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REVIEW

The Paradoxical Web of Pancreatic Cancer Tumor Microenvironment

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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. (*Am J Pathol* 2019, 189: 44–57; <https://doi.org/10.1016/j.ajpath.2018.09.009>)

Pancreatic ductal adenocarcinoma (PDAC) is increasing in incidence and is currently the fourth leading cause of cancer death in the United States, with an estimated incidence of 55,440 in 2018 and 44,330 deaths.¹ By 2030, PDAC is predicted to surpass breast, prostate, and colon cancers to become the second leading cause of cancer death in the United States, following only lung cancer.² PDAC comprises >90% of all pancreatic tumors and has by far the poorest prognosis of all solid tumors.³

Currently, the most promising chance of cure remains surgery, with negative margins in combination with adjuvant therapy. However, most patients present late with localized disease not amenable to surgery at the time of diagnosis. Although there have been multiple recent improvements in the treatment of PDAC, including combination chemotherapies that have improved survival, such as gemcitabine plus capecitabine in the adjuvant setting as well as in advanced disease and the use of fluorouracil, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) in advanced disease, the overall 5-year survival rate remains 8%.^{1,4–6}

PDAC is unique in the fact that there is a significant desmoplastic response generating a large stromal

component, including immune cells, inflammatory cells, growth factors, extracellular matrix, and fibroblasts, which may represent anywhere from 15% to 85% of the tumor.^{7–9} This desmoplastic microenvironment may compromise tumor blood perfusion and oxygen delivery, in turn generating an obstacle for drug delivery. This barrier has been hypothesized to add to the difficulty in finding effective treatments for PDAC and lead to intrinsic resistance to chemotherapy regimens, including gemcitabine.¹⁰ Significant research efforts recently have focused on this tumor-stroma relationship, and this review serves to delineate these components and how they may be used in the pathologic setting and in developing targeted therapeutics.

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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-Kras^{G12D/+};LSL-Trp53^{R172H/+};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.¹⁸

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 colleagues^{18,20} 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 IV α 1 by 2.25-fold, which may contribute to their remodeling of the ECM in the activated setting.²⁰ In addition, they secrete periostin, 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 periostin, leading to an increase in collagen I, transforming growth factor- β 1, 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- β 1 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: $\times 4$ (**A**); $\times 20$ (**B**).

Figure 2 Hematoxylin and eosin (H&E) and trichrome staining of pancreatic tumors arising in two KPC mice recapitulating the dense collagen-rich stroma seen in human pancreatic adenocarcinoma tumors. Scale bars = 100 μ m.

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.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.^{27,28} TFF1 is known to stimulate proliferation of PSCs as well as PSC migration and PDAC cell invasion.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.²⁹

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

Immune Component

Although the TME of PDAC is dominated by dense stroma, it is also replete with immune cells (Figure 3).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.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- α .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.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.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.^{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 (www.clinicaltrials.gov; trial identifier NCT01339754). Trabectedin causes caspase-8-dependent apoptosis and expression of receptors in macrophages, ultimately targeting mononuclear phagocytes.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.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

Figure 3 Depiction of the pancreatic tumor microenvironment throughout progression from pancreatic intraepithelial neoplasia (PanIN) to pancreatic ductal adenocarcinoma (PDAC). CAF, cancer-associated fibroblast; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; CXCR4, chemokine receptor type 4; MDSC, myeloid-derived suppressor cell; PDGF, platelet-derived growth factor; PGF, prostaglandin F; PSC, pancreatic stellate cell; Shh, sonic hedgehog; TGF-β, transforming growth factor-β; Treg, regulatory T cell; VEGFR, vascular endothelial growth factor receptor.

inversely correlated with survival in PDAC.⁴¹ 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.^{42,43} 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.³⁶ 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.³⁵ 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.⁴⁴ 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.⁴⁵ CD4⁺ 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⁺ T cells.⁴⁶ These CD4⁺ Th cells differentiate into two subsets of cells, Th1 and Th2. Th1 cells secrete IL-2 and interferon- γ , 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.⁴⁷ Th1 cells are involved in tumor-killing responses; however, Th2 cells, which are more abundant in PDAC, are tumor promoting. De Monte et al⁴⁸ 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⁺ 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.⁴⁹ This concept was explored in detail by Balachandran et al,⁴⁹ who showed tumors with the highest neoantigen number as well as the most abundant CD8⁺ 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. *In vitro*, collagen I promotes proliferation, migration, and adhesion of PDAC cells.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.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.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.⁵³ The hedgehog ligand activates the PSCs via paracrine effects,

contributing to the increase in stroma and ultimately tumor progression.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.⁵⁵ These now activated PSCs deposit extracellular matrix proteins, resulting in a thick stroma that inhibits access to therapeutic agents.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.^{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.⁵⁸ Olive et al,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 1 in the stroma and proliferation of α -SMA stromal cells and transiently increased blood vessel density in the tumors using a KPC mouse model.⁵⁹ Early-phase studies, however, failed to show a sustained benefit in humans.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.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.^{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.^{62,63} 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.⁶⁴

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.^{65,66} 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.^{67,68} In addition, CTGF stimulates 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.^{69,70} Neesse et al⁷⁰ 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-1 α , Birc6, and X-linked inhibitor of apoptosis.⁷⁰ 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 desmoplasia seen in pancreatic tumors.⁷¹ The structure of HA includes multiple anionic repeats that serve to sequester cations, resulting in osmotic swelling and providing additional support to these tissues.⁷² High levels of HA in PDAC tumors are associated with increased interstitial fluid pressure and desmoplasia, which together generate a barrier to perfusion and, as a result, inhibit systemic drug delivery.^{72–74} HA binds to CD44, a *hayl da herin* which regulates receptor tyrosine kinase and, in turn, impedes intratumoral angiogenesis and induces chemoresistance.⁷⁵ HA is also found in metastatic PDAC lesions.⁷¹

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.^{59,75–77} 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.⁷⁶ 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.⁷⁶ This trial prompted the investigation of PEGPH20 with other agents, including gemcitabine/nab-paclitaxel (NCT02715804 and NCT01839487) and FOLFIRINOX (S1313/NCT01959139), which just closed accrual in July 2017. Hingorani et al⁷⁸ 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 routed in the finding that permanent stroma depletion results in a more aggressive tumor.^{79,80} 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.¹⁷ 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.⁸¹

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 1 Summary of Clinical Trials Targeting PDAC Tumor Microenvironment

Intervention	Target	Phase	Trial identifier	Status	Results	Starting date
GDC-0449 (vismodegib) + gemcitabine	Hedgehog	2	NCT01064622	Completed	No significant difference in response rates, OS, or PFS	2010
PRI-724	Wnt- β -catenin	1b	NCT01764477	Completed	No dose-limiting toxicity in pancreatic cancer trial, some evidence of clinical activity	2015
Nab-paclitaxel + gemcitabine	Nanoparticle albumin-bound paclitaxel	1/2	NCT00398086	Completed	Response rate of 48%, median OS of 12.2 months	2006
PEGPH20 + gemcitabine	HA	1/2	NCT01453153	Completed	Improved PFS and OS (abstract only)	2011
PEGPH20 + gemcitabine + nab-paclitaxel	HA	3	NCT02715804	Recruiting	Response rate and PFS significantly improved in patients with HA high tumors	2016
PEGPH20 + FOLFIRINOX	HA	1/2	NCT01959139	Active, not recruiting	Decreased OS (abstract only)	2013
Bevacizumab + gemcitabine	VEGF-A	3	NCT00088894	Completed	No difference in PFS or OS	2004
Axitinib + gemcitabine	Tyrosine kinase	3	NCT00471146	Discontinued for futility	No difference in OS	2007
MK0752 + gemcitabine	γ Secretase	1/2	NCT01098344	Completed	13 of 19 patients with stable disease, 1 of 19 patients with response	2010
R04929097	γ Secretase	2	NCT01232829	Completed	Well tolerated, but production discontinued by company	2010
R04929097 + cediranib maleate	γ Secretase + VEGF	1	NCT01131234	Completed	No results reported	2010
Ipilimumab	CTLA-4	2	NCT00112580	Completed	No results reported	2005
CP-870,893 + gemcitabine	CD40	1	NCT00711191	Completed	Well tolerated but no phase 2 trial initiated	2008
Trabectedin	FUS-CHOP	2	NCT01339754	Completed	No difference in OS	2011
IPI-926 + FOLFIRINOX	Hedgehog	1	NCT01383538	Completed	Closed early because of poor results from NCT01130142	2011
IPI-926 + gemcitabine	Hedgehog	1b/2	NCT01130142	Completed	Decreased median OS and PFS	2010
FG-3019 (pamrevlumab) + gemcitabine + nab-paclitaxel	CTGF	1/2	NCT02210559	Active, not recruiting	Trend toward increased resectability (abstract only)	2014
Pembrolizumab + paricalcitol	PD-1 + vitamin D receptor	1	NCT02930902	Recruiting	Still recruiting	2016
Paricalcitol	Vitamin D receptor	1b	NCT03300921	Recruiting	Still recruiting	2017
Pembrolizumab + paricalcitol	PD-1 + vitamin D receptor	2	NCT03331562	Recruiting	Still recruiting	2017
Paricalcitol + gemcitabine + nab-paclitaxel	Vitamin D receptor	1	NCT02030860	Active, not recruiting	No results reported	2014
Paclitaxel protein bound + gemcitabine + cisplatin + paricalcitol (neoadjuvant)	Vitamin D	2	NCT03138720	Recruiting	Still recruiting	2017

(table continues)

Table 1 (continued)

Intervention	Target	Phase	Trial identifier	Status	Results	Starting date
Losartan + FOLFIRINOX (neoadjuvant)	Angiotensin receptor (TGF- β 1)	2	NCT01821729	Active, not recruiting	61% R0 resection rate (abstract only)	2013
TH302 + gemcitabine	Hypoxia	2	NCT01144455	Completed	Significantly increased PFS	2010
TH302 + gemcitabine	Hypoxia	3	NCT01746979	Completed	Not reported yet	2012
AV-299 (ficlatuzumab) + gemcitabine + nab-paclitaxel	Hepatocyte growth factor	1	NCT03316599	Recruiting	Still recruiting	2017
VS-4718	FAK	1	NCT02651727	Terminated (company stopped development)	Terminated (company stopped development)	2016
Defactinib + pembrolizumab	FAK + PD-1	1/2A	NCT02758587	Recruiting	Still recruiting	2016
GSK2256098 + trametinib	FAK + MEK	2	NCT02428270	Active, not recruiting	10 of 11 with progressive disease, 1 of 11 with stable disease (abstract only)	2015
Defactinib + pembrolizumab + gemcitabine	FAK + PD-1	1/2	NCT02546531	Recruiting	Not reported yet	2015
Mogamulizumab + MEDI4736 (durvalumab) and mogamulizumab + tremelimumab	CTLA-4 + PD-1 + CCR4	1	NCT02301130	Completed	Not reported yet	2004

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; OS, overall survival; PD-1, programmed cell death 1 receptor; PDAC, pancreatic ductal adenocarcinoma; PEGPH20, PEGylated human recombinant PH20 hyaluronidase; PFS, progression-free survival; TGF- β 1, transforming growth factor- β 1; VEGF, vascular endothelial growth factor.

of PSCs.⁸² 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.⁸² 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, periostin, hepatocyte growth factor, fibronectin, and collagen type I in PSCs.⁸³ In this study, there was also decreased α -SMA, indicating inhibited PSC activation.⁸³ In

preclinical studies, pirfenidone given with gemcitabine reduced tumor growth compared with gemcitabine alone.⁸³ 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.⁸⁴ 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.⁸⁵ There are currently no trials registered using pirfenidone in the setting of PDAC.

Angiotensin Inhibitors

Angiotensin inhibitors, such as olmesartan 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.^{86,87} This finding prompted multiple preclinical

studies using angiotensin inhibitors. Masamune et al⁸⁸ 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.⁸⁹ This was thought to be mediated through transforming growth factor- β 1, 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 FOLFIRINOX 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 -syngeneic 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.⁹⁰ 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.⁹⁰ 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.⁹¹ 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.⁹² TH-302 in combination with gemcitabine improved progression-free survival in preclinical mouse models.⁹² 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.⁹³ 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.⁹⁴

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.⁹⁵ 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.⁹⁶ Treatment with INC280 proved to block metastases better than gemcitabine, but this effect was lost when given in combination.⁹⁶ There is currently a phase 1 trial looking at a combination of gemcitabine, nab-paclitaxel, and ficlatuzumab (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 PYK2 (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.⁹⁷ 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.⁹⁷ 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.^{98–100} Wnt pathway activation is observed in up to 65% of PanINs and increased even more frequently in invasive PDAC.^{101–103} 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-dependent antitumor T-cell immunity.^{105–107} In KPC mouse models, CD40 monoclonal antibody alone as well as with gemcitabine resulted in tumor regression in 30% of mice compared with none of the mice treated with gemcitabine alone.¹⁰⁵ The mechanism of tumor regression in these mice was macrophage-mediated depletion of the stroma through caspase-3 induction and decreased collagen I content. In phase 1 clinical trials, a combination of a CD40 agonist, CP-870, with gemcitabine in advanced PDAC patients resulted in a partial response in 18% of patients and an increase in progression-free survival and median overall survival compared with those treated with gemcitabine alone. Post-treatment biopsies of patients treated with CP-870 and gemcitabine, interestingly, showed a lack of tumor-infiltrating lymphocytes and abundant macrophages.¹⁰⁵

PD-1 binds to PD-1 ligands PD-L1 and PD-L2, resulting in activation and suppression of T-cell activity and allowing for immune escape of tumor cells in the TME. Both PD-1 and PD-L1 are expressed in some PDAC tumors and are associated with poor prognosis.¹⁰⁸ Although targeting PD-1 and PD-L1 has been successful in other tumor types, clinical trials in PDAC did not demonstrate significant response as a monotherapy.^{108,109} However, there are multiple trials using PD-1 and PD-L1 in combination with other drugs. Targeting cytotoxic T-lymphocyte–associated protein 4, another protein receptor that acts as an immune checkpoint expressed in PDAC, with anti–cytotoxic T-lymphocyte–associated protein 4 antibody ipilimumab also showed promise in mouse models, with improved survival; however, this treatment failed to show the same results in phase 2 trials.^{79,110}

Recently, the success of PD-1 blockade has been linked to mismatch repair deficiency.^{111,112} A phase 2 study, reported by Le et al,¹¹² showed patients with mismatch repair–deficient colorectal cancer had a significantly increased immune-related objective response and immune-related progression-free survival compared with mismatch

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.¹¹³ 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>.

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