Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2020 August ; 17(8): 487–505. doi:10.1038/s41575-020-0300-1.

Pancreatic cancer stroma: an update on therapeutic targeting strategies

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related mortality in the Western world with limited therapeutic options and dismal long-term survival. The neoplastic epithelium exists within a dense stroma, which is recognized as a critical mediator of disease progression through direct effects on cancer cells and indirect effects on the tumour immune microenvironment. The three dominant entities in the PDAC stroma are extracellular matrix (ECM), vasculature and cancer-associated fibroblasts (CAFs). The ECM can function as a barrier to effective drug delivery to PDAC cancer cells, and a multitude of strategies to target the ECM have been attempted in the past decade. The tumour vasculature is a complex system and, although multiple anti-angiogenesis agents have already failed late-stage clinical trials in PDAC, other vasculature-targeting approaches aimed at vessel normalization and tumour immunosensitization have shown promise in preclinical models. Lastly, PDAC CAFs participate in active cross-talk with cancer cells within the tumour microenvironment. The existence of intratumoural CAF heterogeneity represents a paradigm shift in PDAC CAF biology, with myofibroblastic and inflammatory CAF subtypes that likely make distinct contributions to PDAC progression. In this Review, we discuss our current understanding of the three principal constituents of PDAC stroma, their effect on the prevalent immune landscape and promising therapeutic targets within this compartment.

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Author contributions

A.N.H. conceived of and drafted the article; R.A.B and A.M. conceived of and provided editorial guidance.

Competing interests

A.M. receives royalties from Cosmos Wisdom Biotechnology for a biomarker assay related to early detection of pancreatic cancer. A.M. is an inventor on a patent that has been licensed to Thrive Earlier Detection. The remaining authors declare no competing interests.

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related mortality in the USA, with a 5-year overall survival of 9% and an estimated 45,750 Americans projected to die from the disease in 2019 (REF.[1]). Globally, in 2018, 458,918 pancreatic cancer diagnoses were made, comprising 2.5% of worldwide cancer cases, and there were 432,242 deaths contributing 4.5% of worldwide cancer-related deaths[2].

Approximately 80% of patients with PDAC have locally advanced or metastatic disease on presentation and are not candidates for curative intent surgery[3], making systemic therapy the mainstay of care. Current chemotherapeutic regimens are based on 5-fluorouracil or gemcitabine and only offer survival times in the range of months in the palliative setting. In the past decade there have been incremental improvements in these regimens, which has extended survival by several months[4,5], but apart from some uncommon exceptions[6–9] there are effectively no FDA-approved targeted therapeutics for PDAC. In an effort to accelerate rational drug development for patients with PDAC, there have been several large-scale gene expression profiling and DNA sequencing efforts to define the molecular landscape of PDAC. These studies have identified at least two molecular subtypes of neoplastic epithelium, with the so-called basal-like (or squamous) subtype carrying a worse prognosis than the classical subtype[10–12]. The digital deconvolution of bulk tumour expression profiling datasets has also demonstrated the presence of activated and normal stromal signatures in samples from patients with PDAC, with the former carrying an independently adverse prognosis and highlighting the contribution of PDAC stroma in disease pathogenesis[13]. This stromal classification scheme was mirrored when the transcriptomes of mouse stromal tissue from patient-derived xenografts (PDXs) were profiled, revealing two major subtypes of PDX-associated stroma[14]. In addition, gene expression profiling of 309 consecutive patients who underwent PDAC resection validated the presence of the basal-like and classical PDAC subtypes within the neoplastic epithelium, and reaffirmed the conspicuous footprint of the PDAC stroma in defining the molecular landscape of PDAC, confirming the same ‘stroma activated’ group of patients and demonstrating a desmoplastic stromal subtype[15]. Both of these stromal subtypes experienced similar overall survival, which was better than the basal molecular subtype but poorer than the classical molecular subtype[15].

The PDAC tumour microenvironment (TME) consists of fibroblasts, endothelial cells, pericytes, neurons, infiltrating immune cells and extracellular matrix (ECM) proteins. As such, a detailed understanding of PDAC stromal biology is critical to the development of novel therapeutics. In this Review, we will discuss the three fundamental components of PDAC stroma: ECM, vasculature and fibroblasts. Our current understanding of these PDAC stroma components will be reviewed with an emphasis on potential therapeutic opportunities. Of note, although immune cells are integral to the TME and we will allude to the pertinent interactions and influence of core stromal elements on the immune system, a detailed discussion of this TME component is beyond the scope of the review.

Extracellular matrix in PDAC

ECM biology in PDAC

A hallmark of PDAC histology is desmoplasia, whereby a fibrotic reaction is caused by an excess of fibroblasts and the deposition of ECM that occupies the bulk of the tumour mass[16–19] (FIG. 1). The ECM is a dense meshwork of structural proteins, adaptor proteins, proteoglycans and enzymes found in all tissues, where it provides biochemical and structural support for tissue homeostasis[20]. In PDAC there is a marked increase in the deposition of ECM. Specifically, type I, III and IV collagens are the main structural proteins constituting PDAC ECM[21], with type IV collagen showing potential as a serum biomarker in predicting survival in the postoperative setting[22]. Pancreatic cancer cells induce a desmoplastic response within the tumour stroma by stimulating stromal fibroblasts to upregulate the expression of collagen family proteins and fibronectin in a paracrine manner[17]. Both primary and metastatic sites in human PDAC have shown prominent desmoplasia and elevated expression of ECM components such as hyaluronic acid and collagens[23]. Moreover, genetically engineered mouse models (GEMM) with different driver mutations have highlighted the role of the tumour epithelium in generating a prominent desmoplastic stroma at secondary organ sites; the KPTC mouse model ($Kras^{LSL-G12D/+}$;$Trp53^{LSL-R172H/+}$;$Tgfbr2^{flox/+}$;$Ptf1a^{Cre/+}$) displayed a stroma with little desmoplasia noted in liver metastases in contrast to prominent liver metastases stromal desmoplasia in the KPC GEMM ($Kras^{LSL-G12D/+}$;$Tp53^{R172H/+}$;$Pdx1^{Cre/+}$)[24]. These data support the idea that a robust desmoplastic reaction is critical in PDAC pathogenesis, which makes therapeutic targeting of ECM components a potential strategy in PDAC.

Collagens are biologically active components of the tumour stroma and not merely structural scaffolding as they have a direct effect on cancer cell proliferation, survival and metastasis[25]. Individual ECM components have been shown to correlate with patient survival in PDAC. For example, in patients with primary human PDAC tumours, the median overall survival was 9.3 months in those with high stromal hyaluronic acid expression compared to 24.3 months in patients with low hyaluronic acid staining[23]. Similarly, the same study showed that high ECM type I collagen levels carried a median overall survival of 6.4 months whereas low levels of ECM type I collagen levels carried a higher median overall survival of 14.6 months. When patient data from a large, publicly available gene expression profiling dataset were stratified according to expression of specific collagens (COL1α2, COL2α1 and COL4α1), it was noted that patients expressing high levels of these fibrillar collagens had a poorer overall survival than those who did not[26]. However, when total collagen was interrogated in this manner, no correlations with patient outcomes were seen, which urges caution when using pan-collagen methods for histological analysis in human PDAC samples. Integrins are a family of transmembrane receptors present on cancer cells that mediate interactions with ECM proteins to affect cancer cell survival, migration, invasion and adhesion[27]. The β1 integrin family in particular is critical in mediating cancer cell interactions with several ECM components, including collagen I, collagen IV and fibronectin. The loss of β1 integrin in human pancreatic cancer cell lines resulted in decreased tumour volume and abrogation of metastasis in orthotopic mouse models[28].

ECM as a barrier to chemotherapy

Data suggest that genetic alterations in the PDAC epithelium affect both collagen architecture and the resulting tissue tension in PDAC. In a preclinical study using PDAC GEMMs, stromal collagen bundles were thicker in the KTC GEMM (*Kras*$^{LSL-G12D/+}$;*Tgfbr2*$^{flox/flox}$;*Ptf1a*$^{Cre/+}$) than in the KPC GEMM, and the former was associated with transforming growth factor-β (TGFβ) signalling loss and increased β1 integrin and STAT3 signalling in tumour epithelium that resulted in increased fibrosis and tissue tension[26]. A separate research group showed that small-molecule inhibition of STAT3 combined with gemcitabine treatment in the KTC GEMM resulted in improved animal survival and smaller tumour volumes than with gemcitabine monotherapy[29]. This treatment did not result in stromal collagen depletion but rather tumour stroma remodelling, which improved in vivo gemcitabine tumour delivery, presumably by alleviating stromal tissue tension. Importantly, the diameter of collagen fibres adjacent to epithelial lesions and levels of biochemical surrogates of tissue tension were greater in TGFβ-high (often *SMAD4* deleted) poorly differentiated PDACs than in well-differentiated or moderately differentiated tumours, which was validated in an independent patient cohort. Taken together, these data introduce the notion that stromal tissue tension is influenced by the underlying genetic makeup of the neoplasm which it surrounds, and this effect might have independent prognostic significance in PDAC.

The PDAC stroma is rich in proteolytic enzymes that contribute to stroma remodelling. This feature has been exploited for drug delivery purposes by engineering an amphiphilic peptide–phospholipid liposome matrix that was responsive to matrix metalloproteinase 2 (MMP2) and loaded with pirfenidone[30], an FDA-approved anti-fibrotic agent used in pulmonary fibrosis[31,32]. When this complex was administered to mice with orthotopically implanted human PDAC xenografts, a marked decrease in tumour collagen I, fibronectin and tenascin C protein levels was observed[30]. Importantly, superior drug penetration in tumours treated with the therapeutic liposome was observed and tumour volumes were substantially reduced in mice treated with gemcitabine and the pirfenidone-loaded MMP2-responsive liposomes compared with mice receiving gemcitabine or pirfenidone alone[30]. In a separate study, Jain and colleagues demonstrated that an angiotensin receptor II inhibitor, losartan, inhibits collagen I production in cancer-associated fibroblasts (CAFs), which limited the desmoplastic response in mouse models of breast, skin and pancreatic cancers[33]. This widely utilized anti-hypertensive drug led to improved interstitial transport of therapeutic nanoparticles and chemotherapeutic agents, with an early-phase clinical trial of 49 patients showing promising results in improving margin-negative resection rates in patients with previously unresectable PDAC[34]. Taken together, these data support an anti-fibrotic strategy in PDAC to counter the desmoplastic reaction and improve chemotherapy tumour penetration.

Alleviating intratumoural pressure to overcome the ECM barrier in PDAC.— The ECM has been posited to pose a barrier to the delivery of chemotherapeutic agents by specifically mediating increased interstitial fluid pressure (IFP) within the TME, which impairs vascular function and tumour permeability. In the KPC GEMM, the IFP of tumours was shown to be nearly ten times greater than the IFP of normal pancreas[35]. The normal

pancreas showed an abundance of vessels >10 mm in diameter, whereas KPC GEMM tumours had no vessels reaching this threshold, raising the possibility that increased IFP leads to intratumoural vessel collapse. Hyaluronic acid is a matrix glycosaminoglycan that is abundant in PDAC stroma and has been linked to elevated IFP[35]. Hyaluronidase encapsulated by polyethylene glycol (pegylated recombinant human hyaluronidase 20 (PEGPH20)) has been formulated to enable improved in vivo half-life and degradation of hyaluronic acid while minimizing the risk of hypersensitivity reactions[36]. The systemic administration of PEGPH20 to tumour-bearing KC ($Kras^{LSL-G12D/+};Pdx^{Cre/+}$) and KPC mice depleted hyaluronic acid in the tumour stroma within 24 h and resulted in decreased intratumoural IFP and an increase in vessel diameter in the mouse models[35]. KPC mice treated with a single cycle of PEGPH20 in addition to gemcitabine experienced a substantial decrease in tumour volume compared to mice that received gemcitabine monotherapy, suggesting that a reduction of IFP enhances tumour perfusion of chemotherapeutic agents. This translated into a significantly decreased incidence of metastasis and increased animal survival in comparison with either agent alone (55 days in the gemcitabine arm versus 91 days in the combination arm; $P = 0.004$)[35]. These findings were replicated by a separate group who demonstrated abundant hyaluronic acid in the stroma of KPC mice tumours and that PEGPH20, administered in vivo, mitigated vascular collapse and increased intratumoural gemcitabine delivery, which translated to a near doubling of animal survival compared with gemcitabine treatment alone[37]. Another group argued that intratumour solid stress, produced by cancer cells proliferating and transmitting stress to the ECM, is the main cause of increased pressure within the TME instead of IFP[38]. This latter group also observed IFP to be an order of magnitude lower than that reported by Provenzano and colleagues[35]. Subsequently, it was shown that the two models converged on the principal that the majority of interstitial PDAC fluid is actually present in a gel-fluid phase[39], an immobile interstitial fluid component in which abundant hyaluronic acid is found and heavily bound to water[40]. These findings explain the marked benefit of PEGPH20 in reducing total intratumoural pressures and vascular collapse (FIG. 2a).

PEGPH20 was assessed in a phase Ib/II clinical trial in combination with modified FOLFIRINOX (mFOLFIRINOX) in 138 treatment-naive patients with metastatic PDAC. Unexpectedly, a detrimental effect was seen in the PEGPH20 arm with a median overall survival of 14.4 months in the chemotherapy alone group versus 7.7 months in the chemotherapy plus PEGPH20 group[41]. Importantly, 45% of patients in the PEGPH20 plus mFOLFIRINOX arm experienced treatment-related serious adverse events in comparison with only 9% in the mFOLFIRINOX arm. As such, patients randomly assigned to mFOLFIRINOX only received a median of eight cycles of chemotherapy while those on the PEGPH20 arm received just four cycles. The increased toxicity and decreased treatment duration seen in the mFOLFIRINOX plus PEGPH20 arm was probably an important factor in this arm experiencing poorer overall survival. Assessment of tumour hyaluronic acid content was not required for study eligibility and was only assessed in a minority of patients but has not been reported to date. Despite these discouraging overall survival results, it is still noteworthy that four out of 55 patients in the chemotherapy plus PEGPH20 arm experienced a complete response, an exceedingly rare occurrence in advanced PDAC[4,5], in contrast to none of the patients in the chemotherapy-alone arm.

A phase III randomized controlled trial (RCT) was initiated, comparing gemcitabine plus nab-paclitaxel with or without PEGPH20 in patients with metastatic PDAC[42]. This trial (HALO-109-301) required high tumour levels of hyaluronic acid in the ECM by immunohistochemistry for patient eligibility in addition to upfront thromboprophylaxis for all participants. However, in 2019 it was reported that this study, unfortunately, did not meet its primary end point of overall survival or the secondary end point of progression-free survival (PFS)[43]. The PEGPH20 plus chemotherapy arm demonstrated an overall survival of 11.2 months compared to 11.5 months in the chemotherapy arm (HR 1.00, $P = 0.9692$). Data regarding outcomes stratified by hyaluronic acid staining have not been published at this time. Nonetheless, the failure of RCTs of PEGPH20 in combination with chemotherapy in patients with metastatic PDAC has led to the discontinuation of the PEGPH20 development programme[44].

Additional ECM therapeutic targets

ECM-derived energy sources.—Human PDAC is a nutrient-poor cancer with generally low levels of glutamine, glucose and glycolytic intermediates in comparison with adjacent normal pancreatic tissue[45]. As a result, PDAC cells have a selective capacity to catabolize large extracellular proteins to meet their amino acid needs, which was shown by the administration of labelled albumin to tumour-bearing mice[46]. Specifically, an ECM rich in type I and type IV collagens can provide a source of proline for PDAC cells, whereby collagens are degraded into peptides, which are subsequently digested to proline[47]. Peptide uptake was enhanced in glucose-limited and glutamine-limited conditions. Once transported inside the mitochondria, proline is converted into glutamate by proline dehydrogenase 1 (PRODH1), where it can enter and fuel the citric acid cycle, providing a rich source of ATP generation in PDAC cells under nutrient-limited conditions. When a competitive inhibitor of PRODH1, deshydroproline, was used to block proline degradation, a decrease in PDAC cell proliferation and survival were noted in vitro. Knockdown experiments of PRODH1 demonstrated decreased tumour volumes in vivo[47]. These studies demonstrate that the ECM is exploited by PDAC tumour cells to maintain a positive energy balance under nutrient-limited conditions. In this context, PRODH1 has emerged as a putative therapeutic target for limiting PDAC cell energy adaptation strategies, and the development of PRODH1 small-molecule inhibitors for preclinical modelling in PDAC is a feasible endeavour[48].

Rho-associated protein kinases.—The Rho-associated protein kinases (ROCKs) 1 and 2 control actomyosin contractility through substrate-level phosphorylation of myosin regulatory genes[49]. ROCK2 protein expression has been shown to increase during human PDAC disease progression, and increased *ROCK1* and *ROCK2* mRNA expression and/or gene amplification correlate with reduced patient survival in The Cancer Genome Atlas dataset[50]. Importantly, conditional ROCK expression in KPC mice resulted in decreased animal survival. ROCK1 and ROCK2 directly promote the invasive abilities of KPC cell lines in an in vitro collagen matrix model by the upregulation of multiple MMPs, leading to matrix remodelling. Treatment of KPC mice with a ROCK inhibitor, fasudil, led to improved animal survival along with increased levels of a surrogate marker for tissue collagen. In a separate study, when fasudil was combined with gemcitabine and nab-paclitaxel, decreased ki67 staining and increased cleaved caspase 3 staining was noted in organotypic KPC–

collagen matrix models[51]. Intravital mouse imaging of subcutaneously implanted KPC tumours revealed that transient pretreatment (priming) of the animal with fasudil led to enhanced disease control with gemcitabine or nab-paclitaxel. These findings were reproduced in an intrasplenic injection model of liver metastasis with the number of macro-metastases markedly reduced in fasudil-pretreated mice who went on to receive combination chemotherapy. Priming with fasudil also increased microvessel density (MVD) in primary and secondary sites, which suggests that increasing vascular routes improves delivery of chemotherapy into the tumour[51]. These data were also upheld in mouse models using primary human PDAC cell lines, where priming with fasudil followed by treatment with a gemcitabine and nab-paclitaxel combination resulted in improved animal survival[51] (FIG. 2b). There are currently no FDA-sanctioned clinical trials involving fasudil in PDAC. Nonetheless, it is an attractive agent given these preclinical data and its off-patent status could potentially make it a cost-effective treatment.

Focal adhesion kinases.—Focal adhesion kinases (FAKs) are non-receptor tyrosine kinases (RTKs) that are activated by ECM receptors including integrins. FAK activation is associated with ECM stiffness in PDAC[52]. Specifically, FAK1 has been shown to be a principal driver in PDAC desmoplasia and the generation of an immunosuppressive TME[53]; Jiang and colleagues showed that the majority of human PDAC epithelia exhibited an increase in FAK expression and activation, which were nearly absent in normal pancreatic epithelium. Furthermore, activated FAK (phosphorylated FAK) correlated highly with decreased tumour infiltration of cytotoxic T cells, increased collagen deposition and infiltration of granulocytes, features consistent with an immunosuppressive TME. Phosphorylated FAK1^{high}CD8^{low} human PDAC tumours carried a substantially worse prognosis than tumours without these features. In the KPC mouse model, treatment with a FAK small-molecule inhibitor reduced tumour fibrosis, progression, metastasis, infiltrating immunosuppressive myeloid populations and ultimately improved animal survival[53]. Importantly, a combination of FAK inhibition with PDL1 and CTLA4 antibodies improved animal survival versus either modality alone resulting in some long-term survivors. An early-phase clinical trial is currently under way to evaluate the maximum tolerated dose and overall response rate of a FAK small-molecule inhibitor, defactinib, in advanced PDAC patients[54] (FIG. 2c). Early results, presented in abstract form, showed stable disease in over half of the 15 patients evaluated, decreased phosphorylated FAK and changes in tumour T cell infiltration in pretreatment versus post-treatment biopsy samples[55]. Further data from the expansion cohort are forthcoming.

Discoidin domain receptor 1.—Discoidin domain receptor 1 (DDR1) is a RTK expressed on PDAC cells that is activated on binding fibrillar collagens and type IV collagen within the TME[56]. Cooperative signals from type I collagen binding to DDR1b and α2β1 integrin on PDAC cells results in E-cadherin to N-cadherin switching and epithelial-to-mesenchymal transition (EMT)[57,58], which contributes to chemoresistance[59] and metastasis[60,61] in PDAC. At a signalling level, type I collagen binding to DDR1 promotes protein tyrosine kinase 2 (PYK2) and pseudopodium-enriched atypical kinase 1 (PEAK1) protumour signalling in human pancreatic cancer cell lines. A small-molecule inhibitor of DDR1, 7rh, resulted in attenuated PYK2 and PEAK1 signalling and decreased migratory

capacity of cancer cells in vitro, while markedly enhancing the survival benefit from chemotherapy in orthotopic and KPC mice[62]. Collagen-mediated DDR1 signalling is also important in gastric carcinoma and *KRAS*-mutated lung cancer, with in vivo administration of 7rh resulting in decreased burden of disease in xenograft models of both malignancies[63,64]. Given the considerable desmoplastic reaction featuring ECM collagens in PDAC, DDR1 blockade might be a potential therapeutic strategy.

In conclusion, the PDAC ECM is a fundamental component of the cancer stroma, orchestrating key components of disease pathogenesis and the overall clinical course. Unfortunately, the most advanced ECM-targeted agent in clinical development for PDAC treatment, PEGPH20, failed to meet clinical trial end points despite sound preclinical data. Although this failure has certainly been disappointing, the ECM features multiple other attractive therapeutic targets based on promising preclinical PDAC models that are currently under clinical investigation.

PDAC-associated vasculature

Angiogenesis biology in PDAC

Nearly five decades ago, Folkman hypothesized that the growth of solid malignancies is dependent on angiogenesis and that anti-angiogenic agents would have therapeutic value[65]. At the time, the new field of tumour angiogenesis was met with great optimism[66] and culminated in the FDA approval of a vascular endothelial growth factor (VEGF)A therapeutic monoclonal antibody, bevacizumab, for colorectal cancer in 2004 (REF.[67]). Since that time, numerous anti-angiogenic therapies have been approved for a variety of cancers such as glioblastoma, non-small-cell lung cancer, renal-cell carcinoma, hepatocellular carcinoma and multiple myeloma. Unfortunately, no anti-angiogenic agent has proven clinically effective in PDAC.

Although the stroma in human PDAC tumours has been proposed to be hypovascular[68], MVD can vary considerably and when decreased is associated with poor survival in an inverse relationship with stromal surface area[69]. In a series of 40 resected human PDAC samples, 67.5% of samples were determined to be positive for VEGF protein expression in the cancer epithelium, which showed a modest correlation with MVD, although VEGF and MVD were independently adverse prognostic factors[70]. Early studies investigating angiogenesis in PDAC demonstrated abundant production of VEGF by human PDAC cell lines and resected tumour tissues[71]. Furthermore, these in vitro studies demonstrated that PDAC cancer cells produce VEGF under the control of activated HIF1α and STAT3 in oxygen-deprived conditions[72,73]. In a separate series of 55 human PDAC resection specimens, HIF1α was expressed in 40% of cases and positively correlated with VEGF expression, metastatic disease and MVD, and was inversely correlated with prognosis[74]. VEGF produced by human PDAC cell lines is functionally active in its ability to promote endothelial cell proliferation in vitro and in large tumours in an immunocompromised mouse xenograft model[75]. Moreover, anti-VEGF strategies were capable of reducing the growth of human PDAC cell lines orthotopically implanted into mice with a marked reduction in tumour MVD[76,77]. However, despite preclinical data suggesting angiogenesis is important in PDAC, the use of anti-angiogenic agents in PDAC has been a clinical failure.

Trials targeting PDAC vasculature

There are currently no FDA-approved anti-angiogenic agents to treat PDAC, despite numerous RCTs over the past decade aimed at evaluating such therapies. A phase II clinical trial of bevacizumab combined with gemcitabine in patients with metastatic PDAC supported the possibility that this could be an active regimen[78]. However, a subsequent phase III RCT of gemcitabine plus bevacizumab versus gemcitabine plus placebo in which 535 patients with advanced PDAC were treated, failed to meet its primary end point of improved overall survival[79]. In another phase III RCT, 607 patients with metastatic PDAC were randomly assigned to receive gemcitabine and erlotinib, an RTK inhibitor acting on the epidermal growth factor receptor and the only other FDA-approved therapy for PDAC at the time, with bevacizumab or placebo[80]. This trial also failed to meet its primary end point as there was no difference in overall survival between the two study arms although the bevacizumab-containing arm showed a statistically significant increase in PFS. The VEGF-trap, aflibercept, was also evaluated in a phase III multicentre RCT in patients with metastatic PDAC in combination with gemcitabine, but the study was halted after a preplanned futility analysis of 427 patients failed to show a difference in overall survival or PFS between the gemcitabine plus aflibercept group and the gemcitabine monotherapy group[81]. Another large phase III RCT involved 632 patients with advanced PDAC that received gemcitabine or gemcitabine plus a potent pan-VEGFR small-molecule inhibitor, axitinib[82,83]. The primary end point of overall survival was not met, and this study was also terminated after an interim futility analysis, further discouraging an anti-angiogenic strategy in PDAC. The multitargeted anti-angiogenic kinase inhibitor sorafenib[84] was also investigated in a multicentre RCT and failed to show an improvement in overall survival[85]. Early-phase clinical trials of other angiogenesis-modulating agents such as lenolidomide[86] also yielded disappointing results in PDAC and are no longer being pursued for this indication. Despite success in other solid malignancies, the repeated failure of anti-angiogenic medications in PDAC requires that the rationales behind their use be revisited if anti-angiogenic agents are ever to benefit PDAC patients.

Rationalizing disappointment in PDAC

Anti-angiogenic therapy results in tumour hypoxia, which promotes chemoresistance, cancer stem cell expansion, invasion and metastasis in multiple in vivo cancer models[87–90]. When human PDAC cell lines were orthotopically implanted into mice and treated with bevacizumab, tumours were eventually able to escape anti-angiogenic treatment and achieve rapid growth rates[91]. Upon isolation of the bevacizumab-resistant cell lines, they displayed an EMT phenotype and overexpression of numerous inflammatory cytokines such as CXCL1, CXCL3, IL-1β, CCL2 and CCL5 in comparison with their parental cell lines. Moreover, using a PDAC GEMM, Aguilera and colleagues found that chronic treatment with a VEGF antibody induced hypoxia and led to increased collagen deposition, epithelial plasticity and metastatic burden[92]. These tumours contained increased levels of fibrillar collagen and collagen signalling, suggesting that VEGF blockade promotes tumorigenicity in PDAC at least in part through hypoxia-induced collagen production and signalling. These data contribute to our understanding of the failure of anti-angiogenic agents in PDAC clinical trials whereby VEGF inhibition decreases MVD within the tumour but ultimately might lead to a more aggressive, mesenchymal-like PDAC with enhanced chemoresistance[59]

and ultimately treatment failure. Given the overwhelming clinical failure of anti-angiogenic agents in PDAC, what role do anti-angiogenic strategies have in the future of PDAC therapeutics?

Anti-angiogenic therapy as a strategy to normalize PDAC vasculature.— Tumour vasculature normalization is a concept in which immature and inefficient blood vessels are pruned by eliminating excess endothelial cells, and therefore unproductive vasculature, enabling reliable delivery of intravenous cancer therapeutics[93,94]. Critically, vessel normalization involves increasing pericyte coverage of the vascular endothelium and reducing vessel leakiness in a paradoxical effort to improve tumour perfusion[95]. In turn, enhanced tumour oxygenation suppresses cancer cell metastasis while promoting differentiation[96].

Various preclinical strategies for vascular normalization have been proposed, exploiting several molecular targets. Semaphorin 3A (SEMA3A) is a molecule secreted by endothelial cells, which participates in promoting physiological vascular normalization by negative regulatory effects on integrins[97]. It is present in precancerous lesions but lost during tumour progression[98], consistent with a dysfunctional, abnormal tumour vasculature late in the disease course. SEMA3A overexpression reduced tumour tissue hypoxia and abrogated the pro-invasive phenotype seen with specific VEGFR2 blockade and treatment with the multikinase-targeted anti-angiogenic agent, sunitinib, in pancreatic neuroendocrine and cervical cancer mouse models[98]. Unfortunately, SEMA3A interaction with its receptor, neuropilin 1, attracts protumoural macrophages[99], which is undoubtedly a disadvantage to a SEMA3A-based approach to tumour vasculature normalization. To circumvent this problem, one study described the generation of a mutant isoform of SEMA3A that does not interact with neuropilin 1 but has a high affinity for the SEMA3A co-receptor, type A plexin[100]. Through interaction with the plexin, this novel molecule resulted in normalization of the tumour vasculature, impairment of metastatic disease progression and improved delivery of gemcitabine in mouse models of pancreatic neuroendocrine tumour and PDAC. Another vascular normalization strategy utilized a small-molecule inhibitor of nucleolin in a PDAC orthotopic mouse model, which resulted in an increase in vessel pericyte coverage and tumour perfusion, culminating in decreased tumour hypoxia and effectively constituting a tumour vessel normalization effect[101]. This approach also resulted in enhanced tumour delivery of gemcitabine with an improved antitumour drug effect. These molecular targets represent potentially powerful clinical strategies to normalize the PDAC tumour vasculature, which might improve chemotherapy delivery to the tumour and decrease PDAC cell aggressiveness that results from tumour hypoxia.

An important added benefit to tumour normalization will be the subsequent administration of more effective chemotherapy regimens currently used in clinical practice. The multidrug PDAC regimen, FOLFIRINOX, is active and contributes to improved overall survival in the curative and metastatic settings in comparison to gemcitabine monotherapy[4,102,103]. Most of the anti-angiogenic trials in PDAC were designed prior to the FOLFIRINOX era at a time when the chemotherapy regimen of choice was single-agent gemcitabine. Hopefully improved drug delivery that accompanies any successful tumour vessel normalization

strategy will result in even greater overall responses with the subsequent administration of FOLFIRINOX instead of gemcitabine monotherapy.

Tumour vasculature normalization as an immunosensitization strategy in PDAC.—In addition to enabling more reliable drug delivery and potentially attenuating tumour hypoxia-associated EMT, tumour vasculature normalization has emerged as an attractive method of promoting antitumour immune responses. Hypoxic conditions increased in vitro PDL1 cell surface protein levels in human prostate and breast cancer cells in addition to murine melanoma cells in an HIF1α-dependent manner[104]. Furthermore, in vitro hypoxic conditions have been shown to impair human macrophage M1 polarization by modulating the expression of costimulatory T cell molecules and secreted cytokine receptors, as the macrophages are pushed towards an immunosuppressive M2 phenotype[105]. Moreover, in a syngeneic orthotopic model of murine breast cancer, a low dose (10 mg/kg body weight) of a VEGFR2-specific antibody (DC101) was more effective than a high dose (40 mg/kg body weight) in improving tumour vessel pericyte coverage and normalizing the vasculature[106]. Low-dose DC101 treatment resulted in the polarization of perivascular tumour-associated macrophages to an M1 phenotype and ultimately promoted the infiltration of T cells into the tumour. When combined with a whole cancer-based vaccination strategy, low-dose DC101 resulted in decreased tumour volumes and improved survival of mice. Given the immune privilege status of PDAC[107], a low-dose VEGF inhibition strategy might be warranted for future assessment.

It has long been appreciated that the VEGF ligand itself might have potent immunosuppressive effects within the TME by altering the tumour cytokine milieu[108], and inhibiting T cell[109] and dendritic cell[110] maturation, leading to the possibility that anti-VEGF agents might have immune sensitizing effects. Prolonged VEGF expression in the tumour vasculature can decrease T cell extravasation by downregulation of ICAM and VCAM1 adhesion molecules on the endothelial cell surface[111]. To that end, treatment with a VEGF antibody was capable of increasing tumour infiltration of adoptively transferred T cells and improving animal survival in a syngeneic mouse model of melanoma[112]. Moreover, the extraction of angiogenesis-related genes from publicly available breast cancer gene expression profile data revealed the presence of favourable and unfavourable PFS groups, with the former showing a marked enrichment for T cell-related genes[113]. When human bladder, breast, liver and ovary PDXs were xenografted into immunocompromised mice an increased level of tumour hypoxia was noted in comparison with the original tumour in patients. This phenotype was reversed in seven of nine PDXs with the adoptive transfer of type 1 T helper cells. Low-integrity, pericyte-poor tumour vasculature might limit the ability of T cells to traffic to the site of a tumour and, by normalizing these vessels, improved tumour T cell infiltration might be possible, thus sensitizing the tumour to immune checkpoint blockade. Additionally, when VEGF blockade was combined with cyclooxygenase 2 blockade in a PDAC GEMM, anti-VEGF-induced EMT and collagen deposition were reversed[114]. The combination treatment also altered the immune landscape by increasing tumour infiltration of cytotoxic T cells while decreasing regulatory T cells. As such, by structurally normalizing the tumour vasculature and blunting the anti-T cell effects

of VEGF signalling, anti-angiogenic therapy has substantial potential to be repurposed as an immunosensitizing approach for PDAC treatment.

In conclusion, although clinical trials using anti-angiogenic strategies in PDAC have been unsuccessful, more recent preclinical models have added new perspectives on how the tumour vasculature might be exploited in the treatment of PDAC. Tumour vessel normalization as a means to improve drug delivery to tumours in addition to sensitization to immune checkpoint blockade offers renewed potential for an anti-vasculature approach to PDAC (FIG. 3).

Intratumoural CAFs in PDAC

CAF heterogeneity

Fibroblasts are supportive cells of mesenchymal origin that are present in substantial quantities in nearly every solid organ. Fibroblasts from each anatomical site have specific gene expression profiles, largely concordant with positional identities in relation to major anatomic axes[115]. They are critical to homeostatic mechanisms governing functional epithelium by providing structural support in addition to secreting soluble factors and ECM proteins[116]. In the setting of cancer, there is an overwhelming body of evidence that demonstrates that CAFs are not mere cellular bystanders but active players during the process of cancer initiation, progression and metastasis[117]. The contribution of CAFs to the biology of PDAC and other carcinomas has generally been held to be tumour-promoting[118], making targeting of CAFs an attractive therapeutic strategy. However, it has also been realized that the functions of CAFs in PDAC biology are more complex than a homogeneous tumour-promoting phenotype[119,120]. As such, a precise characterization of intratumoural CAF heterogeneity is essential to the development of effective therapies targeting PDAC stroma. A molecular categorization of PDAC CAFs has been proposed using a three-dimensional in vitro co-culture system consisting of pancreatic stellate cells (PSCs) and KPC mouse-derived PDAC organoids[121]. PSCs are fibroblasts that are present in the periacinar, periductal and perivascular space of the normal pancreas; they express glial fibrillary acidic protein and display intracellular fat droplets[122]. PSCs expressed α-smooth muscle actin (αSMA) and assumed a myofibroblastic gene expression profile in vitro when directly co-cultured with KPC mouse-derived organoids[121]. These cells were referred to as myofibroblastic CAFs (myCAFs). By contrast, when PSCs and KPC mouse-derived organoids were indirectly co-cultured with an intervening semipermeable membrane, PSCs did not upregulate αSMA, but rather expressed IL-6 and a host of other inflammatory cytokines, and were termed inflammatory CAFs (iCAFs). In human and mouse PDAC tissues, αSMA protein expression was indeed noted in juxtatumoural fibroblasts, whereas IL-6-positive fibroblasts were seen more distal to the tumour edge in a mutually exclusive manner[121]. This model proposes that resident PSCs can give rise to the two major novel CAF subtypes (FIG. 4). In addition, single-cell RNA sequencing (scRNAseq) of human PDAC and intraductal papillary mucinous neoplasm (IPMN) samples has confirmed the presence of myCAF and iCAF stromal fibroblast populations, with the former expressing αSMA and the latter expressing CXCL12 and IL-6 (REF.[123]). Interestingly, the iCAF population was absent in low-grade and high-grade IPMNs whereas myCAFs were abundant

in high-grade IPMNs, potentially reflecting a host protective response to advanced preneoplasia.

Tumour-derived cytokines direct the generation of the two molecular subtypes of CAFs[124]. Specifically, following exposure of PSCs to KPC cancer cell conditioned media or indirectly co-cultured with KPC mouse-derived organoids, the generation of the iCAF expression profile was accompanied by rapid phosphorylation of nuclear p65, a marker of nuclear factor-κB (NF-κB) activation. The dependence of PDAC iCAFs on NF-κB signalling was illustrated when the iCAF gene expression profile was attenuated by treatment with an NF-κB small-molecule inhibitor[124]. In addition, the treatment of mouse and human PSCs with recombinant IL-1α resulted in the expression of inflammatory markers and downregulation of myofibroblast genes such as *Acta2* and *Ctgf*, whereas treatment of PSCs cultured in KPC organoid conditioned media with an IL-1α neutralizing antibody resulted in decreased inflammatory gene expression. IL-1α induced LIF upregulation in PSCs, which in turn activated JAK–STAT signalling and promoted the induction of the iCAF phenotype that was maintained through STAT3 signalling. Treatment with an LIF-neutralizing antibody blocked JAK–STAT activation, whereas neutralization of granulocyte colony-stimulating factor or IL-6 did not reduce JAK–STAT signalling or iCAF marker gene expression[124]. Indeed, our group and another study found that IL-6 and LIF produced by PDAC CAFs acted in a paracrine fashion on pancreatic cancer cells to promote an EMT phenotype, chemoresistance, immune evasion and worsen animal survival in mouse models of PDAC[125,126]. Importantly, LIF protein levels in the serum of patients with PDAC correlated with poorly differentiated tumours and were a better indicator of response to neoadjuvant chemotherapy in patients with PDAC than the most commonly used PDAC tumour marker, CA19–9 (REF.[125]). These data support that LIF might be a potential serum biomarker of response and therapeutic target in PDAC. Conversely, myCAFs displayed increased SMAD2 and SMAD3 phosphorylation, which is evidence of TGFβ signaling[124]. Indeed, immunofluorescence demonstrated αSMA–pSMAD2 double-positive cells in peritumoural fibroblasts in human PDAC and KPC mouse tissues. The myCAF features were recapitulated in vitro when PSCs were treated with recombinant TGFβ1, which also led to IL-1 receptor (IL-1R) negative regulation and attenuation of the iCAF phenotype. These data illustrate distinct mechanisms of myCAF and iCAF genesis in PDAC (FIG. 5).

Another report from Tuveson and colleagues used a scRNAseq approach to profile cellular heterogeneity in several human PDAC tumours and the KPC GEMM[127]. These data reaffirmed the presence of iCAFs and myCAFs in mouse PDAC and, importantly, in human PDAC. However, another putative subtype of CAF was identified — antigen-presenting CAFs (apCAFs). apCAFs overexpressed several major histocompatibility complex (MHC) class II family members, including CD74, which was shown to be co-expressed at the protein level with the pan-CAF marker, podoplanin. Of note, scRNAseq analysis from this report suggests that apCAFs might be transcriptionally distinct from iCAFs and myCAFs. Coincidentally, our group previously demonstrated the existence of an MHC class II molecule-rich CAF population in an scRNAseq dataset that included both KIC (*Kras*$^{LSL-G12D/+}$;*Cdkn2a*$^{fl/fl}$;*Ptf1a*$^{Cre/+}$) and KPC GEMMs[128]. Intriguingly, MHC class II molecule-expressing CAFs demonstrated the ability to induce CD25 and CD69 in co-cultured T cells after having been loaded with ovalbumin peptide[127,129]. These initial data

on apCAFs introduce a promising line of investigation, and further analyses are required to discern why this putative cell type is not capable of attenuating the immune privileged status of PDAC.

In 2019, scRNAseq of viable podoplanin-positive stromal cells from the pancreata of KPP (*Kras*$^{LSL-G12D}$;*Cdkn2a*$^{flox/flox}$;*Pdx1*$^{Cre/+}$) and normal mice was performed[130]. Consistent with prior scRNAseq studies of intratumoural CAF heterogeneity, a CAF population rich in ECM genes was noted (consistent with myCAFs) as was another population rich in inflammatory genes (consistent with iCAFs) in late-stage tumours. Further analyses revealed similar fibroblast populations in normal mouse pancreas and early-stage KPP mice, which give rise to the late-stage iCAF and myCAF populations. Pathway analyses of these populations were consistent with IL-1 and TGFβ signalling, respectively. Interestingly, a mesothelin-expressing population of fibroblasts was noted in the normal mouse pancreas, which expressed MHC class II genes and clustered closely with the previously reported apCAFs[127]. Importantly, this mesothelin-positive and MHC class II-rich population of fibroblasts was previously noted in the normal mouse pancreas by our group and might have given rise to the apCAFs in late tumours[128].

Notably, CAFs can also influence PDAC cancer cell heterogeneity. Using in vitro direct co-culture between human PDAC primary cell lines and CAFs, Ting and colleagues demonstrated that CAFs promote a shift in PDAC cells towards invasive and proliferative phenotypes[131]. High-content digital imaging of RNA in situ hybridization of 195 human PDAC samples (comprised of close to 320,000 single cells) showed that tumour gland ‘units’ exhibited varying ratios of invasive and proliferative cancer cells, associated with differences in stromal abundance, which resulted in distinct clinical outcomes.

iCAFs.—There is strong evidence that the protumorigenic properties of iCAFs are critical to PDAC stromal biology. The biology of iCAFs has formed the basis for numerous ongoing clinical trials in PDAC (TABLE 1). The inflammatory properties of CAFs across multiple cancer types were noted prior to the explicit categorization of PDAC iCAFs and myCAFs by Tuveson and colleagues[121]. Fibroblasts derived from a mouse model of skin carcinogenesis were gene-expression profiled, revealing a pro-inflammatory gene signature that was recapitulated when normal skin fibroblasts were cultured in skin cancer cell conditioned media, implicating a paracrine-mediated process in the generation of iCAFs[132]. In vivo, these inflammatory skin CAFs promoted tumour growth, neovascularization and macrophage recruitment[133]. Short hairpin RNA knockdown of IKKβ demonstrated that the protumour phenotype of these CAFs was also dependent on NF-κB signalling, which in turn required IL-1β paracrine action[132] (FIG. 5).

In the context of PDAC, CAFs were also sorted from KPC mouse tumours and showed an inflammatory gene expression signature that included IL-6 and CXCL1, consistent with reports of PDAC iCAFs displaying marked NF-κB signalling as a result of IL-1α-mediated paracrine activity[134]. Moreover, IL-1 receptor-associated kinase 4 (IRAK4) is highly activated in CAFs of human PDAC and KPC mouse tumours[135] in contrast to normal human pancreatic fibroblasts that display comparatively low phosphorylated IRAK4 levels. IRAK4 activation in CAFs promoted cell proliferation and tumorigenesis, and enhanced stromal

fibrosis in vivo. IL-1β, both autocrine CAF-derived and paracrine tumour-derived, was the critical cytokine responsible for IRAK4 activation in CAFs. Small-molecule inhibition and genetic knockdown of IRAK4 or IL-1R disrupted tumour stroma IL-1β–IRAK4 feed-forward circuitry, leading to decreased tumour collagen content and enhanced sensitivity to gemcitabine in vivo. The expression of several cytokines, such as IL-6, IL-8, CXCL2 and CXCL5, were diminished in response to IRAK4 knockdown and pharmacological inhibition, indicating a dependence on IRAK4 signalling for the iCAF phenotype. Tissue microarray analysis of stromal IL-1β expression was shown to have prognostic significance where low expressors survived for a median of 2.6 years versus high expressors that survived for a median of 1.2 years[135]. Given the abundance of preclinical data implicating IL-1-mediated signalling in iCAF disease pathophysiology, IL-1R blockade is currently undergoing clinical evaluation in combination with standard-of-care chemotherapy for advanced PDAC[136]. Also of note, given the role of JAK–STAT signalling in PDAC iCAF pathogenesis, the treatment of KPC mice with a pharmacological JAK inhibitor as an iCAF targeting strategy resulted in decreased tumour volumes and cancer cell proliferation accompanied by increased αSMA-positive fibroblasts[124]. Unfortunately, ruxolitinib, a JAK1 and JAK2 inhibitor, failed to improve overall survival in two phase III RCTs in patients with locally advanced or metastatic PDAC[137], necessitating further strategies for targeting the protumorigenic iCAF cell type.

The elucidation of specific cell surface markers for iCAFs might reveal readily druggable targets. In an inducible *KRAS* and *TP53* mutant model of PDAC, >70% of stromal cells were positive for αSMA, indicating that the majority of CAFs possess a myofibroblastic phenotype in this GEMM[138]. In this study, platelet-derived growth factor α (PDGFRα)-positive cells made up approximately half of stromal cells, with a third being double-positive for αSMA and PDGFRα. Whether the $\alpha SMA^{+}PDGFR\alpha^{+}$ CAF has phenotypic properties of iCAFs, myCAFS or both is not clear. However, when the $PDGFR\alpha^{+}$ fraction was sorted and expression profiled, numerous cytokines were upregulated in comparison with normal mouse pancreatic fibroblasts, establishing this receptor as a marker of the iCAF phenotype. Serum amyloid A3 (SAA3), a member of the serum amyloid A apolipoprotein family, was markedly upregulated in these pro-inflammatory CAFs and was essential to the protumorigenic function of the $PDGFR\alpha^{+}$ iCAF population. Although wild-type iCAFs promoted tumour growth in an orthotopic mouse model, SAA3-null CAFs inhibited tumour growth. However, this antitumour phenotype was abrogated when SAA3 was knocked down in tumour cells and CAFs, making targeting of SAA3 potentially challenging in practice. Nonetheless, PDGFRα might prove to be a therapeutic target against CAFs. Indeed, an early-phase clinical trial is under way to evaluate the role of a PDGFRα monoclonal antibody, olaratumab, in patients with metastatic PDAC[139].

RNAseq analysis of flow cytometry-sorted FAP^{+} cells revealed increased expression of myofibroblastic and inflammatory genes relative to normal pancreas[140]. When KPC mouse tumours underwent selective depletion of FAP^{+} cells, tumour volumes decreased in a $CD4^{+}$ and $CD8^{+}$ T cell-dependent manner. Treating FAP^{+} cell-depleted KPC mice with PDL1-blocking or CTLA4-blocking antibodies dramatically reduced tumour volumes. When KPC mice were treated with a combination of CXCR4 and PDL1 blockade a marked reduction in tumour volume was also seen. In addition, given that FAP^{+} fibroblasts were found to be the

principal source of CXCL12 (the ligand for CXCR4), iCAFs probably have an important function in dampening the antitumour immune response. In addition, Garg and colleagues orthotopically implanted KPC tumours with normal mouse pancreatic fibroblasts or mouse pancreatic fibroblasts in which the p50 subunit of NF-κB was deleted ($p50^{-/-}$)[141]. They demonstrated that co-injection with $p50^{-/-}$ fibroblasts shrank tumours, increased cytotoxic T cell tumour infiltration and prolonged animal survival. Importantly, NF-κB was shown to mediate CXCL12 secretion in non-neoplastic fibroblasts co-injected with KPC tumour cells, which in turn was responsible for the exclusion of cytotoxic T cells in this model. The non-neoplastic pancreatic fibroblasts with induced NF-κB signalling are consistent with known iCAF biology, and provide another example of iCAFs mediating an anti-immune response in PDAC. CXCL12 is an iCAF-derived molecule, which is an attractive potential target for immunosensitization in PDAC. A phase I/II clinical trial is under way that aims to evaluate the CXCL12 inhibitor NOX-A12 in combination with pembrolizumab in colorectal and pancreatic adenocarcinomas with the primary objective of assessing pretreatment and post-treatment tumour biopsy samples for changes in immune cell infiltrates[142]. In addition, the CXCR4 inhibitor BL-8040 has been combined with pembrolizumab and 5-fluorouracil and leucovorin in a phase II clinical trial of patients with metastatic PDAC who have progressed on first-line gemcitabine-based chemotherapy[143]. Preliminary data reported partial responses in four patients and stable disease in eight patients, accounting for disease control in 12 of 15 evaluable patients with a total accrual goal of 40 patients[144]. Ten patients reported serious adverse events, leading to treatment discontinuation in two of those patients. These early data are reason for cautious optimism as accrual is ongoing.

IL-6 is an inflammatory cytokine secreted by iCAFs in response to paracrine signalling from malignant epithelium[121], which leads to STAT3 activation and enhanced invasion and colony formation of precursor PanIN and PDAC mouse cells in vitro[145]. Treatment of KPC mice with a monoclonal antibody against IL-6R in combination with gemcitabine resulted in decreased cancer cell STAT3 activation, enhanced tumour cell apoptosis, tumour regression and ultimately increased animal survival[146]. Elevated IL-6 levels in human serum is an independent risk factor for the development of extensive hepatic metastases in patients with PDAC[147]. In addition, it has been demonstrated in the KPC GEMM that iCAF-derived IL-6 from the PDAC TME travels to the liver in an endocrine manner and induces STAT3 signalling on binding to IL-6R on hepatocytes[148,149]. Subsequent STAT3 signalling culminates in an influx of locally immunosuppressive myeloid cells and increased hepatocyte ECM deposition, thereby forming the premetastatic niche for PDAC liver metastases. To target IL-6 signalling in PDAC, a clinical trial involving 140 patients with locally advanced or metastatic PDAC is currently under way in which patients are being randomly assigned to receive gemcitabine and nab-paclitaxel plus the IL-6R monoclonal antibody tocilizumab versus chemotherapy alone[150]. Nonetheless, although there have been reports of IL-6 inhibition leading to decreased tumour growth in immunodeficient orthotopic mouse models[151], the limited available clinical data involving IL-6R blockade in combination with chemotherapy have not been encouraging[152].

One study suggests that CAF-derived IL-6 is a major contributor to immune evasion in PDAC[126]. In addition, other preclinical evidence in immunocompetent mouse models supports the use of IL-6R blockade as a sensitization strategy for checkpoint inhibition in

PDAC. IL-6 has been shown to be central in mediating cancer-associated cachexia in KPC animals and impairment of hepatic ketogenesis during caloric restriction in GEMMs and patients with PDAC[153]. The resulting increase in compensatory glucocorticoid levels caused a systemic immunosuppressed state, which in turn contributed to immunotherapy failure in KPC mice. Moreover, IL-6 and numerous other pro-inflammatory genes were shown to be highly expressed in unsorted primary human PDAC CAFs[154]. Furthermore, combined IL-6–PDL1 blockade resulted in decreased tumour volumes in a syngeneic orthotopic PDAC model in a $CD8^+$ T cell-dependent manner. IL-6–PDL1 blockade led to a 35% increase in median survival in a *Kras–Tp53–Brca2*-driven PDAC GEMM, in which increased numbers of circulating type 1 T helper cells were seen as a percentage of total $CD4^+$ T cells in addition to an increase in intratumoural lymphocytes. These data support further clinical testing of dual IL-6 and PDL1 blockade in PDAC with one such trial currently active[155].

myCAFs.—Although CAFs were historically thought to be synonymous with myofibroblasts[156], evidence indicates that αSMA^+ myofibroblasts are a distinct population of CAFs (myCAFs)[121,124,127,128]. Although the existence of distinct αSMA^+ and αSMA^- CAF populations has garnered substantial attention, the functional antitumour activity of αSMA^+ CAFs in the PDAC TME was previously established. In the first instance, with the caveat that tumour-derived sonic hedgehog (Shh) drives a desmoplastic stromal reaction in PDAC, the pharmacological inhibition and tumour-specific genetic ablation of Shh in KPC mice resulted in depletion of stromal fibroblasts from the primary tumour and, ultimately, tumours that featured less differentiation, increased vascularity and metastases, resulting in decreased animal survival[120]. In this model, all αSMA^+ CAFs displayed active Shh signalling and were depleted upon genetic ablation of Shh in the cancer cells, reaffirming the correlation between paracrine Shh signalling and αSMA^+ stromal fibroblasts[157]. In addition to the genetic and pharmacological inhibition of Shh in reducing the desmoplastic response and animal survival, in vivo administration of an Shh agonist resulted in increased numbers of stromal fibroblasts and decreased tumour proliferation[158]. These data provided the mechanistic rationalization for the previous failure of Shh inhibitors in clinical trials, which either did not improve PFS[159] or even worsened overall survival compared with placebo[160]. In parallel, Özdemir and colleagues demonstrated that depletion of αSMA^+ fibroblasts from the stroma of KPC mice resulted in poorly differentiated tumours and shortened animal survival[119]. Low myofibroblast content, assessed in whole tumour sections, was also associated with worse overall survival in human PDAC. The myofibroblast-depleted tumours in KPC mice also had reduced fibroblasts positive for fibroblast-specific protein 1 (FSP1; also known as S100A4 or calvasculin), indicating considerable overlap in the αSMA^+ and $FSP1^+$ CAF populations. However, the number of FAP^+ CAFs was unchanged, indicating a functionally distinct population of CAFs, putatively iCAFs (albeit not named as such when this paper was published). Although a previous study showed poorer outcomes associated with high αSMA stromal fibroblast content in PDAC[161], a desmoplastic stroma was associated with improved patient survival. A separate study showed that poorly differentiated human PDAC tumours had a reduced number of αSMA^+ cells[26]. Taken together, these data prompted a paradigm shift that suggested a subpopulation of CAFs function to constrain tumour growth in PDAC. It is not clear how αSMA^+ CAFs mediate an antitumour phenotype. Although the tumour-promoting iCAF subtype is rich in cytokines,

which might be amenable to therapeutic targeting, the tumour constraining myCAF subtype lacks straightforward therapeutic strategies at this time.

mscCAFs.—The existence of bone marrow-derived mesenchymal stem cells (MSCs) within the TME of several solid tumours has been described, but they comprise a minority of the stromal fibroblast population[162–164]. Functionally, bone marrow-derived MSCs promoted metastasis in an immunodeficient mouse model of multiple subcutaneously implanted breast cancer cell lines in a CCL5-dependent manner[165]. Studies have systematically interrogated human and mouse stroma for the presence of MSCs in the PDAC microenvironment. In ex vivo cultured CAFs from resected human PDAC samples, a small population of MSCs was identified by positivity for four previously used MSC markers: CD44, CD49a, CD73 and CD90 (REF.[166]). Thirteen patient CAF cultures were comprised of a median of 7.3% MSCs with a range of 0.6–20% of the total ex vivo CAF population, which was largely concordant between freshly disassociated tumours. Co-injection of human PDAC cells with primary MSC CAFs (mscCAFs) in an orthotopic mouse model resulted in significantly larger primary tumours ($P < 0.01$) and an increased incidence of liver metastases compared with cancer cells injected alone. This finding was accompanied by an increase in collagen deposition in the mscCAF co-injection tumours. Compared with cancer cells injected alone, there was no significant increase in tumour size or metastases when bulk CAFs were co-implanted with PDAC cells, with even a trend toward decreased tumour size. Granulocyte–macrophage colony-stimulating factor (GM-CSF) was noted to be preferentially expressed in the mscCAFs in comparison with bulk CAFs and drove an EMT and a stem cell-like phenotype in cancer cells in a paracrine fashion. Knockdown of GM-CSF expression in the mscCAFs led to a marked reduction in tumour size and extinguished metastases, thereby identifying the putative cytokine responsible for mscCAF tumour promotion and a valuable target. Importantly, the evidence supporting that GM-CSF-secreting mscCAFs contribute to the polarization of macrophages towards an immunosuppressive phenotype was presented in two simultaneously published reports. Using a PDAC GEMM, the $GR1^+CD11B^+$ macrophage population was shown to suppress antigen-specific T cells[167]. The development of this macrophage population required GM-CSF, as in vivo knockdown of this cytokine blocked tumour development in a $CD8^+$ T cell-dependent manner. In the second study, *KRAS*-mutated pancreatic ductal epithelium was a substantial source of GM-CSF production in the TME, which was required for the recruitment of $GR1^+CD11B^+$ myeloid cells to the tumour stroma[168]. GM-CSF ablation in the neoplastic epithelium resulted in a marked decrease in tumour volume that was also shown to be a $CD8^+$ T cell-mediated process. Additionally, GM-CSF within the TME correlated with poor survival in patients with PDAC[169]. In human PDAC, blocking the CSF1R receptor with the monoclonal antibody cabiralizumab has shown promise; interim analysis of an early-phase clinical trial[170] testing cabiralizumab in combination with a PD1 checkpoint inhibitor, nivolumab, in metastatic solid tumours, including PDAC, showed a 6-month disease control rate of 13% and objective response rate of 10%. Further patient stratification will be required to predict responders to the CSF1R–nivolumab combination for later-stage clinical trials.

Additional therapeutic targets in CAFs

IGF1 and AXL signalling.—PDAC cells signal to pancreatic CAFs via Shh secretion that increases myofibroblast content and the desmoplastic reaction[120]. Shh can also upregulate CAF secretion of insulin-like growth factor 1 (IGF1) and GAS6, causing activation of their respective RTKs, IGF1 receptor (IGF1R) and AXL[171]. These parallel signalling pathways converge on downstream AKT signalling in a non-cell autonomous fashion. Although paracrine signalling between PDAC cells and Shh-activated CAFs caused increased tumour cell mitochondrial performance, proliferation, anchorage-independent growth and resistance to apoptosis, all of these protumorigenic phenotypes were reversed by dual IGFR–AXL pharmacological inhibition in a heterotypic in vitro model, whereas inhibition of a single RTK was ineffective[171]. Of note, a small-molecule AXL inhibitor has been shown to increase gemcitabine sensitivity in a PDAC GEMM[172]. Furthermore, in a KPC orthotopic mouse model, CAFs and tumour-associated macrophages were found to be the main sources of IGF1 in the TME[173]. The blockade of IGF1R with a monoclonal antibody significantly sensitized tumours to gemcitabine, leading to decreased tumour volume and increased cancer cell apoptosis. The translational relevance of these findings was underlined by immunohistochemical analyses demonstrating increased phosphorylated IGF1R staining in 72% of patients with PDAC that were assessed. Moreover, primary human CAF cultures showed increased ex vivo secretion of IGF1 and hepatocyte growth factor, the latter leading to Src activation[174]. In turn, activated IGF1R and Src led to the phosphorylation of the metastatic driver annexin A2. Dual pharmacological inhibition of Shh and hepatocyte growth factor resulted in decreased stromal αSMA content and increased tumour E-cadherin expression in KPC GEMM and orthotopic mouse models. This dual-targeting strategy led to decreased tumour volume and incidence of metastasis whereas single-agent therapy did not show efficacy. Given the well-documented antitumour function of myofibroblasts in PDAC[119,120], it might be prudent to avoid myofibroblast and/or Shh targeting as a strategy to decrease IGF1 production in the PDAC microenvironment. Thus, blocking IGF signalling and avoiding upstream targeting of the Shh-driven myofibroblast phenotype is preferred. Furthermore, there is little evidence that IGF1 is produced by the myCAF population. Rather, scRNAseq data show that the iCAF population is the chief expressor of IGF1 in the TME[128], reinforcing the characterization of iCAFs and myCAFs as protumour and antitumour cell types, respectively.

The largest clinical study assessing IGF1R blockade in PDAC consisted of 800 treatment-naive patients with metastatic PDAC randomly assigned to gemcitabine plus placebo or gemcitabine plus ganitumab, a fully humanized monoclonal antibody against IGF1R[175]. This study did not meet its primary end point of overall survival or secondary end point of PFS and was terminated early after a preplanned futility analysis.

In a separate study, istiratumab, an IGF1R and ERBB3-bispecific antibody, was combined with gemcitabine and nab-paclitaxel in a randomized, placebo-controlled, phase II clinical trial of 88 patients with metastatic PDAC[176]. This study also failed to meet its co-primary end point of improvement in PFS in patients with high IGF1 levels and improvement of PFS in patients with both high serum IGF1 levels and tumours positive for heregulin, a ligand for ERBB3 (REF.[177]). Objective response rate and overall survival also failed to show

improvement in the antibody-containing arm. Preclinical IGF1 pharmacology data suggested the potential failure of single-axis inhibition in PDAC. Moreover, although dual inhibition with epidermal growth factor receptor family blockade was used in two human PDAC IGF1 trials, preclinical studies did not support this combination[178,179]. However, dual blockade strategies using an AXL inhibitor in combination with IGF axis inhibition does have a mechanistic rationale (FIG. 6a). AXL inhibitors have entered clinical trials for various solid malignancies[180] and specifically for PDAC[181] and could reasonably be combined with IGF1R inhibitors in an early-phase clinical trial using enrichment biomarkers for tumour phosphorylated IGF1R and AXL.

Vitamin D receptors.—Gene expression profiling of human PDAC CAFs demonstrated a downregulation of lipid storage genes such as fatty acid-binding proteins relative to normal pancreatic fibroblasts, whereas human and mouse CAFs strongly expressed the vitamin D receptor protein[182]. Treatment of human CAFs with the potent vitamin D analogue calcipotriol resulted in lipid droplet formation and decreased αSMA expression in the majority of samples in vitro. Conditioned media from CAFs induced the expression of multiple protumorigenic genes implicated in survival, EMT and chemoresistance in human PDAC cell lines, an effect that was abrogated when CAFs were pretreated with calcipotriol (FIG. 6b). In the KPC GEMM, the combination of calcipotriol with gemcitabine led to a decreased desmoplastic reaction and tumour volume while increasing MVD and intratumoural accumulation of gemcitabine and ultimately increasing median animal survival by 57%. Moreover, miR-10a-5p has been found to be the top microRNA found in human CAF-derived exosomes[183] and has been implicated in the paracrine tumour-promoting effects of CAFs. The release of miR-10a-5p-containing exosomes was markedly decreased by the treatment of CAFs with vitamin D, elucidating an important mechanism of the antitumour properties of vitamin D in PDAC. Indeed, miR-10a had previously been shown to promote metastatic behaviour of human PDAC cell lines in a zebrafish xenograft model by suppression of the HOXB1 and HOXB3 transcription factors[184]. These preclinical data form a compelling rationale for human clinical trials combining high-dose vitamin D with chemotherapy in PDAC. Moreover, given that vitamin D treatment of CAFs can reduce GM-CSF expression in PDAC cell lines and the role of this cytokine in promoting myeloid-derived suppressor cells in the PDAC microenvironment[167], a strong mechanistic rationale exists for combining vitamin D treatment with checkpoint blockade strategies in PDAC. In a retrospective analysis of a prospective RCT, low-serum 25-hydroxyvitamin D levels were seen in 77% of 256 patients with PDAC[185], although vitamin D levels did not correlate with response to chemotherapy or survival. Signal-seeking prospective clinical trials aimed at assessing the effect of high-dose vitamin D in patients with PDAC undergoing curative resection are currently under way[186,187]. Moreover, early clinical trial data of ten patients with metastatic PDAC, who were treated with nab-paclitaxel, cisplatin, gemcitabine, paricalcitol and the PD1 monoclonal antibody nivolumab, showed a partial response in eight out of ten patients[188]. These encouraging results are being further pursued in an expanded patient cohort[189].

Repurposing ATRA for PDAC treatment.—All-*trans* retinoic acid (ATRA) is a vitamin A derivative that forms the backbone for curative therapy of acute promyelocytic leukaemia

by inducing terminal differentiation of leukaemic cells[190]. There are no solid tumour indications for this agent although several groups have investigated repurposing ATRA for use in PDAC. When PSCs were treated in a three-dimensional culture model with ATRA in a collagen matrix, PSC-driven matrix invasion capacity for pancreatic cancer cell lines was markedly reduced[191]. In vitro, ATRA treatment decreased pancreatic PSC-mediated mechanical organization of the TGFβ-binding protein (latent TGFβ-binding protein 1; LTBP1), which in turn resulted in decreased TGFβ release[192]. The lack of available TGFβ ligand probably contributed to the inactivated, quiescent PSC phenotype. Administration of ATRA to KPC mice resulted in quiescence and reduced mobility of PSCs, decreasing Wnt paracrine signalling and creating a barrier to the invasion of adjacent cancer cells[193]. Consequently, cancer cells showed reduced proliferation and β-catenin nuclear localization, which was reversed by restoring the PSC cancer cell Wnt paracrine loop. Notably, treatment of cancer cells alone with ATRA had no effect on their proliferation in vitro, indicating that ATRA treatment disturbs heterotypic interactions between CAFs and cancer cells in PDAC (FIG. 6c). When ATRA was combined with gemcitabine in KPC mice, there was a marked increase in cancer cell apoptosis in addition to decreased tumour volume and EMT, with multiple signalling pathways implicated, such as Wnt, fibroblast growth factor and Shh[194]. Together, these data have provided the mechanistic rationale for a completed single-arm phase I clinical trial in which standard-of-care gemcitabine and nab-paclitaxel was combined with ATRA in 28 patients with locally advanced or metastatic PDAC[195]. This three-drug regimen proved to be safe and tolerable, with exploratory analysis showing a median survival of 11.66 months compared with the historic control of 8.5 months seen with gemcitabine and nab-palitaxel[5]. A randomized phase II clinical trial comparing gemcitabine and nab-paclitaxel with and without ATRA is being explored.

Future directions

Bringing novel therapeutics to patients with PDAC has been a long-standing and challenging problem. We have reviewed numerous failures in translation, in addition to clinical trials that are currently under way seeking to exploit various aspects of PDAC stromal biology for therapeutic benefit (FIGS 2,6 and TABLE 1). With the low rate of clinical trial enrolment in the USA[196] it is imperative that clinical trials are initiated on the basis of robust preclinical data, optimizing the chances of success. Many PDAC clinical trials have lacked preclinical modelling in either GEMMs or orthotopic PDX models, which are more faithful representations of human disease than decades-old commercial PDAC cell lines[197]. Indeed, the early preclinical PDAC angiogenesis studies used commercial PDAC cell line mouse xenograft models with impressive in vivo results but repeatedly negative clinical trials. In addition, with ever-expanding immune-oncology pipelines it will be important to leverage immunocompetent GEMMs (or humanized PDX models) to confirm antitumour phenotypes and mechanisms. Furthermore, the use of patient-derived PDAC organoid cultures has shown promise in predicting clinical responses to therapies[198] in addition to modelling tumour–stroma interactions[199], although the predictive capabilities of this platform will need to be validated in prospective clinical trials. Moreover, critical new insights into stromal cell heterogeneity will aid in the goal of precise targeting of specific populations of stromal cells (for example, iCAFs or mscCAFs instead of myCAFs). Moreover, the prospective

implementation of serum or tissue biomarkers for the stromal target of interest might prove to be a useful strategy for better patient selection and augmenting the rates of successful clinical trials.

Conclusions

The PDAC stroma is a diverse collection of cellular and non-cellular components that is critical for the pathogenesis of PDAC. Major advancements in understanding the stroma of PDAC have been made and although there are currently no approved stroma-targeting therapeutics, numerous candidates are in preclinical or clinical development. The disappointing results of the PEGPH20 clinical development programme highlights a key factor in clinical translation where a target might be well characterized in preclinical models but the toxicity of the experimental therapeutic impedes the ability to administer standard-of-care chemotherapy (specifically as it relates to the FOLFIRINOX combination trial). Moreover, the repurposing of anti-angiogenic strategies in PDAC for vasculature normalization and tumour immunosensitization has a sound mechanistic rationale and will need to be written into future clinical trials despite apprehension that might persist from previous failures in PDAC anti-angiogenesis clinical trials. Finally, insights into PDAC CAF intratumoural heterogeneity have been a fundamental advancement in our ability to bring CAF-targeting agents to clinical trials, with the iCAF and potentially the mscCAF populations being highly attractive targets.

Acknowledgements

The authors thank D. Primm (UT Southwestern Medical Center) for help in editing this article and C. Kwak (UT MD Anderson Cancer Center) who provided images of mouse tumours. R.A.B. acknowledges funding from the following sources: NIH grants R01 (CA192381) and U54 (CA210181 Project 2), the Effie Marie Cain Fellowship and the Jean Shelby Fund for Cancer Research at Communities Foundation of Texas. A.M. acknowledges funding from the following sources: NCI U24 CA224020, NCI R01CA218004 and NCI R01CA220236.

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Key points

- The tumour microenvironment of pancreatic ductal adenocarcinoma (PDAC) is composed of extracellular matrix (ECM) proteins, tumour vasculature, fibroblasts and immune cells.
- ECM proteins in PDAC can increase intratumoural pressure and act as a barrier to effective drug delivery to the tumour. Clinical trials have aimed to exploit this understanding of the PDAC ECM but have so far failed to show an improvement in patient survival.
- Tumour-associated vasculature has been shown to be important for PDAC disease pathogenesis in preclinical models; however, clinical trials aimed at targeting the PDAC vasculature have not prolonged patient survival.
- Pruning PDAC vasculature (normalization) might present a strategy for improved chemotherapy delivery and host antitumour immune responses.
- Molecular subtypes of pancreatic cancer-associated fibroblasts (CAFs) have been described, most notably inflammatory CAFs and myofibroblastic CAFs, which have been postulated to demonstrate protumour and antitumour properties, respectively.

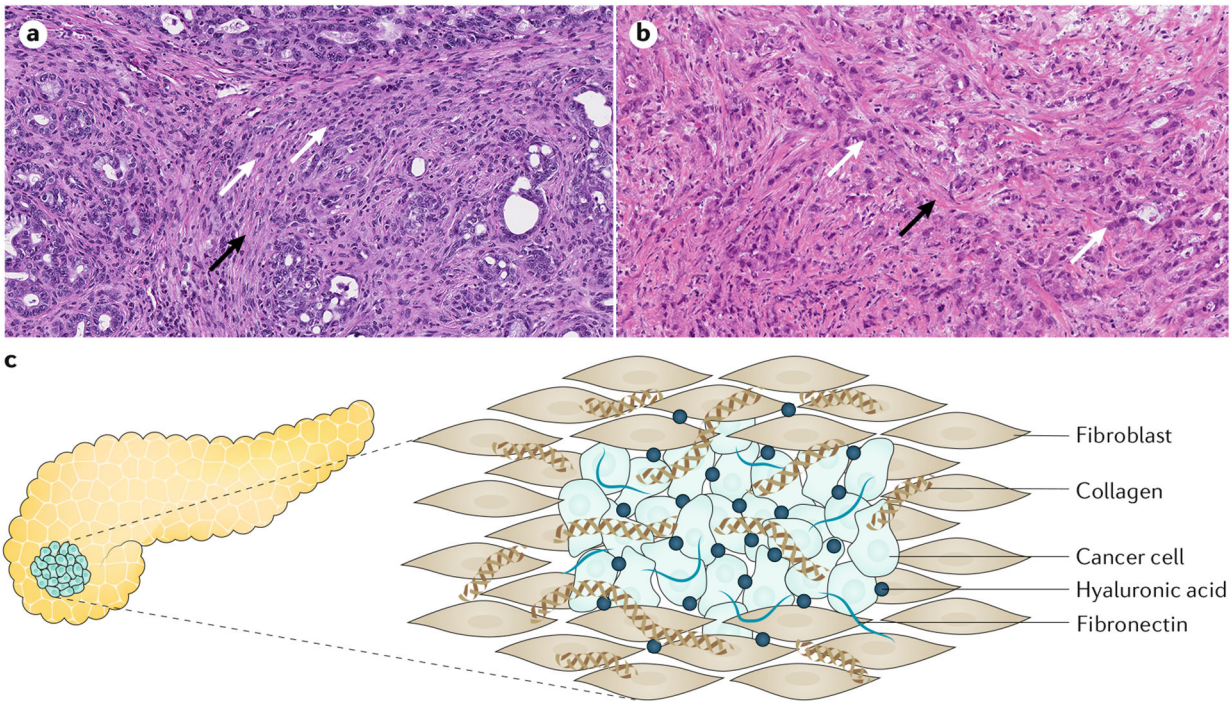

Fig. 1 |. The desmoplastic response is a key feature in human and mouse pDaC.

Haematoxylin and eosin stained sections of pancreatic ductal adenocarcinoma (PDAC) tissues from the KPC genetically engineered mouse model (part **a**) and human (part **b**). Both mouse and human PDAC tissues display an abundance of fibrosis (black arrows) admixed with the malignant epithelium (white arrows). This dense, fibrotic stroma is known as the desmoplastic response and is primarily composed of an abundance of fibroblasts and collagen. Magnification ×20. The schematic of the principal components comprising the desmoplastic response in PDAC (part **c**) shows an increase in fibroblasts, hyaluronic acid and the deposition of extracellular matrix proteins (mainly collagens and fibronectin), which are a hallmark of PDAC desmoplasia. Part **a** courtesy of A. Maitra. Part **b** courtesy of R. Brekken.

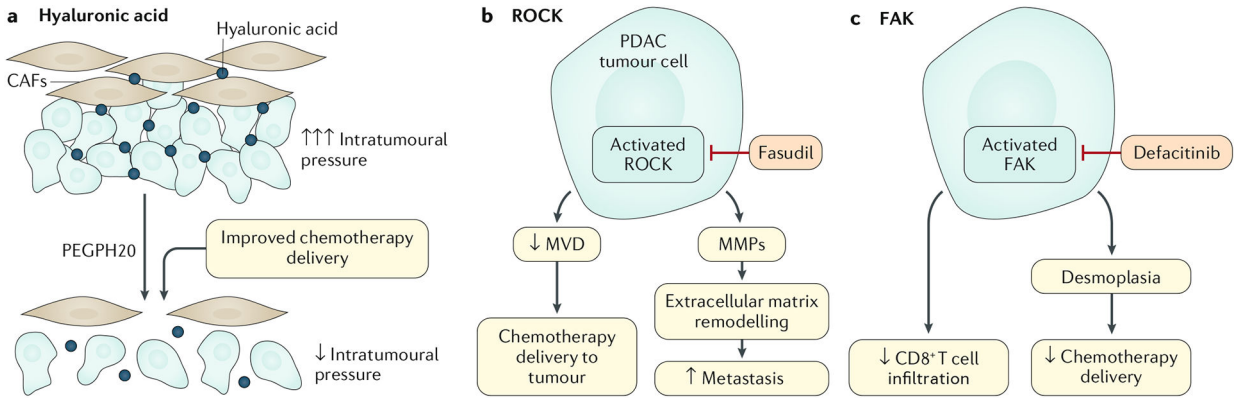

Fig. 2 |. Therapeutic targets in the extracellular matrix of pDaC.

a | Hyaluronic acid degradation by pegylated recombinant human hyaluronidase 20 (PEGPH20) decreases intratumoural pressure, leading to improved chemotherapy delivery in pancreatic ductal adenocarcinoma (PDAC) genetically engineered mouse models. **b** | Activated Rho-associated protein kinase (ROCK) in the PDAC tumour cell can lead to extracellular matrix remodelling through upregulation of matrix metalloproteinases (MMPs). This process favours metastasis and decreased microvessel density (MVD), which then leads to poor tumour perfusion and decreased tumour drug delivery. Fasudil is a small-molecule inhibitor of ROCK with anti-PDAC activity in preclinical models. **c** | When activated, focal adhesion kinase (FAK) can decrease $CD8^+$ T cell infiltration into the PDAC tumour microenvironment and increase the desmoplastic response, which can hinder drug delivery to the tumour. Defactinib is a small-molecule inhibitor of FAK under clinical evaluation for the treatment of PDAC in combination with an immune checkpoint inhibitor[54]. CAF, cancer-associated fibroblast.

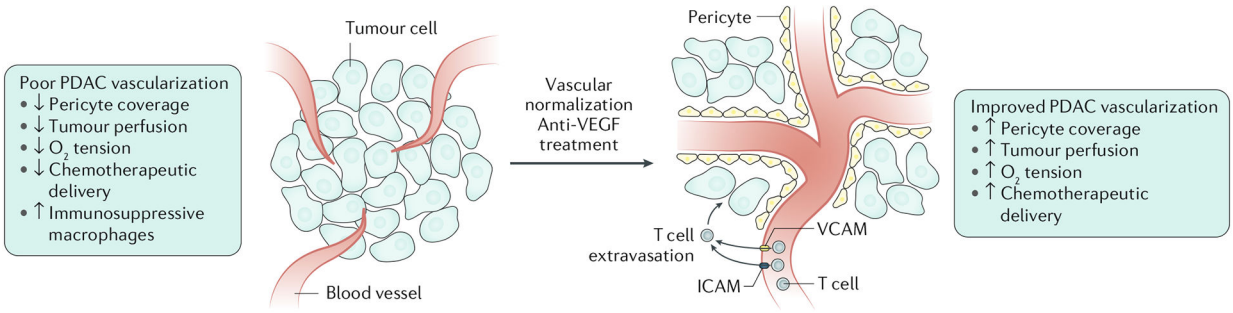

Fig. 3 |. Vascular normalization as a therapeutic strategy in pDaC.

The pancreatic ductal adenocarcinoma (PDAC) tumour microenvironment features an inefficient, leaky vasculature with poor pericyte coverage, which is associated with poor tumour perfusion leading to hypoxia and suboptimal drug delivery. Tumour vasculature normalization by blocking the vascular endothelial growth factor (VEGF)–VEGFR2 axis[106] can improve pericyte coverage and tumour perfusion, leading to decreased hypoxia, increased drug delivery and $CD8^+$ T cell trafficking into the tumour through the action of the leukocyte adhesion molecules ICAM and VCAM.

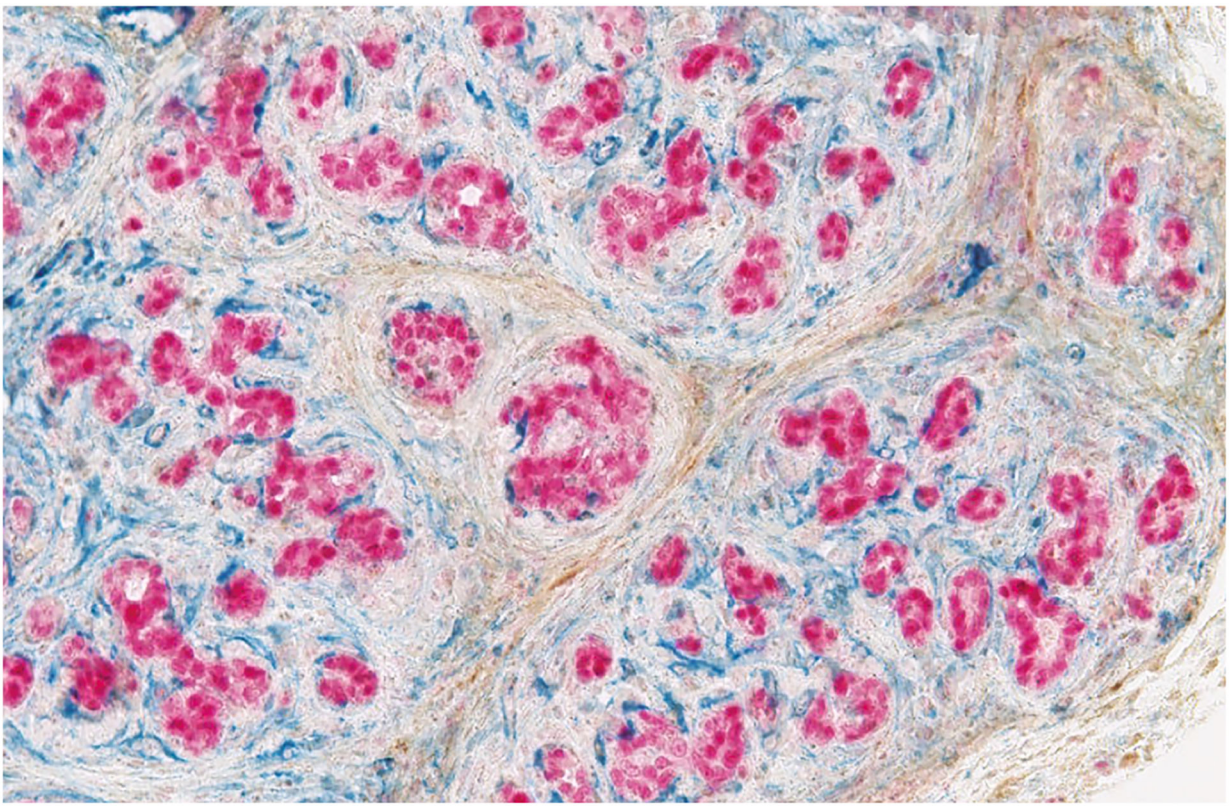

Fig. 4 |. Immunohistochemical analysis of pancreatic ductal adenocarcinoma CaF subtypes. The pancreatic cancer tumour microenvironment features distinct populations of myofibroblastic cancer-associated fibroblasts (CAFs) termed myCAFs and inflammatory CAFs termed iCAFs, which have been shown to be located juxtatumoural and distal to the cancer epithelium, respectively. The image is that of a moribund genetically engineered mouse model of PDAC. Platelet-derived growth factor receptor-α (brown) is a marker of iCAFs whereas α-smooth muscle actin (blue) is a marker of myCAFs. The ductal marker, SOX9 (red), stains for cancer epithelium. Image courtesy of R. Brekken (magnification ×20).

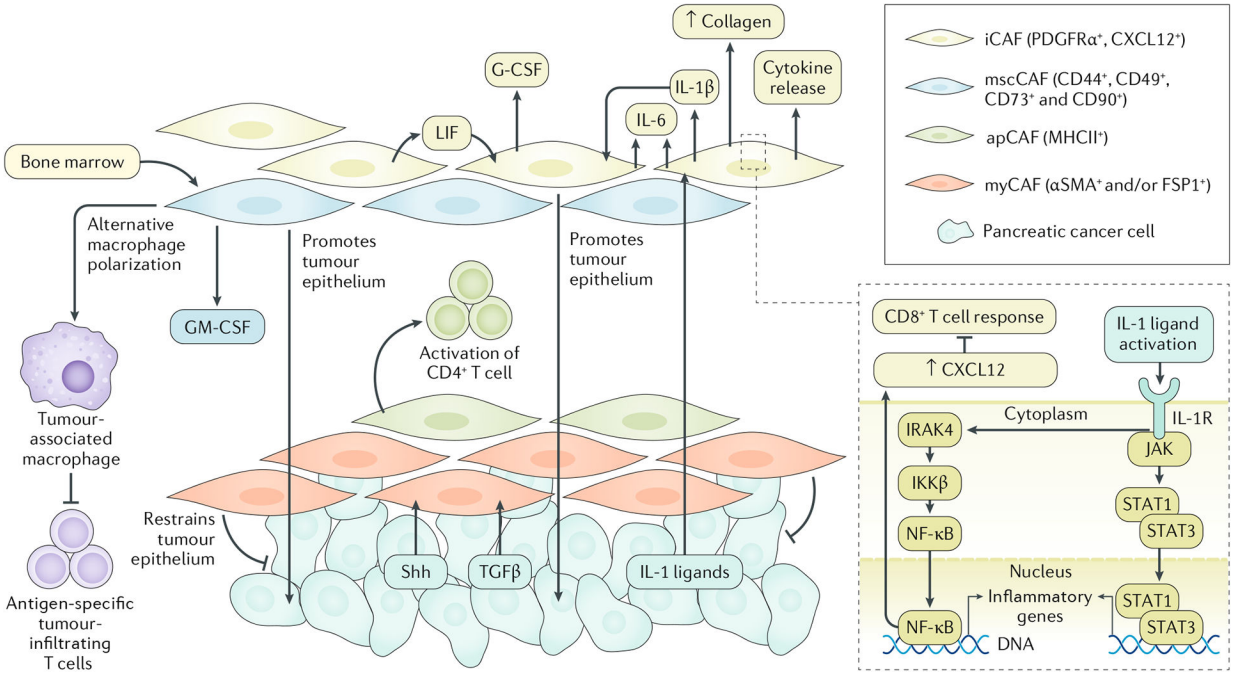

Fig. 5 |. Intratumoural fibroblast heterogeneity in pDaC.

Protumour inflammatory cancer-associated fibroblasts (iCAFs) and antitumour myofibroblastic cancer-associated fibroblasts (myCAFs) are produced when resident pancreatic fibroblasts receive paracrine and cell-contact cues from pancreatic ductal adenocarcinoma (PDAC) cells, respectively , within the tumour microenvironment. Tumour cell-derived IL-1α aids in the generation of iCAFs, which secrete LIF and propagate the iCAF phenotype. IL-1β also acts to maintain the iCAF phenotype through autocrine signalling. iCAFs upregulate a host of inflammatory cytokines, such as IL-6 and granulocyte colony-stimulating factor (G-CSF), in addition to contributing to local collagen deposition. Putative markers for iCAFs are platelet derived growth factor receptor-α (PDGFRα) and CXCL12. When IL-1 receptor (IL-1R) is activated by IL-1 ligands, IL-1 receptor-associated kinase 4 (IRAK4) is also activated, which in turn leads to the activation of IKKβ. These events lead to the liberation of nuclear factor-κB (NF-κB) from inhibitory proteins, enabling it to translocate to the nucleus where it acts as a transcription factor for a host of inflammatory genes that drive the iCAF phenotype[125,133]. iCAFs have also been shown to signal through the JAK–STAT signalling pathway. Cell contact between PDAC cells and juxtatumoural fibroblasts generates myCAFs through transforming growth factor-β (TGFβ) signalling. Putative markers for myCAFs are fibroblast-specific protein 1 (FSP1) and/or α-smooth muscle actin (αSMA). Mesenchymal stem cell-derived cancer-associated fibroblasts (mscCAFs) are recruited from the bone marrow and produce granulocyte–macrophage colony-stimulating factor (GM-CSF) locally in the tumour microenvironment, contributing to polarization of macrophages towards an immunosuppressive phenotype and blunting of a robust T cell response against the tumour. mscCAFs are positive for CD44, CD49, CD73 and CD90. Lastly , major histocompatibility complex (MHC) class II-expressing CAFs (apCAFs) have demonstrated the ability to present antigen to $CD4^+$ T cells. Shh, sonic hedgehog.

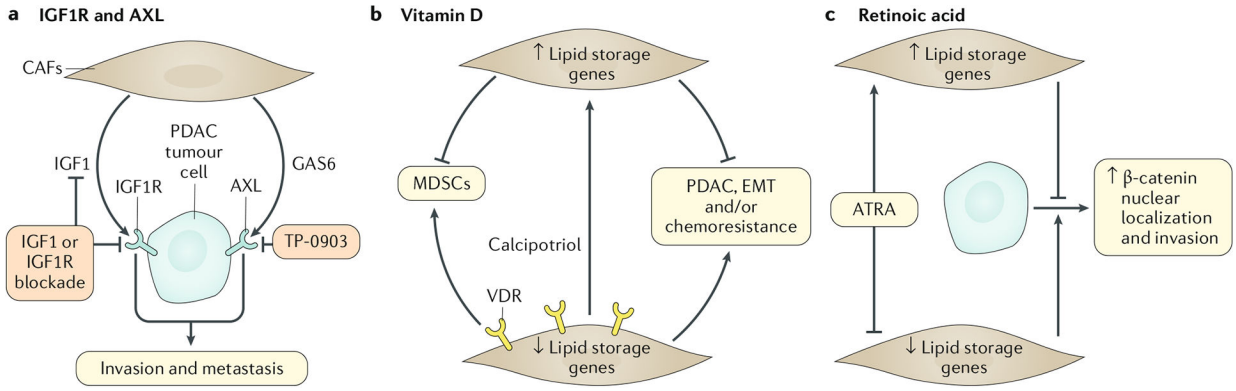

Fig. 6 |. Therapeutic targets in pDaC CaFs.

a | Signalling of insulin-like growth factor 1 (IGF1) receptor (IGF1R) and AXL can lead to invasive and metastatic behaviour of pancreatic ductal adenocarcinoma (PDAC) tumour cells. Clinical data on IGF1R blockade have been unsuccessful, although preclinical data suggest possible merit in dual GAS6–AXL and IGF1–IGF1R blockade. A small-molecule inhibitor of AXL (TP-0903) is now in clinical trials for advanced PDAC[180]. **b** | PDAC cancer-associated fibroblasts (CAFs) have increased vitamin D receptor (VDR) expression and decreased expression of lipid storage genes. When treated with calcipotriol (a synthetic form of vitamin D), these CAFs increase lipid storage gene expression and hinder epithelial-to-mesenchymal transition (EMT), chemoresistance and the action of myeloid-derived suppressor cells (MDSCs). Multiple clinical trials are underway to assess the benefit of vitamin D treatment in PDAC[186,187,189]. **c** | Retinoic acid signalling. PDAC CAFs are induced to assume a lipid storage phenotype when treated with all-*trans* retinoic acid (ATRA), which inhibits β-catenin nuclear localization and invasion of cancer cells. ATRA is being tested in a clinical trial of locally advanced or metastatic PDAC[200].

Table 1 |

Selected ongoing clinical trials targeting the pDaC stroma

Target	Candidate drug and combination regimen	phase of trial	Trial design	Estimated patient enrolment	Primary outcome	Clinical trial reference	preclinical study reference
Focal adhesion kinase	Defactinib plus pembrolizumab plus gemcitabine	I	Non-randomized, open-label	50	Maximum tolerated dose	54	53
IL-1 receptor	Anakinra plus modified FOLFIRINOX regimen	I	Single arm, non-randomized, open-label	13	Number of patients, serious adverse events and adverse events	136	124,135
PDGFRα	Nab-paclitaxel plus gemcitabine with or without olaratumab	Ib/II	Phase Ib, open-label; phase II randomized, double-blinded	186	Number of participants with dose limiting toxicities (phase Ib) and overall survival (phase II)	139	138
CXCR4	BL-8040 (CXCR4 inhibitor) plus pembrolizumab with or without 5-FU and liposomal irinotecan	II	Two-arm, open-label	80	Objective response rate	143,144	140,141
CSF1R	Cabiralizumab plus nivolumab	Ia/Ib	Non-randomized, open-label	295	Safety and objective response rate	155	168,169
IL-6 receptor	Nab-paclitaxel plus gemcitabine with or without tocilizumab	II	Randomized, open-label	140	Overall survival at 6 months	150	145,146
IL-6 and PDL1	Spartalizumab and siltuximab	Ib/II	Non-randomized	42	Maximum tolerated dose	155	153,154
IGF1R	Istiratumab plus nab-paclitaxel and gemcitabine or placebo plus nab-paclitaxel and gemcitabine	II	Randomized, quadruple-blinded	88	Progression-free survival	201	173
AXL	Oral TP-0903 (AXL inhibitor) administered daily for 21 days	I	Non-randomized, parallel assignments	140	Incidence of dose-limiting toxicities and treatment-emergent adverse events	180	171,172
VDR	High-dose oral vitamin D3 supplementation (prior to PDAC surgery)	III	Randomized, open-label, parallel assignments	60	Blood level of vitamin D3 (60-day postoperative mortality is a secondary outcome)	186,187	182
VDR	Nivolumab plus albumin-bound paclitaxel plus paricalcitol plus cisplatin plus gemcitabine	II	Single-arm, open-label	10	Complete response rate	189	167,182

5-FU, 5-fluorouracil; PDAC, pancreatic ductal adenocarcinoma; PDGFRα, platelet-derived growth factor receptor-α; VDR , vitamin D receptor.