HHS Public Access

Author manuscript

Nat Rev Clin Oncol. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Nat Rev Clin Oncol. 2020 September ; 17(9): 527–540. doi:10.1038/s41571-020-0363-5.

The tumour microenvironment in pancreatic cancer — clinical challenges and opportunities

Won Jin Ho, **Elizabeth M. Jaffee**, **Lei Zheng**✉

Abstract

Metastatic pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal solid tumours despite the use of multi-agent conventional chemotherapy regimens. Such poor outcomes have fuelled ongoing efforts to exploit the tumour microenvironment (TME) for therapy, but strategies aimed at deconstructing the surrounding desmoplastic stroma and targeting the immunosuppressive pathways have largely failed. In fact, evidence has now shown that the stroma is multi-faceted, which illustrates the complexity of exploring features of the TME as isolated targets. In this Review, we describe ways in which the PDAC microenvironment has been targeted and note the current understanding of the clinical outcomes that have unexpectedly contradicted preclinical observations. We also consider the more sophisticated therapeutic strategies under active investigation — multi-modal treatment approaches and exploitation of biologically integrated targets — which aim to remodel the TME against PDAC.

Pancreatic cancer, comprising mostly pancreatic ductal adenocarcinoma (PDAC), is an extremely lethal disease[1], with 45,750 estimated deaths in the USA in 2019 (REF.[2]). Symptoms are often non-specific, which means that patients often present at advanced stages. Conventional cytotoxic chemotherapy constitutes the current standard of care for advanced or metastatic PDAC, providing only months of overall survival benefit[3,4].

Carcinogenesis of PDAC involves progressive accumulation of driver mutations, including the oncogene *KRAS*[5] and tumour suppressor gene *TP53* (REF.[6]). These molecular perturbations are accompanied by histological changes that represent the different stages of PDAC development. Morphological evolution begins with the formation of precursor lesions, termed pancreatic intraepithelial neoplasia (PanIN)[7], with increasing histological grades followed by progression to invasive adenocarcinoma. As the cancer develops, it leads to changes in the surrounding tissue stroma. A key function of any non-transformed tissue

✉ Sidney Kimmel Comprehensive Cancer Center, The Skip Viragh Pancreatic Cancer Center for Clinical Research and Care, and The Bloomberg-Kimmel Institute for Immunotherapy at Johns Hopkins University School of Medicine, Baltimore, MD, USA. lzheng6@jhmi.edu.

Author contributions

All the authors contributed to all aspects of manuscript preparation.

Peer review information

Nature Reviews Clinical Oncology thanks I. Garrido-Laguna, A. Maitra, M. Moore and D. Ting for their contribution to the peer review of this work.

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stroma is to provide homeostatic response to injury with its immune, vascular and connective tissue components. However, cancer hijacks such physiological responses to create a favourable tumour microenvironment (TME) for its successful growth[8]. In the words of Harold Dvorak, cancer behaves like “wounds...that never heal”, and stromal transformation is a result of “wound healing gone awry”[9].

Given the clear importance of the TME in tumorigenesis, approaches to target specific features within the TME have garnered much attention. For example, in the past decade advances in immuno-oncology have led to ground-breaking therapeutic options for multiple cancer types. However, even immunotherapeutic strategies, such as immune-checkpoint inhibition, have yielded limited responses in PDACs[10]. Furthermore, therapeutic strategies aimed at ablating the stromal barriers that restrict drug delivery have also demonstrated disappointing and contradictory responses[11,12].

In this Review, we provide an overview of the complexities and the multi-faceted nature of several therapeutic targets within the PDAC microenvironment. We also examine some of the multi-modal strategies that are currently under investigation and designed to overcome the challenges by reprogramming the stroma into an antitumour milieu.

Limitations of targeting desmoplasia

A histopathological hallmark of PDAC is a desmoplastic reaction to the tumour; this hallmark is present in both primary and metastatic tumours[13]. Myofibroblast-like cells in the pancreas (that is, pancreatic stellate cells) are activated by cancer cells to produce fibrosis surrounding the tumour[14,15]. The resultant desmoplasia is known to be responsible for creating a mechanical barrier around the tumour cells, preventing appropriate vascularization and thus limiting exposure to chemotherapy and leading to poor immune cell infiltration[16]. Early research largely stemmed from the idea that the surrounding desmoplasia is tumour promoting (FIG. 1; BOX 1); this view of its role is an imperfect one. The current understanding is that desmoplasia is in fact multi-faceted and that a more holistic approach to targeting the stroma is warranted.

Matrix metalloproteinases

The surrounding extracellular matrix (ECM) has long been implicated in the regulation of cancer progression (for example, migration and invasion). Efforts in the late 1990s and early 2000s focused on the non-specific alteration of the ECM within the surrounding stroma by targeting the proteins that remodel the ECM. Studies showed that proteolytic matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs are differentially expressed between non-transformed pancreas and PDAC tissues, with higher expression of particular MMPs being associated with metastatic disease and/or poorer prognosis[17–19]. Increased expression of MMP2, a type IV collagenase detected within the stromal components in pancreatic cancer specimens, was found to increase invasiveness in vitro and to correlate with the degree of desmoplasia[20–23]. MMP7, a zinc-dependent endopeptidase predominantly expressed by glandular epithelial cells, is overexpressed in PanIN and PDAC[24,25] and contributes to tumour growth and metastasis in a mutant *Kras*-driven mouse model of PDAC[26].

The interplay between stromal cells and cancer cells via MMPs is further exemplified by the role of the tumour cell-associated MMP inducer (EMMPRIN) in stimulating MMP2, MMP9 and EMMPRIN production following co-culture of EMMPRIN-expressing tumour cells and fibroblasts[27]. These observations supported the rationale behind the development and application of multi-MMP inhibitors to suppress cancer progression in the B16 melanoma, colon xenograft and gastric xenograft mouse models[28–30]. Despite the preclinical successes in other cancers and overall tolerability in patients, MMP inhibitors such as marimastat and tanomastat failed to show any significant clinical activity in patients with advanced-stage pancreatic cancer[31–33], suggesting that non-specific targeting of the ECM alone is not effective in pancreatic cancer.

Hyaluronan

A more specific approach to disrupting the ECM within the desmoplastic barrier is targeting hyaluronan, a non-sulfated glycosaminoglycan. Hyaluronan is a major constituent of the stromal ECM, and high deposition of hyaluronan in PDAC is associated with poor prognosis[13,34]. On the basis of this association, researchers investigated an enzymatic approach to targeting the desmoplastic barrier using human recombinant PH20 hyaluronidase (PEGPH20). A 2013 study demonstrated that targeted depletion of hyaluronan led to improved vascular permeability and increased drug delivery in a mouse model of PDAC, leading to improved chemotherapeutic efficacy when used in combination with cytotoxic chemotherapy with gemcitabine[34].

Subsequently, clinical trials investigated the effects of PEGPH20 with two standard-of-care combination chemotherapeutic regimens, gemcitabine plus nab-paclitaxel[12] and FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan and oxaliplatin)[11]. A randomized phase II trial showed that the addition of PEGPH20 enhanced the effects of gemcitabine plus nab-paclitaxel as measured by improved progression-free survival (PFS)[12], but another phase Ib/II trial showed that adding PEGPH20 reduced overall survival in patients receiving FOLFIRINOX[11]. Furthermore, a follow-up phase III trial revealed that combining PEGPH20 with gemcitabine plus nab-paclitaxel did not improve overall survival when compared with gemcitabine plus nab-paclitaxel alone (HR 1.00; $P = 0.97$)[35]. The failure of PEGPH20 to enhance the efficacy of chemotherapy does not necessarily exclude ECM-targeting agents from future anticancer therapeutic developments but suggests that this component of the desmoplastic barrier does not sufficiently account for the ineffectiveness of chemotherapeutics in PDAC.

Sonic hedgehog signaling

Distinct from directly targeting a specific component of the ECM, another approach targeting desmoplasia is to focus on a specific signalling pathway responsible for the development of tumour stroma. The hedgehog signalling pathway is key in pancreas development. During embryogenesis, repression of endodermal Sonic hedgehog (SHH) by inhibin-βB and FGF2 permits expression of *Pdx1* and insulin (*Ins*), which then initiates pancreatic differentiation[36]. The fact that SHH inhibition initiates pancreatic differentiation was corroborated by the observation that an inhibitor of SHH, cyclopamine, promotes heterotopic expansion of pancreatic tissues into adjacent endodermic areas[37].

Unsurprisingly, studies have shown that dysregulated hedgehog signalling leads to pancreatic carcinogenesis. SHH expression under *Pdx1* control results in the development of tubular structures mimicking PanIN in mice genetically engineered to express mutated *Kras*[38]. Also, injury responses in the pancreas induce hedgehog signalling, which then leads to metaplasia with features of PanIN[38]. A 2008 study also showed that hedgehog signalling promotes desmoplasia, and that antibody-mediated inhibition and overexpression of SHH were associated with reduced and increased presence of desmoplasia, respectively[39]. Moreover, SHH overexpression provided paracrine stimulation of stellate cell differentiation and myofibroblast invasion[39].

Given the evidence indicating that SHH contributes to both cell-intrinsic carcinogenesis and desmoplastic processes, inhibition of SHH was investigated as a therapeutic strategy in PDAC. In a mouse model of PDAC driven by *Kras* and *Cdkn2a*, tumours were found to overexpress SHH, and administration of cyclopamine suppressed tumour growth and prolonged survival in cancer-bearing mice[40]. Furthermore, similar to findings with hyaluronan, SHH inhibition in the KPC mouse model (where pancreatic cancer is driven by *Kras* and *Tp53* mutations) led to improved vascular density within the tumour and increased antitumour efficacy of gemcitabine, resulting in improved survival[41].

The results of clinical trials of SHH inhibition, however, have been largely disappointing. A randomized phase II study was stopped early because preliminary results showed that the combination of the SHH inhibitor saridegib and gemcitabine led to a higher rate of progressive disease than did placebo and gemcitabine[42]. A randomized phase Ib/II study showed that the addition of the SHH inhibitor vismodegib to gemcitabine did not improve overall survival or PFS[43]. Another study showed that adding vismodegib to gemcitabine plus nab-paclitaxel did not improve PFS over historical rates observed with chemotherapy alone[44].

Reconciling contradictions

A number of lessons can be learned from the observed contradictions between preclinical and clinical responses and across several clinical trials focused on stromal desmoplasia. First, the potential for toxic effects occurring in humans that were not observed in mice makes incorporating novel therapies into any existing treatment paradigm challenging. In humans, the addition of PEGPH20 to nab-paclitaxel plus gemcitabine was associated with increased rates of thrombotic events and ultimately the need for the use of prophylactic anticoagulation[12]. MMP inhibitors and PEGPH20 were associated with the development of musculoskeletal symptoms, not unexpectedly as both MMP inhibitors and PEGPH20 could in theory alter physiological connective tissue remodelling[12,31,32,45]. Also, higher rates and severity of nausea, vomiting and diarrhoea were seen with PEGPH20 and FOLFIRINOX compared with FOLFIRINOX alone, which led to reduced treatment duration and dose reductions with the combination[11]. With these toxic effects occurring in the setting of novel combinations, the efficacy of standard regimens might be adversely affected by such reduced treatment durations and dose reductions. These examples illustrate that even judicious combinations based on rigorous preclinical studies of novel therapies with known mechanisms of action and safety data can lead to unexpected outcomes in clinical trials.

Second, although the tumour-promoting role of desmoplasia is well established, accumulating evidence demonstrates that desmoplasia is not solely tumour promoting but rather a neutral reactive process to carcinogenesis that also has antitumour functions. Specifically, when studying the role of SHH in KPC mice, *Shh* deletion specifically within pancreatic cancer cells leads to decreased myofibroblastic and desmoplastic content in the stroma in association with reduced survival of the KPC mice, a poorly differentiated histology and increased metastatic ability[46]. The tumour-suppressive effects of SHH signalling were also observed in another study using *Kras*-driven and KPC models with genetic deficiency of *Shh* as well as in mice treated with the SHH inhibitor vismodegib from 5 weeks of age[47]. *Shh*-null KPC tumours were also shown to exhibit significantly increased vascular density and sensitivity to anti-VEGFR therapy[46].

The contradictory observations between the two outcomes of SHH inhibition in mouse experiments can be partly reconciled by the differences in the duration of SHH inhibition. In other words, acute SHH inhibition seems to help in breaking down the barrier to facilitate enhanced drug delivery, but chronic or early SHH inhibition eventually benefits the tumour. The optimal duration of stroma-targeted therapy is unclear at present. Together, these studies suggest that the natural function of the stroma is to restrain tumour growth, tumour angiogenesis and metastatic spread; however, during cancer development, the stromal cells (that is, surrounding fibroblasts) are reprogrammed by tumour cells to support tumour growth[48]. Indeed, the stroma is composed of a variety of different stromal cells that have both antitumour and tumour-promoting functions[49,50].

Of note, the studies did not take into account the heterogeneity of stromal composition in PDAC. Although the stroma-targeted therapies in the preclinical models are tested against a relatively more homogeneous stromal content, previous observations established that patients with PDAC do in fact show a wide range of diversity within the desmoplasia[13]. Variability occurs not only between patients but also within patients, manifested by site-to-site variability. One could speculate that stroma-targeted agents would be beneficial in sites with high stromal density and that they could be harmful in sites with low stromal density. Thus, a valuable approach might be to further develop non-invasive methods such as imaging to characterize or quantify the degree and type of stromal content within the tumour as a key component of trial design[51]. Also, according to consensus clustering of expression levels of key genes, the stroma could be classified as being ‘normal’ or ‘activated’, each portending a different prognosis[52]. Moreover, stromal heterogeneity is not an independent entity; rather, stromal heterogeneity is inherently linked with tumour heterogeneity as it is able to programme tumour behaviour (via quasi-mesenchymal or epithelial phenotype switching)[53]. The tumours of patients with metastatic PDAC on presentation were more likely to have a quasi-mesenchymal signature than an epithelial signature[54]. Interestingly, the quasi-mesenchymal and epithelial subtypes showed different responses to chemotherapy regimens[54], with epithelial phenotype tumours being associated with a longer metastasis-free survival than the quasi-mesenchymal phenotype. Therefore, a possibility exists that therapies aimed at the stroma might yield divergent effects owing to molecular heterogeneity.

Stromal cells

Another approach towards reprogramming the ECM has been to focus on the cells that deposit the components of the ECM. Cancer-associated stromal cells or cancer-associated fibroblasts (CAFs) are a heterogeneous group of cells known to be major producers of ECM proteins. These generally spindle-shaped cells are positive for one or more activated fibroblast markers (for example, fibroblast activation protein (FAP) and a-smooth muscle actin) and classically have been linked with various tumour-promoting functions including tumorigenesis, angiogenesis, immunosuppression and metastasis, as reviewed elsewhere[55].

Targeting of fibroblasts to treat patients with cancer was first assessed using inhibitors targeting FAP The first clinical trial of FAP inhibition was a phase II trial that used a humanized monoclonal antibody, sibrotuzumab, to inhibit CAFs in patients with colorectal cancer. This trial failed to meet its end point, and sibrotuzumab was not investigated further[56].

Small molecule inhibitors of FAP have also been explored in pancreatic cancers. In subcutaneous mouse models of PDAC, the small molecule inhibitor UAMC-1110 did not demonstrate any meaningful activity as a single agent[57]. Similarly, a phase II trial showed that the combination of talabostat and gemcitabine, while relatively well tolerated, had very limited efficacy against metastatic PDAC[58].

Given the lack of success with targeted FAP inhibition, researchers investigated the cellular depletion of activated fibroblasts. On the one hand, genetic deletion of a-smooth muscle actin-expressing fibroblasts in mouse models of PanIN or PDAC led to a disease with a more aggressive phenotype[59], suggesting that fibroblasts naturally have a cancer-restraining function. Also consistent with the tumour-constraining functions of the stroma, pancreatic cancer cells co-cultured with irradiated fibroblasts showed increased invasiveness over pancreatic cells co-cultured with non-irradiated fibroblasts[60]. On the other hand, the adoptive transfer of T cells engineered with chimeric antigen receptors (CARs) specific to FAP (to deplete FAP-expressing CAFs) has been shown to disrupt tumour-promoting desmoplasia and to have antitumour efficacy in a mouse model of lung cancer and in KPC-based pancreatic cancer[61,62], motivating the clinical translation of CAR-T cell therapy against FAP (NCT03932565).

The direct targeting of CAFs, however, is complex and can result in unexpected biological outcomes. Studies on fibroblasts in the pancreatic stroma have revealed the heterogeneity of CAFs by highlighting their phenotypic and functional diversity[49,50,53,63] (FIG. 1). Fibroblasts are cells that typically facilitate homeostatic wound repair, but cancer has the ability to co-opt their function. Specifically, researchers previously discovered that cancer-led signalling via IL-1 or transforming growth factor-β (TGFβ) can differentiate surrounding fibroblasts into inflammatory CAF and myofibroblastic CAF phenotypes, respectively[64]. IL-6 secreted by inflammatory CAFs then provides pro-proliferative effects on the tumour whereas myofibroblastic CAFs are stimulated by TGFβ to produce the surrounding stroma. Subsequently, a third subtype of CAFs was characterized that express MHC class II molecules and have the ability to present antigens to $CD4^+$ T cells, suggesting that some CAFs are important for shaping the antitumour immune responses[49].

Again, instead of simply eliminating the stromal fibroblasts from the TME, a more sophisticated approach might be to exploit the altered microenvironment, particularly the immune TME, that occurs as a result of stromal reprogramming by tumour cells. As proof of concept, a 2019 murine study demonstrated that the addition of PEGPH20 significantly enhanced the effects of cancer-specific vaccines in promoting T cell infiltration into the TME[65]. Another study in KPC mice showed that depletion of FAP^+ CAFs resulted in the immune control of tumour growth and an effective response to immune-checkpoint inhibitors (ICIs)[66]. Both of these studies elucidated the importance of CXCL12–CXCR4 signalling as a means of stromal–immune crosstalk, presenting yet another target for therapy.

Related to these preclinical observations, although not specific to pancreatic cancer, a clinical trial examining the efficacy of combining the antibody targeting PD-1 (pembrolizumab) and the FAP inhibitor talabostat is ongoing (NCT03910660). One small molecule inhibitor of CXCR4, AMD3100, which demonstrated efficacy in the KPC mouse model in combination with anti-PD-1/PD-L1 signalling[66], is now being studied in patients with metastatic pancreatic cancer in combination with an anti-PD-1 inhibitor (NCT04177810). Another CXCR4 antagonist, the peptide-based motixafortide (BL-8040), is also under active investigation to treat PDAC in combination with standard chemotherapy and pembrolizumab, with encouraging results (NCT02826486)[67]. These findings are generally consistent with the perspective that a multi-modal alteration of the TME combining stromal and immune modulation is probably a more appropriate therapeutic approach instead of targeted depletion; however, caution is needed given that the combination of nivolumab, an antibody against PD-1, and the anti-CXCR4 monoclonal antibody ulocuplumab failed to demonstrate efficacy against PDAC (NCT02472977).

Immune compartment targeting in PDAC

Immune cells in the TME have a key role in the development and progression of pancreatic cancer. Inflammation has long been linked with PDAC according to epidemiological studies, as reviewed in depth elsewhere[68]. In a *Kras*^{G12V}-driven model of PDAC, pancreatic inflammation from exposure to caerulein was essential for carcinogenesis[69]. Furthermore, using this model, inflammation was shown to inhibit an oncogene-induced senescence programme that physiologically prevents adult acinar cells or precursor lesions from persistent progression towards invasive carcinoma[70]. In addition, studies have established the Kras-specific immune recognition of mutant *Kras*-driven cancers using a murine lung tumour model[71], T cells from patients with colon cancer[72,73] and T cells from patients with pancreatic cancer[73,74]. These studies have demonstrated a clear link between immunological processes and PDAC carcinogenesis. Despite these findings, PDACs are typically known as immunologically ‘cold’ tumours. Analyses of large PDAC genomic datasets showed that only a subset of pancreatic cancers are immunologically active[75,76]. Studies have identified high tumour mutation burdens exhibiting neoantigenicity to be a key characteristic of inflamed tumours, especially melanomas and lung cancers. However, PDACs have relatively low tumour mutation burdens[77–79], which is consistent with the limited responses observed when PDACs are treated with ICIs[80,81].

Presenting PDAC antigens to the immune system

To overcome the issue of low immune recognition of PDACs, researchers have explored several vaccine therapy approaches to enhance antigen presentation and drive expansion of tumour-specific T cell clones[82] as a way to elicit novel or boost pre-existent immune responses. Strategies targeting PDAC-associated antigens (including telomerase[83], KRAS[84,85], gastrin[86], CEA[87], MUC1 (REF[88]) and mesothelin[89,90]) have included peptide-based vaccines[83–85], virus-based vaccines[87,91], *Listeria*-based vaccines[90], DNA-based vaccines (neoantigens)[92,93] and cell-based vaccines[88,89,94]. On the basis of the results of a multitude of studies, vaccination strategies have now been well established to yield antigen-specific immunological responses in patients with PDAC[83,85,90,95].

Studies have also demonstrated that the use of lethally irradiated allogeneic cell-based vaccines engineered to express granulocyte–macrophage colony-stimulating factor (GM-CSF), such as GM-CSF secreting allogeneic pancreatic tumour cell vaccine (GVAX), successfully recruited immune cell aggregates into the TME with activated signatures and enhanced T cell repertoires[96,97]. Despite positive immunological responses and encouraging findings in early phase trials, many vaccines including TeloVac (telomerase), Primo Vax (telomerase), PANVAC-V (CEA and MUC1) and algenpantucel-L (two allogeneic PDAC cell lines) failed to show significant clinical benefit in phase III trials[82].

The major theme from this series of findings is that vaccination strategies alone might not be sufficient for generating clinically meaningful antitumour effects. Thus, studies are ongoing to explore the effects of combining vaccination strategies with other therapeutic modalities. Importantly, the fact that the overall clinical effect of vaccination strategies is limited despite positive immune recognition of tumours suggests that other immunosuppressive pathways that restrict successful antitumour immune responses are present and could be targeted and reversed.

Targeting immunosuppressive cells to modulate the immune TME

PDAC development is intertwined with multiple types of immunosuppressive cells, including regulatory T (T_{reg}) cells, myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs), and leads to an inherently immunosuppressed TME.

A mechanistic link between *KRAS* mutations and immunosuppressed TME of PDAC has previously been characterized, in which *Kras*^{G12D}-dependent upregulation of GM-CSF can lead to recruitment of $Gr1^{+}CD11b^{+}$ MDSCs and limit antitumour T cell activity[98]. In fact, infiltration of immunosuppressive cells is detected very early in PDAC carcinogenesis. In the *Kras*-driven mouse model of PDAC, T_{reg} cells and MDSCs dominated the immune infiltration in early PanIN; effector T cells were scarce and generally lacking activation[99]. Similarly, in a TGFα-overexpressed *Tp53*-mutated mouse model of pancreatic cancer, MDSCs were detected in premalignant lesions within the pancreas[100].

Analogous to the findings in mouse models, T_{reg} cells are observed in human PanIN and increase with progression to PDAC, and increased prevalence of T_{reg} cells confers poor prognosis for patients with PDAC[101]. In delineating the function of T_{reg} cells and MDSCs, several depletion experiments established T_{reg} cells to be suppressors of antitumour immune

responses, as reviewed elsewhere[102]. However, a precise understanding of MDSCs has been difficult to achieve given their heterogeneity in both mouse and human contexts[103]. Nevertheless, MDSCs — which can be further subtyped into being monocytic or granulocytic — are known to also exert immunosuppressive effects on T cells via arginase, nitric oxide synthase, TGFβ, IL-10 and COX2 (REF.[103]).

In addition to T_{reg} cells and MDSCs, TAMs are known to be involved in PDAC carcinogenesis as their infiltration accompanies KRAS G12D-mediated inflammation[104]. Macrophages have been shown to drive pancreatic acinar-to-ductal metaplasia via secretion of TNF, RANTES and induction of MMP9 (REF.[105]). They also secrete IL-6 to drive progression of early lesions via the JAK-STAT3 signalling pathway[106]. TAMs are not just able to promote cancer growth, they also foster cancer invasiveness by stimulating angiogenesis and inhibit natural killer and T cell function by expressing non-classical MHC class I molecules (for example, HLA-G) and ligands of co-inhibitory receptors PD-1 (PD-L1 and PD-L2) and cytotoxic T lymphocyte antigen 4 (CTLA-4)[107]. In the early stages of carcinogenesis, PanIN interacts with macrophages in the TME via IL-13 to polarize them towards a more immunosuppressive phenotype (that is, an M2 subtype[107]). Moreover, persistence of colony-stimulating factor-1 (CSF-1) in the TME polarizes macrophages towards the M2 subtype (whereas GM-CSF shifts macrophages towards $CD80^+$ MHC class II^{high} proinflammatory macrophages)[108].

Compared with TAMs, tumour-associated neutrophils (TANs) are less mechanistically established with pancreatic carcinogenesis. However, TANs are detected even in PanIN[109], and their presence in the TME is associated with poor prognosis in cancers in general[110]. Importantly, inhibition of TAN infiltration into KPC tumours by knocking out CXCR2, the key chemotactic receptor for neutrophils, resulted in T cell-dependent suppression of tumour growth[111]. Therefore, T_{reg} cells, MDSCs, TAMs and TANs provide targets for immune modulation of the PDAC microenvironment.

Strategies to directly target immunosuppressive cells in the TME have been explored (FIG. 2; TABLE 1). One well-studied example is the incorporation of cyclophosphamide in treatment regimens to target T_{reg} cells. Evidence supports the idea that low-dose cyclophosphamide selectively eliminates T_{reg} cells[112]. Therapeutic strategies have successfully utilized cyclophosphamide in combination with GVAX to augment immune responses to PDAC[89,97,113].

In addition to low-dose cyclophosphamide, CTLA-4 (REF.[114]) and neuropilin-1 (REF.[115]) have been investigated as targets for intratumoural T_{reg} cells. Another example of targeting immunosuppressive cells in the TME, TAMs in particular, is antagonizing the CSF-1 receptor (CSF-1R). The CSF-1R is a member of the receptor protein tyrosine kinase family of growth factor receptors that is expressed by TAMs and MDSCs[116]. Inhibition of CSF-1R has been shown to substantially deplete TAMs and increase the $CD8^+$:$CD4^+$ T cell ratio in mouse models and has demonstrated efficacy in patients with diffuse-type giant cell tumours[108]. In pancreatic cancer models, CSF-1R inhibition resulted in increased expression of immune checkpoints, PD-L1 and CTLA-4, and targeting of PD-1 and CTLA-4 demonstrated superior antitumour efficacy to CSF-1R inhibition alone when used in

combination with CSF-1R inhibition[117]. The utility of CSF-1R in augmenting the antitumour immune response was recapitulated in a study showing its efficacy in combination with GVAX and anti-PD-1 therapy in a liver metastatic mouse model of PDAC[118]. Antibody-based and small molecule inhibition of CSF-1R strategies are actively being investigated clinically in multiple cancer types including PDAC[116].

Another approach to reprogramming immunosuppressive cells in the TME is to target CD40, a costimulatory molecule present on antigen-presenting cells including macrophages. Targeting CD40 with an antibody has been shown to induce TAMs to express higher levels of CD86 and MHC class II molecules and to be tumoricidal against PDAC in KPC mice[119]. Anti-CD40 therapy was also associated with substantial stromal degradation[119], establishing CD40 as another integrated therapeutic target that can modify the TME. Early-phase clinical trials for PDAC of an anti-CD40 antibody with gemcitabine and with or without nivolumab (an anti-PD-1 monoclonal antibody) are ongoing and showing promising results[120]. Other modes of enhancing co-stimulation include targeting STING[121,122] and ICOS[123] signalling. An agonist cellular vaccine of the STING pathway demonstrated anticancer efficacy in multiple murine models of cancer including a metastatic pancreatic cancer model (Panc02)[121]. A STING vaccine, MK-1454, is currently being tested in a phase I clinical trial in lymphoma and solid tumours including PDAC with an overall acceptable safety profile and encouraging efficacy (NCT03010176)[122]. Targeting the ICOS pathway has shown generally promising results in other cancer types with regards to tolerability and efficacy and has been reviewed elsewhere[124]. The use of KY1044, a fully human antibody that depletes $ICOS^{high}$ T_{reg} cells while stimulating $ICOS^{low}$ effector T cells, in solid tumours including PDAC is actively being investigated (NCT03829501)[123].

Modulation of chemokine signalling is another approach to altering the immune TME. CCR2, expressed on myeloid cells, interacts with the multi-functional ligand CCL2 (as well as CCL3 and CCL5) to recruit monocytes into the TME[125]. In mouse models of PDAC, small molecule inhibitors of CCR2 led to blockade of TAM infiltration and improved resistance against tumour progression[125]. This change in the TME also potentiated anti-PD-1 therapy[126]. Thus far, early phase trials using the CCR2 inhibitors PF-04136309 and CCX872 in combination with conventional chemotherapy regimens in patients with PDAC have had varying results (NCT02732938, NCT01413022 and NCT02345408)[127–129]. A phase Ib study of PF-04136309 in combination with gemcitabine and nab-paclitaxel raised concerns about pulmonary toxicity and did not show superior efficacy compared with gemcitabine and nab-paclitaxel alone (NCT02732938)[129], but more encouraging results were reported when both PF-04136409 and CCX872 were combined with FOLFIRINOX (NCT01413022 and NCT02345408)[127,128]. Myeloid-targeted effects of CCX872 were also noted as peripheral monocyte counts at baseline inversely correlated with overall survival (NCT02345408)[127].

The strategy of targeting CXCR2 was explored in order to inhibit TAN infiltration and demonstrated that small molecule inhibition of CXCR2 can abrogate PDAC metastasis, augment T cell infiltration and synergize with anti-PD-1 therapy to extend survival[130]. An orthotopic PDAC model also showed that the chemotherapy response could be enhanced by CXCR2 inhibition[131]. In light of these findings, a phase Ib/II clinical trial investigating

AZD5069, an oral small molecule inhibitor of CXCR2, in combination with the PD-L1 inhibitor durvalumab in patients with PDAC has just completed, demonstrating limited efficacy (with median PFS and overall survival durations of 1.6 months and 2.8 months, respectively) (NCT02583477). Additional studies incorporating CCR2 and CXCR2 inhibition into the treatment paradigm are warranted.

Other clinically investigated immune-oriented methods of targeting the PDAC microenvironment include the use of oncolytic viruses (adenovirus[132], reovirus[133] and herpes simplex virus 1 (REF.[134])), epigenetic modifiers[135] and bispecific antibodies against immune checkpoints[136] (FIG. 2; TABLE 1). A 2018 paper revealed that yet another immune-modulatory agent, pegylated IL-10 (pegilodecakin)[137], has failed to meet the primary end point of overall survival in a phase III trial for treating PDAC in combination with chemotherapy, but the search for the next novel effective therapy for PDAC is far from over; many of these approaches are just beginning to be tested in patients with PDAC. Additionally, even unsuccessful immunotherapeutic agents might benefit from more in-depth preclinical investigation, especially when being tested in novel combinations with other modalities.

Exploiting integrated targets of TME

Despite the aforementioned failures of TME-targeted therapies, exploiting the unique features within the PDAC microenvironment as therapeutic targets warrants further investigation. Prior experiences have made it clear that an entirely stroma-based or immune-oriented approach to treating PDAC is of limited benefit (FIG. 3). Instead, more effective remodelling of the TME might be achieved by building on these prior efforts and exploiting particular points of biological convergence.

Targeting a metabolic convergence to enable TME remodeling

Cancer cell metabolism can be described by the Warburg effect, in which cancer cells maintain high glycolytic activity in order to grow[138]. Cancer cells require glutamine to fuel the tricarboxylic acid cycle for continued anabolic metabolism[139]. Importantly, T cells depend on similar metabolic pathways for successful activation and proliferation[140]. Thus, cancer cells are able to divert the stroma into a tumour-promoting metabolic environment that hinders T cells from providing proper antitumour immune responses.

Using multiple mouse models, one study demonstrated that broad pharmacological blockade of glutamine metabolism enhanced antitumour immune response by augmenting the nutrient availability with which $CD8^+$ T cells can thrive and maintain an activated phenotype[141]. The use of anti-PD-1 ICIs in conjunction with a glutamine antagonist was superior to either therapy alone. Of note, glutamine blockade also led to a significant decrease in the activity of the hexosamine biosynthesis pathway[141]. This pathway is a source of uridine diphosphate N-acetylglucosamine[142], an important substrate for hyaluronan synthesis, and thus has an important role in nutrient sensing in the context of stroma generation[143,144].

Another study fully recapitulated this concept by showing that glutamine antagonism led to a reduction of hyaluronan in the TME, an increase in $CD8^+$ T cell infiltration and improved

sensitivity to anti-PD-1 therapy[145]. Other researchers demonstrated that IFNγ released from $CD8^+$ T cells downregulates the expression of SLC3A2 and SLC7A11, two subunits of the glutamate–cystine antiporter system x_c^-, and impairs the uptake of cystine by tumour cells[146]. Cystine is known to counteract lipid peroxidation, and this study demonstrated that lack of cystine induces tumour cell peroxidation and ferroptosis, another independent mechanism of tumour cell death.

These clear examples show how a point of biological convergence, as related to desmoplasia, cancer metabolism and the immune microenvironment, might be a target in order to optimize therapeutic strategies. A well-studied glutamine antagonist, 6-diazo-5-oxo-L-norleucine (DON), was limited by toxic effects in previous trials, but a novel prodrug form of DON has been developed and is under investigation[147]. Other metabolic targets including the adenosine-generating enzymes CD39 and CD73 (REF.[148]), creatine transporter SLC6A8 (REFS[149,150]) and tryptophan catabolic enzyme IDO1 (REF.[151]) are also under investigation (FIG. 2; TABLE 1).

TME remodelling by targeting the focal adhesion kinase pathway

Studies have established the importance of the focal adhesion kinase (FAK) signalling pathway in shaping the PDAC stroma[152,153]. FAK signalling has long been implicated in processes of wound healing and pathologic fibrosis across various organs[154–158]. FAK has a mechanosensing role in propagating activating signals toward tissue fibrosis within fibrogenic cells such as cardiac myocytes and fibroblasts following stretching or loading[157,159].

In direct relevance to cancer cells, FAK overexpression has been well established as a feature of PDAC, and inhibition of FAK has been shown to suppress pancreatic cancer cell growth, survival and spread[160–165]. Accumulating evidence suggests the importance of FAK in integrating cell–cell or cell–matrix interaction signals with immunomodulation. Specifically, FAK functions downstream of αvβ3 integrin to positively regulate interferon signalling towards expression of PD-L1 upon binding of αvβ3 integrin to ECM[166]. Also within cancer cells, FAK signalling primes a more immunosuppressive TME via recruitment of T_{reg} cells via transcriptional activation of Ccl5 expression[167]. This pathway is bolstered by FAK-induced expression and nuclear translocation of IL-33 (REF.[168]).

Leveraging the biology that converges on FAK signalling, a 2016 study demonstrated that small molecule inhibition of FAK resulted in significantly suppressed tumour growth and increased survival of KPC mice in association with decreased stromal fibrosis and reduced presence of immunosuppressive cells within the TME[152]. Notably, this change in the TME was maximized by combining FAK inhibition with anti-PD-1 therapy, signifying a successful reprogramming of the TME towards immune-responsiveness. Nevertheless, a follow-up study showed that as FAK inhibition led to progressive fibroblast depletion, eventual loss of TGFβ production by the stroma conferred resistance against FAK inhibition through decreased suppression of the STAT3 signalling pathway[153]. As such, the importance of understanding the downstream effects of any therapeutic strategy and how these effects affect the TME cannot be overstated. At least three FAK inhibitors have been clinically tested in PDAC, one of which — defactinib — is being actively studied in PDAC in

combination with the PD-1 inhibitor pembrolizumab (NCT03727880 and NCT02546531)[169–171].

Disrupting TGFβ signalling in the TME

TGFβ is a pleiotropic molecule that generates both tumour-promoting and antitumour effects. Although TGFβ initially suppresses epithelial cell proliferation, it promotes stromal support of cancer and immunosuppression[172]. With regards to immunosuppression, TGFβ induces T_{reg} cells and directly represses several effector T cell functions[172].

In patients with metastatic urothelial cancer, high levels of TGFβ predicted poor response to anti-PD-Ll therapy[173]. Accordingly, inhibition of TGFβ enhanced the actions of ICIs in several mouse models including the KPC model of PDAC[173–175]. In a mouse model of liver metastatic PDAC, combining anti-TGFβ therapy with GVAX was also able to reshape the immune TME with greater infiltration of $CD8^+$ T cells and reduction of T_{reg} cells in association with better survival[176].

Galunisertib, a small molecule inhibitor of TGFβ, has been tested in patients with unresectable PDAC. A randomized phase II trial demonstrated that galunisertib in combination with gemcitabine led to improved overall survival versus gemcitabine alone[177]. The combination of galunisertib and durvalumab was also investigated in patients with metastatic PDAC[178]. Further investigation of galunisertib has since been terminated by the sponsor[179]. Instead, newer generation TGFβ pathway inhibitors, such as TGFβR[180] inhibitors and TGFβ-checkpoint traps[181], are being developed. In addition, as blockade of the angiotensin II type I receptor leads to reduced TGFβ levels in fibroblasts[182,183], the angiotensin receptor blocker losartan was tested both in preclinical models of pancreatic cancer[184] and subsequently in a phase II trial in the neoadjuvant setting in combination with FOLFIRINOX and enabled 69% (30 of 49) of patients with locally advanced disease to have an R0 resection[185]. A randomized phase II trial assessing the effect of losartan in combination with FOLFIRINOX and stereotactic body radiation therapy again in the neoadjuvant setting is ongoing (NCT03563248). Given the multi-faceted nature of TGFβ, the clinical outcome of targeting TGFβ in PDAC is difficult to predict and might depend on how TGFβ inhibition is combined with other modalities. More studies are needed to clarify the utility of TGFβ in treating PDAC.

Conclusions

Accumulating evidence illustrates the importance of understanding the multi-faceted roles of the complex TME components in tumour suppression and progression. Future approaches should therefore prioritize integrated or convergent targets that would reprogramme the TME rather than deplete particular targets. Combination and/or multi-modal strategies that target multiple features of the TME simultaneously might also be successful (FIG. 3). Nevertheless, combination approaches must take into account the complementarity of the targeted pathways. When developing novel therapeutic strategies we should investigate whether there are TME features that are organ specific and should be considered when treating metastatic cancers (for example, differences of primary versus hepatic versus lung). In addition, we should be aware of the feedback responses that occur with any treatment and

consider how they should be leveraged in a combinatorial fashion, taking into account how the preceding conventional chemotherapy and/or radiation will affect the efficacy of a therapeutic strategy of interest. Another important point to consider is how we can inform therapeutic decisions on the basis of individualized characterization of the TME in an individual patient. Given the relatively low incidence of PDACs, future trials should involve deep profiling of the TME and personalization of therapeutics when possible to accelerate progress towards more effective treatment strategies. This goal is ever closer to becoming a reality as multiplexed imaging, immunophenotyping and mutational analysis tools are increasingly high-throughput. Many trials have failed, but given the progress in our understanding of the PDAC microenvironment and the emerging strategies we have reasons to be hopeful for the future successful treatment of pancreatic cancers.

Acknowledgements

W.J.H. is the recipient of an ASCO Young Investigator Award and an AACR Incyte Immuno-Oncology Research Fellowship, and is supported by NIH T32CA00971-38.

Competing interests

W.J.H. could potentially receive patent-related royalties from Rodeo Therapeutics. E.M.J. receives commercial research grants from Aduro Biotech, Amgen, Bristol–Myers Squibb, Corvus and Hertix, has ownership interest in Aduro Biotech, and is a consultant and/or advisory board member for Achilles Therapeutics, Adaptive Biotechnologies, CStone Pharmaceuticals, Dragonfly Therapeutics, Genocea and the Parker Institute for Cancer Immunotherapy. She is a member of the National Cancer Advisory Board and the Chief Medical Advisor for the Lustgarten Foundation. L.Z. receives grant support from Amgen, Bristol–Myers Squibb, Halozyme, Inxmed, iTeos, Merck and NovaRock, and received royalties for licensing GVAX to Aduro Biotech. He is a paid consultant and/or advisory board member for Akrevia, Alphamab, Biosion, Da tare vive, Found atio n Med icin e, F usun Biopharmaceutical, Mingruzhiyao, NovaRock and Sound Biologics, and holds shares in Alphamab and Mingruzhiyao.

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Box 1 | Limitations in preclinical assessment of novel therapies

Studying therapies that target features within the tumour microenvironment (TME) requires that 1) the cancer resides within an intact biological stroma (that is, in vivo tissue space) and 2) the cancer is recognized by the surrounding stroma and the immune system as self. Therefore, the most ideal models for preclinical testing of TME-targeted therapies consist of syngeneic transplantation of cancer cells or sporadic models of carcinogenesis rather than any in vitro culture systems or xenograft models. Early studies in the 1980s commonly used a mouse model of pancreatic cancer that was generated in C57BL/6 mice using a local implantation of the carcinogen 3-methylcholanthrene[186]. The cell line established from this model, Panc02, can be syngeneically transplanted to assess therapeutics. Given the method of carcinogenesis, the Panc02 cell line, unsurprisingly, harbours numerous mutations (586 missense, 19 stop gains and 32 indels)[187]. While Panc02 exhibits a stop-gain mutation in *Smad4* it does not have mutations in *Kras* or *Tp53* (REF.[187]). Based on these genetic differences between Panc02 and the majority of human pancreatic ductal adenocarcinomas that bear *KRAS* and *TP53* mutations[188], successful translation of the findings in syngeneic models based on Panc02 are limited. Nonetheless, one of the first proof-of-concept animal models demonstrating the benefit of STING agonist vaccination in cancer was the Panc02 model[121].

To overcome the limitations of the Panc02 model, genetically engineered mouse models were developed in which $Kras^{G12D}$ and $Tp53^{R172H}$ mutations were inserted under Cre recombinase expression driven by the pancreas-specific promoter *Pdx1* (the ‘KPC model’)[189]. In fact, most of the TME-oriented studies that have led to clinical trials in pancreatic cancer in the past decade have utilized the KPC model[16,41,47,66,119,152,174]. In all of these studies, however, the most common method of assessing therapeutic efficacy has been to begin therapy at the time when ultrasound shows that a minimum tumour size is reached at the primary pancreatic site (for example, 5–10 mm in diameter). The mice are then followed for survival and maximal reduction of tumour size via ultrasound measurements. Although this method is perhaps the most accurate way to recapitulate the real-life heterogeneity in disease progression and metastatic spread, the model fails to emulate how most of the therapies are tested in clinical trials in which patients are enrolled with metastatic disease and very commonly after prior lines of therapy. Many studies have demonstrated that the TME in the primary site is different from that of metastatic sites and that prior therapy reprogrammes the tumour[13,51,53,54]. Furthermore, reliable biological or molecular correlates are often not defined during the preclinical stage as it is challenging to do so. Without defining key correlates a priori, additional development of or gain of insight from the failed therapy becomes even more limited. To improve the chances of translational success of preclinical findings, it might be preferable, in some cases, to design the preclinical model to mimic the disease state in which the therapy is to be tested. One such example is a murine pancreatic tumour model in which metastatic liver disease is modelled by the intraportal injection of KPC cells via the splenic vasculature[190]. Alternatively, neoadjuvant trial paradigms in humans enable evaluation of an intact TME and enable a deeper understanding of the effects of therapy,

allowing ‘reverse’ translations (that is, analysis of clinical correlates informing preclinical target or therapy development).

Key points

- Therapeutic approaches to target stromal desmoplasia, a histopathological hallmark of pancreatic ductal adenocarcinoma, have classically focused on depleting the stromal constituents; results have been generally disappointing, owing to the multi-faceted nature of tumour stroma.
- Isolated strategies to overcome specific immune targets have also met with limited success, likely owing to the presence of multiple immunoregulatory pathways within the pancreatic ductal adenocarcinoma microenvironment.
- In recognition of the functional complexity of the tumour microenvironment (TME), combining complementary stromal-targeted and immune-targeted treatment modalities to leverage the changes in the TME offers a more rational treatment approach.
- Points of biological convergence, such as stromal–immune crosstalk, including glutamine metabolism, focal adhesion kinase and transforming growth factor-β signalling, are promising targets for remodelling the TME into an antitumour milieu.

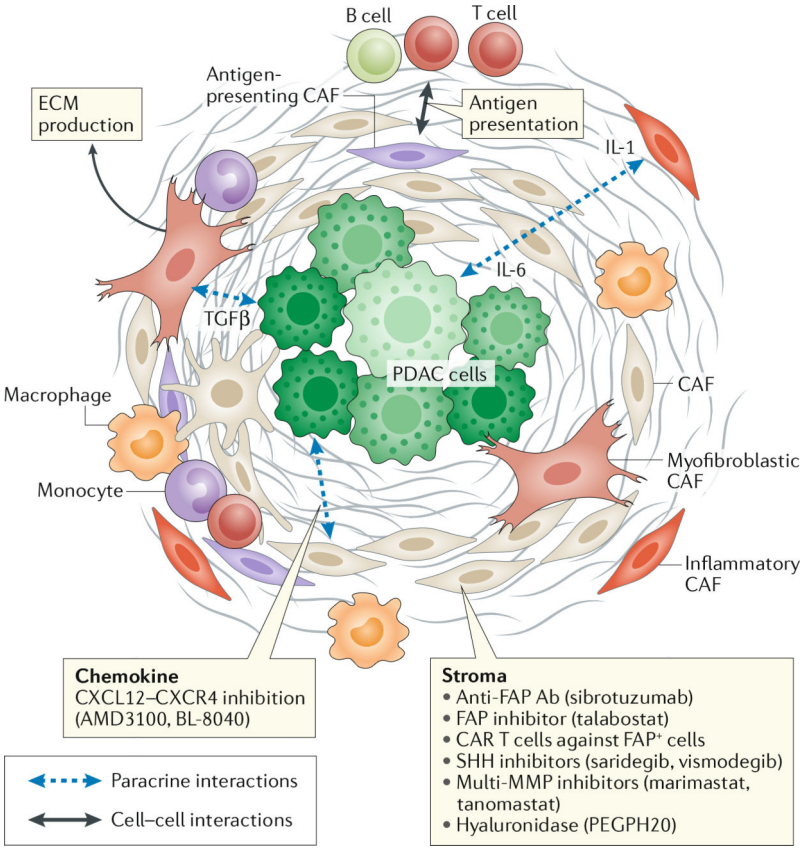

Fig. 1 |. Targeting PDAC-associated stroma.

The role of the stroma to either promote or resist tumour formation and progression is influenced by the surrounding signals. Both cell–cell and paracrine interactions between cancer-associated fibroblasts (CAFs) and cancer cells are involved in programming the stroma. CAFs, key constituents of the pancreatic ductal adenocarcinoma (PDAC) stroma, are heterogeneous, and include myofibroblastic, inflammatory and antigen-presenting subtypes. Fibroblasts in proximity to cancer cells are induced by transforming growth factor-β (TGFβ) from cancer cells into myofibroblastic CAFs, producing the mechanical barrier that can be both tumour promoting and antitumour. Inflammatory CAFs, located in the stroma away from the cancer cells, are reprogrammed by cancer-secreted IL-1 to produce cytokines and chemokines (for example, IL-6), which further promote cancer growth. The subsequently developed antigen-presenting CAFs, which express MHC class II molecules, modulate the immune cells in the stroma. Approaches to deconstruct the stroma have included the use of matrix metalloproteinase (MMP) inhibitors, hyaluronidase, Sonic hedgehog (SHH) inhibitors, fibroblast activation protein (FAP) targeting agents and CXCR4 inhibitors. Ab, antibody; CAR, chimeric antigen receptor; ECM, extracellular matrix.

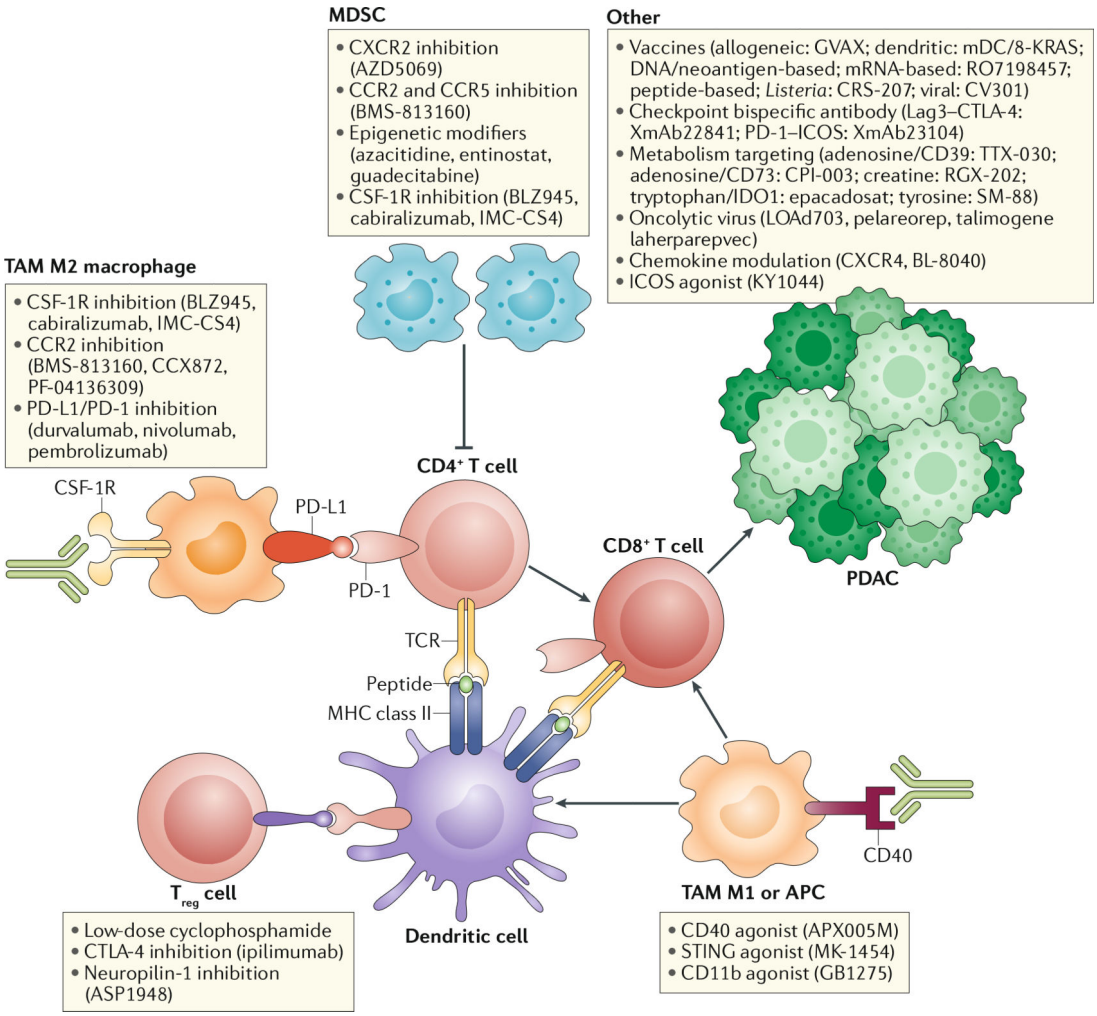

Fig. 2 |. Myeloid and T_{reg} targeting strategies to treat PDAC.

Antigen-presenting machinery relying on dendritic cells or inflammatory macrophages (TAM M1) and supported by helper T cells ($CD4^+$ T cells) steers the antitumour immune response to eliminate pancreatic ductal adenocarcinoma (PDAC) — for example, via cytotoxic T cells ($CD8^+$ T cells). However, myeloid-derived suppressor cells (MDSCs), anti-inflammatory tumour-associated macrophages (TAM M2), and regulatory T (T_{reg}) cells regulate these processes via several inhibitory pathways, establishing an immunosuppressive tumour microenvironment. Many strategies to abrogate or overcome these immunological targets have been proposed. Clinically tested approaches are listed in the corresponding boxes with the specific types and names of the agents in parentheses. APC, antigen-presenting cell; CSF-1R, colony-stimulating factor-1 receptor; CTLA-4, cytotoxic T lymphocyte antigen 4; GVAX, granulocyte–macrophage colony-stimulating factor secreting allogeneic pancreatic tumour cell vaccine; TCR, T cell receptor.

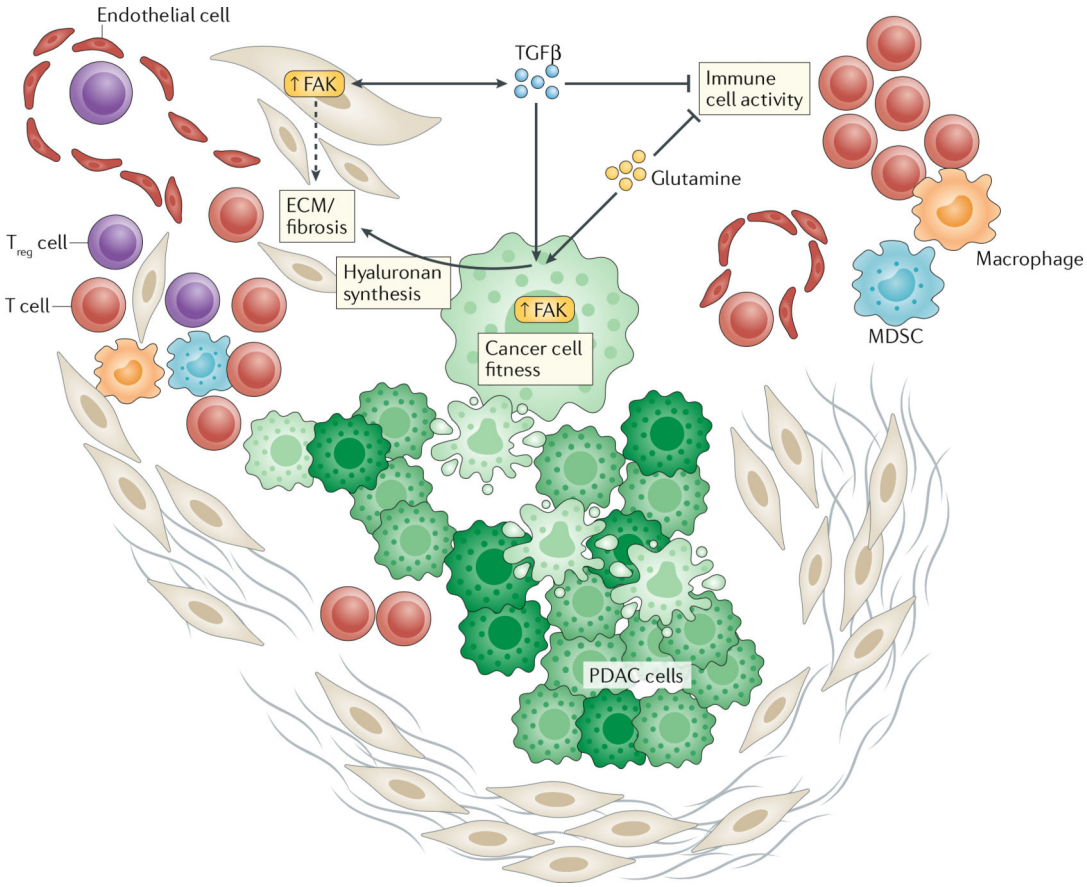

Fig. 3 |. Remodelling the PDAC microenvironment.

Pancreatic ductal adenocarcinoma (PDAC) is classically surrounded by desmoplastic stroma composed of cancer-associated fibroblasts and extracellular matrix (ECM). The stroma provides a dense mechanical barrier (both antitumour and tumour promoting) against vascularization, immune cell trafficking and cancer invasiveness. The tumour microenvironment is also characterized by the presence of multiple immunosuppressive pathways. Exploiting biologically integrated targets of the stroma (such as glutamine metabolism, transforming growth factor-β (TGFβ) and focal adhesion kinase (FAK) signalling) and the immunosuppressive pathways is the most likely approach to remodel the tumour microenvironment into an effective antitumour environment. MDSC, myeloid-derived suppressor cell; T_{reg} cell, regulatory T cell.

Table 1 |

Clinically investigated novel immune-modulating agents for PDAC

Category	Mechanism	Agent	Clinical trial	Phase	Notes
Chemokine inhibition	CCR2 and CCR5 inhibitor (small molecule)	BMS-813160	NCT03184870	I/II	With chemotherapy or anti-PD-1 (nivolumab)
	CCR2 inhibitor (small molecule)	CCX872	NCT02345408	Ib	Monotherapy
		PF-04136309	NCT02732938	Ib/II	With chemotherapy
	CXCR2 inhibitor (small molecule)	AZD5069	NCT02583477	I/II	With anti-PD-L1 (durvalumab)
	CXCR4 inhibitor (small molecule)	Motixafortide (BL-8040)	NCT02826486	II	With chemotherapy and anti-PD-1 (pembrolizumab)
Epigenetic modifier	Hypomethylating agent	Azacitidine	NCT01845805	II	Adjuvant setting
	DNMT inhibitor	Guadecitabine	NCT03257761	I	With anti-PD-L1 (durvalumab)
	HDAC inhibitor	Entinostat	NCT03760614	I	With chemotherapy
Cytokine	Pegylated IL-10	Pegilodecakin	NCT02923921	III	With chemotherapy
Checkpoint inhibition	Bispecific LAG3–CTLA-4 Ab	XmAb22841	NCT03849469	I	With anti-PD-1 (pembrolizumab)
	Bispecific PD-1–ICOS Ab	XmAb23104	NCT03752398	I	Monotherapy
Co-stimulator agonism	CD40 agonist (mAb)	APX005M	NCT03214250	I/II	With chemotherapy and anti-PD-1 (nivolumab)
	STING agonist (mAb)	MK-1454	NCT03010176	I	With anti-PD-1 (pembrolizumab)
	ICOS agonist	KY1044	NCT03829501	I/II	With anti-PD-L1 (atezolizumab)
Myeloid-specific agents	CSF-1R inhibitor (small molecule)	BLZ945	NCT02829723	I/II	With anti-PD-1 (spartalizumab)
	CSF-1R inhibitor (mAb)	Cabiralizumab	NCT03336216	II	With chemotherapy and anti-PD-1 (nivolumab)
		IMC-CS4	NCT03153410	I	With GVAX, low-dose Cy and anti-PD-1 (pembrolizumab)
	CD11b agonist (mAb)	GB1275	NCT04060342	I/II	With chemotherapy or anti-PD-1 (pembrolizumab)
Vaccines	Allogeneic GM-CSF-secreting cells	GVAX	NCT03190265	II	With GVAX, low-dose Cy and anti-PD-1 (nivolumab) and anti-CTLA4 (ipilimumab)
	Listeria-based vaccine	CRS-207			
	Dendritic cell-based vaccine	mDC3/8-KRAS	NCT03592888	I	Adjuvant setting
	Personalized DNA (neoantigen)		NCT03122106	I	Adjuvant setting
	Personalized mRNA	RO7198457	NCT03289962	I	With anti-PD-L1 (atezolizumab)
	Based on two recombinant poxviruses	CV301	NCT03376659	I/II	With anti-PD-L1 (durvalumab)
Metabolism targeting	Adenosine-generating enzyme CD39 (mAb)	TTX-030	NCT03884556	I	With chemotherapy or anti-PD-1 (pembrolizumab)
	Adenosine-generating enzyme CD73 (mAb)	CPI-006	NCT03454451	I	With anti-PD-1 (pembrolizumab)
	SLC6A8 (creatine transporter) inhibitor	RGX-202	NCT03597581	I	With chemotherapy

Category	Mechanism	Agent	Clinical trial	Phase	Notes
	Tyrosine hydroxylase inhibitor	Racemetyrosine (SM-88)	NCT03512756	II/III	With chemotherapy
	IDO1 inhibitor	Epacadostat	NCT03006302	II	With GVAX, low-dose Cy and anti-PD-1 (pembrolizumab)
Oncolytic virus	Adenovirus (intratumoural injection)	LOAd703	NCT02705196	I/II	With chemotherapy and anti-PD-L1 (atezolizumab)
	Reovirus (intravenous injection)	Pelareorep	NCT03723915	II	With anti-PD-1 (pembrolizumab)
	Type I herpes simplex virus (intratumoural injection)	Talimogene laherparepvec	NCT03086642	I	Monotherapy

Ab, antibody; CSF-1R, colony-stimulating factor-1 receptor; CTLA-4, cytotoxic T lymphocyte antigen 4; Cy, cyclophosphamide; DNMT, DNA methyltransferase; GM-CSF, granulocyte–macrophage colony-stimulating factor; GVAX, GM-CSF secreting allogeneic pancreatic tumour cell vaccine; HDAC, histone deacetylase; mAb, monoclonal antibody; PDAC, pancreatic ductal adenocarcinoma.