Pancreatic ductal adenocarcinoma (PDAC) is increasing in incidence and is projected to become the second leading cause of cancer death in the United States. Despite significant advances in understanding the disease, there has been minimal increase in PDAC patient survival. PDAC tumors are unique in the fact that there is significant desmoplasia. This generates a large stromal compartment composed of immune cells, inflammatory cells, growth factors, extracellular matrix, and fibroblasts, comprising the tumor microenvironment (TME), which may represent anywhere from 15% to 85% of the tumor.

It has become evident that the TME, including both the stroma and extracellular component, plays an important role in tumor progression and chemoresistance of PDAC. This review will discuss the multiple components of the TME, their specific impact on tumorigenesis, and the multiple therapeutic targets.

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This article is a part of a review series on benign and neoplastic pancreatic lesions from their pathologic to molecular profiles and diagnoses.
Pancreatic Tumor Biology

The development of PDAC has been shown to progress because of an activating mutation in the KRAS oncogene, resulting in acinar to ductal metaplasia, followed by subsequent progression through increasing grades of pancreatic intraepithelial neoplasia (PanIN) and ultimately PDAC after acquiring additional somatic mutations in multiple tumor suppressor genes, including p16/CDKN2A, TP53, and SMAD4, and the overexpression of HER2-2/neu in mouse models.11-14 The progression from acinar cell to PDAC is accompanied by a profuse fibrotic stromal desmoplasia, which is the basis of a complex tumor microenvironment (TME) (Figure 1). This microenvironment is heterogeneous and is composed of a cellular and acellular component. The cellular component includes stroma, cancer-associated fibroblasts, myofibroblasts, pancreatic stellate cells (PSCs), blood vessels, and immune cells. The acellular component is made up of collagen, fibronectin, and multiple soluble factors, including cytokines, chemokines, and growth factors residing in the extracellular matrix (ECM).7-9,15 This microenvironment is not static and is constantly changing composition. Thus, this is a challenging system to generate in animal models. However, the LSL-KrasG12D/LSL-Trp53R172H/+;Pdx-1-Cre (KPC) mouse model of PDAC, introduced in 2005, recapitulates the dense stromal reaction and many of the key features of the immune microenvironment observed in human PDAC16 (Figure 2). The generation of this mouse model of PDAC has significantly advanced the study of the complex microenvironment of PDAC as well as potential therapeutic targets.

Stroma

Pancreatic Stellate Cells

Pancreatic stellate cells, first identified in 1998 in normal rat pancreatic tissue and later in normal human pancreas, function to maintain connective tissue architecture but are quiescent.17 In the normal human pancreas, PSCs comprise approximately 4% to 7% of the parenchymal cells and contain cytoplasmic lipid droplets containing vitamin A in the quiescent state.18 When activated, PSCs play an integral role in synthesis and deposition of ECM.19

PSCs have been isolated from patients with both chronic pancreatitis and PDAC.19 Chronic inflammation of the pancreas activates PSCs, resulting in loss of vitamin A droplets, a myofibroblast-like phenotype; expression of cytoskeletal protein α-smooth muscle actin (α-SMA); and deposition of excessive ECM.17,18,20 Activated PSCs have been identified in both human and mouse samples in regions containing pancreatic fibrosis.21

In the setting of carcinogenesis, these activated PSCs can be observed as early as in the preneoplastic PanIN stages.15,17 Apte and colleagues18,20 noted colocalization of α-SMA with procollagen mRNA in PDAC samples, which was the first suggestion that activated PSCs were responsible for the majority of collagen in the stroma of human PDAC tumor samples. They further showed that, in vitro, human PDAC conditioned medium resulted in activation of PSCs and an increase in proliferation of these cells.22 In this setting, the PSCs not only deposit excessive ECM, but also foster a network of inflammatory cells, acinar cells, and transformed cancer cells.19,23,24 Activated PSCs show differential expression of multiple genes, including an up-regulation of matrix metalloproteinase-3 by 32.25-fold and a down-regulation of the basement membrane component, collagen type IVα1 by 2.25-fold, which may contribute to their remodeling of the ECM in the activated setting.20 In addition, they secrete peristin, a cell adhesion protein that stimulates cancer cell growth and confers resistance of these cancer cells to starvation and hypoxia.25 The activated PSCs are involved in an autocrine loop with peristin, leading to an increase in collagen I, transforming growth factor-β1, and fibronectin, ultimately resulting in increased chemoresistance.20,25

PSCs activated in the setting of carcinogenesis show an increase in proliferation and migration mediated by platelet-derived growth factor. The increased production of fibronectin and collagen I is mediated by transforming growth factor-β1 and fibroblast growth factor 2.17,18,21,22 In addition, cyclooxygenase-2, expressed by PSCs, is important for the interaction between PSCs and PDAC cells. Studies using conditioned medium from cancer cells

Figure 1  A: Overview of hematoxylin and eosin section: pancreatic ductal adenocarcinoma with desmoplastic fibrous response. B: Desmoplastic environment, including acute and chronic inflammation. Circle indicates a partially invaded islet. Original magnification: ×4 (A); ×20 (B).
resulted in an increased expression of cyclooxygenase-2 and proliferation of PSCs. Furthermore, a cyclooxygenase-2 inhibitor prevented PSC proliferation in the same conditioned medium.\textsuperscript{26} Members of the trefoil factor (TFF) protein family, including TFF1 and TFF2, normally excreted by gastrointestinal mucosa in response to damage, are both expressed in the setting of PDAC.\textsuperscript{27,28} TFF1 is known to stimulate proliferation of PSCs as well as PSC migration and PDAC cell invasion.\textsuperscript{26} TFF2 is known to increase PDAC cell migration via the chemokine receptor type 4, which has elevated expression in both PanINs and PDAC. It has also been implicated in metastasis via downstream activation of Akt and extracellular signal-regulated kinase pathways.\textsuperscript{29}

\textit{In vivo} studies have shown that patients whose PDAC tumors have fibrotic foci have shorter survival than those without.\textsuperscript{30} In addition, high \(\alpha\)-SMA/collagen ratios in pancreatic tumors have also been correlated with worse prognosis.\textsuperscript{31}

### Immune Component

Although the TME of PDAC is dominated by dense stroma, it is also replete with immune cells (Figure 3).\textsuperscript{32} This presence of immune cells, which can also be found in conjunction with inflammation, has been tied to the transition from normal pancreas to PDAC. This is demonstrated by the increased incidence of PDAC reported in patients with chronic pancreatitis.\textsuperscript{33} Chronic inflammation in these patients is postulated to occur because of persistent activation of the immune response after release of inflammatory mediators, including IL-6 and tumor necrosis factor-\(\alpha\).\textsuperscript{34} These immunoinflammatory cells exhibit altered function and ultimately result in production of immunosuppressive signals, as well as inflammatory cytokines that promote tumor progression and invasion.\textsuperscript{35} Specifically, M2 tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells appear in precursor lesions of PDAC and persist through invasive cancer, inducing immunosuppression in mouse models.\textsuperscript{36}

#### Tumor-Associated Macrophages

TAMs have been identified in many tumor types, and the distribution is associated with prognosis. In PDAC, tumor cells induce differentiation and education of the macrophages to the M2 phenotype (characterized by CD163 and CD204), and in turn, enhance the progression of tumor growth, angiogenesis, and metastasis.\textsuperscript{37,38} TAMs have been studied as a therapeutic target in several tumor types, including PDAC. A phase 2 trial targeting TAMs with trabectedin in patients with metastatic pancreatic cancer was recently completed (\textit{www.clinicaltrials.gov}; trial identifier NCT01339754). Trabectedin causes caspase-8-dependent apoptosis and expression of receptors in macrophages, ultimately targeting mononuclear phagocytes.\textsuperscript{39} There are multiple other potential therapeutic targets of TAMs, including colony-stimulating factor 1 receptor, which is expressed by macrophages. Blockade of colony-stimulating factor 1 receptor in mouse models reprogrammed the TAMs and enhanced antigen presentation as well as antitumor T-cell immune responses.\textsuperscript{40} Decoy receptor 3, also referred to as tumor necrosis factor receptor superfamily member 6b, is overexpressed in PDAC, and therefore is an additional therapeutic candidate. Expression of decoy receptor 3 is
inversely correlated with survival in PDAC. Decoy receptor 3 down-regulates expression of major histocompatibility complex-II and human leukocyte antigen-DR on macrophages, making it a potential target to modulate macrophages in PDAC. There needs to be additional preclinical studies done with these potential targets.

Myeloid-Derived Suppressor Cells

MDSCs are a mixture of immature myeloid cells and comprise two types of cells, polymorphonuclear granulocytic MDSCs and mononuclear monocytic MDSCs. However, identification remains difficult because of their lack of defining surface markers. Tumor cell production of granulocyte-macrophage colony-stimulating factor attracts immature myeloid cells and skews differentiation toward MDSCs. The MDSCs then suppress both CD4⁺ and CD8⁺ T cells, mitigating the CD8⁺ T-cell immune surveillance, and allow for expansion of immunosuppressive regulatory T cells. MDSCs increase as PDAC progresses from preinvasive lesions to invasive disease in mouse models, and circulating levels have been correlated to a more advanced stage in human PDAC patients. Thus, the inhibition of MDSCs is a potential therapeutic target in PDAC. Selective depletion of granulocytic MDSCs in mouse models enhances apoptosis of tumor cells with an increased infiltration of CD8⁺ T cells. There are various methods to inhibit MDSCs in patients with PDAC that need to be further explored.

Tumor-Infiltrating T Cells

The stroma of PDAC is rich in CD3⁺ T lymphocytes, of which the major components are CD4⁺ helper T (Th) cells, CD8⁺ T cells, and CD4⁺CD25⁺forkhead box P3 regulatory
T cells.\textsuperscript{45} CD4\textsuperscript{+} T cells, which are found in pancreatic tumors, promote PanIN formation and KRAS-driven PDAC development by secreting IL-17, suppressing the antitumor activity of CD8\textsuperscript{+} T cells.\textsuperscript{46} These CD4\textsuperscript{+} Th cells differentiate into two subsets of cells, Th1 and Th2. Th1 cells secrete IL-2 and interferon-\gamma, responsible for the cell-mediated immune response. Th2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13 and are implicated in the humoral immune response.\textsuperscript{47} Th1 cells are involved in tumor-killing responses; however, Th2 cells, which are more abundant in PDAC, are tumor promoting. De Monte et al\textsuperscript{19} showed that elevated Th2/Th1 ratios within the tumor-infiltrating cells are a negative survival marker in patients with stage IIB/III pancreatic cancer. There are multiple CD4\textsuperscript{+} T-cell targets as well as targets against the multiple cytokines mentioned above that are beyond the scope of this review.

Recently, T-cell immunity has been noted in tumors of long-term PDAC survivors.\textsuperscript{48} This concept was explored in detail by Balachandran et al,\textsuperscript{49} who showed tumors with the highest neoantigen number as well as the most abundant CD8\textsuperscript{+} T-cell infiltrates were associated with the longest-term PDAC survivors. They found neoantigen-rich hotspots, including MUC16, that will allow future research using directed neoantigen targeting as a potential therapy.

### Extracellular Matrix

The acellular component of stroma (alias, the ECM) comprises 90% of the PDAC tumor mass. The ECM is composed of collagen, fibronectin, and multiple soluble factors, including cytokines, chemokines, and growth factors secreted by PSCs. These factors provide structural support and promote differentiation, remodeling, and carcinogenesis. \textit{In vitro}, collagen I promotes proliferation, migration, and adhesion of PDAC cells.\textsuperscript{50} It has also been shown to interact with both collagen IV and integrin receptors on the surface of PDAC cells, promoting proliferation, maintaining a migratory phenotype, and avoiding apoptosis.\textsuperscript{51}

The various components of the TME work in concert to maintain tumor integrity, survival, and propagation. There are a slew of targets that are actively under investigation that aim to induce tumor regression and prolong patient survival.

### Clinical Targets of the TME

#### Hedgehog

The hedgehog pathway, although not expressed in the developing pancreas, is activated in premalignant lesions as well as PDAC tumors.\textsuperscript{52} This pathway is active in embryonic development as well as stem cell regulation. The hedgehog ligand, sonic hedgehog, is expressed in increasing amounts as premalignant pancreatic lesions progress.\textsuperscript{53} The hedgehog ligand activates the PSCs via paracrine effects, contributing to the increase in stroma and ultimately tumor progression.\textsuperscript{54} Specifically, pancreatic cancer cells produce sonic hedgehog, which, in turn, binds to its receptor on PSCs, Patched1, and activates intracellular signaling by removing the inhibitory effects of smoothened, leading to activation and translocation of the transcription factor Gli1 into the nucleus. Gli1 regulates genes implicated in cell differentiation, proliferation, and apoptosis found in extracellular matrix proteins.\textsuperscript{55} These now activated PSCs deposit extracellular matrix proteins, resulting in a thick stroma that inhibits access to therapeutic agents.\textsuperscript{56}

The hedgehog pathway was thus an attractive target for treatment, and multiple strategies for interrupting the pathway have been tested in an attempt to deplete the stroma. There have been several preclinical studies using hedgehog targeting in pancreatic cancer. Alkaloid cyclopamine and its derivatives, including IPI-926, smoothened antagonists, and 5E1 sonic hedgehog blocking antibodies, have all been studied.\textsuperscript{56,57} The most promising targeted therapy, IPI-926, is a semisynthetic small-molecule inhibitor derivative of the alkaloid cyclopamine that inhibits the hedgehog pathway by binding to and inhibiting Smoothened and thus keeping Gli inactive.\textsuperscript{58} Olive et al,\textsuperscript{59} in an exciting preclinical study, showed IPI-926 enhanced perfusion of gemcitabine within the pancreatic tumor and improved survival. In this study, IPI-926 decreased collagen I in the stroma and proliferation of \(\alpha\)-SMA stromal cells and transiently increased blood vessel density in the tumors using a KPC mouse model.\textsuperscript{59} Early-phase studies, however, failed to show a sustained benefit in humans.\textsuperscript{60} This outcome was similar to multiple other hedgehog-targeted agents, which showed promise in preclinical studies, but failed to improve survival in early clinical trials.

#### Secreted Protein Acidic and Rich in Cysteine

Secreted protein acidic and rich in cysteine (SPARC; alias osteonectin) is a glycoprotein secreted by osteoblasts during bone formation. It binds calcium-initiating mineralization and promotes mineral crystal formation. In the setting of PDAC, SPARC is an extracellular protein expressed in the stroma, implicated in worse prognosis. In preclinical studies, SPARC showed a high affinity for nab-paclitaxel, which is paclitaxel conjugated with albumin nanoparticles in an attempt to increase delivery to the pancreas. Nab-paclitaxel is now used in combination with gemcitabine as the standard of care in the adjuvant setting.\textsuperscript{61} This enhanced binding of nab-paclitaxel to the SPARC-rich stroma is thought to increase delivery of the drug to tumor cells. In these studies, nab-paclitaxel, given in combination with gemcitabine, acted directly on stromal cells, resulting in stromal depletion. In addition, increased blood vessel diameter and increased expression of Nestin, an endothelial cell marker, were noted. These findings ultimately resulted in increased efficacy of gemcitabine.\textsuperscript{5,61} Furthermore, in clinical trials using gemcitabine in combination with...
nab-paclitaxel, increased expression of SPARC in tumor tissue was correlated with improved median overall survival compared with those with minimal SPARC expression. However, recently, new data show nab-paclitaxel is also effective in SPARC-deficient models, suggesting its effect may not be entirely dependent on stroma targeting and thus requires more studies to delineate the mechanism.

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is a profibrotic extracellular protein expressed in the stroma of pancreatic adenocarcinoma. Increased expression of CTGF has been documented in chronic pancreatitis and pancreatic adenocarcinoma. In the setting of PDAC, expression is mediated by chemokine signaling through CXC proteins. These proteins then bind to growth factors and integrins, which promote fibrosis, collagen deposition, cancer progression, and metastasis. In addition, CTGF simulates PSC proliferation and ECM protein production in the PDAC stroma.

Thus, CTGF became a promising therapeutic target for the treatment of PDAC. The monoclonal antibody, FG-3019, as well as multiple antagonists that block the interaction of CTGF with its receptor, including SB225002, were effective in preclinical studies in improving tumor response to chemotherapy as well as increasing survival in murine models. Neesse et al showed increased rates of tumor cell apoptosis when FG-3019 was combined with gemcitabine in mouse models. Although the gross appearance of the tumor stroma was unchanged in these studies, there was significant down-regulation of both prosurvival and antiapoptotic proteins, including Psen1, Ubqln2, hypoxia-inducible factor-1z, Birc6, and X-linked inhibitor of apoptosis. There are currently ongoing phase 1/2 trials looking at the efficacy of FG-3019 in conjunction with gemcitabine and nab-paclitaxel (NCT02210559) in advanced unresectable pancreatic cancer.

Hyaluronan

Hyaluronan (HA) is a nonsulfated polysaccharide glycosaminoglycan found in the PDAC stromal matrix. HA in collaboration with collagens generate the fibrotic desmosplasia seen in pancreatic tumors. The structure of HA includes multiple anionic repeats that serve to sequester cations, resulting in osmotic swelling and providing additional support to these tissues. High levels of HA in PDAC tumors are associated with increased interstitial fluid pressure and desmosplasia, which together generate a barrier to perfusion and, as a result, inhibit systemic drug delivery. HA binds to CD44, a hyaladherin which regulates receptor tyrosine kinase and, in turn, impedes intratumoral angiogenesis and induces chemoresistance. HA is also found in metastatic PDAC lesions. Because of these previously mentioned findings, HA is a promising therapeutic target in the treatment of PDAC. PEGylated human recombinant PH20 hyaluronidase (PEGPH20), an enzyme that degrades hyaluronan, has had promising results in both mouse and human phase 1 and 2 trials. In these early-phase studies, PEGPH20 increased tumor vascular patency, leading to improved delivery of gemcitabine, albeit transiently. In NCT01453153, patients with advanced PDAC treated with a combination of gemcitabine and PEGPH20 had improved overall as well as progression-free survival compared with patients treated with gemcitabine alone. When stratified by level of HA expression in tumor samples, those with high HA benefitted the most. However, thrombotic events were common in this study and, thus, going forward, anticoagulants were given in the combination. This trial prompted the investigation of PEGPH20 with other agents, including gemcitabine/nab-paclitaxel, FOLFIRINOX (S1313/NCT01959139), which just closed accrual in July 2017. Hingorani et al recently published results from the phase 2 study examining PEGPH20 in combination with gemcitabine and nab-paclitaxel in patients with advanced PDAC. In patients with HA high tumors (>50% HA staining of tumor surface), progression-free survival was significantly improved in those treated with the PEGPH20; and median overall survival was 11.5 versus 8.5 months for patients treated with PEGPH20, gemcitabine, and nab-paclitaxel versus those treated with gemcitabine and nab-paclitaxel alone.

The incremental successes and failures thus far with both PEGPH20 and hedgehog inhibitors, respectively, targeting stromal depletion as a treatment for pancreatic cancer suggest a need to further study the tumor-stromal biology. It is thought that the stroma, in addition to generating a physical barrier to therapies, may also have tumor suppressive properties. This idea is rooted in the finding that permanent stroma depletion results in a more aggressive tumor. Thus, stroma modulation rather than depletion may prove the key to developing effective targeted therapies.

Vitamins A and D

A hallmark of activated PSCs in PDAC is a loss of retinol-storing cytoplasmic lipid droplets. The treatment of activated PSCs in culture as well as in PDAC mouse models with vitamin A analogs reduced paracrine signaling of PSCs via restoration of secreted frizzled-related protein 4 and ultimately down-regulation of Wnt–β-catenin signaling. This ultimately resulted in reduced proliferation, migration, and invasion of cancer cells. This finding leads to similar preclinical trials using vitamin D after transcriptome analysis of human PSCs, which found high levels of vitamin D receptor. The activation of vitamin D receptor resulted in a reprogramming of the stroma through a dormant, less tumorigenic phenotype.
<table>
<thead>
<tr>
<th>Intervention</th>
<th>Target</th>
<th>Phase</th>
<th>Trial identifier</th>
<th>Status</th>
<th>Results</th>
<th>Starting date</th>
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<td>2017</td>
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<td>No results reported</td>
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<td>Vitamin D</td>
<td>2</td>
<td>NCT03138720</td>
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<td>2017</td>
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of PSCs. PDAC mouse models treated with a combination of calcitriol, a vitamin D analog, and gemcitabine lead to a less inflammatory, more quiescent stroma; enhanced delivery of the gemcitabine; and improved survival than those treated with gemcitabine alone. There are currently five active clinical trials looking at vitamin D analogs in combination with other chemotherapy or targeted drugs for the treatment of early as well as late PDAC (NCT02930902, NCT03300921, NCT03331562, NCT02030860, and NCT03138720).

Pirfenidone

Pirfenidone, a known antifibrotic agent originally used to treat pulmonary fibrosis, decreases the expression of several fibrosis mediators, including transforming growth factor-β and collagen. In in vitro studies, treatment with pirfenidone decreased PSC proliferation, invasion, migration, ECM components, and tumor growth. This was evident from decreased expression of platelet-derived growth factor-A, perioestin, hepatocyte growth factor, fibronectin, and collagen type I in PSCs. In this study, there was also decreased α-SMA, indicating inhibited PSC activation. In preclinical studies, pirfenidone given with gemcitabine reduced tumor growth compared with gemcitabine alone. Pirfenidone has also been studied in combination with chemotherapeutic drugs, such as gemcitabine in the form of β-cyclodextrin matrix metalloproteinase-2 responsive liposome, where it more efficiently targeted the delivery of the drugs to the tumor cells and increased perfusion of the chemotherapy agents to the tumors. However, this has not been validated in preclinical models yet. Pirfenidone has also been combined with N-acetyl cysteine in preclinical models, resulting in decreased stroma and increased drug efficacy. There are currently no trials registered using pirfenidone in the setting of PDAC.

**Angiotensin Inhibitors**

Angiotensin inhibitors, such as olmasartan and losartan, used currently for treatment of hypertension, have shown some promise in pancreatic cancer. Angiotensin II has been shown to stimulate PSC proliferation, migration, and ECM production through protein kinase C and the epidermal growth factor–extracellular signal-regulated kinase pathways. This finding prompted multiple preclinical studies.

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**Table 1** (continued)

<table>
<thead>
<tr>
<th>Intervention</th>
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<th>Trial identifier</th>
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<td>FAK + PD-1</td>
<td>1/2A</td>
<td>NCT02758587</td>
<td>Recruiting</td>
<td>Still recruiting</td>
</tr>
<tr>
<td>GSK2256098 + trametinib</td>
<td>FAK + MEK</td>
<td>2</td>
<td>NCT02428270</td>
<td>Active, not recruiting</td>
<td>10 of 11 with progressive disease, 1 of 11 with stable disease (abstract only)</td>
</tr>
<tr>
<td>Defactinib + pembrolizumab + gemcitabine</td>
<td>FAK + PD-1</td>
<td>1/2</td>
<td>NCT02546531</td>
<td>Recruiting</td>
<td>Not reported yet</td>
</tr>
<tr>
<td>Mogamulizumab + MEDI4736 (durvalumab) and mogamulizumab + tremelimumab</td>
<td>CTLA-4 + PD-1 + CCR4</td>
<td>1</td>
<td>NCT02301130</td>
<td>Completed</td>
<td>Not reported yet</td>
</tr>
</tbody>
</table>

Clinical trial descriptions may be found at https://clinicaltrials.gov.

CHOP, doxorubicin, cyclophosphamide, vincristine, and prednisone; CTGF, connective tissue growth factor; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; FAK, focal adhesion kinase; HA, hyaluronan; 05, overall survival; PD-1, programmed cell death 1 receptor; PDAC, pancreatic ductal adenocarcinoma; PEGPH20, PEGylated human recombinant PH20 hyaluronidase; PFS, progression-free survival; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.
studies using angiotensin inhibitors. Masamune et al demonstrated that olmesartan reduced the activated PSC marker, α-SMA, and collagen deposition as well as decreased tumor growth in mouse models. Similarly, losartan reduced the density of α-SMA-positive cells, decreased collagen and hyaluronan expression, enhanced chemotherapy efficacy, and attenuated hypoxia in orthotopic mouse models. This was thought to be mediated through transforming growth factor-β1, CTGF, and endothelin-1, all of which control PSC production of ECM. There is a currently accruing phase 2 trial looking at FOLFIRINOX in combination with losartan before radiation therapy versus FOLFRINIOX and radiation therapy alone to evaluate the role of losartan as a chemotherapy sensitizer in PDAC patients (NCT01821729).

There are conflicting data from a study that used a -synergic orthotopic mouse model with angiotensin II receptor deficiency in pancreatic fibroblasts. The tumors in these angiotensin receptor 2 knockout mice grew larger compared with -control wild-type mice. This was followed up with co-culture of angiotensin receptor 2 overexpressing fibroblasts with pancreatic cancer cells, which resulted in vascular endothelial growth factor (VEGF)–mediated attenuation of cancer cell growth. These conflicting results suggest a need for further studies investigating the role of angiotensin II in the PDAC stroma, which will hopefully be elucidated in the current ongoing preclinical and clinical trials.

**Hypoxia and Angiogenesis**

The dense stroma of PDAC in combination with accumulation of ECM proteins result in impaired tumor vasculature and hypoxia. In response, PDAC cells have adapted to this hypoxic environment, resulting in hypoxic resistant tumor cells. As a result, this generates a barrier to pharmacodelivery. Thus, hypoxia-activated cytotoxic prodrugs, such as TH-302, are attractive targets. TH-302 is a 2-nitroimidazole prodrug that is activated only under hypoxic conditions, resulting in the radical anion to undergo irreversible fragmentation, releasing the active drug, which is an alkylating cytotoxic agent. TH-302 in combination with gemcitabine improved progression-free survival in preclinical mouse models. Phase 2 trials supported the preclinical data, with progression-free survival, tumor response, and CA19-9 response all significantly improved with gemcitabine plus TH-302 in patients with advanced PDAC. This combination has now moved on to phase 3 trials (NCT01746979).

Angiogenesis is imperative for progression of all tumor types; therefore, it is a potential therapeutic target. One of the most well-studied therapeutic targets of angiogenesis is VEGF. Bevacizumab, an anti-VEGF antibody, has shown significant benefits in the treatment of metastatic colorectal cancer. However, a phase 3 trial looking at bevacizumab given with gemcitabine, which included patients with advanced pancreatic cancer, showed no survival benefit. Thus, the role of VEGF in angiogenesis in the setting of PDAC must be further delineated to use it successfully as a therapeutic target.

In addition to VEGF, the hepatocyte growth factor—c-MET pathway has also been implicated in tumor angiogenesis. In the setting of the PDAC TME, PSCs secrete hepatocyte growth factor, which binds to receptor c-Met on cancer cells, leading to dimerization and phosphorylation of the receptor and downstream signaling, resulting in proliferation and migration of PDAC cells. The inhibition of this pathway with a neutralizing antibody, INC280, decreased neoangiogenesis and decreased tumor growth in xenogenic and syngeneic mouse models when given alone as well as in combination with gemcitabine. Treatment with INC280 proved to block metastases better than gemcitabine, but this effect was lost when given in combination. There is currently a phase 1 trial looking at a combination of gemcitabine, nab-paclitaxel, and ficituzumab (AV-299), a hepatocyte growth factor monoclonal antibody in patients with advanced PDAC; however, there are no results yet (NCT 03316599).

**Focal Adhesion Kinase**

Focal adhesion kinases (FAKs), including FAK1 and PYSK2 (FAK2), are nonreceptor tyrosine kinases elevated in human PDAC tissue, and they correlate with increased fibrosis as well as poor CD8+ cytotoxic T-cell infiltration. The inhibition of FAK in combination with VS-4718 limited tumor progression and increased survival in KPC mice associated with reduced tumor fibrosis and decreased tumor-infiltrating immunosuppressive cells. In addition, the inhibition of FAK in these mice sensitized previously unresponsive tumors to T-cell immunotherapy and programmed cell death 1 receptor (PD-1) agonists. A phase 1 study of VS-4718 is currently suspended because of funding (NCT0265172); however, multiple other studies looking at FAK inhibitors in combination with PD-1 (NCT02758587), trametinib (NCT02428270), pembrolizumab, and gemcitabine (NCT02546531) are still active.

**Wnt/β-Catenin Pathway**

Wnt/β-catenin signaling is crucial for normal embryonic development and the maintenance of adult tissue. Dysregulation of this signaling pathway leads to initiation and progression of multiple cancers, including PDAC, as well as aggressive tumor biology. Several Wnt/β-catenin inhibitors have shown success in vivo and are now in preclinical trials. One example, PRI-724, a second-generation molecule that targets coactivators CBP and P300 by inhibiting expression of the apoptosis inhibitor BIRC5, is currently in clinical trials in patients with advanced or...
metastatic PDAC as an adjunct to gemcitabine (NCT01764477).104

Immune Cells

Inflammation and the resulting immune response have long been thought to play a role in PDAC, given the fact that chronic pancreatitis is a risk factor for developing PDAC. Many immune costimulatory factors and checkpoint regulators have been implicated in PDAC and are part of the stroma. However, unlike in other tumor types, there has been little clinical success with immunotherapy in PDAC, possibly because of the hypoxic and, therefore, immunosuppressive environment resulting from the dense stroma. Despite this, the role of inflammation and immune response in disease progression is currently being studied.

CD40, a cell surface molecule and member of the tumor necrosis factor receptor family, plays a role in immune regulation, apoptosis, and the production of macrophage-necrosis factor receptor family, plays a role in immune response and immune-modulation and thus make strides in pro-

repair—proficient colorectal cancers (40% and 78% versus 0% and 11%, respectively).

Mismatch repair—deficient noncolorectal cancers demonstrated similar results, with an immune-related objective response rate of 71% and an immune-related progression-free survival rate of 67%. The reasons for the disappointing results of PD-1 blockade in clinical trials may be because of the fact that only 2% of pancreatic cancers have mismatch repair deficiency.111

Another postulated reason for the disappointing results of PD-1, PD-L1, and immune checkpoint inhibitors is CXCL12. CXCL12 is a ligand for chemokine receptor type 4, secreted by cancer-associated fibroblasts, which ultimately binds to tumor cells, resulting in the depletion of CD8+ T cells and generating an immunosuppressive environment. In studies in which cancer-associated fibroblasts were depleted and CXCL12 delivery was targeted by a competitive blocker, cytotoxic T cells were increased within the stroma and PDAC tumor growth was inhibited.112 Administration of a chemokine receptor type 4 inhibitor, plerixafor (AMD3100), in KPC mice induced a rapid T-cell response and acted synergistically with PD-L1 antibody to inhibit tumor growth.113 Thus, the combination of immune checkpoint inhibitors with targeted drugs, such as the chemokine receptor type 4 inhibitor, which reverses immunosuppression in the TME in animal models, is an attractive therapeutic regimen and is currently being investigated in clinical trials. One such trial is the phase 1 study, NCT02301130, looking at mogamulizumab in combination with durvalumab as well as tremelimumab in advanced solid tumors, including PDAC.

Conclusions

The TME, including the stroma and extracellular component, clearly plays an important role in the progression and chemoresistance of PDAC. Although there have been many promising targets against PDAC taking advantage of the TME that have made it to early clinical trials (Table 1), none has borne out to significantly improve clinical outcomes or become standard of care. Multiple targeted approaches are currently under investigation, which will potentially provide a better combination of stromal depletion, drug delivery, and immune modulation, and thus make strides in prolonging survival in patients with PDAC.

Supplemental Data

Supplemental material for this article can be found at https://doi.org/10.1016/j.ajpath.2018.09.009.

References


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