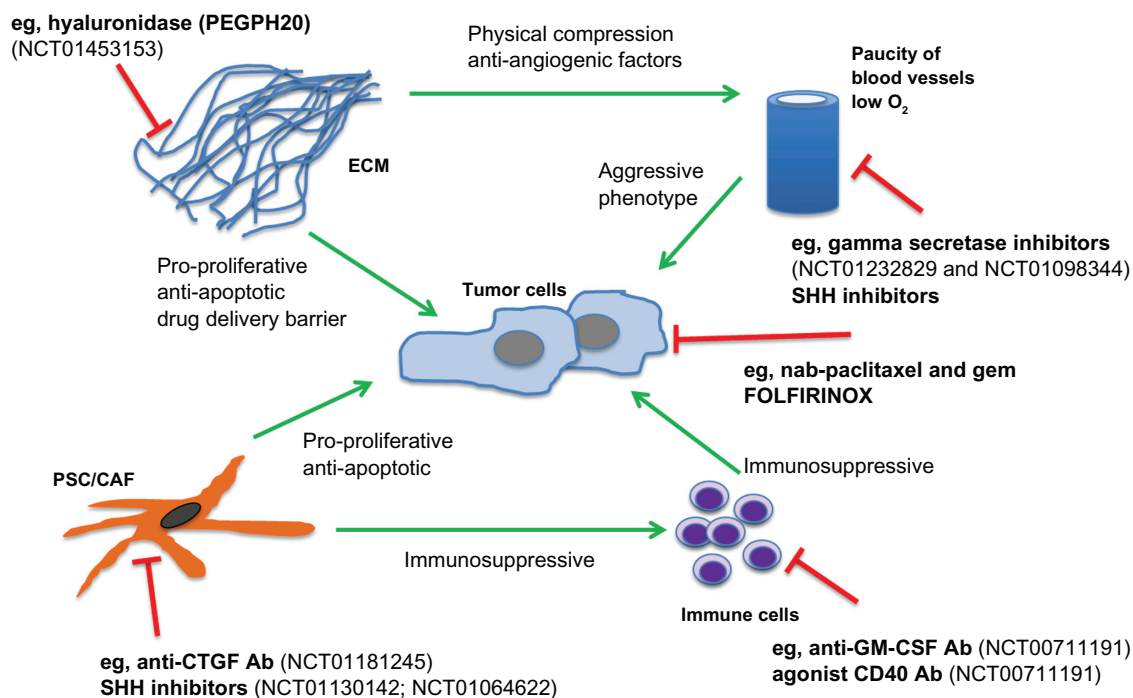
The traditional PanIN-PDA sequence (“ductal carcinogenesis”) has recently been challenged by the description of an alternative route of pancreatic carcinogenesis known as “acinar ductal carcinogenesis”. Careful histopathologic investigations by Esposito et al identified tubular complexes within areas of acinar ductal metaplasia that form atypical flat lesions and may bypass the common PanIN precursor stages and directly evolve to invasive cancer.<sup>66–68</sup> Histologic analysis of sporadic PDA cases confirmed the presence of tubular complexes in almost 80% of cases, and atypical flat lesions were also detected in cases of familial pancreatic cancer. These exciting findings need to be confirmed in larger series of sporadic pancreatic cancer cases and may provide novel insights into the development of PDA, with potentially profound implications in future diagnostic and preventive algorithms. Interestingly, earlier studies in GEMMs of pancreas cancer had also pointed towards a putative role of the acinar cell compartment in driving carcinogenesis with and without concomitant inflammation.<sup>69–72</sup>

## Role of the stroma in pancreatic cancer

Histologically, PDA is an extremely stroma-rich and hypovascular tumor, and indeed, most of the pancreatic tumor mass consists of activated (myo)fibroblasts, immune cells, and extracellular matrix components, such as collagen, desmin, fibronectin, and hyaluronic acid.<sup>9,73,74</sup> Over recent years, it has become increasingly evident that the complex network of soluble cytokines, growth factors, proteases, and extracellular matrix components collaboratively interact within the tumor microenvironment, sustaining and driving

cancer cell proliferation, invasion, early metastasis, and therapeutic resistance.<sup>75–80</sup> An important subtype within the stromal population is pancreatic stellate cells, which have emerged as pancreas-specific myofibroblasts and share morphologic and functional characteristics with hepatic stellate cells.<sup>81–83</sup> Activated pancreatic stellate cells secrete profibrotic proteins abundantly and interact with tumor cells in multiple ways to establish and maintain the pronounced desmoplastic reaction in PDA (Figure 2).<sup>84–86</sup> The clinical relevance is highlighted by histologic characteristics of patient samples, suggesting that the extent of the stromal reaction correlates with shortened survival in patients undergoing surgery.<sup>87</sup> The complex cellular and biochemical interactions of pancreatic stellate cells and cancer cells have limited faithful *in vitro* investigation to traditional two-dimensional coculture assays in the laboratory. Therefore, intensive efforts are currently being made to establish three-dimensional or organotypic culture systems where cancer cells and stromal cells can be grown within a reconstituted extracellular matrix gel to study the tumor-stromal crosstalk and test novel compounds.<sup>88,89</sup>

The advent of various GEMMs of pancreas cancer has marked a milestone for the scientific community in understanding the biological implications of the tumor stroma and provides ample opportunities for preclinical testing of novel agents directed against cell-autonomous and noncell-autonomous targets.<sup>90–93</sup> The most commonly used pancreatic cancer GEMM bears an activating *K-ras*<sup>G12D</sup> allele that is conditionally activated in pancreatic progenitor cells by crossing mice with transgenic strains that express Cre recombinase in pancreatic lineages (PdxCre or p48Cre). These mice are referred to as “KC” mice and develop murine PanIN lesions with 100% penetrance, and progress to PDA with a long latency.<sup>94</sup> The addition of a dominant negative mutation in the tumor suppressor gene *p53* (*Trp53*<sup>R172H/+</sup>) greatly accelerates pancreatic tumor development and penetrance at an early age, and these mice are accordingly termed “KPC” mice.<sup>95</sup> In contrast with traditional xenograft tumors, GEMMs faithfully recapitulate human PDA, including the presence of abundant tumor stroma and comorbidities such as cachexia, jaundice, metastasis to distant sites, and activation of biochemical pathways. Although the KPC is a faithful mouse



**Figure 2** Schematic of tumor microenvironment crosstalk and interdependence in PDA, with a particular focus on novel experimental therapeutic interventions and clinical trials. **Notes:** Left bottom panel: PSC and CAF exert immunosuppressive, growth-promoting, and antiapoptotic effects on tumor cells, and can be targeted by inhibition of SHH and CTGF. Upper left panel: ECM components providing a scaffold for tumor cells, creating barriers for drug delivery, and providing a variety of prosurvival signals for tumor cells. Upper right panel: Tumor vessels are compressed by dense tumor stroma, and vessel density is low due to antiangiogenic factors in the ECM scaffold. The hypoxic environment causes an aggressive tumor phenotype, and tumor vasculature can be targeted by SHH and gamma secretase inhibitors. Lower right panel: immune cells create an immunosuppressive microenvironment allowing pancreatic tumors to progress, and immunotherapeutic approaches such as agonist CD40 antibodies or anti-GM-CSF antibodies reverse this phenotype. Novel agents/regimens directly targeting tumor cells are nab-paclitaxel + gemcitabine and FOLFIRINOX. Selected ongoing and recently completed clinical trials are mentioned by National Clinical Trial (NCT) number, and details can be obtained online at <http://clinicaltrials.gov/>. The FOLFIRINOX protocol includes folinic acid, fluorouracil, irinotecan, and oxaliplatin.

**Abbreviations:** Ab, antibody; CAF, cancer-associated fibroblasts; CTGF, connective tissue growth factor; ECM, extracellular matrix; GM-CSF, granulocyte-macrophage colony-stimulating factor; PDA, pancreatic ductal adenocarcinoma; PSC, pancreatic stellate cells; SHH, Sonic Hedgehog; nab-paclitaxel, albumin-bound paclitaxel.

model of PDA, the resources and staffing required to maintain, image, and treat a large cohort of animals is substantial and beyond the practical abilities of a single laboratory.

The first ultrasound-guided and controlled preclinical study in KPC mice was performed by Ken Olive in the Tuveson laboratory and targeted the profibrotic Sonic Hedgehog (SHH) pathway. SHH plays important roles during pancreas organ development, and is re-expressed during malignant transformation to activate and expand stromal rather than epithelial cells, thus promoting desmoplasia in pancreatic carcinogenesis.<sup>96–98</sup> Strikingly, pharmacologic inhibition of SHH by the smoothed inhibitor IPI-926 resulted in marked stromal depletion and increased microvessel density and patency, paralleled by significantly improved delivery of several chemotherapeutics in the KPC mouse model.<sup>99</sup> Although the intrinsic effects of SHH inhibition on chemosensitivity could not be excluded, this study identified the tumor stroma as a biophysical barrier for drug delivery. Thus, the tumor microenvironment, in particular the dense tumor stroma, is now considered to be a potential reason for the failure of most systemic therapies in PDA. Indeed, hypoenhancing masses are visualized in PDA patients whenever contrast-enhanced imaging is used, and poor perfusion has been associated with an aggressive phenotype.<sup>100</sup>

## Novel targets in the microenvironment of pancreatic cancer

The biophysical role of the pancreatic tumor stroma as a barrier to drug delivery has been the focus of intensive clinical and preclinical research over the last 3 years, and also attracted attention in other tumor entities.<sup>101,102</sup> Surprisingly, an SHH inhibitor IPI-926 (saridegib, Infinity, MA, USA) and GDC-0449 (vismodegib, Genentech, CA, USA) both failed in Phase II clinical trials, and investigations are still ongoing to comprehend the discrepancy between the clinical and preclinical data. However, the SHH signaling cascade remains an intriguing and widely investigated pathway in PDA, and pharmacologic inhibition may still be a beneficial therapeutic option in the future, depending on the specific compound and cotreatments.<sup>103</sup>

Other solid and soluble components of the tumor microenvironment have been targeted in order to break down the extracellular matrix scaffold, relieve vessel compression, and increase drug accumulation within the tumor (Figure 2). One prominent example is the enzymatic depletion of hyaluronic acid, a glycosaminoglycan, by human recombinant PEGylated hyaluronidase (PEGPH20). Pancreatic cancers are extremely rich in the megadalton form of hyaluronic

acid, and the solvation of water by hyaluronic acid is thought to be responsible for the high interstitial fluid pressure in PDA that results in compression of intratumoral blood vessels.<sup>104,105</sup> The Hingorani group has shown that treatment with PEGPH20 in murine pancreas tumors results in decreased levels of intratumoral fluid pressure in PDA, and this group and ours have noted that treatment with PEGPH20 results in the re-expansion of blood vessels and improved drug delivery, accompanied by slowing of tumor growth and prolonged survival in KPC mice.<sup>106,107</sup> The results of a Phase I/II dose-escalation study (NCT01453153) with PEGPH20 in combination with gemcitabine in 28 patients with previously untreated stage IV pancreatic cancer were presented at the 2013 annual scientific meeting of the American Society of Clinical Oncology, and suggested promising efficacy, particularly in patients with a high intratumoral content of hyaluronic acid.<sup>108</sup>

Connective tissue growth factor (CTGF/CCN2) is a pleiotropic growth factor that is overexpressed in human and murine pancreas tumors. Therapeutic inhibition of CTGF using a monoclonal human antibody (FG-3019, Fibrogen, CA, USA) resulted in significantly increased induction of tumor cell apoptosis in KPC mice when combined with gemcitabine.<sup>78</sup> Notably, neither stromal depletion nor increased drug delivery was observed. Rather, stromal-derived CTGF impinged on the antiapoptotic machinery in tumor cells, and the X-linked inhibitor of apoptosis protein was down-regulated upon treatment with FG-3019. Finally, cotreatment with FG-3019 and gemcitabine resulted in slowing of murine tumors and prolonged survival in KPC mice.<sup>78</sup> FG-3019 in combination with gemcitabine and erlotinib (an epidermal growth factor receptor tyrosine kinase inhibitor) is currently being investigated in a Phase I safety and bioactivity study in patients with locally advanced and metastasized PDA (NCT01181245).

Secreted protein acidic and rich in cysteine (SPARC) is overexpressed by cancer-associated fibroblasts and represents another intriguing target in PDA. Results from a Phase I/II clinical study showed that patients with high stromal SPARC levels responded better to nab-paclitaxel and gemcitabine (median survival 17.8 months versus 8.1 months for low SPARC),<sup>109</sup> suggesting that the albumin-binding protein SPARC may act as a novel biomarker for PDA that retains nab-paclitaxel to accumulate the drug intratumorally. In contrast, two independent studies identified high expression of SPARC as a negative prognostic marker for patients with resectable and unresectable PDA.<sup>110,111</sup> Our group has undertaken several preclinical studies regarding SPARC and

the efficacy and mechanism of action of nab-paclitaxel in combination with gemcitabine. We reported remarkable therapeutic efficacy for nab-paclitaxel in highly treatment-resistant KPC tumors. Importantly, nab-paclitaxel was much better tolerated than cremophor-paclitaxel and could be administered in more than four-fold higher concentrations. Moreover, we identified a synergistic effect of nab-paclitaxel through reactive oxygen species-mediated degradation of the primary gemcitabine-metabolizing enzyme, cytidine deaminase. Therefore, combinations of nab-paclitaxel and gemcitabine resulted in increased intratumoral gemcitabine levels.<sup>112</sup> However, genetic ablation of SPARC in the KPC model neither resulted in decreased intratumoral (nab-) paclitaxel levels nor altered the response to treatment.<sup>113</sup> Nonetheless, elevated serum SPARC levels may play a role in paclitaxel uptake in certain PDA patients and could be investigated noninvasively in PDA patients prior to starting nab-paclitaxel-based chemotherapies.<sup>113</sup> The exact function of SPARC and the mechanism of action of nab-paclitaxel remains a subject of intense clinical<sup>114</sup> and preclinical investigation,<sup>115</sup> and will help to systematically evaluate the predictive power of different *in vivo* models by rigorous comparison with the human data. To this end, tissue analysis of the MPACT trial is anxiously awaited by the field and should provide more answers on this exciting topic.

Partly owing to the hypovascular state of PDA, hypoxia is considered a hallmark feature that may predict more aggressive behavior and impair the response to therapies by providing a niche for slow-cycling, highly drug-resistant cells.<sup>116–118</sup>

Chemotherapeutic agents such as TH-302 are selectively activated in the hypoxic tumor microenvironment and are currently being investigated in preclinical<sup>119</sup> and several clinical trials in PDA patients (NCT01746979, NCT00743379, NCT01144455, NCT01833546). Hypoxia is also known to stimulate the Notch signaling pathway, and gamma secretase inhibitors are currently under early clinical investigation in PDA patients (NCT01232829, NCT01098344). Experimental data in human cell lines and GEMMs underscore the therapeutic potential of Notch inhibitors in inducing treatment responses.<sup>120,121</sup>

The field of cancer immunotherapy is rapidly evolving and has recently provided fascinating insights into pancreatic carcinogenesis with potential therapeutic implications for patients. For instance, tumor-derived granulocyte-macrophage colony-stimulating factor has been ascribed a central role in mediating a proinflammatory and immunosuppressive tumor microenvironment in PDA, and abrogation

of granulocyte-macrophage colony-stimulating factor blocked tumor development by inhibition of Gr-1<sup>+</sup> CD11b<sup>+</sup> and recruitment of cytotoxic CD8<sup>+</sup> T-cells into the tumor microenvironment.<sup>122,123</sup> However, immune surveillance does not inevitably depend on therapy-induced T-cells. A combined Phase I preclinical-clinical study (NCT00711191) investigated the effects of a CD40 agonist antibody in 21 patients with metastatic PDA, and showed promising clinical activity, with tumor regression in some patients.<sup>124,125</sup> Mechanistically, activated macrophages, but not activated T-cell-infiltrated tumors, induced tumor cell death and depleted the tumor stroma.<sup>124</sup> This is the first clear example that shows how closely interconnected the immune cell and stromal cell compartment is in PDA, and suggests that critical cross points between immune and stromal cells must be interrupted in order to achieve robust treatment responses.

## Conclusion and future perspectives

Tumor-stromal interactions are highly complex and contribute to the key hallmarks of cancer, such as sustained proliferative signaling, angiogenesis, activation of invasion, and metastasis, as well as extracellular matrix remodeling.<sup>126</sup> More recently, the tumor microenvironment has been increasingly appreciated as being instrumental in mediating resistance to therapy in PDA and other cancers, thus opening up numerous avenues for therapeutic exploration, for both the biophysical and biochemical approaches described above.

Emerging evidence of metabolic reprogramming driven by oncogenic K-ras,<sup>56</sup> and the dependency on autophagy,<sup>51</sup> a catabolic pathway degrading cellular organelles and macromolecules, highlight additional metabolic targets that will be investigated in the near future. Further, exciting data have been reported recently by the Fearon group in Cambridge, UK, showing that a subtype of stromal cells expressing fibroblast activation protein- $\alpha$  not only cause failure of immune surveillance in murine tumors but may also contribute to tumor syndromes, such as cachexia and anemia,<sup>127</sup> symptoms that are most relevant for patient well-being and survival.

A critical and clinically relevant issue remains the dynamics of tumor cell dissemination, and whether PDA metastasizes early or late during disease progression. Whereas deep sequencing data for human PDA suggested a long latency (on average 17 years) for the occurrence of distant metastases,<sup>71</sup> a computational analysis of 228 PDA patients supported the notion that spread of malignant cells represents an early event during carcinogenesis, and most patients may harbor distant metastases at earlier disease stages than previously anticipated.<sup>128</sup> Provocative lineage tracing

experiments in GEMMs of pancreatic cancer proposed a mechanism by which single mutant cells detach from the basement membrane by epithelial-to-mesenchymal transition to enter the blood circulation prior to the development of frank malignancy. The disseminated cells seeded the liver and showed stem cell properties, a process that was further promoted by pancreatic inflammation.<sup>129</sup>

These studies must be confirmed independently but may transform our understanding of the evolution of pancreatic cancer and prioritize our efforts toward investigational clinical trials that compare neoadjuvant cytotoxic and anti-metastatic therapies with upfront surgery. Also, patients at risk for developing pancreatic cancer (eg, Peutz–Jeghers syndrome, hereditary pancreatitis, familial pancreatic cancer) should be considered for evaluation of anti-inflammatory and antimetastatic therapeutic approaches. Moreover, these studies may open new avenues to understand the genetic, epigenetic, and microenvironmental determinants that may explain long-term survivors and those who never develop metastases.<sup>130</sup>

GEMMs of PDA that recapitulate important aspects of the tumor microenvironment are critical tools for investigating tumor-stromal interactions in the laboratory and for testing novel compounds that target components of the microenvironment prior to clinical testing. Given the long list of failed clinical trials in the past, it remains speculative which compounds will make the difference for patients with pancreatic cancer. Therefore, it is timely for the field to consider including these GEMMs prior to evaluating therapies in the clinic.

To enhance our molecular understanding of treatment success or failure, clinicians should seek to obtain pretreatment biopsies from patients via endoscopic ultrasound-guided fine needle aspiration biopsy or endoscopic ultrasound-guided core biopsy. Limitations in the quantity and quality of biopsy samples requires optimized approaches to obtain tissue specimens for histologic and immunohistochemical analysis of stromal, inflammatory, and parenchymal tissue components,<sup>131</sup> and post-treatment biopsies are highly desirable for monitoring the effects of treatment on tumor biology and to prospectively explore potential biomarkers. Contrast-enhanced endoscopic ultrasound combined with elastography is an additional noninvasive technique that may provide useful information before and during therapies.<sup>114,132,133</sup> Further, experimental molecular imaging approaches in various mouse models of pancreas cancer have recently identified potentially promising candidates, such as plectin-1, cathepsins, and

the tight-junction protein claudin-4, that could be deployed for early detection,<sup>134–137</sup> and should now be rigorously evaluated in the clinical setting to improve early diagnosis of PDA.

To conclude, the last few years have seen a virtual explosion of knowledge in the field of basic and translational pancreatic cancer research, and we are hopeful that the continuing effort to translate these findings to the clinic will eventually benefit our patients. Targeting the tumor microenvironment provides a novel and much needed vantage point of attack, and we anticipate that several of the components within the tumor microenvironment described here will be exploited to achieve robust treatment responses for this recalcitrant tumor.

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## Disclosure

The authors report no conflicts of interest in this work.

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