The Pancreas Cancer Microenvironment

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is a common and lethal malignancy resulting in more than 250,000 deaths per year worldwide. Despite extensive efforts, cytotoxic and targeted therapies have provided only limited efficacy for patients with PDA to date. One contributing factor to the failure of systemic therapies may be the abundant tumor stromal content that is the characteristic of PDA. The PDA stroma, aptly termed the tumor microenvironment, occupies the majority of the tumor mass, and consists of a dynamic assortment of extracellular matrix components and nonneoplastic cells including fibroblastic, vascular, and immune cells. Recent work has revealed that the PDA stroma supports tumor growth and promotes metastasis and simultaneously serves as a physical barrier to drug delivery. Accordingly, methods that alter stromal composition or function, for instance interference with the vasculature via Notch/Hedgehog pathway inhibition or relief of vascular compression by hyaluronidase, are under active investigation. Here, we will review our current understanding of the PDA tumor microenvironment, and highlight opportunities for further exploration that may benefit patients. Clin Cancer Res; 18(16); 4266–76. ©2012 AACR.

Tumor Microenvironment—Achilles’ Heel of Pancreatic Cancer?

Pancreatic ductal adenocarcinoma (PDA) is an aggressive malignant disease of the exocrine pancreas with a 5-year survival rate of less than 5% (1). In the United States, it represents the fourth-leading cause of cancer-related deaths with an estimated 43,920 new cases and 37,390 deaths in 2012 (2). The majority of patients initially present with advanced and metastatic disease with only 10% to 15% of patients being candidates for surgical resection. Unfortunately, postsurgically, most patients still relapse despite adjuvant systemic therapies (3). This dismal prognosis is a result of the late diagnosis of the disease, the lack of biomarkers allowing early screening, the early metastatic dissemination, and ultimately the resistance to systemic therapies.

Recent years have seen significant advances in the treatment for many tumor types, including melanoma, lung, and colorectal cancer based on the rational design of targeted therapies directed at molecular alterations arising in cancer cells (4). Unfortunately, similar success has not occurred in PDA, which remains a lethal disease. Gemcitabine, the current standard-of-care chemotherapeutic, was approved mainly on the basis of patient benefit and produced only a modest increase in survival (5). Even targeted therapy approaches have had limited success so far. Indeed, the only other drug approved is for the EGFR receptor (EGFR) tyrosine kinase inhibitor erlotinib (Tarceva; Genentech), which, when combined with gemcitabine, increased overall survival from 5.91 to 6.24 months (6). A promising classical combination chemotherapy approach recently reported is FOLFIRINOX (oxaliplatin, irinotecan, leucovorin, and 5-fluorouracil), which achieved a significant survival benefit for patients with metastatic PDA compared with gemcitabine (11.1 vs. 6.8 months; ref. 7). Unfortunately, FOLFIRINOX is only suitable for patients with a good performance status due to increased toxicity. Therefore, new approaches are sorely needed for the vast majority of patients with PDA.

What are the reasons that most conventional and targeted therapies fail to provide substantial response rates in pancreatic cancer? The challenges faced by oncologists in the treatment of pancreatic cancer may in part be explained by the diverse influences exerted by the microenvironment on the cancer cells. Intriguingly, there is a huge discrepancy between the relative success and effectiveness of therapies, including gemcitabine, reported in preclinical assays (cell culture and xenograft mouse models) and subsequent failure in human PDA (8). Revealing the underlying molecular mechanisms of the microenvironment–tumor cell cross-talk is challenging due to the heterogeneous nature of the PDA stroma. Importantly, the generation of genetically engineered mouse models (GEMM) for pancreatic cancer that faithfully recapitulate the human disease, including resistance to gemcitabine, has enabled new approaches to understand the importance of the...
tumor microenvironment (TME) in disease pathogenesis and therapeutic response (9–11). These GEMMs are founded on early genetic analyses that revealed the presence of a single point mutation in the \( \text{KRAS} \) oncogene in more than 90% of the human PDA specimens (12). Subsequent genetic manipulation of the orthologous gene in mice showed that this mutation was sufficient to initiate the formation of premalignant ductal transformation (pancreatic intraepithelial neoplasia, PanIN). Further studies showed that the loss or mutation of tumor suppressor genes commonly acquired during human disease progression (\( \text{Tp53} \) and \( \text{Ink4a/Arf} \)) cooperates with \( \text{Kras} \) in mice to promote invasive cancer (13–15). More insight into the underlying genetic alterations in pancreatic cancer is provided by Iacobuzio-Donahue and colleagues in this \( \text{CCR Focus} \) section (16).

Pancreatic ductal adenocarcinoma is one of the most stroma-rich cancers. It is not uncommon for stromal components to outnumber cancer cells, as illustrated in Fig. 1. PDA stroma is very heterogeneous and comprises cellular and acellular components, such as fibroblasts, myofibroblasts, pancreatic stellate cells, immune cells, blood vessels, extracellular matrix (ECM), and soluble proteins such as cytokines and growth factors. The TME is not a static entity; rather, it is constantly changing in composition, especially in the progression from preneoplastic PanIN to invasive PDA. We aim to outline the current evidence for TME influences on multiple aspects of PDA. These include proliferation and survival, metastasis, resistance to therapy, and escape from immune control. We have limited our analysis to those studies carried out in the most relevant GEMMs or orthotopic tumor models as well as clinical data because...
Stromal Fibroblasts—The Pancreatic Stellate Cell

Pancreatic stellate cells (PaSCs) were identified in 1998 as a rare stromal cell type in the healthy pancreas (17, 18). Their periacinar star-shaped morphology, characteristic marker protein expression, and storage of fat droplets rich in vitamin A resembled hepatic stellate cells and inspired the name. Under homeostatic conditions, PaSCs are quiescent, and their physiologic role has yet to be delineated. Acute and chronic inflammatory conditions cause activation of PaSCs, which is characterized by morphologic changes, increased proliferation, deposition of ECM, and expression of α-smooth muscle actin (α-SMA) as well as the loss of the fat droplets (19). On the basis of the observation that activated PaSCs are detected in areas with high collagen content, it was postulated that PaSCs may be causally involved in the pathogenesis of pancreatic fibrosis (20). Although the embryonic origin of PaSCs has not yet been addressed, mesenchymal cells in the bone marrow are a likely source for PaSCs in adult mice after injury (pancreatitis and partial pancreatectomy) and in 7,12-dimethylbenz(a)anthracene (DMBA)-initiated sarcomatoid pancreatic tumors (21–23).

The scarcity of PaSCs and their limited life span in culture has prompted the generation of immortalized PaSC lines from human, rat, and mouse pancreata (24–30; Table 1). Such immortalized PaSCs have enabled the dissection of important cross-talk pathways between PaSCs and neoplastic PDA cells by coculturing in monolayers or 3-dimensional models. Indeed, it was recently reported that all-trans retinoic acid induced the quiescence of PaSCs, and this led to decreased proliferation and survival of pancreatic cancer cells in 3-dimensional coculture and in a GEMM (31). Therefore, PaSCs represent a resource that may be harnessed to explore the tumor-promoting aspects of tumor fibroblasts in PDA.

Cocultures of PaSCs and PDA cells have generally shown an enhancement of pancreatic cancer cell proliferation and migration by release of growth factors and cytokines (32). In vivo studies corroborate those findings, revealing that the coinjection of PaSCs with tumor cells in orthotopic models of PDA increases tumor size and causes a higher incidence of metastasis (25, 33). In a subsequent study, Xu and colleagues investigated the role of PaSCs in the metastatic process and found that they orchestrate metastatic dissemination by comigrating with neoplastic cells to potentially establish the appropriate metastatic niche or “soil” (34). Two recent publications may provide an additional explanation for the enhanced tumorigenicity of tumor cell/PaSC cotransplants. In vitro experiments showed that PaSCs increase the stem cell phenotype of pancreatic cancer cells and suggest that pharmacologic targeting of PaSCs could have unrecognized additional benefits (35, 36). The identification of major signaling pathways activated in PaSCs in response to contact with cancer cells will be an interesting platform on which to develop therapies targeting PaSCs. For instance, the mitogen-activated protein kinase pathway plays a prominent role in the response to mitogenic stimuli of which platelet-derived growth factor (PDGF) seems most potent (37, 38). Other potential targets include stimulators of the fibrinogen program such as fibroblast growth factor, downstream effectors generated by transforming growth factor β, connective tissue growth factor, and EGF. Additional insights may be gleaned through investigation of pathways known to be relevant for hepatic stellate cell activation (39). Finally, targeting specific pathways germane for PaSC–neoplastic cell cross-talk may also modulate other aspects of the TME, including the vascular and immune system.

Extracellular Matrix as an Obstacle to Therapy

Pancreatic ductal adenocarcinoma is histologically characterized by the abundance of ECM, commonly also referred to as desmoplasia (Fig. 1A–D). ECM components include collagen, fibronectin, proteoglycans, and hyaluronic acid, as well as catalytically active enzymes and proteases. The accumulation of ECM components distorts the normal architecture of pancreatic tissue inducing an abnormal configuration of blood and lymphatic vessels (40–42). One factor potentially contributing to therapeutic resistance

### Table 1. Overview of published pancreatic stellate cell lines of human, mouse, or rat origin

<table>
<thead>
<tr>
<th>Name of cell line</th>
<th>Source</th>
<th>Method of immortalization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLT-PaSC</td>
<td>Primary human from chronic pancreatitis</td>
<td>SV40 large T antigen + hTERT</td>
<td>26</td>
</tr>
<tr>
<td>25HPaSC</td>
<td>Human PDA</td>
<td>SV40 large T antigen + hTERT</td>
<td>25</td>
</tr>
<tr>
<td>PS-1</td>
<td>Human pancreas (transplantation tissue)</td>
<td>hTERT</td>
<td>24</td>
</tr>
<tr>
<td>irPaSC</td>
<td>Sprague-Dawley rat</td>
<td>SV40 large T antigen</td>
<td>28</td>
</tr>
<tr>
<td>SAM-K</td>
<td>Male Wistar rat</td>
<td>SV40 large T antigen</td>
<td>29</td>
</tr>
<tr>
<td>SIPS</td>
<td>Male Wistar rat</td>
<td>Spontaneous immortalization</td>
<td>27</td>
</tr>
<tr>
<td>LTC-7 and LTC-14</td>
<td>Male LEW.1W</td>
<td>SV40 large T antigen</td>
<td>30</td>
</tr>
<tr>
<td>imPaSC</td>
<td>C57BL/6 mouse</td>
<td>SV40 large T antigen</td>
<td>28</td>
</tr>
</tbody>
</table>
in PDA may be the rigidity of the ECM that compresses blood vessels, leading to reduced perfusion that ultimately impedes the delivery of drugs to neoplastic cells. Indeed, we previously reported that the concentration of an active intracellular metabolite of gemcitabine, 2',2-difluorodeoxy-cytidine triphosphate (dFdCTP), was high in stroma-poor subcutaneous or orthotopic xenografts/syngeneics but hardly detectable in stroma-rich PDA tumors in a GEMM (11). Further analysis revealed that transplanted tumors exhibited an increased vascular content and function as compared with primary GEMM tumors and human PDA. Because sonic hedgehog (SHH) signaling has been shown to be restricted to the stromal compartment and to enhance the desmoplastic reaction (43, 44), our laboratory reasoned that pharmacologic inhibition of the Shh pathway may have a positive impact on gemcitabine delivery. As predicted, the combination of a smoothened inhibitor (IPI-926) with gemcitabine caused depletion of tumor stroma and resulted in increased microvessel density and patency (11). This alteration of the TME was paralleled by significantly enhanced intratumoral concentrations of dFdCTP, transient disease stabilization, and a survival benefit (11). Several clinical trials have been initiated as a result of this and are recruiting patients to investigate the mechanism and treatment effect of pharmacologic SHH inhibitors in patients with pancreatic cancer (http://clinicaltrials.gov; NCT01195415, NCT01064622, NCT01130142, and NCT01096732). Unfortunately, Infinity Pharmaceuticals announced in January 2012 that it was halting its phase II trial of the smoothened inhibitor IPI-926 plus gemcitabine (NCT01130142). This is very surprising in light of the encouraging results of 31% partial response rate (10% for gemcitabine only) reported in a previous phase Ib trial (ASCO 2011, abstract 4114). An analysis of this trial is under way by the investigators conducting this trial.

Secreted protein acidic and rich in cysteine (SPARC) represents another proposed target to facilitate depletion of the tumor stroma in pancreatic cancer. SPARC is over-expressed by fibroblasts in the TME of human and murine PDA (Fig. 1C) and has been shown to inversely correlate with survival (45, 46). A novel drug formulation consisting of paclitaxel associated with albumin [Abraxane (Celgene) or nab-paclitaxel] has been hypothesized to accumulate in and potentially deplete PDA tumor stroma via binding of albumin to SPARC-positive fibroblasts, thus representing a mechanism for targeting a specific cell type within the PDA TME (47). The first clinical trial of gemcitabine in combination with nab-paclitaxel showed a promising overall survival of 12.2 months, and the subset of patients with elevated SPARC expression was correlated with increased survival in this study. The potential role of SPARC as a predictive biomarker for positive responsive to nab-paclitaxel and gemcitabine contrasts with a separate report that showed poor prognosis for patients who had SPARC-enriched tumors resected and received standard adjuvant therapy (45, 46). In this trial, patients with high SPARC levels had a mean overall survival of 17.8 months as compared with 8.1 for low SPARC (48). A preclinical study in a GEMM from our laboratory confirmed the remarkable efficacy of nab-paclitaxel in combination with gemcitabine, although in contrast to the data originating from patient-derived xenografts, we did not observe stromal depletion in our preclinical setting. Instead, we reported a mechanism involving impaired gemcitabine metabolism due to reactive oxygen species–mediated degradation of cytidine deaminase (49). Furthermore, in depth investigations are required to elucidate the exact role of SPARC as a novel biomarker for patients with PDA, in particular, whether treatment with nab-paclitaxel represents the sole determinant for its prognostic impact.

Another possible strategy to relieve vessel compression and aid drug delivery is to enzymatically break down the ECM scaffold. Many cancers are rich in hyaluronan (HA), a megadalton glycosaminoglycan that retains water due to its high colloid osmotic pressure (Fig. 1D; ref. 50). This provides elasticity to connective tissue in healthy organs, but excessive HA accumulation in solid tumors raises interstitial fluid pressure and compresses blood vessels. We and others have recently shown in a GEMM of PDA that enzymatic remodeling of the ECM is indeed a promising avenue. Hyaluronan degradation by hyaluronidase PEGPH20 decreased interstitial fluid pressure in murine PDA tumors (51), as shown previously in a prostate cancer xenograft model (52). Consequently, increased vessel patency, drug delivery, and survival were observed (51, 53). It will be interesting to see if this concept holds true in human pancreatic cancer when the results of an ongoing phase I/II trial of hyaluronidase (PEGPH20) plus gemcitabine will be released (NCT01453153).

The Conundrum of Hypovascularity in Pancreatic Cancer

As alluded to in the previous paragraph, the vasculature in PDA is profoundly affected by the excessive desmoplasia. As a consequence, vascular dysfunction represents a major obstacle to pharmacodelivery. Moreover, how cancer cells maintain their nutrient demands to fuel the rapid growth despite the lack of adequate perfusion is elaborated upon in the accompanying article by Le and colleagues in this CCR Focus section (54). The discovery of the hypovascularity and perfusion impairment has broken with the general assumption of an "angiogenic switch" required for tumor progression (55, 56). Unlike pancreatic neuroendocrine tumors, which are clearly dependent on angiogenic factors, their exocrine counterparts seem to thrive without the requirement for excessive angiogenesis (11, 57). In fact, hypovascularity and perfusion impairment have long served as diagnostic tools in the imaging of pancreatic masses (58, 59), but mechanisms behind these histopathologic features have not been fully elucidated.

Transcriptional analysis has previously shown a gradient of angiogenic activation from normal pancreas to PDA (60). Consistent with this finding, components of the prototypically angiogenic VEGF pathway are highly expressed in tumor cells and associated endothelia (61, 62). Despite this,
tumor samples show substantially lower microvessel densities (MVD) than those of the normal pancreas (Fig. 2; ref. 11, 57). Although VEGF immunostaining is positively correlated with MVDs, it has limited association with patient survival (63, 64). Despite these contradictory findings, antiangiogenic therapy was tested in PDA. Initial approaches targeted matrix metalloproteinases using marimastat and BAY 12-9566 as well as the integrins αVβ3 and αVβ5 using cilengitide. These compounds did not provide any clinical benefit in trials (65–67). More recently, targeted agents such as bevacizumab, an anti-VEGFA monoclonal antibody, have been investigated in advanced pancreatic cancer in combination with gemcitabine and did not improve survival compared with gemcitabine plus placebo in a randomized phase III trial (5.8 vs. 5.9 months; ref. 68). In addition, there was no significant benefit to overall survival in combining bevacizumab with erlotinib and gemcitabine compared with the combination of the latter 2 compounds (7.1 vs. 6.0 months; ref. 69). Bevacizumab has also been evaluated in other combinations including with docetaxel and with concurrent capetitabine and radiation without any proven benefit, although certain studies are still under way (70, 71). VEGF receptor inhibition using axitinib in combination with gemcitabine also had no beneficial effect on overall survival (72). In addition, the kinase inhibitor sorafenib, which targets VEGFR as well as PDGFR, c-KIT, RafI, and FLT3, was found to be inactive in advanced pancreatic cancer (73). Likewise, discouraging results testing sunitinib in a preclinical trial in the LSL-KrasG12D results testing sunitinib in a preclinical trial in the advanced pancreatic cancer (73). Likewise, discouraging kinase inhibitor sorafenib, which targets VEGFR as well as beneficial effect on overall survival (72). In addition, the axitinib in combination with gemcitabine also had no still under way (70, 71). VEGF receptor inhibition using without any proven benefit, although certain studies are also been evaluated in other combinations including with docetaxel and with concurrent capetitabine and radiation without any proven benefit, although certain studies are still under way (70, 71). VEGF receptor inhibition using axitinib in combination with gemcitabine also had no beneficial effect on overall survival (72). In addition, the kinase inhibitor sorafenib, which targets VEGFR as well as PDGFR, c-KIT, RafI, and FLT3, was found to be inactive in advanced pancreatic cancer (73). Likewise, discouraging results testing sunitinib in a preclinical trial in the LSL-KrasG12D, p53<sup>fl/fl</sup>/Ptf1a-Cre model add to mounting evidence of angiogenic independence and dominance of tumor-driven angiostasis of PDA (57). This phenotype suggests that endogenous inhibitors in the microenvironment might exert an overriding angiostatic effect during the natural history of PDA. Many of these factors are generated from plasma and ECM proteins by proteases, which are frequently upregulated in tumor and stellate cells (PaSCs; ref. 74). For example, angiostatin and endostatin are produced by PDA and detected at high concentrations in patients’ circulation (75, 76). Moreover, whereas activated PaSCs are ostensibly proangiogenic, their coculture with tumor cells robustly increases endostatin levels, showing the angiostatic potential of such heterotypic interactions (77).

On the whole, antiangiogenic therapies have proved not to be a viable option for pancreatic cancer, and although therapeutic delivery may contribute to these failures, alternative approaches targeting the PDA vasculature remain attractive and potentially feasible. Two scenarios are possible: If PDA maintains itself on frugal use of restricted resources as a consequence of limited perfusion, it may be conceivable that impairing perfusion even more could tip the balance toward widespread hypoxic necrosis (78). In contrast, increasing tumor perfusion may seem counterintuitive but could synergize with cytotoxic therapy to increase the intratumoral drug delivery and response.

**Is PDA Hypoxic?**

The previous paragraph laid the groundwork for this question. Most solid tumors contain areas of below-optimal oxygen concentration (hypoxia). This occurs as a result of inefficient tumor vascular supply and a high metabolic need for oxygen (79). Many studies have provided evidence that hypoxic cells are more resistant to both chemotherapy and radiotherapy and can increase their invasive and metastatic potential, ultimately creating a more aggressive disease (80, 81). The ability of cancer cells to survive under these hypoxic conditions results from the ability to co-opt pathways necessary for embryonic development under hypoxic conditions. The main pathway involved in the hypoxic response is the hypoxia-inducible factor (HIF) pathway (82). HIF can induce a wide range of gene products controlling cellular metabolism and energetics, cell survival, migration, and pH (83). The HIF transcription factors also direct the transcription of many angiogenic growth factors (84).

Considering that the hypovascular nature of PDA has a significant impact on perfusion and drug delivery (11), it would be reasonable to assume a hypoxic state. However, direct evidence is sparse and the majority of publications have used surrogate markers for measuring hypoxia, such as necrosis or expression of HIF target genes (85–87). Only one small study, involving 7 patients with pancreatic cancer, has directly measured the oxygen pressure during pancreaticoduodenectomy by inserting needle electrodes. This study revealed a dramatic reduction in oxygenation of tumor tissue versus normal pancreas (88). Interestingly, preclinical work in an orthotopic model of pancreatic cancer has shown a lack of correlation between MVD and hypoxia, perhaps suggesting that a hypovascular pancreatic tumor is not directly linked to hypoxia (89). Nevertheless, this work did predict more aggressive behavior, including a more metastatic phenotype in hypoxic tumors. Clinical
work is under way to assess the prognostic significance of these results in patients with pancreatic cancer, using the hypoxia probe, pimonidazole, administered 24 hours before surgery (NCT01248637).

The reason hypoxia poses a challenge to the field of anticancer therapeutics is that it provides a niche for slow-cycling, highly drug-resistant cells, which may be identical to the proposed cancer stem cells (90, 91). Thus, standard chemotherapy agents fail because they are unsuccessful at targeting the cell within the hypoxic TME, which might be those that most need to be eliminated (92). In addition, hypoxic conditions are also known to stimulate the Notch signaling pathway, and it has recently been shown that pancreatic cancer cells can be sensitized by Notch inhibition (78). Thus, the hypovascular state of PDA could be exploited by novel therapeutic approaches such as hypoxia-activable prodrugs (93). Further investigations are required into the downstream Notch targets that are important for tumor cell survival under hypoxic conditions. Currently, a clinical trial is investigating the benefit of combining Notch pathway inhibition and gemcitabine (NCT01232829 and NCT01098344).

Out of Balance—Immune Cells in PDA

As is the case with other cancer types, inflammation seems to be crucially linked to PDA development as exemplified by chronic pancreatitis being a major risk factor (94). However, the molecular details are still obscure and are just beginning to be elucidated (95, 96). A comprehensive analysis of the immune cell composition of PanIN and PDA in the LSL-Kras<sup>G12D</sup>;Pdx1-Cre mice defined an important baseline for future studies (97). Use of enzymatic tumor digestion followed by fluorescence-activated cell sorting analysis revealed that immune cells make up roughly 50% of the tumor cell mass (Fig. 3 illustrates similar findings by immunofluorescence). From this study, it is...
apparent that immunosuppressive cell types, such as regulatory T cells and myeloid-derived suppressor cells, are predominant, with hardly any CTLs infiltrating the tumors. This paints a picture of a striking imbalance in protumorigenic and antitumorigenic immune cells. To add to the complexity, a recent study revealed a new immunosuppressive cell type in the stroma of PDA and other cancers. This cell expresses fibroblast activation protein \( \alpha \) (FAP\(\alpha\)), and FAP\(\alpha\) cell ablation resulted in immunologic control of tumor growth in several subcutaneous tumor models (98).

Successful immunotherapy depends on the cancer cells expressing proteins that can be recognized as altered by the immune system. These fall in 2 categories: Tumor-associated antigens are nonmutated self proteins that are aberrantly regulated (overexpressed or expressed in other tissues or oncofetal antigens), whereas tumor-specific antigens are generated as a consequence of the mutational events in neoplastic cells and are de novo antigens. The goal is to induce high-affinity cytotoxic T cells (CTL or CD8 T cells) without causing autoimmunity. Antigens targeted in immunotherapy clinical trials in PDA have included MUC1, mesothelin, KRAS, carcinoembryonic antigen, survivin, and telomerase, as well as whole tumor cells engineered to express granulocyte macrophage colony-stimulating factor [GM-CSF; reviewed in Dodson and colleagues (99)].

In the first phase I clinical trial using irradiated allogeneic GM-CSF–secreting tumor vaccine the treatment was well tolerated and found to be safe for use in humans (100). This result warranted a larger phase II trial to investigate the disease-free and overall survival after surgical resection followed by chemoradiation and vaccination, which reported a median survival of 24.8 months (101).

Another approach is to pulse dendritic cells with tumor antigens \( \text{ex vivo} \) and reinfuse them into patients. Muc1-pulsed dendritic cells were evaluated in a phase I/II trial in patients with resected pancreatic and biliary tumors. The vaccine transiently increased the percentages of functional

### Table 2. Summary of past and current clinical trials targeting components of the TME

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Current phase</th>
<th>Trial identifier</th>
<th>Status</th>
<th>Median survival</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Erlotinib + gemcitabine</td>
<td>III</td>
<td>NCT00026338</td>
<td>Completed</td>
<td>6.24 vs. 5.91 mo</td>
<td>6</td>
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<tr>
<td>Oxaliplatin + irinotecan + leucovorin + 5-FU</td>
<td>II/III</td>
<td>NCT00112658</td>
<td>Completed</td>
<td>11.1 vs. 6.8 mo</td>
<td>7</td>
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<tr>
<td>GDC-0449 + gemcitabine</td>
<td>II</td>
<td>NCT01064622</td>
<td>Active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPI-926 + gemcitabine</td>
<td>II/III</td>
<td>NCT01130142</td>
<td>Stopped</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>nab-Paclitaxel + gemcitabine</td>
<td>II/II</td>
<td>NCT00398086</td>
<td>Completed</td>
<td>12.2 mo</td>
<td>48</td>
</tr>
<tr>
<td>PEGPH20 + gemcitabine</td>
<td>II/II</td>
<td>NCT01453153</td>
<td>Active</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marimastat + gemcitabine</td>
<td>III</td>
<td>N/A</td>
<td>Completed</td>
<td>5.44 vs. 5.39 mo</td>
<td>65</td>
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<tr>
<td>Clengitide + gemcitabine</td>
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<td>N/A</td>
<td>Completed</td>
<td>6.7 vs. 7.7 mo</td>
<td>66</td>
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<tr>
<td>BAY 12-9566</td>
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<td>Completed</td>
<td>3.7 vs. 6.6 mo</td>
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<tr>
<td>Bevacizumab + gemcitabine</td>
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<td>N/A</td>
<td>Completed</td>
<td>5.8 vs. 5.9 mo</td>
<td>68</td>
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<tr>
<td>Bevacizumab + erlotinib + gemcitabine</td>
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<td>N/A</td>
<td>Completed</td>
<td>7.1 vs. 8.0 mo</td>
<td>69</td>
</tr>
<tr>
<td>Bevacizumab + docetaxel</td>
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<td>N/A</td>
<td>Completed</td>
<td>4.1 vs. 5.4 mo</td>
<td>70</td>
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<tr>
<td>Radiotherapy + capcitabine</td>
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<tr>
<td>Axitinib + gemcitabine</td>
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<td>NCT00471146</td>
<td>Completed</td>
<td>8.5 vs. 8.3 mo</td>
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</tr>
<tr>
<td>Sorafenib + gemcitabine</td>
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<td>N/A</td>
<td>Completed</td>
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<td>RO4929097</td>
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<td>Irradiated allogeneic GM-CSF–secreting tumor vaccine</td>
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<tr>
<td>MUC1 peptide-loaded dendritic cell vaccine</td>
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<td>Completed</td>
<td>26 mo</td>
<td>102</td>
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<td>Ipilimumab</td>
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<td>NCT00112580</td>
<td>Completed</td>
<td>N/A</td>
<td>103</td>
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<tr>
<td>CP-870,893 + gemcitabine</td>
<td>I</td>
<td>NCT00711191</td>
<td>Completed</td>
<td>7.4 mo</td>
<td>104</td>
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<td>Mechanistic studies</td>
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<tr>
<td>GDC-0449 + gemcitabine</td>
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<td>NCT01195415</td>
<td>Active</td>
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<td>GDC-0449</td>
<td>II</td>
<td>NCT01096732</td>
<td>Active</td>
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<tr>
<td>Preoperative pimonidazole</td>
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<td>NCT01248637</td>
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CD4 and CD8 T cells as well as regulatory T cells. Four of 12 (33%) patients in this study were alive 5 years after surgery, with a median survival of 26 months (range, 13–69 months; ref. 102). Both of these studies compare favorably in terms of the median survival for resected pancreatic cancer, which is normally between 11 and 20 months. A third approach is the use of blocking/neutralizing antibodies such as ipilimumab, which targets CTLA-4, a surface protein expressed by activated T cells that confers inhibitory signals. Unfortunately, ipilimumab as a single agent was found to be ineffective in a phase II trial in locally advanced and metastatic pancreatic cancer. However, one of the patients on this study experienced significant delayed regression of the primary tumor and 20 hepatic metastases, which may merit further investigation (103).

GEMMs are an ideal system to evaluate immune therapeutic approaches in PDA; however, few reports exist to date on this topic. This may be because the tumor antigens for PDA are unknown, making the tracing of immune responses very difficult. Nonetheless, a recent immunotherapy study used the “KPC” GEMM (LSL-KrasG12D;LSL-P53R172H;Pdx1Cre) to evaluate whether activation of antigen-presenting cells via stimulation of CD40 would result in increased tumor antigen presentation and priming of effector T cells (104). Treatment with agonistic anti-CD40 achieved tumor stabilization and even regression in KPC mice but was surprisingly T-cell independent. Instead, tumor control was exerted by the activated macrophages targeting the fibrotic stroma. Furthermore, an early-phase clinical trial with anti-CD40 antibody showed promising results in patients (104).

Conclusions and Future Outlook

The influences of the stroma in pancreatic cancer are as manifold as its components (Fig. 4). This curse may be turned into a blessing as this complexity also provides numerous avenues for therapeutic exploration (clinical trials mentioned in this review are summarized in Table 2). Accumulating evidence suggests that the extensive desmoplastic reaction may be, at least, partly responsible for the innate chemoresistance in pancreatic tumors by creating barriers that fence off tumor cells from circulating active therapeutic compounds. Breaching this stromal barrier represents a promising strategy to improve the delivery and efficacy of cytotoxic drugs in the future. Therapeutic benefit may be gained by strategies aimed at depleting the desmoplastic stroma, exploiting the poor vasculature, or activating the immune system to target tumor cells. We anticipate that future therapies will have to be tailored to target several of the described components of the microenvironment to achieve long-lasting therapeutic response.

Disclosure of Potential Conflicts of Interest

D.A. Tuveson is a Senior Group Leader at the Cancer Research UK Cambridge Research Institute. No potential conflicts of interests were disclosed by the other authors.

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